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## Effects of aging on responsiveness and inflammation in the airways of patients with bronchial asthma

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**Abstract :** Ventilatory function, cellular composition in bronchoalveolar lavage (BAL) fluid and release of chemical mediators from leucocytes were examined in 25 older asthmatics (mean age 66.7 years) and 25 younger asthmatics (mean age 45.1 years) in relation to clinical asthma types. 1. Ventilatory function tests showed that the values of ventilatory parameters such as %MMF,  $\% \dot{V}_{50}$  and  $\% \dot{V}_{25}$  were lower in older subjects than in younger subjects, and a significant difference was found in the  $\% \dot{V}_{50}$  value between the two age groups. Regarding clinical asthma types, the values of %MMF,  $\dot{V}_{50}$ , and  $\dot{V}_{25}$  were significantly lower in both younger and older subjects with type II. 2. In analysis of cellular composition in BAL fluid, the proportion of BAL lymphocytes was significantly lower in type II older subjects than in younger subjects with the same type. A significantly increased proportion of BAL neutrophils was observed in both younger and older subjects with type II. 3. The release of leukotriene C<sub>4</sub> (LTC<sub>4</sub>) from leucocytes was significantly lower in type II older subjects than in younger subjects with the same type, and LTC<sub>4</sub> release in patients with type II was also significantly lower than that in those with other asthma types in both younger and older subjects. The release of histamine and leukotriene B<sub>4</sub> (LTB<sub>4</sub>) from leucocytes was not significantly different between the two age groups.

These results demonstrate that ventilatory function, cellular composition in BAL fluid, and the release of chemical mediators from leucocytes are affected by aging and clinical asthma types.

**Key words :** Ventilatory function, Cellular composition in BAL fluid, chemical mediators, aging, clinical asthma type

## Introduction

Bronchial hyperresponsiveness to various stimuli is one of the characteristics of asthma. The hyperresponsiveness of bronchi is affected by the degree of IgE-mediated allergic reaction, and pathophysiological changes of airways associated with inflammatory cell infiltration (1-3). These two factors, IgE-mediated allergic reactions and pathophysiological changes of airways, play major roles in characterizing asthma attacks of each subject. Furthermore, these two factors seem to change quantitatively and qualitatively with aging (4-6).

Recently, the pathophysiological changes of the airways of asthmatics have been extensively studied by analyzing humoral and cellular composition in bronchoalveolar lavage (BAL) fluid (7-11). It has been demonstrated that concentrations of chemical mediators such as histamine,  $LCT_4$ , and  $LT B_4$  are increased in BAL fluid of asthmatics (12,13). Our previous studies have shown that cellular composition in BAL fluid of asthmatics changes with aging (14, 15).

Ventilatory function is speculated to be affected by aging. In healthy subjects, ventilatory dysfunction in the small airways is often observed in elderly. There are few reports on changes of ventilatory function in asthmatics with aging.

In the present study, clinical features by elderly patients with bronchial asthma were studied by observing ventilatory function, cellular composition in BAL fluid, and release of chemical mediators from leucocytes, and the results were compared with those in younger asthmatics.

## Subjects and Methods

The subjects were 25 asthmatics over the age of 60 (older patients). Of these, 15 were females and 10 were males. The mean age was 66.7 years with a range of 61 to 74 years. The mean value of serum IgE was  $316 \pm 476$  IU/ml ( $\pm$ SD). Twenty five patients with asthma under the age of 59 (15 females and 10 males) were selected as control subjects. Their mean age was 45.1 years (range 24 to 58 years) (younger patients). The mean value of serum IgE was  $426 \pm 599$  IU/ml.

The subjects were classified into three asthma types according to clinical symptoms, as previously described (16-20). The criteria were:

Type Ia. Simple bronchoconstriction type: Patients with symptoms such as wheezing and dyspnea which are mainly elicited by bronchoconstriction.

Type Ib. Bronchoconstriction + hypersecretion type: Patients with symptoms due to hypersecretion (more than 100 ml/day of expectoration), in addition to bronchoconstriction.

Type II. Bronchiolar obstruction type: Patients with symptoms mainly elicited by bronchiolar obstruction.

