◎原 著

IgE-mediated allergy enhances and glucocorticoids inhibit the generation of leukotrienes B4 and C4 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 71 patients with asthma (42 with atopic and 29 with nonatopic asthma) and 23 healthy controls. Of these patients, 22 had SDIA (steroid-dependent intractable asthma). 1. The generation of LTB4 and LTC4 by leucocytes was significantly more enhanced in patients with atopic, non-SDIA asthma than in healthy subjects, but not in patients with nonatopic asthma. The generation of LTB4 and LTC4 in atopic asthma was significantly more decreased in patients with SDIA than in those with non-SDIA. 2. The LTC4 generation was significantly larger in attack stage than in attack-free stage of patients with atopic and nonatopic asthma when they had not SDIA. However, no significant difference was found in LTB4 generation between attack and nonattack stages in these patients. 3. In patients with SDIA, no significant differences were observed in the generation of LTB4 and LTC4 between attack and nonattack stages. The results suggest that IgE-mediated allergy and asthma attacks enhance and glucocorticoids inhibit the generation of LTB4 and LTC4 by leucocytes.

key words : bronchial asthma, LTB4, LTC4, IgE-mediated allergy, glucocorticoids

Introduction

Leukotrienes are potent pro-inflammatory mediators contributing to pathophysiological changes of the airways in asthma. Cysteinyl leukotrienes (cysLTs) display bronchoconstrictory effects[1], increase mucus formation[2] and bronchial wall edema[3]. The cysLTs are mainly generated by eosinophils in the late asthmatic reaction[4]. The amount of cysLTs produced is related to the eosinophil activation state[5]. Leukotriene B4 (LTB4) stimulates neutrophil chemotaxis and activation of the cells, leading to the release of

mediators, emzymes, and superoxides[6]. LTB4 selectively increases the number and percentage of neutrophils in the human lung[7]. It has been shown that neutrophil inflammation enhances bronchial hyperresponsiveness[8,9]. 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[10].

In the present study, generation of LTC4 and LTB4 by peripheral leucocytes stimulated with Ca ionophore A 23187 was examined in patients with asthma in relation to IgE-mediated allergy and glucocorticoid therapy.

Subjects and Methods

The subjects of this study was 71 patients (43 females and 28 males) with asthma and 23 healthy subjects (12 females and 11 males, mean age 54.3 years). The mean age of patients with asthma was 61.5 years (range 27-74 years) and mean level of serum IgE was 461 IU/ml (range 2−5195 IU/ml). Asthma was diagnosed according to the criteria of the American Thoracic Society (ATS)[11]. Among all subjects with asthma, 42 were atopic, as shown by a positive RAST score for inhalant allergens, and 29 were nonatopic, whose mean serum IgE level was under 250 IU/ml and RAST score for inhalant allergens was all negative. Twelve of 42 patients with atopic asthma and 10 of 29 with nonatoppic asthma had steroid-dependent intractable asthma (SDIA).

The generation of leukotrienes, LTC4 and LTB4, by peripheral leukocytes was assessed by a method previously reported[12]. 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 5×10^6/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 μ l of 50% ethanol. The HPLC analysis for LTB4 and LTC4 was performed by a method described by Lam, et al[13]. The results were expressed as ng/5×10^6 cells.

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

Table 1 shows the characteristics of patients with atopic and nonatopic asthma. The number of patients with positive RAST score for inhalant allergens was significantly larger in patients with atopic asthma than in those with nonatopic asthma. The mean of serum IgE was significantly higher in atopic asthma than in nonatopic. The generation of LTB4 by leucocytes stimulated with Ca ionophore A23187 was significantly higher in patients with atopic, non-SDIA (87.1 ± 43.9 ng/5×10^6 cells) (mean±SD) compared to the generation in healthy subjects (43.1 ±

24.2 ng／5 ×10^6 cells) ($p<0.001$). A significant difference was found in LTB4 generation between atopic subjects with SDIA and non-SDIA. However, there was no significant difference in LTB4 generation between patients with nonatopic asthma and healthy controls (Fig. 1).

The generation of LTC4 by leucocytes was significantly larger in patients with atopic, non-SDIA (79.3±27.0 ng／5 ×10^6 cells) than in healthy subjects (5.1±5.2 ng／5 ×10^6 cells) ($p<0.001$). The LTC4 generation in patients with atopic, non-SDIA was significantly higher in patients with atopic, SDIA ($p<0.001$), and in those with nonatopic, non-SDIA ($p<0.001$) and SDIA ($p<0.001$) (Fig. 2).

