◎原著

Leukotrienes B4 and C4 generation by peripheral leucocytes in patients with asthma

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Summary: The generation of leukotrienes B4 (LTB4) and C4 (LTC4) by leucocytes stimulated with Ca ionophore A23187 was examined in 16 patients with asthma (8 with atopic and 8 with nonatopic asthma) and 12 healthy controls. 1. The LTB4 generation by leucocytes was not significantly different between patients with asthma and healthy controls. The generation of LTC4 was significantly larger in patients with asthma than in healthy controls. The LTC4 generation was also significantly larger in patients with attacks (58.4 ± 38.5 ng/5x10^6 cells) than in those without attacks (23.3 ± 25.9 ng/5x10^6 cells)($p<0.05$). 2. In atopic asthma, the LTC4 production was significantly larger in patients with attacks (84.7 ± 35.4 ng/5x10^6 cells) compared to the production in those without attacks (40.4 ± 27.2 ng/5x10^6 cells)($p<0.02$). However, the production of LTB4 was not significantly different between attack and attack-free stages. 3. In nonatopic asthma, the LTC4 production was also significantly higher in patients with attacks (32.2 ± 26.3 ng/5x10^6 cells) than in those without attacks (12.2 ± 3.5 ng/5x10^6 cells)($p<0.05$). However, the LTB4 production was not significantly different between attack and nonattack stages. 4. The LTC4 production was significantly larger in atopic asthmatics compared to the production in nonatopic subjects both in attack and nonattack stages. These results suggest that the generation of LTC4 by leucocytes of patients with asthma is closely related to IgE-mediated reaction and asthma attacks.

key words: bronchial asthma, LTB4, LTC4, IgE-mediated reaction, asthma attacks

Introduction

Asthma is characterized by airway inflammation. Antigen challenge produces both early and late asthmatic response[1)]. The late asthmatic reaction is closely related to airway inflammation[2,3)]. Inflammatory cells

such as lymphocytes, neutrophils and eosinophils, and a number of cytokines including leukotrienes released from these cells participate in the late asthmatic reaction. Among inflammatory cells, activated T lymphocytes and eosinophils play an important role in induction and persistence of the reaction[4].

Leukotrienes are potent pro-inflammatory mediators contributing to pathophysiological changes of the airways in asthma. Cysteinyl leukotrienes (cysLTs) display bronchoconstrictory effects[5], increase mucus formation[6] and bronchial wall edema[7]. The cysLTs are mainly generated by eosinophils in the late asthmatic reaction[8]. The amount of cysLTs produced is related to the eosinophil activation state[9]. Leukotriene B4 (LTB4) stimulates neutrophil chemotaxis and activation of the cells, leading to the release of mediators, emzymes, and superoxides[10]. LTB4 selectively increases the number and percentage of neutrophils in the human lung[11]. It has been shown that neutrophil inflammation enhances bronchial hyperreponsiveness[12,13]. LTB4 is mainly generated by neutrophils. Preincubation of human neutrophils with granulocyte/macrophage-stimulating factor (GM-CSF) results in a modest increase in LTB4 production in response to the chemotactic peptide[14].

In the present study, generation of LTC4 and LTB4 by peripheral leucocytes stimulated with Ca ionophore A 23187 was examined in patients with asthma.

Subjects and Methods

The subjects of this study was 16 patients (12 females and 4 males) with asthma and 12 healthy subjects (7 females and 5 males, mean age 56.2 years). The mean age of patients with asthma was 62.8 years (range 52-74 years) and mean level of serum IgE was 211 IU/ml (range 61-835 IU/ml). Asthma was diagnosed according to the criteria of the American Thoracic Society (ATS)[15]. Among 16 subjects with asthma, 8 were atopic, as shown by a positive RAST score for inhalant allergens, and 8 were nonatopic, whose mean serum IgE level was under 200 IU/ml and RAST score for inhalant allergens was all negative.

The generation of leukotrienes, LTC4 and LTB4, by peripheral leukocytes was assessed by a method previously reported[16]. 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 μ g) 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 dried up to solid. The solid was dissolved with 250 μ l of 50% ethanol. The HPLC analysis for LTB4 and LTC4 was performed by a method described by Lam, et al[17]. The results were expressed as ng/$5x10^6$ cells.

Pulmonary function test was carried out in all patients using a Chestac 33 (Chest Co, Japan) linked to a computer, when they were attack-free.

