● 原著

気管支喘息における気管支肺胞洗浄液中のヒスタミン およびロイコトリエン C₄ レベルについて

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Histamine and leukotriene C₄ levels in bronchoalveolar lavage (BAL) fluid in patients with bronchial asthma

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【Summary】

The levels of histamine and leukotriene C₄ (LTC₄) in bronchoalveolar lavage (BAL) fluid and the release of these mediators from BAL cells stimulated by Ca ionophore A 23187 were examined in 22 asthmatic subjects. In BAL fluid, histamine was detectable in 9 (40.9%) and LTC₄ was detectable in 10 (45.5%) of the 22 asthma patients. BAL cells from patients with detectable histamine (DH), many of whom were evaluated as atopic, released more histamine and less LTC₄ than those of patients without DH. In contrast, BAL cells from patients with detectable LTC₄ (DL), many of whom were assessed as nonatopic, released more LTC₄ and less histamine than those of patients without DL. These results suggest that histamine and LTC₄ levels differ in relation to the IgE-mediated allergic reaction.

Key words : histamine,
leukotrienes,
BAL fluid,
bronchial asthma

【概 要】

ヒスタミンおよびロイコトリエン C₄ (LTC₄) は、気管支攣縮性の化学伝達物質であり、気管支喘息では IgE

抗体に mediate される即時型アレルギー反応の際に、肥満細胞や好塩基球およびその他の細胞から遊離される。本研究では、気管支喘息症例を対象に、気管支肺胞洗浄法 (BAL) により得られた液性成分中のこれらの化学伝達物質濃度を測定し、Ca ionophore A 23187 刺激時の BAL 細胞および末梢血白血球からのこれらの化学伝達物質遊離との関連について、若干の検討を加えた。対象は気管支喘息 22 例 (平均年齢 56.2 歳, 平均血清 IgE 値 589 IU/ml) であった。BAL 液中のヒスタミンおよび LTC₄ 濃度は、それぞれヒスタミン自動分析装置および HPLC により測定した。また、BAL 細胞 (10⁶) および末梢血白血球 (5×10⁶) からのヒスタミンおよび LTC₄ 遊離は、Ca ionophore A 23187 (1 μg) 添加後、37°C, 15 分間 incubate した後その遊離量を測定した。

成績: BAL 液中のヒスタミン濃度が測定し得た症例 (DH) は、22 例中 9 例 (40.9%) であり、また LTC₄ が測定し得た症例 (DL) は 10 例 (45.5%) であった。BAL 液中ヒスタミンが測定し得た症例の多くは、血清 IgE 値の上昇, RAST 陽性などの所見からアトピー型と診断され、その BAL 細胞からは LTC₄ よりもヒスタミンがより多く遊離された。一方、BAL 液中の LTC₄ が測定し得た症例の多くは、血清 IgE 低値, アレルゲンによる皮内反応陰性, RAST 陰性などの所見から非アトピー型と診断され、その BAL 細胞からは、ヒスタミンよりも LTC₄ がより多く遊離された。末梢血白血球からのヒスタミン, LTC₄ 遊離もほぼ同様の傾向を示したが、DH 症例におけるヒスタミン遊離を除き有意の差はみられなかった。以上の結果は、気道内ヒスタミンおよび LTC₄ 濃度は、ある程度 IgE 系反応と関連があることを示唆しているものと考えられた。

I. Introduction

Asthma attacks may occur within 30 min from exposure to inciting agents (immediate asthmatic reaction: IAR). They may be induced at 6 to 8 hours (late asthmatic reaction: LAR) after bronchial allergen challenge. Chemical mediators, such as histamine and leukotrienes, play the predominant roles in the IAR, while airway inflammation is the predominant factor in the LAR. The mechanism underlying the onset of asthma is divided into two types, atopic and nonatopic, on the basis of the presence or absence of the IgE-mediated allergic reaction¹⁾, although several reports have suggested that asthma is almost always induced by this reaction^{2,3)}.

