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Release of histamine and leukotrienes C_4 and B_4 from peripheral leucocytes and bronchoalveolar cells and bronchial hyperresponsiveness in patients with asthma

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Abstract : The proportions of cells in bronchoalveolar lavage (BAL) fluid, the release of histamine, leukotrienes C_4 and B_4 from leucocytes and BAL cells, and bronchial reactivity to methacholine were examined in 40 patients with asthma in relation to patient age.

1. The proportions of lymphocytes and neutrophils in BAL fluid were higher in older patients over age 60 than in younger patients between 20 and 49. The proportions of eosinophils and basophilic cells in BAL fluid were higher in younger patients than in older patients, and the difference in the proportion of BAL basophilic cells was significant between the two groups ($p<0.05$).
2. The release of histamine from BAL cells was significantly higher in younger patients than in older patients ($p<0.001$). The release of LTC_4 from BAL cells was higher in older patients compared to younger patients.
3. Bronchial reactivity to methacholine was higher in younger patients than in older patients. The number of patients reactive to low concentration of methacholine (390 μg/mℓ or less) was larger in younger patients (12/16 ; 75.0%) than in older patients (5/14 ; 35.7%).

These results suggest that bronchial hyperresponsiveness changes with aging, accompanied by changes in the release of chemical mediators from BAL cells and in the proportion of BAL cells.

Key words : BAL cells, histamine, leukotrienes, bronchial hyperresponsiveness, asthma

Introduction

Bronchial asthma is characterized by bronchial hyperresponsiveness, in which eicosanoids including leukotrienes C_4 and B_4 and lymphokines released from cells in the airways participate. Our previous studies have demonstrated that bronchial hyperresponsiveness is related to the proportion of cells in bronchoalveolar lavage (BAL) fluid and the release of chemical mediators from BAL cells[1,2].

Airway reactivity of asthma is also affected by aging. Our previous studies have shown that IgE-mediated allergic reactions, such as immediate skin reactions to various allergens, the production of IgE antibodies to inhaled allergens, basophil histamine release, and the bronchial challenge test with inhaled allergens, are affected by aging[3-9]. Furthermore, we have shown that the proportion of inflammatory cells in the airways changes with age[10], and that the proportion of BAL cells related to bronchial hyperresponsiveness is different between atopic and non-atopic sathma[2].

In the present study, the relationships between bronchial hyperresponsiveness and the release of histamine and leukotrienes, LTC_4 and LTB_4 from peripheral leucocytes and BAL cells were examined in patients with asthma.

Subjects and methods

The subjects were 40 patients with asthma (20 females and 20 males, mean age 51.4 years, range 21−73 years). The mean level of serum IgE was 782 IU/mℓ (range, 6−4134 IU/mℓ). They were all non-smokers. The subjects were divided into 2 groups according to their ages : 20−49 and over 60 years.

Bronchoalveolar lavage (BAL) was performed as previously reported[11-16]. Informed consent for the BAL procedure was obtained from all subjects. Briefly, the aspirates obtained using a bronchofiberscope were filtered through a sterile steel mesh and centrifuged at 1200 rpm for 10 min at 4℃. The resulting cell pellet was resuspended in Tris ACM. Smear preparations were made from the cell suspension, and these were stained with May Giemsa. A differential cell count was performed on 500 cells, excluding epithelial cells.

In the assessment of the release of histamine and leukotrienes C_4 (LTC_4) and B_4 (LTB_4) from peripheral leucocytes, cells were separated by counterflow centrifugation elutriation[17], and the number of the cells was adjusted to 5×10^6/mℓ in Tris ACM. Ca ionophore A23187 (1 μg) was added to the cell suspension (1mℓ). The mixed solution was incubated for 15 min at 37℃, and concentrations of histamine (in supernatant fluid and cells) and LTs C_4 and B_4 (in supernatant fluid) were measured. The release of histamine and LTC_4 and LTB_4 from BAL cells was performed by the method previously described[1]. The histamine content was analyzed by an automated spectrofluorometric histamine analysis system (Technicon)[18], as reported previously[5,6]. The results were expressed as a percentage of total histamine release. In assessing the release of LT_S C_4 and B_4 from leucocytes, the cell suspension (1mℓ) was incubated with Ca ionophore A 23187, and 4mℓ of 100% ethanol was then added. After the mixed solution was centrifuged, the supernatant fluid was vacuum dried and resuspended in 250 μl of high-performance liquid chromatography (HPLC) solvent (CH_3 CN/H_2 O=1 : 1). The resuspended solution was subjected to HPLC

(c−18 reverse-phase column, detection at 280 nm). The results were exprssed as ng/10^6 cells. In this study, the mean recovery rate at BAL was 27.1 ± 11.6% (±SD). The total number of cells aspirated into the BAL fluid was 7.21 ± 9.8 × 10^6.

