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# Release of histamine and leukotriene C 4 from bronchoalveolar cells in patients with bronchial asthma. A role of histamine in atopic asthma.

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Abstract : To clarify the main humoral triggering factor (histamine and/or leukotriene) of the early stage of asthma attacks, the release of histamine and leukotriene C 4 (LTC4) from bronchoalveolar lavage (BAL) cells stimulated with Ca ionophore A23187 was examined in 7 patients with atopic asthma, and the results were compared to those in 7 nonatopic asthma patients. 1. The proportion of BAL basophilic cells was significantly higher in atopic patients than in nonatopic patients ($p<0.05$). 2. The content of histamine in BAL fluid was significantly higher in atopic (2.3mcg/mℓ) comparted to that in nonatopic patients ( 0 mcg/mℓ)($p<0.001$). The content of LTC4 was high in nonatopic (2.4ng/mℓ) than in atopic patients (0.5ng/mℓ), however, this was not significant. 3. The release of histamine from BAL cells was 32.6% in atopic and 0 % in nonatopic patients, and this was significant ($p<0.001$). The release of LTC4 from BAL cells was significantly higher in nonatopic (11.3ng/$10^6$cell) than in atopic (3.5ng/$10^6$cell)($p<0.02$).

The results demonstrate that histamine play more important role in atopic patients as a main triggering factor of attacks than LTC4. In contrast, in nonatopic patients, LTC4 is more predominant than histamine during early stage of asthma attacks.

Key words : Histamine, LTC4, BAL cells, atopic asthma

## Introduction

In the onset mechanism of asthma, humoral factors such as histamine and leukotrienes in the early stage of asthma attacks[1-4], and cellular components such as lymphocytes, neutrophils, eosinophils, and basophils in the late stage have been shown

to play important roles[5-13]. Particularly, release of histamine and leukotrienes in the early stage are important as the early triggering factor of attacks, since pathophysiological changes in the airways of asthma, such as bronchoconstriction, mucus hypersecretion and edema of mucous membrane, are at first introduced by the release of these chemical mediators from tissue mast cells[14].

Asthma is classified into two types, atopic and nonatopic, based on the presence or absence of an IgE-mediated allergic reaction[15]. Atopic asthma is often observed in young patients, having specific IgE antibodies to allergens. In contrast, nonatopic asthma is often seen in late onset adult patients, and shows low serum IgE levels and negative IgE antibodies to allergens. This suggests that the main triggering factor of asthma attacks are different between atopic and nonatopic asthma patients.

In the present study, to clarify the main triggering factor of attacks, release of histamine and leukotrienes from inflammatory cells in the airways was examined in atopic asthma patients, and the results were compared to those in nonatopic asthma patients.

## Subjects and Methods

Seven patients with atopic asthma were selected in this study to examine the main humoral triggering factor (histamine and/or leukotrienes) of IgE-mediated allergic reaction of atopic asthma. They were all sensitive to house dust mite, showing a positive RAST score ( 2 + or more) to the allergen. Seven nonatopic asthma patients were selected as control subjects. Their serum IgE levels were under 200 IU/mℓ and RAST score to various allergens was negative.

Bronchoalveolar lavage (BAL) was performed according to a previously reported method[12-14] when the subjects were attack-free. Informed consent for this BAL procedure was obtained from all study subjects. The aspirate obtained by BAL were filtered through a sterile steel mesh, and the filtrates were centrifuged at 300 *g* for 10 min at 4 C. The cell pellet was resuspended in Tris ACM. After the number of cells was adjusted to $10^6$ cells/mℓ in Tris ACM, Ca ionophore A23187 ( 1 mcg) was added to the cell suspension. The mixed solution was then incubated for 15 min at 37℃ and centrifuged at 300 *g* for 10 min at 4 ℃. The histamine content of both the cells and supernatant fluid were analyzed by perchloric acid precipitation and assayed with automated spectrofluorometric histamine analysis system (Technicon Instruments Co). Histamine release was expressed as a percentage of total histamine. The HPLC analysis for extraction and quantification of LTC4 was performed by a method described by Lam et al[16]. The extraction of leukotrienes was performed by a method using a C18 Sep-Pak (Walters Associates). The concentration of LTC4 was analyzed by an HPLC system, Model 510 (Walters Associate), equipped with an ultraviolet detector. The column used was a 5 *mm* × 10 *cm* Radial-Pak cartridge (Shimazu). The results were expressed as ng/$10^6$ cells. 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 the number of basophilic cells was calculated. The results were expressed as percentages of the total number of cells. In this study, the mean recovery rate at BAL was 28.8 ± 11.8% (± SD). The total