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

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

Results

The generation of LTB4 by leucocytes stimulated with Ca ionophore A23187 was higher in patients with asthma despite the presence and absence of attacks than in healthy controls. However, there was no significant difference in LTB4 generation between patients with asthma and healthy controls. The generation of LTC4 by leucocytes was significantly larger in asthma patients with (58.4 ± 38.5 ng/$5x10^6$ cells) (mean $\pm$ SD)($p<0.001$) and without attacks (23.3 ± 25.9 ng/$5x10^6$ cells) ($p<0.05$) compared to the genera-

tion in healthy subjects (4.4. ± 4.7 ng/5x10^6 cells). The LTC4 generation was also significantly larger in patients with attacks than in those at attack-free stage ($p<0.05$), as shown in Fig. 1.

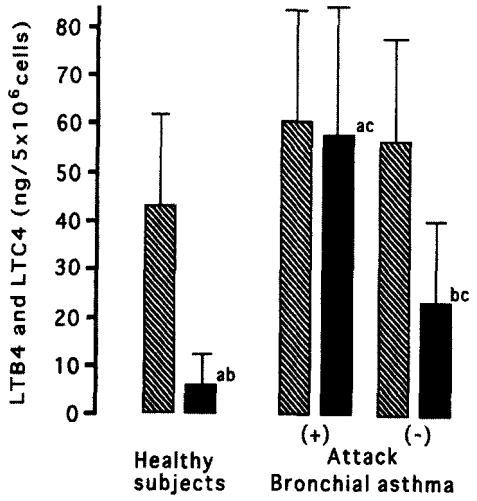

Fig.1. Generation of leukotrienes B4 (LTB4)(▨) and C4 (LTC4)(■) by leucocytes in healthy subjects and patients with asthma. a;$p<0.001$, b;$p<0.05$, c;$p<0.05$.

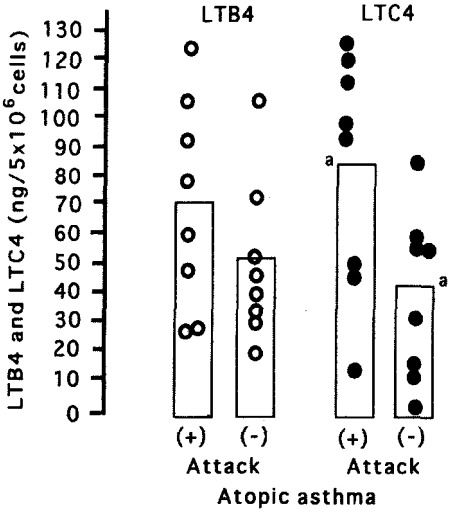

FIg.2. Generation of leukotrienes B4 (LTB4) and C4 (LTC4) by leucocytes in patients with atopic asthma. a;$p<0.02$.

In atopic asthma, the production of LTB4 by leucocytes was higher in patients with attacks than in those without attacks, however, this was not significant. The LTC4 production was significantly larger in asthmatics with attacks (84.7 ± 35.4 ng/5x10^6 cells) compared to the production in those without attacks (40.4 ± 27.2 ng/5x10^6 cells)($p<0.02$)(Fig.2). In patients with nonatopic asthma, there was no difference in LTB4 production between attack and attack-free stages. However, the LTC4 production in nonatopic asthma was also significantly larger in patients with attacks (32.2 ± 26.3 ng/5x10^6 cells) than in those without attacks (12.2 ± 3.5 ng/5x10^6 cells) ($p<0.05$)(Fig.3).

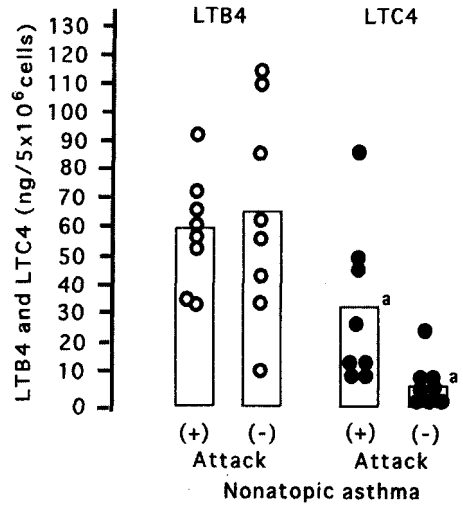

Fig.3. Generation of leukotrienes B4 (LTB4) and C4 (LTC4) by leucocytes in patients with nonatopic asthma.a;$p<0.05$.