The generation of LTB4 and LTC4 by leucocytes was generally low in patients with SDIA, and there were no significant differences in the generation of LTB4 and LTC4 by leucocytes of these patients between attack and attack-free stages (Fig. 3,4). The generation of LTB4 and LTC4 by leucocytes of patients with non-SDIA was generally higher compared to the generation in those with SDIA. The LTC4 generation was significantly higher in attack stages than in attack-free stages of both atopic ($p<0.02$) (Fig. 5) and nonatopic asthma ($p<0.05$) (Fig. 6). However, no significant difference was present in the LTB4 generation between attack and attack-free stages.

Table 1. Characteristics of patients with atopic and nonatopic asthma

Asthma type	SDIA	Mean age (years)	RAST(+)	S-IgE(IU/ml)
Atopic	(+)	62.4	10/12 (83.3%)	525 [ab] (80-1187)
	(-)	60.1	28/30 (93.3%)	750 [cd] (40-5195)
Nonatopic	(+)	57.9	0/10	107 [ac] (2-215)
	(-)	65.2	0/19	139 [bd] (10-236)

SDIA: steroid-dependent intractable asthma, RAST(+); number of patients with positive RAST score for inhalant allergens. a; $p<0.001$, b and d; $p<0.01$, c; $p<0.05$.

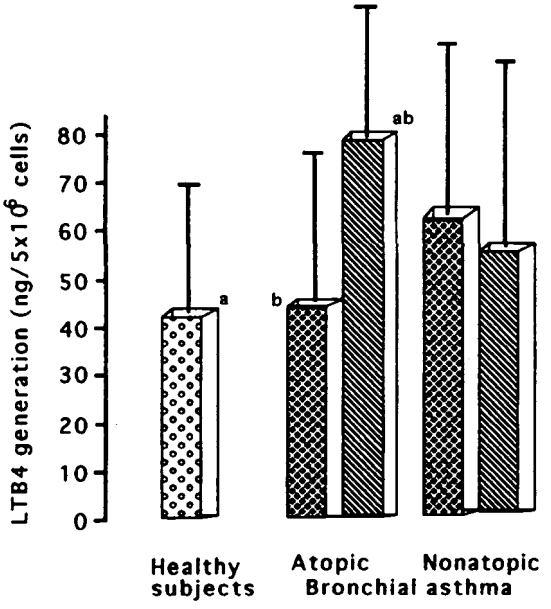

Fig. 1. Generation of leukotriene B4 (LTB4) by leucocytes in healthy subjects and atopic and nonatopic patients with non-SDIA (▨) and SDIA (■) at attck-free stage. a ; $p<0.001$, b ; $p<0.01$.

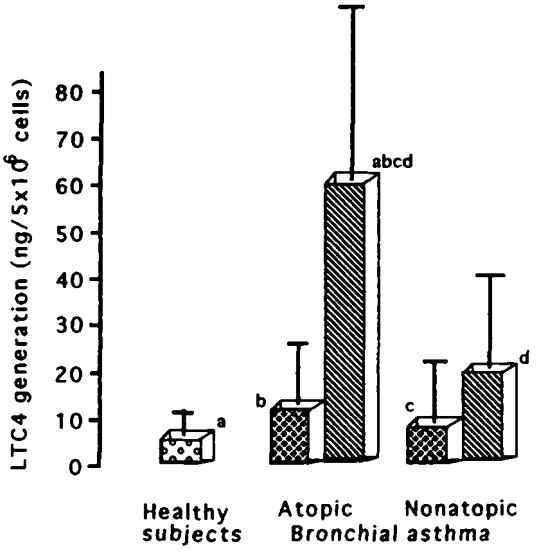

Fig. 2. Generation of leukotriene C 4 (LTC 4) by leucocytes in healthy subjects and atopic and nonatopic patients with non-SDIA (▨) and SDIA (▩) at attck-free stage. a, b, c, and d ; $p<0.001$