number of cells aspirated into BAL fluid was $8.21 \pm 10.2 \times 10^6$.

The level of serum IgE was measured by the radioimmunosorbent test (RIST), and IgE antibodies against allergens were assessed by the radioallergosorbent test (RAST).

Statistically significant difference of the mean was evaluated using the unpaired Student't test. A value of $p<0.05$ was regarded as significant.

## Results

Table 1 shows characteristics of atopic and nonatopic asthma patients. Serum IgE level was remarkably higher in atopic asthma than in nonatopic asthma. However, this difference was not significant. Cellular composition of BAL fluid in both atopic and nonatopic subjects was shown in Table 2. The proportions of BAL lymphocytes, neutrophils, and eosinophils were not significantly different between atopic and nonatopic asthma patients. The proportion of BAL basophilic cells was significantly higher in atopic patients than in nonatopic patients ($p<0.05$).

Table 1. Characteristics of patients with bronchial asthma studied

| Asthma type | No of patients | Mean age (years) | RAST 2+< to HD | serum IgE (IU/ml) |
|---|---|---|---|---|
| Atopic | 7 | 48.0 (36-62) | 7 | 1202 (170-4134) |
| Nonatopic | 7 | 52.0 (22-63) | 0 | 91 (18-174) |

HD; house dust

Table 2. Cellular composition of BAL fluid in patients with asthma studied

| Asthma type | No of patients | BAL cells (%) Mac | Lym | Neut | Eos | Bas |
|---|---|---|---|---|---|---|
| Atopic | 7 | 83.5 ±11.2 | 11.6 ± 8.3 | 2.4 ±2.1 | 2.3 ±1.9 | 0.15[a] ±0.13 |
| Nonatopic | 7 | 77.4 ± 7.9 | 18.9 ± 7.9 | 2.9 ±2.6 | 0.9 ±1.0 | 0.03[a] ±0.05 |

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

The content of histamine in BAL fluid was significantly higher in atopic patients (mean ± SD : 2.3 ± 1.5mcg/mℓ) than in nonatopic patients (mean 0 mcg/mℓ)($p<0.001$)(Fig. 1). In contrast, the content of leukotrienes C 4 (LTC4) was higher in nonatopic patients (2.4 ± 2.6ng/mℓ) compared to that in atopic patients (0.5 ± 1.3ng/mℓ). However, this was not significant (Fig. 2).

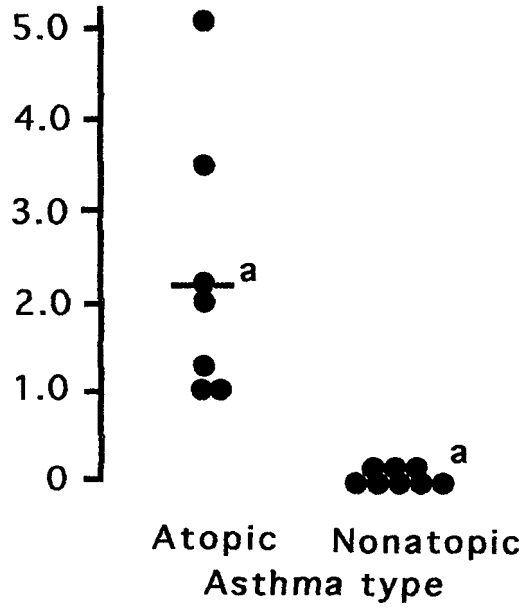


Fig. 1. Histamine content in BAL fluid in patients with asthma. a ; $p<0.001$.