Bronchoalveolar lavage (BAL) was performed by a previously reported method (16-20). The BAL examination was carried out when the patients were free of attack after informed consent was obtained from all study subjects. The aspirates obtained by a bronchofiberscope were filtered through a sterile steel mesh and centrifuged at 1200 rpm for 10 min at 4°C. Smear preparations made from the cell suspension were stained with May Giemsa. Cell differentiation was

performed by observing 500 cells, excluding epithelial cells. In this study, the mean recovery rate at BAL was  $26.4 \pm 8.2\%$  ( $\pm$ SD). The total cell number aspirated into BAL fluid was  $7.84 \pm 9.6 \times 10^6$ . The results were expressed as a percentage of the total cells.

For the experiments on chemical mediator release from leucocytes, the number of cells was adjusted to  $5 \times 10^6 / \text{ml}$  in Tris ACM after leucocytes were separated by a counterflow centrifugation elutriation (21). Ca ionophore A23187 ( $1 \mu\text{g}$ ) was added to the cell suspension, the suspension was incubated for 15 min, and the concentration of histamine (in the supernatant and cells) and leukotrienes C<sub>4</sub> and B<sub>4</sub> (in the supernatant only) were measured. The histamine concentration was assayed by an automated spectrofluorometric analysis system (22) after perchloric acid precipitation, as previously reported (23, 24). The results were expressed as a percentage of total histamine release. For measurement of LTs C<sub>4</sub> and B<sub>4</sub>, the cell suspension was incubated with Ca ionophore A23187, and then 4 ml of 100% ethanol was added. After the suspension was centrifuged, the supernatant was vacuum dried and resuspended in 250  $\mu\text{g}$  of high performance liquid chromatography (HPLC) solvent (CH<sub>3</sub> CN/H<sub>2</sub> O=1 : 1). The resuspended solution was subjected to HPLC (c-18 reversed-phase column, with detection at 280 nm) and the results were expressed as ng/ $5 \times 10^6$  cells.

Ventilatory function using a Box Spiro 81-S (Chest CO.) was measured in all subjects when they were free of attack. Six ventilatory parameters, %FVC, FEV<sub>1.0</sub>%, %PEFR, %MMF,  $\dot{V}_{50}$ , and  $\dot{V}_{25}$ , were compared between younger and older subjects.

## Results

### 1. Ventilatory function

The values of six ventilatory parameters were generally lower in older patients than in younger patients. The difference in these parameters except  $\dot{V}_{50}$  was, however, not significant between the two age groups. The  $\dot{V}_{50}$  value was significantly lower in older patients than in younger patients ( $P < 0.05$ ) (Fig. 1). Regarding clinical asthma types, there was no significant difference in the values of ventilatory parameters between older and younger patients. In older subjects, the values of %MMF,  $\dot{V}_{50}$  and  $\dot{V}_{25}$  were significantly lower in subjects with type II than in those with types Ia and Ib. In contrast, in younger subjects, the values of FEV<sub>1.0</sub>%, %MMF,  $\dot{V}_{50}$  and  $\dot{V}_{25}$  were significantly lower in patients with type II than in those with types Ia and Ib. These results suggest that ventilatory parameters, particularly %MMF,  $\dot{V}_{50}$  and  $\dot{V}_{25}$  were low in both younger and older patients with type II asthma (Table 1).

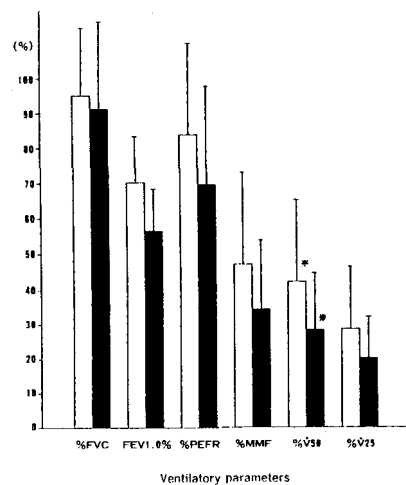


Fig. 1. Comparison of ventilatory function between younger ( $\square$ ) and older asthmatics ( $\blacksquare$ ). \*  $p < 0.02$ .

