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IgE-mediated allergy and cigarette smoking enhance the generation of leukotrienes B₄ (LTB₄) and C₄ (LTC₄) by leucocytes and bronchial hyperresponsiveness in patients with atopic asthma

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Abstract : Influences of IgE-mediated allergy and cigarette smoking on pathophysiology, evaluated by bronchial hyperresponsiveness and the generation of LTB₄ and LTC₄, of asthma were examined in 69 patients with asthma sensitive to inhalant allergens such as house dust mite and *Candida albicans*.

1. Bronchial hyperresponsiveness was significantly higher in previous and current smokers of asthmatics than in never-smokers. 2. The generation of leukotrienes B₄ (LTB₄) and C₄ (LTC₄) by leucocytes was significantly larger in patients with serum IgE over 350 IU/ml than in those with serum IgE less than 150 IU/ml (LTB₄ : $p < 0.01$, LTC₄ : $p < 0.05$). 3. Long term glucocorticoid regimen suppressed the generation of LTC₄, but not bronchial hyperresponsiveness and LTB₄ generation. 4. Bronchial hyperresponsiveness was not significantly correlated with patient age in these patients with atopic asthma. The results demonstrate that IgE-mediated allergy significantly enhances the generation of LTB₄ and LTC₄ by leucocytes, and cigarette smoking significantly enhances bronchial hyperresponsiveness in patients with atopic asthma.

Key words : IgE-mediated allergy, bronchial hyperresponsiveness, LTB₄, LTC₄, asthma, cigarette smoking

Introduction

Asthma is clinically classified into two types, atopic and non-atopic, on the basis of the presence or absence, respectively, of IgE-mediated reactions^{1,2}. Atopic asthma is caused by IgE-mediated allergy, which is clinically evaluated by serum IgE levels, skin reactivity, radio-allergosorbent test (RAST) score for inhalant allergens, basophil histamine release^{3,4} and bronchial challenge test with corresponding allergens. Patients with atopic asthma often have a family history of allergic disease, suggesting that IgE-mediated allergy is closely related to a family history of allergic diseases.

Bronchial hyperresponsiveness is one of the characteristics demonstrating the pathophysiology of asthma. Our previous studies have shown that bronchial hyperresponsiveness decreased significantly as age at onset increased in patients without a family history, and further that responsiveness was significantly higher in patients who were >60 years of age at onset who had a family history than in those who did not⁵.

It has been shown that relationship between smoking and allergy is complex⁶. During the first three years of life, both prenatal and post-natal exposure to environmental tobacco smoke (ETS) appears to have an adjuvant effect on allergic sensitization⁷⁻⁹. Exposure to cigarette smoke increases sensitization to food allergens in a few years of life¹⁰, but not associated with sensitization to inhaled allergens^{10,11}.

In the present study, influences of IgE-mediated allergy and cigarette smoking on bronchial hyperresponsiveness and the generation of leukotrienes B₄ (LTB₄) and C₄ (LTC₄) by peripheral leucocytes were studied in patients with atopic asthma.

Subjects and Methods

The subjects of this study were 69 patients (28 females and 41 males, mean age 64.3 years) with atopic asthma showing a positive RAST score for inhalant allergens such as house dust mite and *Candida albicans*. Of them, 28 patients were previous and current smokers (all males, mean age 66.2 years, who had a history of smoking with 40.5 pack-years) and residual 41 were never-smokers. Further, 21 patients had long-term glucocorticoid regimen for more than 10 years.

Five milliliters 6% dextran (molecular weight 200,000 kDa) (Nacalai Tesque Inc.) were added to 20 mL heparinized peripheral blood, and the resultant mixture incubated for 1 h at room temperature. The density was adjusted to 5×10^6 cells \cdot mL⁻¹, and the cells were then stimulated with 1 μ g calcium ionophore A23187. LTC₄ and LTB₄ were quantified by means of high-performance liquid chromatography, as described by Lam et al¹².