Bronchial reactivity (BR) to methacholine was examined by a Astograph (TCK6100H, Chest Co.) one week before the BAL examination. Various concentrations of methacholine (49, 98, 195, 390, 781, 1563, 3125, 6250, 12500 and 125000 $\mu g/m\ell$) were prepared for bronchial challenge according to the method used by Chai et al[19]. An increase of total respiratory resistance (Rrs) after methacholine inhalation was observed by the oscillation method. A methocholine concentration causing a significant increase in Rrs was assessed as Cmin (minimum concentration). All medications were stopped 12 hours prior to the examination.

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

Results

Table 1 shows the proportion of cells in bronchoalveolar lavage (BAL) fluid in the two age groups. The proportions of eosinophils and basophilic cells in BAL fluid were higher in patients between the ages of 20 and 49 than in those over the age of 60, and differnce in the proportion of BAL basophilic cells was significant ($p<0.05$). In contrast, the proportions of lymphocytes and neutrophils in BAL fluid were higher in patients over age 60 than in those between 20 and 49. However, there was no significant difference in the proportion of these cells between the two age groups.

Table 1. Cellular composition of BAL fluid in patients with bronchial asthma divideed into two age groups

Patient age (years)	Mean age (years)	BAL cells (%) Mac	Lym	Neut	Eos	Bas
20-49	37.5	78.8 +13.4	10.4 +6.0	1.6 +2.0	8.5 +11.4	0.21[a] +0.3
60+	65.4	69.8 +20.2	20.4 +15.2	5.8 +15.2	4.0 +8.7	0.07[b] +0.17

Mac;macrophages, Lym;lymphocytes, Neut;neutrophils, Eos;eosinophils, Bas;basophils. a and b;$p<0.05$.

The release of histamine and leukotrienes C_4 (LTC_4) from peripheral leucocytes was higher in patients between 20 and 49 than in those over age 60. However, this was not significant. The release of leukotriene B_4 (LTB_4) was higher in patients over 60 compared to those between 20 and 49, as shown in Fig. 1. The difference was not significant.

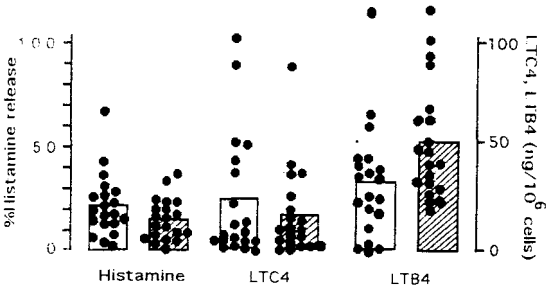

Fig. 1. The release of histamine, LTC_4 and LTB_4 from peripheral leucocytes in asthma patients between the ages of 20 and 49 (□) and over the age of 60 (▨)

Figure 2 shows the release of histamine and LTC_4 and LTB_4 from BAL cells. The amount of histamine release from BAL cells was significantly larger in patients between 20 and 49 than in those over 60 ($p<0.001$). The release of LTC_4 from BAL cells was higher in patients over 60 compared to those between 20 and 49. This was not significant. There was no significant difference in the release of LTB_4 from BAL cells between the two age groups.

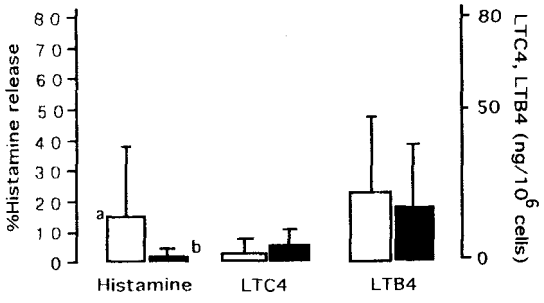

Fig. 2. The release of histamine, LTC_4 and LTB_4 from BAL cells in asthma patients between the ages of 20 and 49 (□) and over the age of 60 (■). a and b ; $p<0.001$.

Figure 3 shows bronchial reactivity (BR) to methacholine in the two age groups. The BR was in general higher in patients between 20 and 49 than in those over 60. In 12 (75.0%) of 16 patients between 20 and 49, minimum concentration of methacholine (Cmin) inducing bronchospasm was 390 $\mu g/m\ell$ or less. In contrast, BR with the Cmin of 390 $\mu g/m\ell$ or less was observed in 5 (35.7%) of the 14 patients over 60.