Table 1. Ventilatory function in younger and older asthmatics in relation to clinical asthma types

Asthma type	Age (yr)	No of patients	Ventilatory parameters					
			%FVC	FEV <sub>1.0%</sub>	%PEFR	%MMF	$\dot{V}_{50}$	$\dot{V}_{25}$
Ia	0-59	10	91.6 <sup>a</sup> ±18.9	76.5 <sup>a</sup> ±10.9	96.3 <sup>c</sup> ±25.5	58.2 <sup>e</sup> ±27.5	51.9 <sup>g</sup> ±23.8	36.8 <sup>i</sup> ±19.9
	60+	9	101.1 ±16.3	70.4 ±10.6	76.9 ±23.0	37.9 <sup>b</sup> ±13.1	33.2 <sup>d</sup> ±13.9	24.9 <sup>f</sup> ±11.1
Ib	0-59	10	104.0 ±15.2	74.5 <sup>b</sup> ±7.5	93.1 <sup>d</sup> ±24.0	50.9 <sup>f</sup> ±18.9	44.6 <sup>h</sup> ±16.7	30.5 <sup>j</sup> ±12.9
	60+	9	78.8 ±29.5	71.3 <sup>a</sup> ±7.9	74.1 ±29.7	39.6 <sup>c</sup> ±19.3	34.4 <sup>e</sup> ±18.7	23.7 <sup>g</sup> ±13.5
II	0-59	5	83.6 ±23.0	51.1 <sup>ab</sup> ±10.2	55.8 <sup>cd</sup> ±16.2	17.3 <sup>ef</sup> ± 8.1	12.7 <sup>gh</sup> ± 6.8	8.9 <sup>ij</sup> ±5.4
	60+	7	77.9 ±29.1	56.9 <sup>a</sup> ±13.5	55.6 ±24.8	17.5 <sup>bc</sup> ± 5.2	13.0 <sup>de</sup> ± 6.9	8.6 <sup>fg</sup> ±3.5

Ia: simple bronchoconstriction type; Ib: bronchoconstriction + hypersecretion type; II: bronchiolar obstruction type. <sup>a</sup>Mean±SD. a, d: p < 0.02; b, e, f, g, h, i, j: p < 0.01; c, r: p < 0.05; A, c, E, G: p < 0.02; B, D, F: p < 0.01.

The mean value of each ventilatory parameter was compared between younger and older patients. The ratio of the mean value in older patients to the mean value in younger patients (Y/O ratio) was from 0.9 to 1.0 in all ventilatory parameters of type II asthma patients. The Y/O ratio of ventilatory parameters was higher than 1.2 in older patients with types Ia and Ib, suggesting that the values of %MMF, % $\dot{V}_{50}$  and % $\dot{V}_{25}$  are more decreased in older patients with types Ia and Ib than in those with type II (Fig. 2).

2. Cellular composition in BAL fluid

Cellular composition in BAL fluid of older patients was similar to that of younger patients, and the proportion of macrophages, lymphocytes, neutrophils and eosinophils in BAL fluid was not significantly different between the two age groups (Fig. 3). In patients classified by clinical symptoms, the proportion of lymphocytes in BAL fluid of type II asthma was significantly lower in older patients than in younger patients (p < 0.05). There was no significant difference

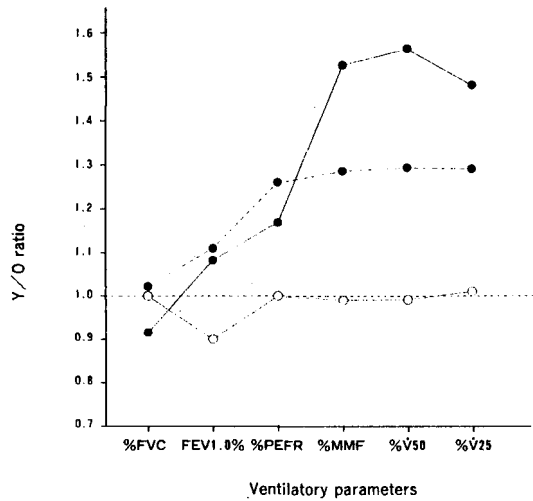


Fig. 2. Ratio of mean value of each ventilatory parameter in older subjects to mean value in younger subjects (Y/O ratio) in Ia. Simple bronchoconstriction type (●—●), Ib. bronchoconstriction + hypersecretion type (●---●), and II. bronchiolar obstruction type (○—○).

