◎原 著

Difference in the onset mechansisms of attacks between atopic and nonatopic asthma. A role of leukotriene C 4

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Abstract : Concentrations of main bronchoconstricting chemical mediators, histamine and leukotriene C 4 (LTC 4), were measured in bronchoalveolar lavage (BAL) fluid, and when cells (peripheral leukocytes and BAL cells) were stimulated by Ca ionophore A23187, in 7 atopic and 7 nonatopic asthma patients. 1 . The proportion of basophilic cells was significantly larger in atopic than in nonatopic asthma ($p<0.05$), however no significant difference was present in the other BAL cells between the two asthma types. 2 . Concentration of histamine in BAL fluid was significantly higher in atopic than that in nonatopic asthma, however, difference in that of LTC 4 was not found between them. 4 . The release of LTC 4 from BAL cells was higher in nonatopic than that in atopic asthma, but this was not significant. In contrast, the release of histamine was significantly higher in atopic compared to that in nonatopic asthma ($p<0.001$) when the cells were stimulated by Ca ionophore A23187.

These results suggest that both histamine and LTC 4 participate in the onset mechanism of atopic asthma, and only LTC 4 in that of nonatopic asthma.

Key words : Histamine, LTC 4 , atpic, nonatopic, BAL cells

Introduction

Bronchial asthma is divided into two types, atopic and nonatopic, based on the presence or absence of IgE－mediated allergic reaction[1]. Atopic asthma is often observed in younger patients and nonatopic in older patients. Our previous studies have shown that the role of histamine during triggering events of asthma attacks is different between younger and older patients with asthma[2].

In atopic asthma, bridging of IgE receptors on mast cell membrane is caused by allergen, followed by release of chemical mediators such as histamine and leukotriene C 4 (LTC 4)[3-7]. These mediators, especially

histamine, induce pathophysiological changes of airways such as bronchoconstriction, mucus hypersecretion and edema of mucous membreane. Thus, histamine is one of the most important mediators relating to the onset mechanisms of atopic asthma attacks. In contrast, the onset mechanism of nonatopic asthma is still unclear in relation to participation allergic reactions.

Leukotriene C 4 is also one of the chemical mediators having bronchoconstricting action and participates in the onset mechanisms of asthma attacks. However, it is not well known in what kind of asthma attacks LTC 4 predominantly participates.

This study was performed to clarify the difference in action of histamine and LTC 4 during onset periods of attacks between atopic and nonatopic asthma.

Subjects and methods

The subjects were 7 patients (4 females and 3 males) with atopic asthma and 7 (4 females and 3 males) with nonatopic asthma. All subjects with atopic asthma showed positive RAST score for house dust mite (HDm). In contrast, patients with negative RAST score for inhalant allergens and low levels of serum IgE (less than 200 I U/ mℓ) were evaluated as those with nonatopic asthma. Characteristic of these patients was shown in Table 1 .

Tabel 1 . Characteristics of patients with bronchial asthma studied

Asthma type	No of patients	Age (years)	Serum IgE (IU/ml)	FEV1.0%
Atopic	7	52.3	455 (198-1057)	65.7 ± 9.5
nonatopic	7	52.0	91 (18-174)	62.9 ± 3.1

Histamine content in bronchoalveolar lavage (BAL) fluid, from peripheral leukocytes and BAL cells stimulated by Ca ionophore A23187, was analyzed by perchloric acid precipitation and analyzed with an spectrofluorometric analysis system (Technicon Instruments Co.), as previously described[8-10]. The release of histamine from peripheral leukocytes stimulated by Ca ionophore A23187 was observed by a whole blood method, as previously reported. Regarding the release of histamine from BAL cells, Ca ionophore A23187 (1 μg/mℓ) was added to the cell suspension, after the number of BAL cells was adjusted to 10^6 cells/mℓ in Tris ACM. The mixed solution was then incubated for 15 min at 37 ℃ and centrifuged at 300g for 10 min at 4 ℃. Histamine release was expressed as a percentage of total histamine.

The HPLC analysis for extraction and quantification of leukotriene C 4 (LTC 4) was performed by a method by Lam et al[6,11]. The extraction of LTC 4 was performed using a CIS Seppak (walters Associates). The concentration of LTC 4 was analyzed by an HPLC system, Model 510 (Walters Associates), equipped with an ultravioletdetector. The column used was a 5 mm x10cm Radial-Pak cartridge (Shimazu). The results were expressed as ng/10^6 cells.

