◎原 著

The role of leukotrienes B4 (LTB4) and C4 (LTC4) in pathophysiology of asthma in the elderly. Relationship to bronchial hyperresponsiveness and FEV1.0%

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Abstract: The generation of leukotrienes B4 (LTB4) and C4 (LTC4) by leucocytes stimulated with Ca ionophore A23187 was examined in 67 patients with asthma. 1. The generation of leukotriene B4 (LTB4) by leucocytes was significantly more increased in patients with asthma than in those with pulmonary emphysema (PE) and healthy subjects. The generation of leukotriene C4 (LTC4) was also signficantly more increased in patients with asthma compared to the generation in those with PE and healthy subjects. The generation of both LTB4 and LTC4 was significantly more increased in patients with PE than in healthy subjects. 2. The generation of both LTB4 and LTC4 was larger in patients with asthma over age 70 than in those of other age groups, however, the difference was not significant. 3. The generation of LTC4 was significantly higher in attack stage than in non-attack stage in all age groups of patients with asthma, however, the generation of LTB4 was not significantly different between the two stages. 4. Bronchial hyperresponsiveness to methacholine in patients with asthma tended to decrease with aging, and the bronchial hyperresponsiveness was to a certain extent correlated with the generation of LTB4, but not LTC4. 5. The generation of LTC4 was correlated with value of FEV1.0%.

These results may suggest that both LTB4 and LTC4 participate in the pathophysiology of asthma in the elderly.

Key words: asthma, LTB4, LTC4, FEV1.0%, bronchial hyperresponsiveness

Introduction

Leukotrienes (LTB4 and cysLTs) are potent pro-inflammatory mediators related to pathophysiological changes of the airways in asthma. LTB4 is mainly generated by neutrophils[1]. LTB4 stimulate neutrophil chemotaxis and activation of the cells, andselectively increases the number and percentage of neutrophils in the human lung[2]. It has been demonstrated that neutrophil inflammation enhances bronchial hyperresponsiveness[3-5]. Our previous studies have shown that a significant correlation between LTB4 generation by leucocytes and the degree of bronchial hyperresponsiveness to methacholine is observed in patients with asthma[5]. Cysteinyl leukotrienes (cysLTs) are mainly produced by eosinophils[6], and the amount of cysLTs generated is related to the eosinophil activation state[7]. CysLTs display bronchoconstrictory effects[8], increase mucus formation[9], and bronchial wall edema[10].

Our previous studies have shown that enhanced leukotriene generation from peripheral leukocytes is observed in patients with COPD, and the presence of specific IgE antibodies against inhalant allergens enhances LTC4 generation, bronchial hyperresponsiveness, and the relationship between LTC4 generation and airway obstruction[11]. Our studies have also shown that increased generation of LTC4 is suppressed by perilla seed oil (α -linolenic acid) supplementation[12,13].

In this study, the role of LTB4 and LTC4 was observed in elderly patients with asthma in relation to asthmatic cycle, bronchial hyperresponsiveness, and FEV1.0%.

Subjects and Methods

The subjects of this study is 67 patients with asthma (40 females and 37 males, mean age 62.1 years, range 27-86 years), 17 with pulmonary emphysema (all males, 68.8years, 56-81 years) and 20 healthy subjects (11 females and 9 males, 51.7 years, 32-91 years). Asthma was diagnosed according to the criteria of the American Thoracic Society (ATS)[14]. The subjects were divided into 4 groups according to patient age : <49, 50-59, 60-69, and 70+ years.

The generation of leukotrienes, LTB4 and LTC4, by peripheral leucocytes was assessed by a method peviously reported[6,11]. Buffy coat was separated by adding a quarter volume of 6% dextran and followed by being left 1 hour at room temperature. After the number of the cells was adjusted to $5x10^6$/ml in Tris ACM, Ca ionophore A23187 (1 μg) was added to the cell suspension. The mixed solution was incubated for 15 min at 37℃, and centrifuged at 3000 rpm for 30 min after the addition of 4 times volume of pre-chilled ethanol (finally 80% ethanol). Supernatant was taken into the syringe filter (Toyo Roshi Co, Japan), and the filtrate was decompressed and dryed up to solid. The solid was dissolved with 250 μl of 50% ethanol. The HPLC analysis for LTB4 and LTC4 was performed by a method described by Lam, et al.[15]. The results were expressed as ng/$5x10^6$ cells.