Bronchial responsiveness to methacholine was assessed in all subjects. Respiratory resistance (Rrs) during continuous inhalation of methacholine in stepwise increments was measured using a TCK 6100 Astograph (Chest Co)¹³. Methacholine was serially diluted two-fold with saline (in 10 dose steps from 25 mg \cdot mL⁻¹ to 49 μ g \cdot mL⁻¹) and prepared for bronchial challenge, which proceeded according to the method of Chai et al.¹⁴. The increase in Rrs after methacholine inhalation was measured using the forced oscillation technique. The minimum dose of methacholine (Cmin) inducing bronchoconstriction was used as an indicator of bronchial hyperresponsiveness.

Serum IgE was measured by radioimmunosorbent test (RIST), and IgE antibodies specific to aeroallergens including house dust mite, pollens and moulds were measured using 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

Bronchial hyperresponsiveness was not significantly correlated with serum IgE levels. In contrast, cigarette smoking influenced bronchial hyperresponsiveness: the responsiveness was significantly higher in previous and current smokers of asthmatics with serum IgE less than 150 IU/ml and between 151 and 350 IU/ml than in never-smokers. However, in patients with serum IgE over 351 IU/ml, a significant difference was not found between ex-smokers and never-smokers (Fig. 1).

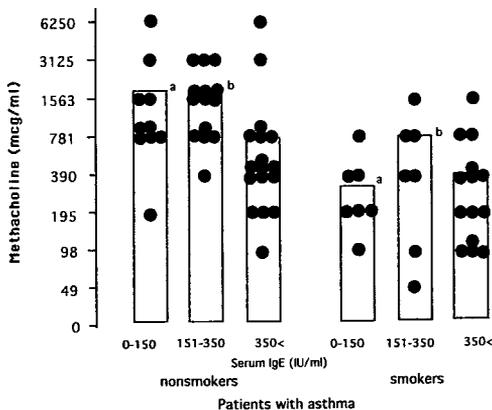


Fig.1. Comparison between bronchial hyperresponsiveness to methacholine and serum IgE level in patients with asthma in relation to cigarette smoking. a: $p < 0.001$, b: $p < 0.01$.

The generation of LTB₄ was in general larger in ex-smokers than in never-smokers, however, there was not a significant difference in LTB₄ generation between ex-smokers and never-smokers. The LTB₄ generation significantly increased as serum IgE level increased in never-smokers, and the amount of LTB₄ was significantly larger in patients with serum IgE over 351 IU/ml compared with the amount in those with serum IgE less than 150 IU/ml ($p < 0.01$) and

between 151 and 350 ($p < 0.02$). However, the LTB₄ generation was not correlated with serum IgE levels in ex-smokers (Fig. 2).

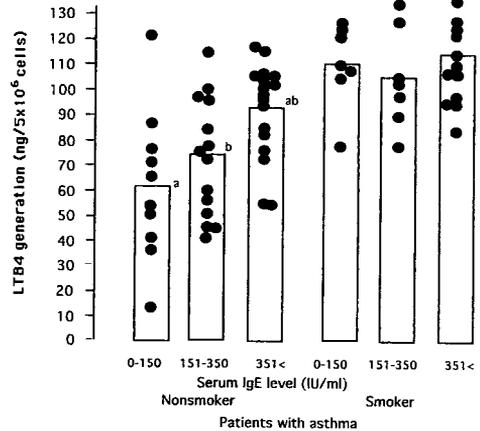


Fig.2. Correlation between leukotriene B₄ generation by leucocytes and serum IgE level in patients with asthma in relation to cigarette smoking. a: $p < 0.01$, b: $p < 0.02$.

The generation of LTC₄ by leucocytes was significantly higher in patients with serum IgE levels over 351 IU/ml than in patients less than 150 IU/ml of serum IgE ($p < 0.05$) and in those between 151 and 350 IU/ml ($p < 0.05$). A significant difference was not found in LTC₄ generation between ex-smokers and never-smokers (Fig. 3).