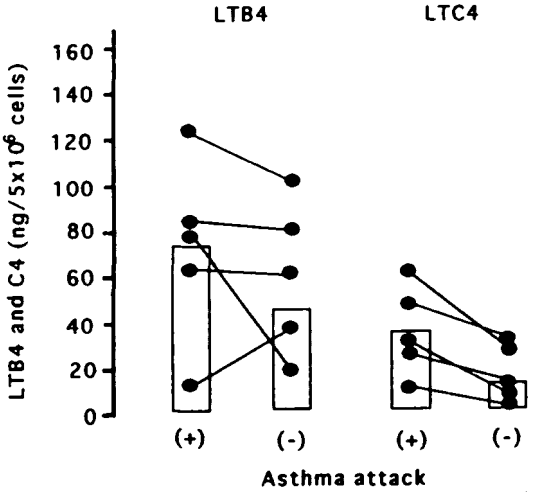

Fig. 3. Generation of leukotrienes B 4 (LTB 4) and C 4 (LTC 4) by leucocytes in patients with atopic SDIA in relation to asthmatic cycle.

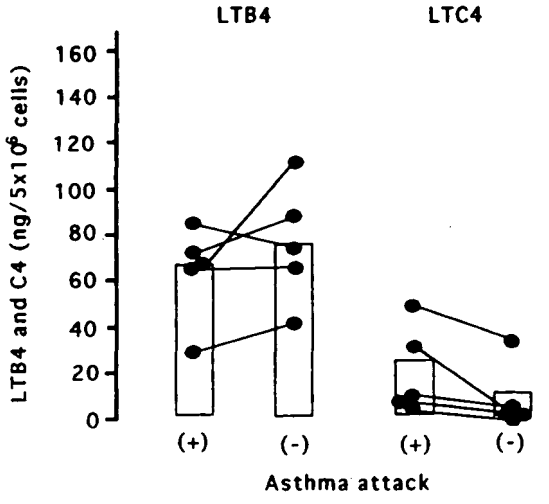

Fig. 4. Generation of leukotrienes B 4 (LTB 4) and C 4 (LTC 4) by leucocytes in patients with nonatopic SDIA in relation to asthmatic cycle.

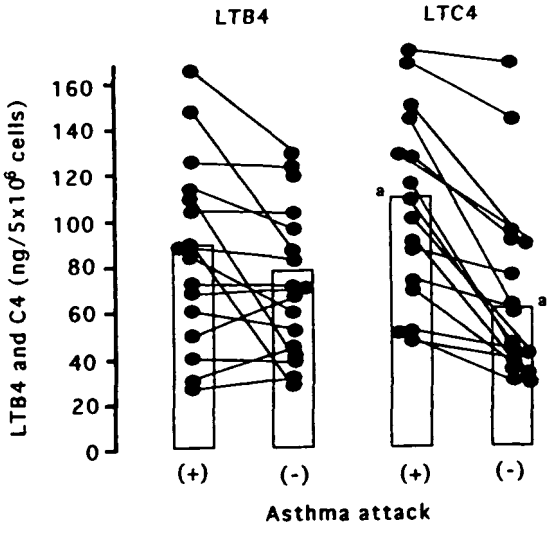

Fig. 5. Generation of leukotrienes B 4 (LTB 4) and C 4 (LTC 4) by leucocytes in patients with atopic non-SDIA in relation to asthmatic cycle. a ; $p<0.02$.

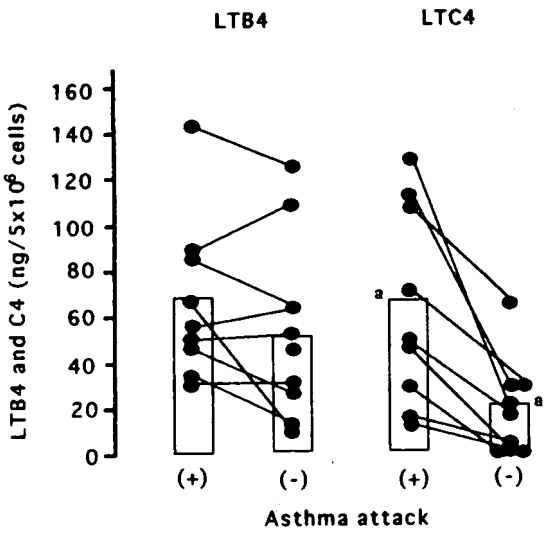

Fig. 6. Generation of leukotrienes B 4 (LTB 4) and C 4 (LTC 4) by leucocytes in patients with nonatopic non-SDIA in relation to asthmatic cycle. a ; $p < 0.05$.

Discussion

Asthma is characterized by inflammatory changes of the airways, in which 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[14].