Bronchial reactivity to methacholine was measured by an Astograph (TCK 6100, Chest Co). Different concentrations of methacholine (49, 98, 195, 390, 781, 1563, 3125, 6250, 12500 and 25000 μg/ml) were prepared for bronchial challenge according to the method used by Chai et al.[16]. The increase of total respiratory resistance (Rrs) after methacholine

inhalation was measured by the oscillation method[17]. A mthacholine concentration causing a significant increase in Rrs was assessed as Cmin (minimum concentration). All medications were stopped 12 hours prior to examination.

Ventilatory function tests using a Box Spiror 81-S (Chest Co, Japan) were carried out in all subjects when they were attack-free.

IgE antibodies against inhalant allergens, house dust mite, cockroach, and Candida albicans, were estimated by radioallergosorbent test (RA ST), snd serum level of IgE was measured by radioimmunosorbent tset (RIST).

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

Table 1 shows the characteristics of patients with asthma studied here. The mean level of serum IgE was higher in patients under age 49 compared with the level in other age groups. However, the differences were not significant. The frequency of patients with a positive RAST against inhalant allergens was more than 50% in all age groups.

Table 1. Characteristics of patients with asthma studied

Age, years	No of patients	Mean age years	IgE (IU/ml)	positive* RAST(%)
<49	8	40.2	1201	6/8(75.0%)
50-59	20	55.7	392	20/15(75.0%)
60-69	21	62.6	339	11/21(52.4%)
70+	18	74.0	390	11/18(61.0%)

*positive RAST score against inhalant allergens

The generation of LTB4 and LTC4 was compared among healthy subjects, patients with pulmonary emphysema (PE), and those with asthma. The LTB4 and LTC4 generation was significantly larger in patients with asthma than in those with PE and healthy subjects. The generation of both LTB4 and LTC4 in patients with PE was significantly higher than the generation in healthy subjects (Fig. 1).

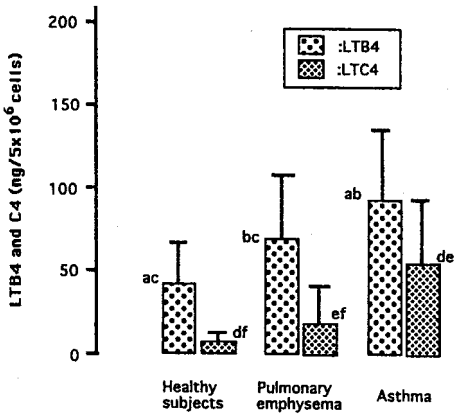

Fig.1. Generation of LTB4 and LTC4 by leukocytes in healthy subjects, patients with pulmonary emphysema, and those with asthma. a and d:p<0.001, b and e:p<0.01, c and f:p<0.02.

The generation of LTB4 by leucocytes was the highest (96.2 ±32.8 ng/5x10^6 cells) in patients with asthma over age 70, however, there were not significantly differences among four age groups (Fig. 2). The LTC4 generation

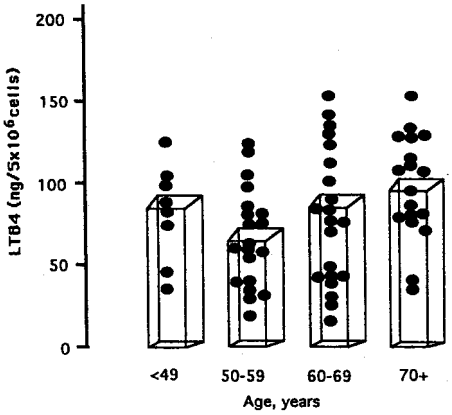

Fig.2. Generation of LTB4 by leukocytes in patients with asthma

by leucocytes was also the highest (57.6±48.

9 ng/5x10^6 cells) in elderly patients over age 70 compared with the generation in other age groups (Fig. 3).