In atopic asthma, the attacks are triggered by allergen-IgE antibody binding on tissue mast cell membrane, in which chemical mediators such as histamine and leukotrienes are released from the cells⁴⁻⁷⁾, followed by inflammatory cell infiltration⁸⁻¹⁴⁾. Histamine and leukotrienes induce transient bronchoconstriction^{15,16)}, these mediators being released from mast cells in the IAR, and from inflammatory cells in the LAR¹⁷⁻¹⁹⁾.

These mediators have been implicated in the pathogenesis of asthma; their participation in asthma has been suggested by their presence in

bronchoalveolar lavage (BAL) fluid²⁰⁻²²⁾. It has been shown that mediator levels in BAL fluid from atopic asthma subjects are higher after allergen challenge than levels in control subjects, including nonatopic asthma subjects, atopic subjects who do not have asthma, and normal subjects^{20,23,24)}; these findings show that there is significant variability in asthma patients regarding mediator levels in BAL fluid²⁵⁾.

In the present study, to clarify the role of mediators in the asthma attacks of each patient, we compared histamine and leukotriene C₄ (LTC₄) levels in BAL fluid in asthma patients, and we compared correlations between these levels and the release of these mediators from BAL cells and peripheral leucocytes.

II. Subjects and methods

The subjects of this study were 22 asthma patients (16 females and 6 males; mean age 56.2 years, range 22~74 years). The level of serum IgE was 589 IU/ml (range, 18~4,134 IU/ml). Of the 22 patients, 7 (31.8%) were sensitive to inhalant allergens. All patients were non-smokers. In this study, patients showing either a positive RAST score of 2+ or more to inhalant allergens or total serum IgE level more than 500 IU/ml were assessed as atopic, and those with negative skin reaction to

Table 1 Characteristics of patients with bronchial asthma studied

Case	Age (years)	Sex	Serum IgE (IU/ml)	Skin reaction to allergens	RAST score to allergens	Asthma type
1	33	F	33	—	—	N
2	36	F	170	HD, Rag, Asp	HD 3+	A
3	41	M	1,932	HD	HD 2+	A
4	57	F	199	HD	HD 2+	A
5	50	M	578	HD	HD 2+	A
6	48	F	1,200	HD, Rag	HD 3+	A
7	62	F	202	HD	HD 2+	A
8	43	M	4,134	HD, Silk	HD 2+	A
9	62	M	1,132	—	—	A
10	74	F	35	—	—	N
11	56	F	18	—	—	N
12	68	F	116	—	—	N
13	69	F	110	—	—	N
14	22	F	139	—	—	N
15	60	F	174	—	—	N
16	64	F	211	Silk	—	?
17	53	F	69	—	—	N
18	49	F	66	—	—	N
19	63	M	141	—	—	N
20	61	F	30	—	—	N
21	64	F	1,545	HD	HD 1+	A
22	74	M	726	Asp	—	A

HD : house dust, Rag : ragweed, Asp : Aspergillus, A : atopic, N : non-atopic

various allergens and serum IgE level less than 200 IU/ml were evaluated as nonatopic, as shown in Table 1.

Bronchoalveolar lavage (BAL) was performed in all subjects by a previously reported method^{26,27)}, when they were asymptomatic. Informed consent for the BAL procedure was obtained from all subjects. Briefly, after the bronchoscope was routinely wedged in a segment of the right middle lobe, 4×50 ml aliquots of sterile isotonic saline at 37°C were introduced into the segment, and were immediately aspirated into siliconized glass containers. After filtration through a sterile steel mesh, the filtrates were centrifuged at 1,200 rpm for 10 min at 4°C. The cell pellet was used for BAL cytology, and the supernatant for measuring histamine and leukotriene C₄ levels.

BAL cytology was performed by observing 500 cells excluding epithelial cells, on smear preparations made from the cell pellets and stained with

May Giemsa. The results were expressed as percentages of the total number of cells.