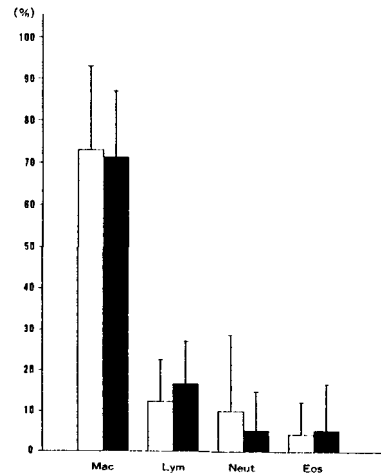


Fig. 3. Cellular composition in BAL fluid of younger (□) and older asthmatics (■). Mac, macrophages; Lym, lymphocytes, Neut, neutrophils; Eos, eosinophils.

in the proportion of BAL cells in types Ia and Ib between the two age groups. The proportion of macrophages and lymphocytes in type II older patients was significantly lower than those in types Ia and Ib older patients. In contrast, a significantly increased proportion of neutrophils in BAL fluid was observed in older patients with type II than in those with types Ia and Ib. In younger patients, the proportion of neutrophils in BAL fluid was also significantly higher in type II asthma patients than in those with type Ia ( $p < 0.05$ ). There was no significant difference in the proportion of eosinophils between the two age groups and among the three clinical asthma types (Table 2).

Table 2. Cellular composition in BAL fluid of younger and older asthmatics in relation to clinical asthma types

Asthma type	Age (yr)	BAL cells (%)			
		Mac	Lym	Neut	Eos
Ia	0-59	79.2±15.1 <sup>a</sup>	13.9±12.9	1.7±2.2 <sup>c</sup>	5.1±10.9
	60+	83.9± 8.4 <sup>a</sup>	12.8± 8.5	1.4±1.0 <sup>d</sup>	1.8± 3.2
Ib	0-59	77.6±13.0	12.9±12.2	5.6±5.5	3.9± 4.4
	60+	59.7±17.6	20.2±12.9 <sup>b</sup>	5.5±5.8 <sup>e</sup>	13.9±15.7
II	0-59	70.4±12.1	19.2±12.0 <sup>a</sup>	10.0±12.2 <sup>c</sup>	0.5± 0.4
	60+	47.7±25.4 <sup>b</sup>	6.8± 4.4 <sup>b</sup>	38.3±28.1 <sup>d</sup>	7.1± 4.5

Ia: simple bronchoconstriction type; Ib: bronchoconstriction + hypersecretion type; II: bronchiolar obstruction type. \*Mean±SD. Mac, macrophages; Lym, lymphocytes; Neut, neutrophils; Eos, eosinophils. a:  $p < 0.05$ ; a, d:  $p < 0.001$ ; b, c:  $p < 0.05$ ; e:  $p < 0.01$ .

### 3. Release of chemical mediators

Release of histamine, leukotrienes C<sub>4</sub> and B<sub>4</sub> (LTC<sub>4</sub> and LTB<sub>4</sub>) from peripheral leucocytes stimulated with Ca ionophore A 23187 was compared between younger and older patients. The release of histamine and LTB<sub>4</sub> was higher in younger patients than in older patients. There was, however, no significant difference in the release of histamine, LTB<sub>4</sub> and LTC<sub>4</sub> between the two age groups. Regarding clinical asthma types, the release of histamine was high in younger patients compared to older patients in all

clinical asthma types, although there was no significant difference between the two age groups. The release of LTB<sub>4</sub> was high in younger patients with types Ib and II, but the release was not significantly different from older patients. The release of LTC<sub>4</sub> was significantly lower in older patients than in younger patients in type II asthma ( $p < 0.05$ ). The LTC<sub>4</sub> release in type II older patients was significantly lower than in older patients with type Ia ( $p < 0.05$ ) and type Ib ( $p < 0.05$ ). There was no significant difference in the release of LTC<sub>4</sub> of younger patients among the three clinical asthma types (Table 3).

Table 3. Release of chemical mediators from leucocytes stimulated by Ca ionophore A23187

Asthma type	Age (yr)	No of patients	Histamine release	LTB <sub>4</sub> (ng/5×10 <sup>6</sup> cells)	LTC <sub>4</sub>
Ia	0-59	9	19.9±17.5 <sup>*</sup>	44.2±29.5	16.2±23.7
	60+	9	16.6±18.9	48.2±27.1	10.7± 8.1 <sup>a</sup>
Ib	0-59	7	20.9±11.9	44.1±34.7	4.9± 7.6
	60+	7	14.7±13.4	38.2±14.1	14.4±10.8 <sup>b</sup>
II	0-59	5	9.9± 6.6	52.6±11.8	7.3± 4.2 <sup>c</sup>
	60+	5	16.8±14.3	30.1±17.4	2.0± 1.5 <sup>abc</sup>

Ia: simple bronchoconstriction type; Ib: bronchoconstriction + hypersecretion type; II: bronchiolar obstruction type. \* Mean±SD. a, b, c:  $p < 0.05$ .