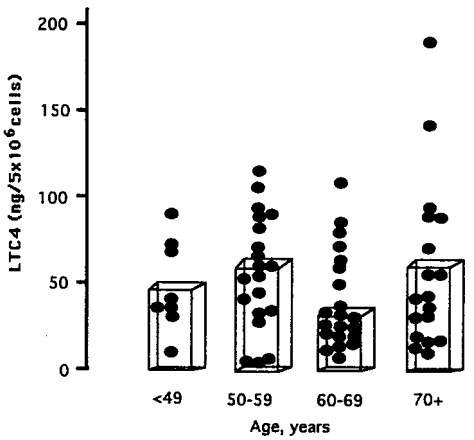

Fig.3. Generation of LTC4 by leukocytes in patients with asthma

The generation of LTB4 during attack stages was larger than the generation in non-attack stages in patients under age 49. The same tendency as the generation in these patients was observed in other age groups. However, the difference in LTB4 generation between attack and non-attack stages was not significant in all age groups (Fig. 4). In contrast, the generation

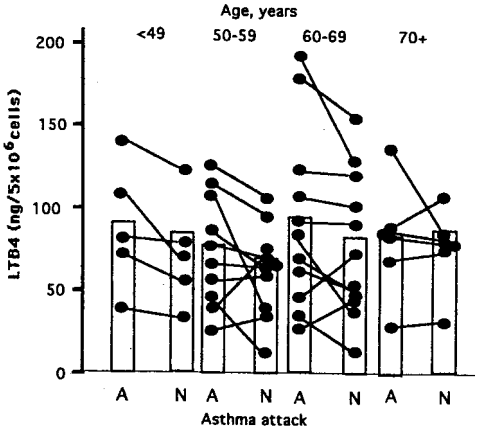

Fig.4. Generation of LTB4 by leukocytes in patients with asthma in relation to asthmatic cycle. A:attack stage, N: non-attack stage

of LTC4 during attack stages was significantly larger than the generation in non-attack stages in all age groups (Fig. 5).

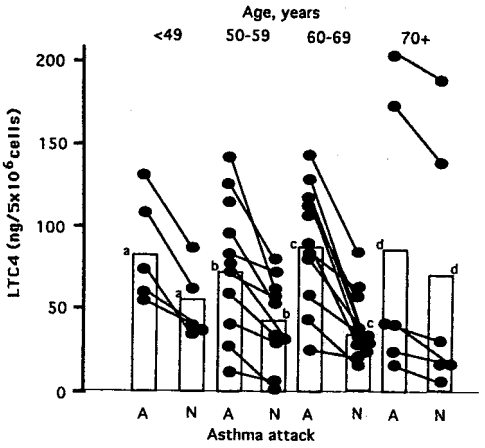

Fig.5. Generation of LTC4 by leukocytes in patients with asthma in relation to asthmatic cycle. A:attack stage, B:non-attack stage. a and b:p<0.02, c:p<0.001, d:p<0.05.

Bronchial hyperresponsiveness (BH) to methacholine showed a tendency to decrease with aging, as shown in Fig. 6. A correlation between

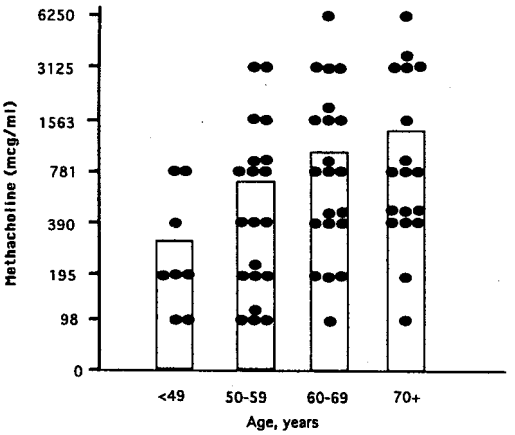

Fig.6. Bronchial hyperresponsiveness to methacholine in patients with asthma

BH to methacholine and the generation of LTB4 by leucocytes was found in patients with asthma. Only 2 (11.8%) of 17 patients with BH to methacholine more than 1563 mcg/ml showed an increased generation of LTB4 more than 100 ng/6x10^6 cells. In contrast, 16 (45.7%) of 35 patients with BH to methacholine less than 390

mcg/ml showed an increased LTB4 generation more than 100 ng/5x10^6 cells (Fig. 7) Any significant correlation was not observed between