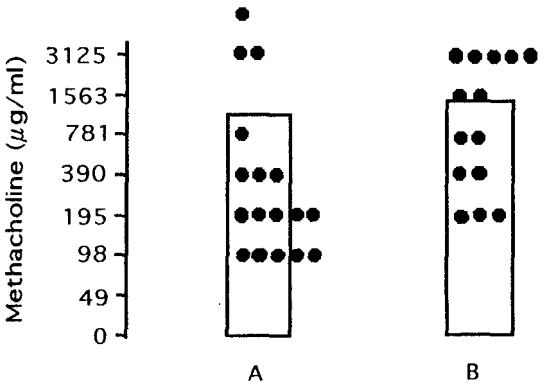

Fig. 3. Bronchial reactivity to methacholine in asthma patients between the ages of 20 and 49 (A) and over the age of 60 (B)

Discussion

Bronchial hyperresponsiveness is one of the main characteristics in bronchial asthma. It is well known that bronchial hyperreactivity is closely related to eicosanoids and lymphokines released from cells in the airways and the proportion of these cells. In IgE-mediated allergic reactions associated with asthma, the release of chemical mediators such as histamine and leukotrienes occurs early in the asthma attacks, and inflammatory cell infiltration in the airways occurs at a late stage. These two events play important roles in the onset of asthma. In the inflammatory process, lymphocytes[20-22], neutrophils[23,24], eosinophils[25-28] and basophils or mast cells[29,30] have been observed in the airways by analyzing the cellular components of BAL fluid. These humoral and cellular factors in the onset of asthma may be affected by age and by serum IgE levels[5,6,31].

In the present study, we examined the composition of cells in BAL fluid, the release of histamine and leukotrienes from leucocytes and BAL cells, and bronchial hyperresponsiveness in relation to patient

age. Regarding BAL cells, the proportion of eosinophils and basophilic cells in BAL fluid were higher in younger patients between 20 and 49 than in older patients over age 60, and the difference in the proportion of BAL basophilic cells was significant. In contrast, the proportions of lymphocytes and neutrophils in BAL fluid were higher in older patients than in younger patients, although this was not significant. These results suggest that cellular composition of BAL fluid changes with aging.

The release of histamine from BAL cells was significantly larger in younger patients than in older patients. While the release of LTC_4 from BAL cells was higher in older patients than in younger patients. These results demonstrate that histamine is more significant in the onset mechanisms of younger patients than in older patients. However, clinical significance of LTC_4 and LTB_4 in the onset mechanisms of older patients was not different from that of younger patients.

Bronchial reactivity to methacholine was higher in younger patients compared to older patients. Number of patients reactive to low concentration of methacholine (390 $\mu g/m\ell$ or less) was larger in younger patients than in older patients. The results show that bronchial hyperresponsiveness shows a tendency to decrease with aging.

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気管支喘息における末梢血白血球および気管支肺胞洗浄液中の細胞からのヒスタミンおよびロイコトリエンC_4，B_4遊離と気道過敏性

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気管支喘息を対象に，気管支肺胞洗浄（BAL）液中の細胞の出現頻度，末梢血白血球およびBAL細胞からのヒスタミン，ロイコトリエンC_4，B_4遊離，気道過敏性などについて，患者年齢との関連のもとに検討を加えた。

1．BAL液中のリンパ球および好中球頻度は，60才以上の老齢症例で，20－49才の若青年症例に比べやや高い傾向が見られたが，推計学的には有意の差は見られなかった。BAL液中の好酸球，好塩基細胞の頻度は，若青年症例で，老年症例に比べて高く，特に好塩基性細胞の頻度には両症例群間に有意の差が見られた（$P<0.05$）。

2．BAL細胞からのヒスタミン遊離は，若青年症例で，老年症例に比べ有意に多い傾向が見られた（$P<0.001$）。一方，BAL細胞からのロイコトリエンC_4遊離は，若青年症例に比べ，老年症例で多い傾向が見られたが，両症例群間に有意の差は見られなかった。

3．メサコリンに対する気道の反応性は，全般的に若青年症例で，老年症例に比べより強い傾向を示した。そして，390 $\mu g/m\ell$またはそれ以下の低濃度のメサコリンに反応を示す症例は，若青年症例では16例中12例（75.0%），老年症例では14例中5例（35.7%）であった。

以上の結果より，気道過敏性は，BAL細胞からの化学伝達物質遊離の変化やBAL細胞の頻度の変化とともに，加齢の影響を受けることが明らかとなった。

キーワード：BAL細胞，ロイコトリエン，ヒスタミン，気道過敏性，気管支喘息