To 1 ml of the cell suspension (10⁶ cells/ml) in Tris ACM, 1 µg of the Ca ionophore A 23187 was added, and the mixed solution was incubated at 37°C for 15 min. For the histamine release experiments, the mixed solution was centrifuged, and histamine content in the cell pellet and supernatant was measured with an automated fluorometric histamine analysis system (Technicon)^{17,18,20)}. The results were expressed as percentages of the total histamine content. For the LTC₄ release experiments, 4 ml of 100% ethanol was added to the mixed solution. The solution was then air dried, and resuspended in 250 µl of high-performance liquid chromatography (HPLC) solvent (CH₃CN/H₂O=1:1). The solution was then subjected to HPLC (c-18 reverse phase column, detection 280 nm). The results were expressed as ng/10⁶ cells. The levels of histamine and LTC₄ in BAL fluid were

measured by the same procedures. The concentrations of histamine less than $1 \mu\text{g}$ and LTC_4 less than 1 ng in BAL fluid were assessed as being not detectable by these procedures. In this study, the mean recovery rate at BAL was $26.8 \pm 10.2\%$ ($\pm\text{SD}$), and the total cell number was $9.5 \pm 9.8 \times 10^6$.

The release of histamine and LTC_4 from peripheral leucocytes was observed by using cells separated by counterflow centrifugation elutriation with a JE-6 B rotor (Beckmen)^{18,29,30}. After the cells (5×10^6) were incubated with the Ca ionophore A 23187 at a concentration of $1 \mu\text{g/ml}$ at 37°C for 30 min, the release of histamine and LTC_4 were measured by the methods described above.

Immediate skin reaction was observed following intradermal injection with 0.02 ml of commercial allergen extracts. The reaction was evaluated at 30 min after the injection, and wheals more than 9 mm long or the flares more than 20 mm in diameter was regarded as positive.

The level of serum IgE was determined by radioimmunosorbent test (RIST). IgE antibodies for allergens were evaluated by radioallergosorbent test (RAST).

III. Results

1. Histamine levels in BAL fluid

Histamine was detectable in the BAL fluid of 9 of the 22 (40.9%) asthma patients, and was not detectable in the remaining 13. The LTC_4 level was lower in patients with detectable histamine (DH) in the BAL fluid than in those without DH, but the difference was not significant. The level of serum IgE was significantly higher in patients with DH than in those without DH ($p < 0.05$). A positive RAST score of 2+ or more to allergens was observed only in patients with DH. Thus, patients with DH had the characteristics of high serum IgE levels and a positive RAST score, many of them being assessed as atopic (Table 2).

The proportion of cells, macrophages, lymphocytes, neutrophils, and eosinophils in BAL fluid was not significantly different in patients with and without DH (Table 3).

2. Histamine release from BAL cells and peripheral blood

The release of histamine from BAL cells induced by the Ca ionophore A 23187 was significantly higher in patients with DH than in those without DH

Table 2 Characteristics of patients with detectable histamine (DH) in BAL fluid

DH	No. of patients	In BALF		Serum IgE (IU/ml)	RAST to allergen
		His ($\mu\text{g/ml}$)	LTC_4 (ng/ml)		
+	9	$2.1 \pm 1.4^{*a}$	0.99 ± 1.9	$1,064 \pm 1,238^b$	7
-	13	0 ^a	2.24 ± 2.45	86 ± 52^b	0

DH : detectable histamine in BAL fluid, His : histamine, *Mean $\pm$ SD

^a $p < 0.001$, ^b $p < 0.05$

Table 3 Cellular composition in BAL fluid of patients with detectable histamine (DH)

DH	No. of patients	BAL cells (%)			
		Mac	Lym	Neut	Eos
+	9	78.7^{*} ± 12.8	17.0 ± 1.1	2.2 ± 1.8	2.1 ± 1.7
-	13	76.6 ± 13.4	16.9 ± 7.2	5.0 ± 8.4	1.3 ± 1.9

DH : detectable histamine in BAL fluid. *Mean $\pm$ SD

Mac : macrophages, Lym : lymphocytes, Neut : neutrophils, Eos : eosinophils

Fig. 1 Release of histamine (●) and LTC₄ (○) from BAL cells stimulated by Ca ionophore A 23187 in asthmatic subjects with and without detectable histamine in the BAL fluid. Significant difference between a and a' at $p < 0.001$.