Leukotriene B4 and cysLTs, LTC 4 , LTD 4 and LTE 4 , play an important role in pathophysiology of the airways of bronchial asthma. A number of factors can influence LTB 4 production as well as cysLTs. LTB 4 has a chemotactic action for neutrophils as well as interleukin 8 (IL 8), which causes bronchial hyperresponsiveness and airway neutrophil accumulation[15].

LTC4 production is almostly exclusively due to eosinophils[8, 9, 16]. Eosinophils appear to be important in asthma pathophysiology. Accumulation of the cells into the airways often associated with increased production of LTC 4 [17]. The amount of LTC 4 production by eosinophils depends not only on the number of the cells but also on the degree of activation[18]. When antigen was challenged into the airways, the concentration of LTC 4 increased strongly correlated with the number of eosinophils migrated into the airways, suggesting that antigen causes recruitment and activation of the cells[19].

In the present study, the generation of LTB 4 and LTC 4 by leucocytes stimulated with Ca ionophore A 23187 was examined in patients with asthma in relation to IgE-mediated allergy and glucocorticoid therapy. It has been shown that stimulation with ionophore A23187 induced a significantly higher leukotriene C 4 generation from granulocytes of asthmatic children than from granulocytes of healthy controls[5]. They also demonstrated that granulocytes from patients with a history of severe asthma displayed a higher LTC 4 formation than granulocytes from patients with less severe disease.

Our results also showed that the LTB 4 and LTC 4 generation was significantly larger in patients with atopic, non-SDIA than in healthy controls. However, the generation of LTB 4 and LTC 4 was not significantly more increased in patients with nonatopic asthma than in healthy subjects. In atopic asthma, the LTB 4 and LTC 4 generation were significantly more suppressed in patients with SDIA than in those with non-SDIA. The results suggest that the generation of LTB 4 and LTC 4 by leucocytes is influenced by IgE-mediated allergy and glucocorticoid reg-

imen. Furthermore, the generation of LTC4 by leucocytes was significantly higher in attack stage than in attack-free stage in patients with non-SDIA. The results are consistent with the data showing enhanced production of LTC 4 by activated eosinophils during antigen challenge[19]. Regarding asthma type, the generation of LTC 4 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[20, 21]. Parallel patterns of increase of IL-5 and eosinophils imply the possibility of a bidirectional interaction between them[22, 23]. These results may suggest a possibility that LTC 4 generation is more closely related to IgE-mediated reaction than to other reaction, and influenced by glucocorticoid regimen.

References

1. Barnes NC, Piper PJ, and Costello JF : Comparative actions of inhaled leukotriene C 4 , leukotriene D 4 and histamine in normal human subjects. Thorax 39 : 500－504, 1984.
2. Marom ZJ, Shelharmer MK, Bach DR, et al. : Slow-reacting substances, leukotriene C 4 and D 4 , increase release of mucus from human airways in vitro. Am Rev Respir Dis 136 : 449－451, 1982.
3. Lewis RA, and Robin JL: Arachidonic acid derivatives as mediators of asthma. J Allergy Clin Immunol 76 : 259－263, 1985.
4. Shaw RJ, Cromwell O, and Kay AB : Preferential generation of leukotriene C 4 by human eosinophils. Clin Exp Immunol 56 : 716－722, 1984.
5. Schauer U, Eckhartt A, Muller R, et al. : Enhanced leukotriene C 4 production by peripheral eosinophilic granulocytes from children with asthma. Int Arch Allergy Appl Immunol 60 : 201－206, 1989.
6. Sha'afi RI, Naccache PH, Molski TF, et al. : Cellular regulatory role of leukotriene B 4 : its effects on cation homeostasis in rabbit neutrophils. J Cell Physiol 108 : 401－408, 1981.
7. Martin TR, Pisstorese BP, Chi EY, et al. : Effects of leukotriene B 4 in the human lung : recruitment of neutrophils into the alveolar spaces without a change in protein permeability. J Clin Invest 84 : 1609－1619, 1989.
8. O'Byrne PM, Leikauf GD, Aizaw H, et al. : Leukotriene B 4 induces airway hyperresponsiveness in dogs. J Appl Physiol 59 : 1941－1946, 1985.
9. Pauwels RA, Kips JC, Peleman RA, et al. : The effect of endotoxin inhalation on airway responsiveness and cellular influx in rats. Am Rev Respir Dis 141 : 540－545, 1990.
10. Busse WW : Leukotrienes and inflammation. Am J Respir Crit Care Med 157 : 5210－5213, 1998.
11. American Thoracic Society : Definition and classification of chronic bronchitis, asthma, and polmonary emphysema. Am Rev Respir Dis 85 : 762－768, 1962.
12. Sakakibara H, Hirose K, Matsushita K, et al. : Effect of supplementation with eicosapentaenoic acid ethyl ester, MND-21, on generation of leukotrienes by Calcium ionophore-activated leukocytes in bronchial asthma. Jpn J Assoc Thorax Dis 33 : 396－412, 1996.
13. Lam S, Chan H, LeRiche JC, et al. : Release of leukotrienes in patients with bronchial asthma. J Allergy Clin Immunol