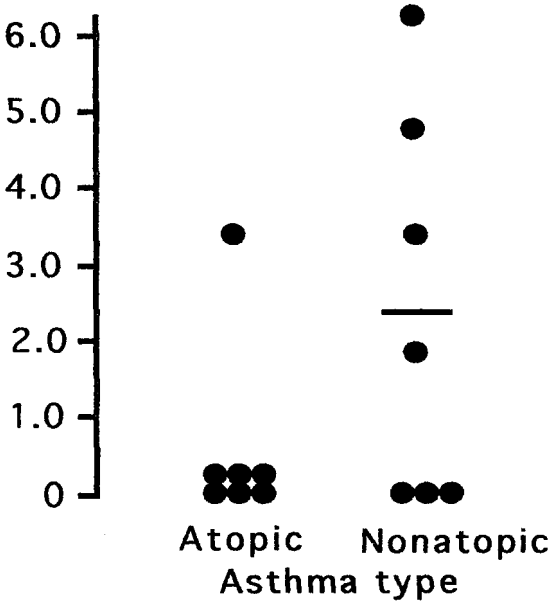


Fig. 2. LTC4 content in BAL fluid in patients with asthma.

The release of histamine from BAL cells was 32.6 ± 7.8% in atopic patients and 0 % in nonatopic patients. The histamine release from BAL cells was significantly higher in atopic than in nonatopic patients ($p<0.001$)(Fig. 3). The release of LTC4 from BAL cells was significantly higher in nonatopic (11.3 ± 5.8ng/$10^6$ cells) than atopic patients (3.5 ± 3.0ng/$10^6$ cells)($p<0.02$)(FIg. 4).

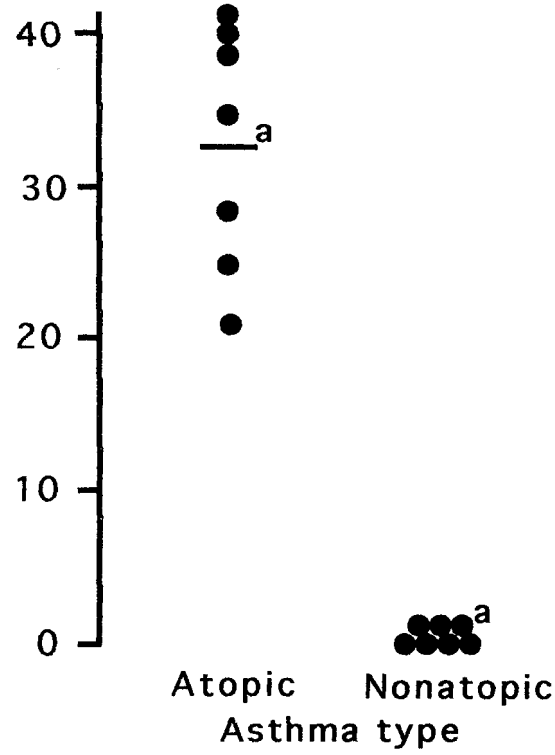


Fig. 3. Release of histamine from BAL cells in patients with asthma. a ; $p<0.001$.