There was significant difference in LTC4 generation between atopic and nonatopic subjects with asthma. During attack stages, the LTC4 generation was significantly larger in atopic asthmatics compared to the generation in nonatopic subjects ($p<0.001$). When they were attack-free, the generation of LTC4 was also significantly higher in atopic than in nonatopic asthmatics ($p<0.05$).

Discussion

Leukotriene B4 and cysLTs, LTC4, LTD4 and LTE4, play an important role in pathophysiology of the airways of bronchial asthma. A number of factors can influence LTB4 production as well as cysLTs. LTB4 has a chemotactic action for neutrophils as well as interleukin 8 (IL8), which causes bronchial hyperresponsiveness and airway neutrophil accumulation[18].

LTC4 production is almostly exclusively due to eosinophils[8,9,19]. Eosinophils appear to be important in asthma pathophysiology. Accumulation of the cells into the airways often associated with increased production of LTC4[20]. The amount of LTC4 production by eosinophils depends not only on the number of the cells but also on the degree of activation[21]. When antigen was challenged into the airways, the concentration of LTC4 incresed strongly correlated with the number of eosinophils migrated into the airways, suggesting that antigen causes recruitment and activation of the cells[22].

In the present study, the generation of LTB4 and LTC4 by leukocytes stimulated with Ca ionophore A 23187 was examined in patients with asthma in relation to asthma type and asthmatic cycle. IT has been shown that stimulation with ionophore A23187 induced a significantly higher leukotriene C4 generation from granulocytes of asthmatic children than from granulocytes of healthy controls[9]. They also demonstrated that granulocytes from patients with a history of severe asthma displayed a higher LTC4 formation than granulocytes from patients with less severe disease.

Our results also showed that the LTC4 generation was significantly larger in patients with asthma than in healthy controls. Furthermore, the generation of LTC4 by leucocytes was significantly higher in attack stage than in attack-free stage. The results are consistent with the data showing enhanced production of LTC4 by activated eosinophils during antigen challenge[22]. Regarding asthma type, the generation of LTC4 by leucocytes was significantly larger in atopic asthma compared to the generation in nonatopic disease in both attack and attack-free stages. An important role of interleukin 5 (IL-5) in IgE-mediated allergic reactions has been shown by several investigators[23,24]. Parallel patterns of increase of IL-5 and eosinophils imply the possibility of a bidirectional interaction between them[25,26]. These results mat suggest a possibility that LTC4 generation is more closely related to IgE-mediated reaction than to other reaction. However, LTB4 generation by leucocytes of patients with asthma was not significantly different between atopic and nonatopic asthma, and between attack and attack-free stage.

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気管支喘息における末梢血白血球のロイコトリエン B4 および C4 産生能

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気管支喘息 16 例（アトピー型喘息 8 例，非アトピー型喘息 8 例）を対象に，Ca ionophore A23187 刺激時の末梢血白血球のロイコトリエン B4 (LTB4) および C4 (LTC4) 産生能について検討した。1．LTB4 産生能は，喘息症例と健康人の間に有意の差は見られなかった。LTC4 産生能は，健康人に比べ喘息症例で有意に高い傾向が見られた。また，喘息症例では，非発作時 (23.3 ± 25.9ng/5x10^6 cells) に比べ発作時 (58.4 ± 38.5 ng/5x10^6 cells) に有意に高い値を示した ($p<0.05$)。アトピー型喘息では，非発作時 (40.4 ± 27.2ng/5x10^6 cells) に比べ発作時 (84.7 ± 35.4ng/5x10^6 cells) に有意の高値を示したが ($p<0.02$)，LTB4 産生に関しては両者間に有意の差は見られなかった。3．非アトピー型喘息においても同様，非発作時 (12.2 ± 3.2ng/5x10^6 cells) に比べ発作時 (32.2 ± 26.3ng/5x10^6 cells) ）で有意の高値であったが ($P<0.05$)，LTB4 では有意差は見られなかった。4．LTC4 産生能は，発作時、非発作時とも，アトピー型喘息で非アトピー型喘息に比べ有意に高いことが示唆された。

以上の結果より，喘息症例における白血球の LTC4 産生能は IgE に mediate される反応と密接な関連がある可能性が示唆された。