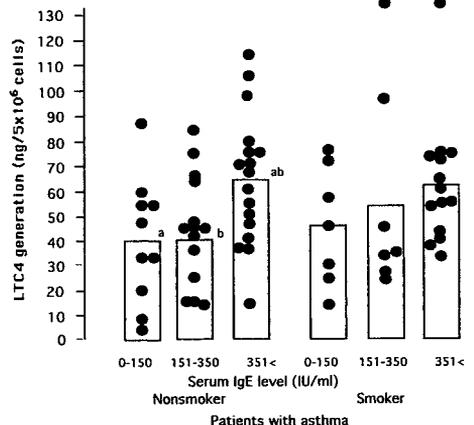


Fig.3. Correlation between leukotriene C₄ generation by leucocytes and serum IgE level in patients with asthma in relation to cigarette smoking. a and b: $p < 0.05$.

The LTC₄ generation was significantly lower in ex-smokers with long-term glucocorticoid regimen than in those without glucocorticoid therapy, but the difference in LTC₄ generation between patients with and without long-term glucocorticoid regimen was not significant in never-smokers. Influences of long-term glucocorticoid regimen were not observed in bronchial hyperresponsiveness and LTB₄ generation (Table 1).

Table 1. Effects of long-term glucocorticoid regimen on bronchial hyperresponsiveness, and LTB₄, LTC₄ generation in patients with asthma

	Long-term glucocorticoid regimen		Difference
	-	+	
Nonsmoker			
No	28	13	
Prednisolone (mg/day)	4.8 ± 2.1 ^a	-	
Bronchial hyperresponsiveness*	1168 ^b	1709 ^c	NS
LTB ₄ generation (ng/5x10 ⁶ cells)	82.5	69.1	NS
LTC ₄ generation (ng/5x10 ⁶ cells)	61.5	30.8	p<0.001
Smoker			
No	20	8	
Prednisolone (mg/day)	8.8 ± 3.2 ^a	-	
Bronchial hyperresponsiveness*	457 ^b	298 ^c	NS
LTB ₄ generation (ng/5x10 ⁶ cells)	115.2	111.8	NS
LTC ₄ generation (ng/5x10 ⁶ cells)	66.1	50.1	NS

a:p<0.01, b:p<0.05, and c:p<0.02. *Bronchial hyperresponsiveness to methacholine (μg / ml). NS: not significant

A significant correlation was not observed between bronchial hyperresponsiveness and patient age in both ex-smokers and never-smokers, as shown in Fig. 4 and Fig. 5.

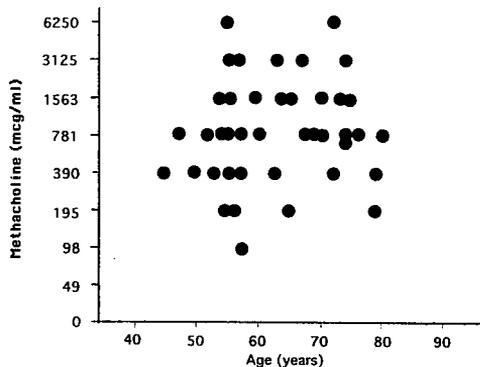


Fig. 4. Correlation between bronchial hyperresponsiveness to methacholine and age in nonsmokers of patients with asthma.

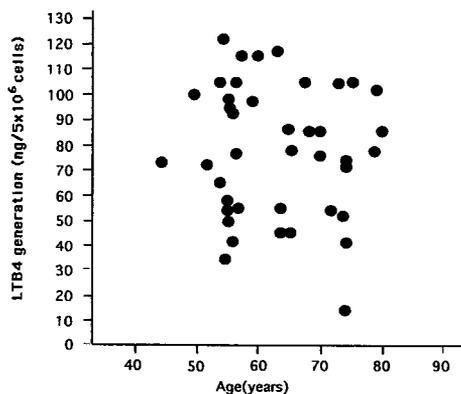


Fig. 5. Correlation between leukotriene B₄ generation by leucocytes and age in nonsmokers of patients with asthma