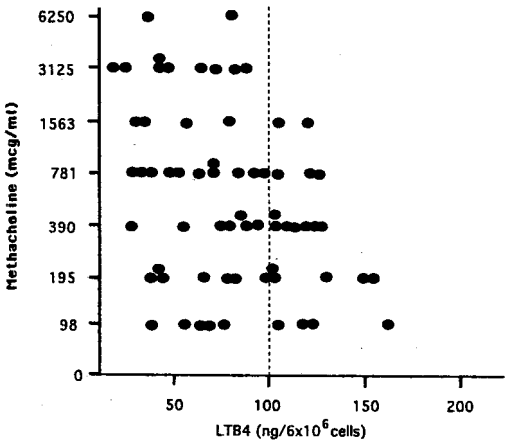

Fig.7. Correlation between bronchial hyperresponsiveness to methacholine and the generation of LTB4 by leucocytes in patients with asthma

LTC4 generation and BH to methacholine, as shown in Fig. 8.

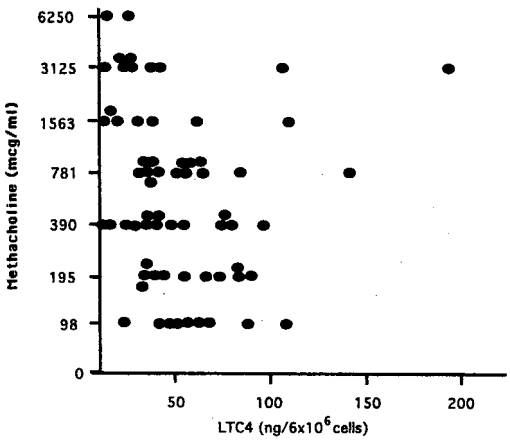

Fig.8. Correlation between bronchial hyperresponsiveness to methacholine and the generation of LTC4 by leucocytes in patients with asthma

The LTB4 generation was not related to the value of FEV1.0% in patients with asthma (Fig.9).

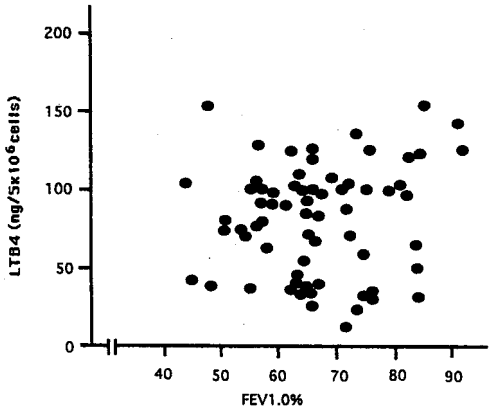

Fig.9. Correlation between the generation of LTB4 by leucocytes and FEV1.0% in patients with asthma

While an increased LTC4 generation was to a certain extent related to the value of FEV1.0% in patients with asthma : 10 (83.3%) of 12 asthmatics with an increased generation of LTC4 more than 80 ng/5x10^6 cells showed the value of FEV1.0% less than 70% (Fig. 10).

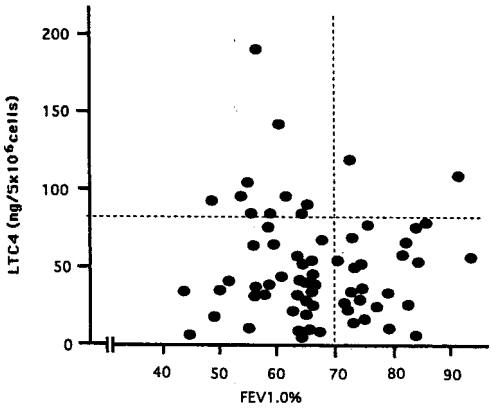

Fig.10. Correlation between the generation of LTC4 by leucocytes and FEV1.0% in patients with asthma

Discussion

It has been well known that leukotriene B4 and cysLTs, LTC4, LTD4 and LTE4, play an important role in pathophysiology of the airways of bronchial asthma. LTB4, which is mainly produced by neutrophils, has a chemotactic action for neutrophils as well as interleukin 8 (IL8).