### Discussion

The mechanism of onset of asthma and the pathophysiological changes of airways are closely related to clinical features of patients with bronchial asthma. These two factors participating in clinical symptoms have been speculated to be affected by aging (4-6). In the present study, factors showing the clinical characteristics of asthma, ventilatory function, cellular composition in BAL fluid, release of chemical mediators, were examined in relation to clinical asthma types associated with the pathophysiological changes of airways and to aging.

It has been demonstrated that ventilatory

function in small airways is decreased as the age is higher in healthy subjects. In this study, the values of  $\%MMF$ ,  $\% \dot{V}_{50}$  and  $\% \dot{V}_{25}$  which may represent ventilatory dysfunction in small airways, showed a tendency to be lower in older patients than in younger patients, and a significant difference was found in the value of  $\% \dot{V}_{50}$  between younger and older patients. Regarding clinical asthma types, the Y/O ratio of ventilatory parameters was high in types Ia and Ib asthma patients, suggesting that ventilatory function in types Ia and Ib older asthma patients may be more decreased rather than those with type II. In type II asthmatics, ventilatory function is remarkably decreased in both younger and older patients. In fact, the values of ventilatory parameters such as  $\%MMF$ ,  $\% \dot{V}_{50}$  and  $\% \dot{V}_{25}$  were significantly lower in type II patients than in those with other clinical asthma types in both younger and older subjects. The results show that aging and the pathophysiological changes of airways related to clinical asthma types may influence ventilatory function in asthmatics.

Cellular composition in BAL fluid has been extensively studied in relation to airway inflammation in asthmatics (1–3). Blood cells, lymphocytes, neutrophils, eosinophils and basophils which migrate from the bloodstream into local allergic sites, are considered to play a major role in the inflammatory process (7–13). There are little data on cellular composition of BAL fluid in elderly asthmatics. Our results from this study reveal that there is no significant difference in the proportion of these blood cells in BAL fluid between younger and older subjects. In the subjects classified by clinical symptoms, the proportion of lymphocytes in BAL fluid was significantly lower in older subjects with

type II than in younger subjects with same type. There was no significant difference in the proportions of other blood cells in BAL fluid between the two age groups. While a significantly higher proportion of neutrophils was found in both younger and older subjects with type II. The results show that cellular composition in BAL fluid is affected by aging and clinical asthma types.

It has been reported that chemical mediators in BAL fluid are increased in asthmatics (23), and the release of chemical mediators from leucocytes are also increased in some asthmatics (12, 13). Our data from this study reveal that there is no significant difference in the release of histamine and  $LTB_4$  between younger and older subjects. In the release of  $LTC_4$ , the release was significantly lower in type II older subjects than in younger subjects with the same type, and this  $LTC_4$  release in type II asthma was also significantly lower than in types Ia and Ib in older subjects. The results demonstrate that the release of chemical mediators are different between the two age groups and among three clinical asthma types.

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#### 気管支喘息における気道の反応性および炎症反応におよぼす加齢の影響について

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25例の老年者気管支喘息と25例の若壮年者喘息症例を対象に、換気機能、気道内細胞成分の頻度、白血球からの化学伝達物質遊離などの気道反応と関連した要素について、喘息の臨床病型との関連のもとに検討を加えた。1. %MMF, % $\dot{V}_{50}$ , % $\dot{V}_{25}$ などの換気パラメーターは老年症例において全般的に低く、% $\dot{V}_{50}$ では若壮年症例に比べ有意の低値を示した。臨床病型別では、II型喘息において、両年齢群とも他の病型に比べ%MMF,

% $\dot{V}_{50}$ 及び% $\dot{V}_{25}$ 値は有意に低い傾向が見られた。

2. 気管支肺胞洗浄 (BAL) 液中のリンパ球頻度は、II型の老年症例において若壮年症例に比べ有意に低い値を示した。BAL液中好中球頻度は、いずれの年齢群においても、他の病型に比べ有意に高い値を示した。3. 白血球からのLTC<sub>4</sub>遊離は、II型の老年症例において若壮年症例に比べ、また他の臨床病型に比べ有意に低い値を示した。ヒスタミンおよびLTB<sub>4</sub>遊離には両年齢群間に有意の差は見られなかった。

これらの結果は、換気機能、気道内細胞成分の頻度、白血球からの化学伝達物質遊離などが、加齢や臨床病態により影響を受けることをある程度示しているものと考えられた。

キーワード : 換気機能, 気道内細胞成分, 化学伝達物質, 加齢, 臨床病型