Bronchoalveolar lavage (BAL) was performed according to the method previously described[12-15]. Informed consent for BAL examination was accepted by all subjects. After the aspirate were centrifuged at 300g for 10 min at 4 ℃, the cell pellet was resuspended in Tris ACM. BAL cytology was performed by observing 500 cells, excluding epithelial cells, on smear preparations which were made from BAL cell suspensions and stained with May-Giemsa. Regarding mast

cells and basophils in BAL fluid, 1000 cells were observed and number of basophilic cells was calculated. In this study, the mean recovery rate at BAL was 31.4% in atopic and 25.3% in nonatopic asthma.

The level of total IgE in sera was measured by radioimmunosorbent test (RIST) and IgE antibodies for inhalant allergens were estimated by radioallergosorbent test (RAST).

Results

1. Bronchoalveolar lavage (BAL) cells

Table 2 shows the proportion of BAL cells in atopic and nonatopic asthma patients. The proportion of basophilic cells was significantly higher in atopic compared to that in nonatopic asthma ($p<0.05$). The proportions of the other BAL cells were not significantly different between two asthma types.

Regarding absolute counts of eosinophils and basophilic cells, the number of the two BAL cells was larger in atopic than in nonatopic, however, these differences were not significant (Fig. 1).

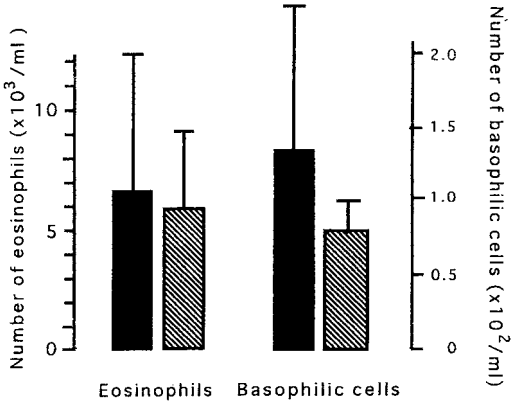

Fig. 1 Absolute counts of eosinophils and basophilic cells in BAL fluid of atopic (■) and nonatopopic asthma (▨).

Table 2. Cellular composition of BAL fluid of atopic and nonatopic asthma

Asthma type	Recovery rate (%)	No of total cells (x10⁶)	BAL cells (%) Mac	Lymph	Neut	Eos	Bas
Atopic	31.4 ±19.8	8.4 ±4.2	75.8 ±11.7	16.0 ±9.4	2.5 ±3.3	5.5 ±9.4	0.25[a] ±0.27
Non-atopic	25.3 ± 4.6	7.4 ±1.7	77.4 ± 7.8	18.9 ±8.0	2.7 ±2.6	0.9 ±1.0	0.02[a] ±0.03

Mac;macrophages, Lympn;lymphocytes, Neut;neutrophils, Eos;eosinophils, Bas;basophilic cells. a:p<0.05

2. Histamine release

%Histamine release from peripheral leukocytes was higher in atopic (26.0±15.9%) than in nonatopic asthma (16.8±3.7%), but this was not significant (Fig. 2).

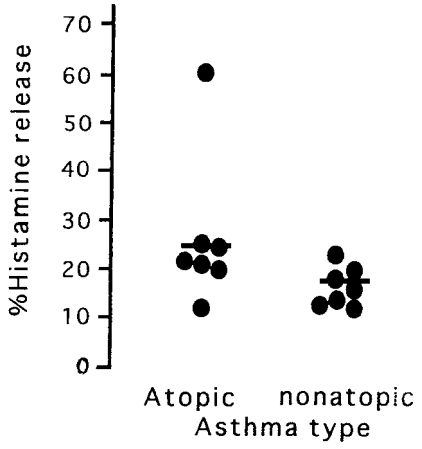

Fig. 2 Histamine release from peripheral leucocytes in atopic and nonatopic asthma

Histamine content in BAL fluid was significantly higher in atopic than in nonatopic asthma patients ($p<0.001$), in whom histamine was not detectable by a fluorometric method (Table 3).