In contrast, LTC4, which is almostly generated by eosinophils[3,4], has bronchoconstrictory action[8], increase mucus formation[9], and induces accumulation of eosinophils into the airways[18].

In the present study, the role of LTB4 and LTC4 in the elderly patients with asthma was examined in relation to asthmatic cycle, bronchial hyperresponsiveness, and the value of FEV1.0%. The generation of both LTB4 and LTC4 by leucocytes was significantly higher in patients with asthma and in those with PE than in healthy subjects. The results shows the possibility that both LTB4 and LTC4 participate in the pathophysiology of asthma and pulmonary emphysema.

The generation of LTB4 by leucocytes was the largest in patients over age 70 compared to the generation in other age groups. Although the difference in the LTB4 generation among four age groups was not significant, the generation of LTB4 was considerably large even in patients over age 70. The LTC4 generation in patients over age 70 was also considerably large compared to the generation in those under age 49. These results demonstarate that both LTB4 and LTC4 participate in the onset mechanism of asthma in the elderly. Regarding the LTB4 and LTC4 generation relating to asthmatic cycle, a significant difference was found in the LTC4 generation between attack and non-attack stages : a significant increase in LTC4 generation was observed during asthma attacks. However, there was no significant difference in LTB4 generation between attack and non-attack stages. The results suggest that LTC4 is closely related to asthma attcks associated with bronchoconstriction.

Bronchial hyperresponsiveness to methacholine was related to a certain extent to the LTB4 generation by leucocytes in patients with asthma, but not to the LTC4 generation. Our previous studies have shown that leukotriene C4 production by leucocytes is associated with immunoglobulin E-mediated allergy and asthma exacerbations, and further that generation of LTB4 is closely related to bronchial hyperresponsiveness to methacholine in patients with asthma[5]. The results suggest that an increased production of LTB4 by leucocytes is closely related to an increase in bronchial hyperresponsiveness.

The FEV1.0% value was related to a certain extent to LTC4 generation in patients with an increased production of LTC4, but not to LTB4 production, suggesting that LTC4 is corrrelated with bronchoconstriction. These results obtained here show that LTB4 and LTC4 play an important role in asthma in the elderly.

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高齢者気管支喘息の臨床病態におけるロイコトリエンＢ４，Ｃ４の役割．気道過敏性とFEV1.0%との関連

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気管支喘息67例を対象に，Ca ionophore A 23187刺激時の白血球からのロイコトリエンＢ４（LTB4）およびＣ４（LTC4）の産生を観察し，高齢者喘息の臨床病態におけるLTB4，LTC4の役割について検討した。1．LTB4の産生は，気管支喘息において肺気腫および健康人に比べ有意に高い値を示した。LTC4の産生も同様に気管支喘息において，肺気腫，健康人に比べ有意に高い値を示した。LTB4，LTC4いずれの産生も，肺気腫症例において健康人と比べ有意に高い値が観察された。2．LTB4，LTC4の産生は，70才以上の喘息症例において，他の年齢層の症例に比べより高い値が示されたが，推計学的な有意差は見られなかった。3．高齢者を含めいずれの年齢層の気管支喘息においても，LTC4の産生は発作時に，非発作時に比べ有意の亢進が見られたが，LTB4に関しては発作時，非発作時の間に有意の差は見られなかった。4．気道の過敏性は，年齢が高くなるにつれて低下する傾向が見られた。そして，この気道の過敏性はLTB4産生とある程度関連していることが示されたが，LTC4との関連は見られなかった。5．FEV1.0%値は，ある程度LTC4産生亢進と関連があることが示された。

以上の結果より，70才以上の喘息においても，LTB4，LTC4がその発作時病態に重要な役割を果していることが示唆された。