Fig. 2 Release of histamine (●) and LTC₄ (○) from peripheral leucocytes stimulated by Ca ionophore A 23187 in asthmatic subjects with and without detectable histamine in the BAL fluid. Significant difference between a and a' at $p < 0.05$.

($p < 0.001$). The release of LTC₄ from the BAL cells of patients with DH was lower than the release shown in those without DH; however, this difference was not significant (Fig. 1). The release of histamine from peripheral leukocytes was significantly higher in patients with DH than in those without DH ($p < 0.05$), but there was no significant difference between the release of LTC₄ from peripheral leukocytes in patients with and without DH (Fig. 2).

3. Leukotriene C₄ levels in BAL fluid

Leukotriene C₄ (LTC₄) in BAL fluid was detectable in 10 of the 22 patients. In these 10 patients with detectable LTC₄ (DL) in BAL fluid, the level of histamine was significantly lower than the level in those without DL ($p < 0.05$). The level of serum IgE was also significantly lower in patients with DL whose level was less than 200 IU/ml than in those without DL ($p < 0.02$). Eight of these 10 patients showed negative skin reaction to allergens, and nine had no IgE antibodies to inhalant allergens. Thus, many of the patients with DL were assessed as

Table 4 Characteristics of patients with detectable LTC₄ (DL) in BAL fluid

DL	No. of patients	In BALF LTC ₄ (ng/ml)	His (μg/ml)	Serum IgE (IU/ml)	RAST to allergen
+	10	4.3±1.5**	4.8± 9.7 ^b	86±52 ^c	1
-	12	0 ^a	18.0±16.3 ^b	1,009±1,008 ^c	6

DL : detectable leukotriene C₄ in BAL fluid, His : histamine*Mean±SD. ^a p<0.001, ^b p<0.05, ^c p<0.02**Table 5** Cellular composition in BAL fluid of patients with detectable LTC₄ (DL)

DL	No. of patients	BAL cells (%)			
		Mac	Lym	Neut	Eos
+	10	74.7* ±15.4	18.3 ± 9.4	5.3 ±9.5	1.7 ±2.2
-	12	79.1 ±10.7	15.9 ± 9.4	2.7 ±2.0	1.6 ±1.5

DL : detectable LTC₄ in BAL fluid. *Mean±SD

Mac : macrophages, Lym : lymphocytes, Neut : neutrophils, Eos : eosinophils

Fig. 3 Release of histamine (●) and LTC₄ (○) from BAL cells stimulated by Ca ionophore A 23187 in asthmatic subjects with and without detectable LTC₄ in the BAL fluid. Significant difference between a and a' at p<0.05, and between b and b' at p<0.05.

nonatopic (Table 4). There was no significant difference between numbers of each BAL cell type in patients with and without DL (Table 5).

4. Leukotriene C₄ release from BAL cells and peripheral leucocytes

The release of histamine from BAL cells in the BAL fluid was significantly lower in patients with

DL than in those without DL (p<0.05). In contrast, the LTC₄ release from BAL cells in patients with DL was significantly higher than the release in those without DL (p<0.05) (Fig. 3).

The release of histamine from peripheral leucocytes was lower in patients with DL than in those without DL, but the difference was not significant. The LTC₄ release from peripheral leucocytes was

Fig. 4 Release of histamine (●) and LTC₄ (○) from peripheral leucocytes stimulated by Ca ionophore A 23187 in asthmatic subjects with and without detectable LTC₄ in the BAL fluid.

higher in patients with DL than in those without DL; however, this difference was not significant (Fig. 4).

IV. Discussion

Of various mediators, histamine and LTC₄ (or LTD₄, LTE₄) are well known to induce the bronchoconstriction^{15,16} that is characteristic of asthma. These mediators are released from mast cells⁴⁻⁷) and airway inflammatory cells in patients with bronchial asthma¹⁷⁻¹⁹). The presence of these mediators in the BAL fluid of patients with asthma has been shown²⁰⁻²²), and increased levels of these mediators have also been demonstrated in patients with bronchial asthma^{20,23,24}). These findings indicate that histamine and LTC₄ are closely related to the mechanism underlying the onset of asthma. The levels of histamine and LTC₄ released into BAL fluid differ among asthma patients, however, the reason for this is not known²⁵).

