◎原 著

Characteristics of humoral and cellular factors participating in onset mechanism of asthma in relation to clinical types classified by symptoms.

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Abstract : Characteristics of each asthma type classified by clinical symptoms and findings were studied in 72 patients with bronchial asthma. 1. Ventilatory function tests showed that the values of %MMF, $\%\dot{V}_{50}$ and $\%\dot{V}_{25}$ were significantly lower in patients with bronchiolar obstruction (type II) compared to the values of those with simple bronchoconstriction type (type Ia) and those with bronchoconstriction + hypersecretion (type Ib). 2. The proportion of neutrophils in bronchoalveolar lavage (BAL) fluid was significantly higher in type II than in type Ia and type Ib. Several patients with type Ib showed higher proportion of BAL eosinophils. 3. The release of LTC_4 from leucocytes was significantly lower in type II compared with type Ia and type Ib. There was no significant difference in the release of histamine and LTB_4 among the three asthma types.

Key words : Bronchial asthma, Asthma classification, BAL fluid, chemical mediators.

Introduction

Bronchial asthma is characterized by transient bronchoconstriction, accompanied with edema of mucous membrane and hypersecretion. The pathophysiological changes in bronchial asthma can be observed by clinical symptoms during asthma attacks. In adult asthma cases, the attacks tend to be chronic and severe. Therefore, it is often difficult for physicians to control their attacks with usual medication except corticosteroid hormone.

Our previous studies have shown that bronchial asthma can divided into three

clinical types according to the symptoms and findings (1−3). In the present studies, characteristics of each asthma type were examined by analyzing cellular composition of bronchoalveolar lavage (BAL) fluid and release of chemical mediators from leucocytes.

Subjects and Methods

The subjects were 72 patients with bronchial asthma (45 females and 27 males). The mean age of the subjects was 55.5 years (range ; 26−76 years). The mean conccentration of total IgE was 372 IU/mℓ (range ; 48−3200 IU/mℓ). Of 72 subjects, 26 (36.1%) had been on corticosteroid therapy for longer than two years, and 19 (26.4%) were sensitive to house dust mite. They were all nonsmokers, because smokers were excluded before analysis of data in this study.

Bronchoalveolar lavage (BAL) was performed according to the method previously described (4, 5) during attack-free stages. Informed consent for the BAL examination was accepted by subjects. After the aspirates were centrifuged at 1200 rpm for 10 min at 4℃, the cell pellet was resuspended in Tris ACM. Smear preparations were made using the cell suspension. The slides were air-dried and stained with May-Giemsa. A differential cell count was carried out on 500 cells excluding epithelial cells. In this study, the mean recovery rate at BAL was 26.8±9.9% (mean ±SD).

The subjects were classified into three types of asthma according to their clinical symptoms and findings (1−3). The cases, whose symptoms, wheezing and dyspnea, were assessed to be elicited mainly by bronchoconstriction, were evaluated as Ia. simple bronchoconstriction type. The cases, whose symptoms were due to hypersecretion (more than 100mℓ/day of expectoration), in addition to bronchoconstriction, were evaluated as Ib. bronchocon-striction + hyperssecretion type. The subjects, whose symptoms were assessed to be caused by bronchiolar obstruction, in addition to bronchoconstriction or hypersecretion, were estimated as II. bronchiolar obstruction type.

The release of chemical mediators, histamine, leukotriene B_4 (LTB_4) and leukotriene C_4 (LTC_4), from peripheral leukocytes was examined by stimulation with Ca ionophore A 23187 (1.0 μg/mℓ). Cells were separated by counterflow centrifugation elutriation using a JE-6B rotor (Beckman) (6, 7). Venous blood (17.5mℓ) was drawn into a test tube containing 2.5mℓ of 0.1 M EDTA. After centrifugation for 8 min at 500 rpm, the supernatant was removed, and the precipitate was transferred into the rotor with the buffer (pH 7.2, osmotic pressure 325 mOsm/kgH_2O) containing 1/10 M phosphate buffer, 0.14 M NaCl and 2% (w/v) bovine serum albumin (6). The sample flowing out of the rotor for 8 min at a flow rate of 4.5mℓ/min was collected into a test tube. After the rotor was washed out with the buffer for 10 min, the procedure for cell separation was started at a flow rate of 6mℓ/min. The flow rate was increased by 1mℓ per 4 min, followed by the collection of cells into a test tube. The final flow rate was 15mℓ/min. The experiments were performed under 4℃ at 2000 rpm (8). The cells separated at flow rate of 4.5 to 10 mℓ/min were applied for the experiment of histamine release and cells at flow rate 11 to 15mℓ/min for the experiments of leukotrienes release. The histamine content of the cells and the supernatant fluid was analyzed by perchloric acid precipitation and assayed by an automated spectrofluorometric histamine analysis system (Technicon) (9 − 11). The results were expressed as a percent release of

the total histamine content. The release of leucotrienes was evaluated by measuring the content of leukotrienes in the supernatant by a HPLC and the results were expressed as ng/10^6 cells.