Table 3. Concentrations of histamine and LTC4 in BAL fluid of atopic and nonatopic asthma

Asthma type	Histamine (%)	LTC4 (ng/ml)
Atopic	3.2 ±3.6[a]	1.3 ±1.4
Nonatopic	0[a]	3.5 ±2.1

p:a<0.001

As shown in Fig. 3, histamine release from BAL cells was also significantly higher in atopic compared to the release in nonatopic asthma ($p<0.001$)

3. Leukotriene C4 release

LTC4 content was higher in nonatopic than in atopic asthma, however, significant difference was not present between them (Table 3.). In contrast, LTC4 release from BAL cells was observed when the cells were stimulated by Ca ionophore A231877, and the release was higher in nonatopic (13.0±4.6ng /10^6 cells) than in atopic asthma (9.1±6.2 ng/10^6 cells), however, this was not significant (Fig. 3)

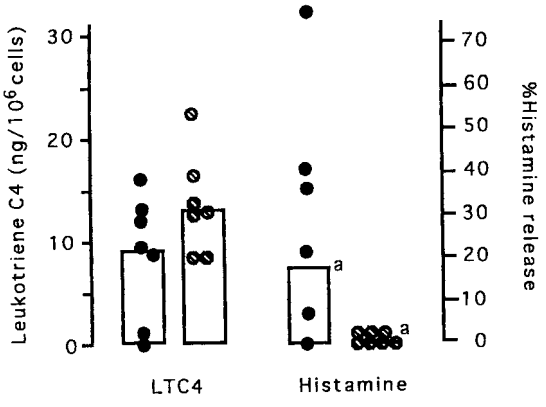

Fig. 3 LTC4 and histamine release from BAL cells in atopic (●) and nonatopic (⊘) asthma. a:$p<0.001$.

Discussion

Bronchial asthma is classified into two types, atopic and nonatopic, based on the presence or absence of IgE-mediated allergic reaction. However, despite of the presence or absence of IgE-mediated reaction, chemical mediators such as histamine and LTC4, which are released from tissue mast cells during the time of immediate asthmatic reaction (IAR), and from inflammatory cells during late asthmatic reaction (LAR), play an important role in the onset mechanisms of asthma attacks[16-18]. In recent years, many kinds of antiallergic drugs inhibiting actions of histamine and LTC4 have been developed. Thus, it is very important for physicians to know roles of chemical mediators such as histamine and LTC4 in asthma attacks of each patient.

We carried out the present study to clarify that the way in which the release of chemical mediators, histamine and LTC4, from BAL cells in asthma patients are different between the two asthma types, atopic and nonatopic. Thus, the release of histamine and LTC4 was observed in the airways of atopic and nonatopic asthma patients. Regarding inflammatory cells in the airways, the proportion of basophilic cells was significantly higher in atopic than in nonatopic asthma. It has been noted that activated T cells and eosinophils participate in the onset mechanisms of asthma attacks[19] and furthermore, it has been suggested that neutrophils in the airways may be related to asthma attacks[20,21]. However, the proportions of the other BAL cells except basophilic cells were not different between the two asthma types. The results

relating to the release of chemical mediators showed that histamine largely participates in attacks of patients with atopic asthma. However, histamine seemed not to be related to attacks of patients with nonatopic asthma. LTC 4 , which has strong action inducing bronchospasm mainly during LAR, was significantly released from BAL cells of both atopic and nonatopic asthmatics when the cells were stimulated with Ca ionophore A23187, and the release was higher in nonatopic than in atopic asthma, although this difference was not significant. These results suggest that LTC 4 induce pathophysiological changes including bronchospasm in the airways of both atopic and nonatopic asthma patients. In application of anti-allergic agents for the treatment of asthma, drugs inhibiting actions of histamine and/or LTC 4 should be selected in atopic asthma, drugs mainly inhibiting action of LTC 4 in nonatopic asthma.

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アトピー型および非アトピー型気管支喘息の発作発症機序の差異について…ロイコトリエンC4の役割について

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気管支喘息患者14人(アトピー型，非アトピー型各７人)につき気管支肺胞洗浄(BAL)液及び気管支肺胞洗浄細胞と末梢血白血球をカルシウムイオノファA23187で刺激し，主要な気管支収縮メディエーターであるヒスタミンとロイコトリエンC４(LTC４)の濃度を測定した。１．BAL中細胞の比率では好塩基球のみ非アトピー型に比べアトピー型で優位に高い値であった。($p<0.05$)
２．BAL液のヒスタミンの濃度はアトピー型で有意に高い値であったが，ロイコトリエンC４はアトピー型，非アトピー型で有意な差を認めなかった。３．BAL細胞からのカルシウムイオノファA23187刺激によるロイコトリエンC４産生はアトピー型に比べ非アトピー型で高い値であったが，有意差は認めなかった。一方，同刺激によるヒスタミン遊離は非アトピー型よりアトピー型で有意に高値であった。($p<0.001$)。以上の結果よりアトピー型の気管支喘息の発症機序にはヒスタミン，ロイコトリエンC４の両者が，非アトピー型に於いてはロイコトリエンC４のみが主として関与していること可能性が示唆された。