Discussion

In atopic asthma, IgE-mediated allergy play an important role in pathogenesis of the disease. Our previous studies demonstrate that the leucocytes of patients with atopic asthma generated significantly more LTC₄ than those of patients with nonatopic asthma, suggesting that LTC₄ production is more closely related to IgE-mediated allergy than to other asthmatic reaction¹⁵⁾. Further, it has been shown that the leucocytes of patients with relatively more severe hyperresponsiveness to methacholine produced significantly more LTB₄ than those of patients who are less hyperresponsive. Fujimura et al. showed that interleukin-8 induces bronchial hyperresponsiveness as well as airway neutrophil accumulation in guinea-pigs in vivo, and that this may be partly mediated by the release of LTB₄. Although hyperresponsiveness to methacholine also significantly correlated with leukotriene C₄, this correlation was weaker than that between methacholine hyperresponsiveness and LTB₄ production.

In the present study, influences of IgE-mediated allergy, cigarette smoking, and long-term regimen of glucocorticoids, as one of the agents

influencing the pathophysiology of asthma, were examined in patients with atopic asthma. At first, serum IgE levels were to a certain extent correlated with the generation of LTB₄ and LTC₄ in never-smokers of asthmatics, but not in ex-smokers. Furthermore, Serum IgE levels were not correlated with bronchial hyperresponsiveness. The results suggest that cigarette smoking influences the generation of LTB₄ and LTC₄ enhanced by IgE-mediated allergy. Socondarily, it was demanstrated in this study that cigarette smoking enhanced bronchial hyperresponsiveness, and the hyperresponsiveness was significantly higher in ex-smokers of asthmatics with serum IgE less than 150 IU/ml and between 151 and 350 IU/ml compared with hyperresponsiveness in never-smokers. However, a significant difference in bronchial hyperresponsiveness between ex-smokers and never-smokers was not observed in patients with serum IgE over 351 IU/ml. The results reveal that the influence of smoking on bronchial hyperresponsiveness was smaller than the influence of IgE-mediated allergy. The amount of LTB₄ and LTC₄ generated by leucocytes appeared to be larger in ex-smokers than in never-smokers, but this difference was not significant. Thirdly, long-term glucocorticoid regimen suppressed the generation of LTC₄ in never-smokers of asthmatics, but not in ex-smokers. The regimen of glucocorticoids was not correlated with bronchial hyperresponsiveness. The results obtained here suggest that IgE-mediated allergy and cigarette smoking enhances the generation of LTB₄ and LTC₄, and brronchial hyperresponsiveness, which is closely related to an increase in the generation of LTB₄.

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IgE系アレルギー反応はアトピー型気管支喘息の白血球ロイコトリエンB4 (LTB4) およびC4 (LTC4) 産生を促進させ、喫煙は気道過敏性を亢進させる

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ハウスダストあるいはカンジダ抗原に対して特異的IgE抗体が陽性を示す気管支喘息69例を対象に, IgE系反応と喫煙の, 気道過敏性および白血球の

LTB4, LTC4産生に対する影響について検討を加えた。1. 気道過敏性は, 非喫煙者に比べ喫煙者でより高度であることが明らかにされた。2. 白血球のLTB4, LTC4産生は, 血清IgE値が351単位/ml以上の非喫煙症例で150単位以下および151 - 350単位/mlの症例に比べ有意に高い値を示した (LTB4 : $p < 0.01$, LTC4 : $p < 0.05$)。3. 長期間の副腎皮質ホルモン投与は, LTC4産生を抑制したが, 気道過敏性およびLTB4産生には影響しなかった。4. 今回のようなアトピー型喘息では, 気道過敏性と年齢の間には有意の相関は見られなかった。以上の結果より, アトピー喘息では, IgE系反応は白血球のLTB4, LTC4産生を促進させ, また喫煙は気道過敏性を促進させることが明らかになった。