The level of total IgE in sera was measured by radioimmunosorbent test (RIST).

Results

1. Ventilatory function in each clinical asthma type

To demonstrate the characteristics of airway disorder in each asthma type, ventilatory function test was carried out in all subjects during attack-free stages.

Table 1 presents the results of ventilatory function in each asthma type. The value of each ventilatory prameter was generally low in type II and marked difference was found in %MMF, $\%\dot{V}_{50}$ and $\%\dot{V}_{25}$ between type Ia or Ib and type II (Table 1).

Table 1. Ventilatory function in patients with bronchial asthma in relation to clinical type

Clinical type	%FVC	$FEV_{1.0\%}$	%PEFR	%MMF	$\%\dot{V}_{50}$	$\%\dot{V}_{25}$
Ia	93.4 ±19.2	69.1[a] ±13.0	80.7 ±26.2	49.6[b] ±24.0	41.2[d] ±20.2	30.7[f] ±17.6
Ib	104.1 ±20.8	68.6 ± 9.9	81.6 ±28.0	43.6[c] ±21.7	36.7[e] ±19.2	26.5[g] ±14.7
II	81.3 ±26.4	60.5[a] ±12.1	64.2 ±22.7	17.3[b,c] ± 8.1	12.0[d,e] ± 7.0	9.3[f,g] ± 6.5

Ia :simple bronchoconstriction type, Ib :bronchoconstriction + hypersecretion type, II :bronchiolar obstruction type.
a : $p<0.05$, b,c,d,f,g : $p<0.001$. e : $p<0.01$.

2. Characteristics of the subjects studied

The subjects were classified into three clinical asthma types. No significant difference was present in the mean age among three asthma types. Serum concentration of total IgE was lowest in type II asthma, although any significant difference was not present among them (Table 2).

Table 2. Characteristics of patients with bronchial asthma classified by clinical symptoms

Clinical type	Age, years Mean	Range	No	Serum IgE (IU/ml)
Ia	53.8	26-74	35	429 ± 543*
Ib	56.7	28-70	25	384 ± 511
II	58.2	37-76	12	180 ± 89

*Mean ± sd

3. Cellular composition in BAL fluid of each asthma type

The proportion of macrophages in BAL fluid was significantly higher in type Ia (78.6±14.9%) than in type II (61.5±22.0%) ($p<0.01$). The proportion of BAL lymphocytes was similar from 14.1% to 15.9% in three asthma types, and no significant difference was found among them. Marked increase in the proportion of BAL neutrophils was observed in type II cases, whose ventilatory parameters such as %MMF, $\%\dot{V}_{50}$, and $\%\dot{V}_{25}$ representing small airway dysfunction was very low. The proportion of BAL neutrophils (20.5±25.1%) in type II cases was significantly higher compared with that in type Ia (2.1±2.6%) ($p<0.001$) and type Ib (4.8±7.1%) ($p<0.01$). The proportion of BAL neutrophils in type Ib was greater than the proportion in type Ia, although no significant difference was found between them. Relating to eosinophils, the proportion in type Ib was highest (18.1±11.3%) among the three asthma types. Several cases with high proportion of eosinophils, who were cases with so-called intrinsic asthma accompanied with eosinophilia and hypersecretion in the airways, were included in type Ib. No significant

difference was, however, present in the proportion of BAL eosinophils among them (Fig. 1).

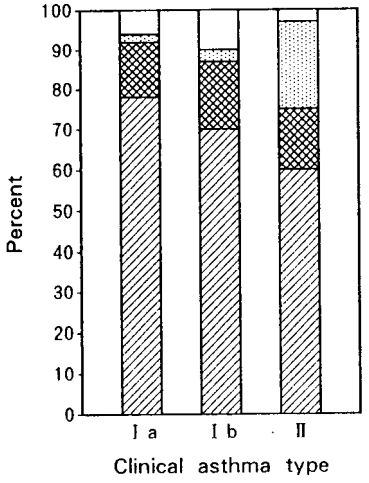

Fig. 1. Cellular composition in BALF of patients with bronchial asthma in relation to clinical type. ▨ : macrophages, ▩ : lymphocytes, ⁘ : neutrophils, □ : eosinophils

4. Release of chemical mediators from leucocytes

Histamine release from leucocytes induced by Ca ionophore A23187 was the highest in type Ia (17.0±14.8%) and the lowest in type II (12.0±9.2%), although there was no significant difference between them. The release of LTC_4 from leucocytes by Ca ionophore A23187 was significantly higher in type Ia (11.5±10.7ng/10^6cells) than in type II (4.1±4.1ng/10^6cells) ($p<0.05$). The value of LTC_4 induced by Ca ionophore A23187 was 8.7±6.9ng/10^6 cells in type Ib. The release of LTB_4 from leucocytes was 39.5±20.9ng/10^6cells in type Ia, 37.1±21.3ng/10^6cells in type Ib and 39.1±21.2ng/10^6 cells in type II. The values were similar and no significant difference was foud in the release of LTB_4 among the three asthma types (Fig. 2).