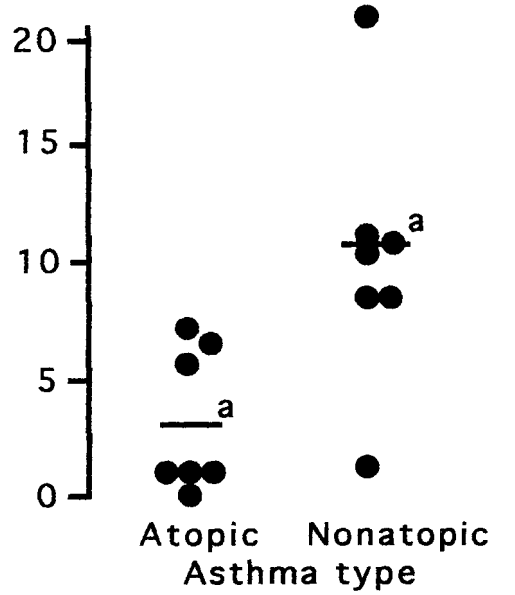


Fig. 4. LTC4 release from BAL cells in patients with asthma. a ; $p<0.02$.

## Discussion

Asthma can be clinically classified into two types, atopic and nonatopic by the onset mechanism of attacks. Although there are some reports suggesting that asthma is almost always associated with some type of IgE-related reaction[17, 18], there are many asthma patients who are clinically regarded as nonatopic, showing negative skin test, low serum IgE levels, and negative RAST score to various allergens. Nonatopic asthma clinically diagnosed is often observed in late onset (over the age of 40 years) adult patients. Their attacks often become severe and intractable[19, 20] within a couple of years from onset of asthma. The onset mechanism of attacks, particularly, triggering event in early stage of attacks in nonatopic asthma is still unclear. In contrast, in atopic asthma, IgE-mediated allergic reaction was triggered by bridging of IgE receptors on mast cell membrane, followed by release of chemical mediators such as histamine and leukotrienes[1-4, 21-23].

In recent years, focus has been placed on inflammatory cell infiltration in the airways as a main onset mechanism of asthma[5-14]. However, release of chemical mediators such as histamine and leukotrienes has been considered to be the main mechanism of the onset of early stage of asthma attacks. The mechanism is important as triggering factor of asthma attacks.

In the present study, to clarify whether a main triggering factor in the early stage of attacks is different between atopic and nonatopic asthma, release of histamine and leukotrienes from BAL cells was examined in these two different asthma types. The results revealed that the release of histamine from BAL cells was significantly higher in atopic than in nonatopic asthma. In contrast, the release of LTC4 from BAL cells was significantly higher in nonatopic asthma compared to that in atopic asthma. These results suggest that histamine is more important as triggering factor of attacks in atopic asthma than LTC4, and that LTC4 is more important than histamine in nonatopic asthma in relation to early stage of attacks.

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**気管支喘息患者の気管支肺胞細胞からのヒスタミンとロイコトリエンＣ４遊離－アトピー性喘息におけるヒスタミンの役割について－**

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気管支喘息発作初期に関与する液性因子（ヒスタミン，ロイコトリエン）の役割を明らかにするために，気管支肺胞洗浄（BAL）細胞からのカルシウムイオノフォアA23187によるヒスタミンとロイコトリエンＣ４（LTC４）遊離の検討を行った。対象はアトピー性喘息患者７名，非アトピー性喘息患者７名とした。１．BAL液中の好塩基性細胞の比率は，アトピー性喘息患者において有意に高値を示した。２．BAL液中ヒスタミン濃度は非アトピー性喘息患者（０mcg／mℓ）に比して，アトピー性喘息患者（2.3mcg／mℓ）において有意に高値を示した。一方，BAL液中LTC４濃度は，アトピー性喘息患者（0.5ng／mℓ）に比して非アトピー性喘息患者（2.4ng／mℓ）において，高値を示したが，有意差は見られなかった。３．BAL細胞からのヒスタミン遊離は非アトピー性喘息患者（０％）に比して，アトピー性喘息患者（32.6％）において有意に高値を示した。LTC４遊離はアトピー性喘息患者（3.5ng／$10^6$細胞）に比して，非アトピー性喘息患者（11.3ng／$10^6$細胞）において有意に高値を示した。

以上より，喘息発作初期に関与する液性因子としては，アトピー性喘息患者においてはヒスタミンが重要であり，非アトピー性喘息患者においてはLTC４が，重要であることが，明らかになった。