81: 711−717, 1988.
14. Walker C, Kaegi MK, Braun P, et al. : Activated T cells and eosinophilia in bronchoalveolar lavages from subjects with asthma correlated with disease severity. J Allergy Clin Immunol 88 : 935−942, 1991.
15. Fujimura M, Xiu Q, Tsujiura M, et al. : Role of leukotriene B4 in bronchial hyperresponsiveness induced by interleukin- 8 . Eur Respir J 11 : 306−311, 1998.
16. Weller PF, Lee CW, Foster DW, et al. : Generation and metabolism of 5-lipogenase pathway leukotrienes by human eosinophils : Predominant production of leukotriene C4. Proc Natl Acad Sci USA 80 : 7626−7630, 1983.
17. Underwood DC, Osborn RR, Newsholme SJ, et al. : Persistent airway eosinophilia after leukotriene (LT) D 4 administration in the guinea pigs. Am J Respir Crit Care Med 154 : 850−857, 1996.
18. Silberstein DS, and David JR: The regulation of human eosinophil function by cytokines. Immunol Today 166 : 129−141, 1987.
19. Sedgwick JB,Calhoun WJ, Gleich GJ, et al. : Immediate and late airway response of allergic patients to segmental antigen challenge. Am Rev Respir Dis 144 : 1274−1284, 1991.
20. Jarjour NN, Calhoun WJ, Becky Kelly EA, et al. : The immediate and late allergic response to segmental bronchopulmonary provocation in asthma. Am J Respir Crit Care Med 155 : 1515−1521, 1997.
21. Okhawara Y, Lei X-F, Strampfli MR, et al. : Cytokine and eosinophil responses in the lung, peripheral blood, and bone marrow compartment in a murine model allergen-induced airway inflammation. Am J Respir Cell Med Biol 16 : 510−520, 1997.
22. Dubucquoi S, Desreumaz P, Janin A et al. : Interleukin synthesis by eosinophils : association with granules and immunoglobulin-dependent secretion. J Exp Med 179 : 703−708, 1994.
23. Sulakvelidze I, Inman T, Rerecich T, et al. : Increases in airway eosinophils and in terleukin- 5 with minimal bronchoconstriction during repeated low-dose allergen challenge in atopic asthmatics. Eur Respir J 11 : 821−827, 1998.

Ⅰ型アレルギー反応および副腎皮質ホルモンは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産生に影響をおよぼす

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気管支喘息71例および健康人23名を対象に、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）の産生能について検討を加えた。なお、71例中ステロイド依存性重症難治性喘息（ＳＤＩＡ）は22例であった。１．アトピー性、非ＳＤＩＡ症例におけるＬＴＢ４、ＬＴＣ４産生は、健康人と比べ有意に高い値を示したが、非アトピー性喘息では健康人との間に有意の差は見られなかった。また、アトピー性喘息では、ＳＤＩＡ症例において非ＳＤＩＡ症例に比べ、ＬＴＢ４、ＬＴＣ４産生が有意に抑制されていた。２．非ＳＤＩＡ症例では、アトピー性、非アトピー性を問わず、ＬＴＣ４産生は、非発作時に比べ発作時に有意に亢進した状態であった。しかし、ＬＴＢ４産生には、非発作時、発作時との間に有意の差は見られなかった。３．ＳＤＩＡ症例では、ＬＴＢ４、ＬＴＣ４産生と発作との有意の関連は見られなかった。

以上の結果より、IgE にmediate されるアレルギー反応や喘息発作はＬＴＢ４、ＬＴＣ４産生に促進的に、また副腎皮質ホルモンは抑制的に作用することが示唆された。