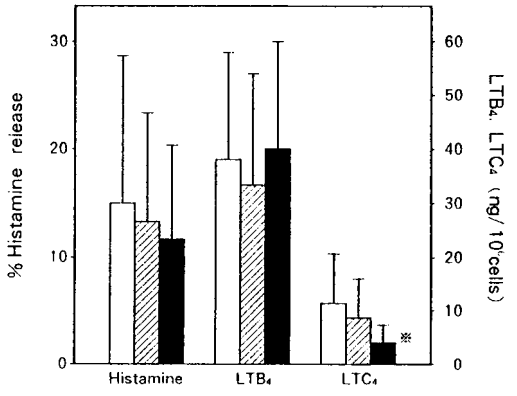

Fig. 2. Release of chemical mediators from leucocytes of patients with bronchial asthma in stimulation with Ca ionophore A23187. □: type Ia, ▨: type Ib, ■ : type II
※Significant difference from type Ia at $p<0.05$

Discussion

Recently, the role of inflammatory response including inflammatory mediators and cells has be noticed in the pathogenesis of bronchial asthma. Inflammatory mediators have been demonstrated to increase in BAL fluid during the time of the immediate asthmatic response (IAR), and that of the late asthmatic response (LAR) (12, 13), which is associated with inflammatory cells. It has been reported that an increased number of lymphocytes (14-16) and eosinophils (17, 18) in BAL fluid was observed in asthma patients. While there are only a few reports on the changes in number of neutrophils in BAL fluid of asthmatics (19). Boichot et al (20) have observed bronchopulmonary response to acetylcholine and 5-hydroxytryptamine is

enhanced 3−4 hr and 18−24 hr after antigen exposure of sensitized animals. At the same time, they showed a significant increase in the number of neutrophils in BAL fluid 3−4 hr after the exposure of sensitized animals to antigen, which was associated with a significant eosinophilia at 18-24 hr. Thus, inflammatory mediators and cells participate in the pathogenesis of human asthma.

Asthma symptoms are variegated. Some patients show strong wheezing with dyspnea during their asthma attacks. Other patients show no or a little wheezing inspite of dyspnea, and several patients have a lot of expectoration. Our previous studies have demonstrated that bronchial asthma can be classified into three clinical types according to their symptoms and findings (1-3). In this study, to analyze the characteristcs of each asthma type, cellular composition in BAL fluid and the release of chemical mediators from leucocytes of each clinical asthma type were compared among the three clinical asthma types. Ventilatory function test demonstrated that the values of %MMF, $\%\dot{V}_{50}$ and $\%\dot{V}_{25}$, which are estimated to represent the dysfunction of the small airways, were markedly decreased in type II cases. There was no significant difference in ventilatory function beween type Ia and type Ib, although the subjects in both types showed an obstructive ventilatory pattern with a decrease in $FEV_{1.0}$.

The data from the present study reveal that the proportion of neutrophils in BAL fluid was significantly higher in type II (bronchiolar obstruction type) compared with types Ia and Ib, demonstrating the participation of neutrophils in bronchiolar obstrution. The proportion of eosinophils was increased in some cases of type Ib, which included several so-called intrinsic asthma accompanied with eosinophilia and hypersecretion in the airways. Relating to release of chemical mediators, the release of LTC_4 was significantly lower in type II than in type Ia, suggesting that type II cases are characteristic of higher proportion of neutrophils in BAL fluid and less release of LTC_4. The release of histamine and LTB_4 from leucocytes showed no significant difference among the three asthma types.

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気管支喘息の臨床分類とその細胞性および液性因子の特徴

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気管支喘息72例を対象に，その臨床病態の特徴を，気道炎症性の細胞および化学伝達物質の観察により検討した。

1．気道反応の特徴を換気機能の面から検討すると，II細気管支閉塞型において，ＭＭＦ，$\dot{V}_{50}$や$\dot{V}_{25}$などの小ないし細気管支領域の換気障害を示すパラメーターの値は，Ia型，Ib型に比べ有意の低下を示した。

2．気道細胞反応では，II型において，他の臨床病型に比べ，BAL液中好中球の出現頻度の有意の増加が観察された（Ia，$p<0.001$；Ib，$p<0.01$）。好酸球の出現頻度は，Ib過分泌型において著しい増加傾向を示す症例が見られたが，その平均出現頻度には３病型間に有意の差は見られなかった。

3．好中球からのメジエーター遊離では，ヒスタミン遊離は，Ia単純性気管支攣縮型において最も高い値が示されたが，推計学的には３病型間に有意の差は見られなかった。ロイコトリエンC_4では，Ia型においてII型に比べ有意に高い遊離が観察された（$p<0.05$）。ロイコトリエンB_4遊離には，３病型間に差は見られなかった。