In the present study, we investigated the presence of histamine and LTC₄ in the airways in asthma patients. With the spectrofluorometric method^{17,18,26}) used in this study, histamine was detectable in the BAL fluid of 9 of the 22 (40.9%) asthma patients. Of these 9 patients with detectable histamine (DH) in BAL fluid, 7 were sensitive to house dust mites. One of the two remaining patients had a high serum IgE, of more than 500 IU/

mI, and was assessed as atopic. Thus, 8 of the 9 patients with DH were evaluated as atopic. The release of histamine from BAL cells and peripheral leucocytes was significantly higher in patients with DH than in those without DH. Regarding the 13 patients without DH in the BAL fluid, 10 showed negative skin reactions to various allergens, and all 13 showed negative RAST scores, of 1+ or less, to allergens. The level of serum IgE was under 200 IU/mI in 10 of the 13 patients. Thus, many of these patients were evaluated as nonatopic. The levels of serum IgE were significantly different in patients with and without DH in the BAL fluid. The release of LTC₄ from BAL cells was rather higher in patients without DH than in those with DH. These results suggest that many of the patients with DH were atopic, and that histamine was the major mediator.

Leukotriene C₄ in BAL fluid was detectable in 10 of the 22 (45.5%) patients. All of the patients with detectable LTC₄ (DL) in BAL fluid had a low level of serum IgE, of less than 200 IU/mI, and negative skin reactions to various allergens (except for 2 subjects), while only one patient showed a positive RAST to house dust mites. The levels of serum IgE and BAL histamine were significantly lower in patients with DL than in those without DL. In this study, dose-response curves of histamine and LTC₄ release were examined by three different

concentrations, 1, 5, and 10 $\mu\text{g/ml}$ of Ca ionophore A 23187, and 1 $\mu\text{g/ml}$ of the agent was selected as most suitable concentration for the experiment. Histamine release from BAL cells in patients with DL was also significantly lower than that in patients without DL. There was, however, no significant difference between the release of histamine from peripheral leucocytes in patients with and without DL. In contrast, the release of LTC₄ was significantly higher in patients with DL in the BAL fluid than in those without DL. The release of LTC₄ from peripheral leucocytes was higher in patients with DL than in those without DL, although the difference was not significant. These results suggest that many of the patients with DL were nonatopic, and that LTC₄ was the major mediator.

Histamine and LTC₄ levels have been reported to be increased in atopic asthmatics after allergen challenge²³⁻²⁵. Both histamine and LTC₄ participate in the IAR of atopic subjects, but the levels of these mediators are different in relation to LAR²⁵. In this study, many of the patients with DH in the BAL fluid were evaluated as atopic, and their BAL cells released more histamine than LTC₄ on stimulation with the Ca ionophore A 23187. In contrast, many of patients with DL were assessed as nonatopic, and their BAL cells released more LTC₄

than histamine. Their peripheral leukocytes showed almost the same tendencies as the BAL cells. It is, however, unclear whether these differences in histamine and LTC₄ levels are related to the LAR, as has been reported by Wenzel et al.²⁵; the difference may be related to an IgE-mediated reaction. Further studies are necessary to clarify the mechanism underlying these differences.

V. Conclusion

The levels of histamine and leukotriene C₄ (LTC₄) in bronchoalveolar lavage (BAL) fluid were measured in 22 patients with bronchial asthma. Histamine was detectable in 9 (40.9%), and LTC₄ was detectable in 10 (45.5%) of the 22 patients. Many of the patients with detectable histamine (DH) in the BAL fluid were assessed as atopic; their BAL cells released more histamine and less LTC₄ than those of patients without DH. In contrast, many of the patients with detectable LTC₄ (DL) in the BAL fluid were evaluated as nonatopic; their BAL cells released more LTC₄ and less histamine than those of patients without DL. These findings show that histamine and LTC₄ levels differ in relation to the IgE-mediated allergic reaction.

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