
◎原 著

Effects of aging on bronchoalveolar lavage (BAL) cells in patients with asthma

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Abstract : Effects of aging and glucocorticoid therapy on bronchoalveolar lavage (BAL) cells, particularly lymphocytes, neutrophils and eosinophils, were examined in 81 patients with asthma. 1. The proportion of BAL lymphocytes tended to increase with aging in asthmatics under age 69 years, and the proportion was significantly higher in patients with asthma between the ages of 60 and 69 than in those under age 39 and between the ages of 40 and 49. 2. The proportions of BAL neutrophils and eosinophils were not related to aging. 3. The proportion of BAL lymphocytes was higher in patients without glucocorticoid therapy than in those with steroid-dependent intractable asthma (SDIA) in those under age 69 years. In patients between the ages of 50 and 59, the proportion of BAL lymphocytes was significantly higher in patients without glucocorticoids than in those with SDIA. 4. The proportion of BAL neutrophils was higher in patients without glucocorticoid therapy than in those with SDIA, and the difference was significant in patients between the ages of 60 and 69. 5. The proportion of BAL eosinophils was not related to glucocorticoid therapy. These results suggest that the proportion of BAL lymphocytes and neutrophils is affected by aging and glucocorticoid therapy, but not BAL eosinophils.

Key words : aging, asthma, glucocorticoid therapy, BAL cells

Introduction

In recent years the number of asthmatics in the elderly has been increasing. The feature of asthma in the elderly is different from that of

young patients in relation to the participation of IgE-mediated allergy and pathophysiology in the airways. Asthma is characterized by bronchial hyperresponsiveness related to such pathophysiological changes in the airways as bronchospasm, bronchial wall edema, and mucus

hypersecretion. It is known that inflammatory cell infiltration of the airways following the release of chemical mediators such as histamine and leukotrienes has been recognized as a major mechanism relating to the onset mechanism of asthma¹⁻³. In the inflammatory process, lymphocytes, neutrophils, eosinophils, and basophils and mast cells have been observed in the airways of patients with asthma, by analyzing BAL fluid⁴⁻⁸. Of these inflammatory cells, lymphocytes^{2,9}, neutrophils^{10,11}, eosinophils^{4,12}, basophils and mast cells¹³ are considered to play an important role in this inflammatory process.

The proportion of the cells in bronchoalveolar lavage (BAL) fluid is affected by various factors such as medication, disease severity and aging. Our previous studies have shown that the proportion of BAL lymphocytes is significantly reduced and the proportion of BAL neutrophils is significantly increased in steroid dependent intractable asthma (SDIA) compared to non-SDIA patients, and that these changes in the proportion of BAL cells are related to suppressed ventilatory function and a reduction in the release of histamine and leukotriene C4 (LTC4) from BAL cells^{14,15}.

In the present study, the number and proportion of BAL cells were examined in patients with asthma in relation to glucocorticoid therapy and aging.

Subjects and Methods

The subjects in this study were 81 patients with asthma (49 females and 32 males, mean age 52.9 years, range 21-74 years). The mean of serum IgE level was 508 IU/ml (range 11-4124 IU/ml) and the frequency of patients with positive RAST against inhalant allergens was 33.3% (27/81). Of these, 40 had steroid-dependent intractable asthma (SDIA) and had been treated

with glucocorticoids for more than 2 years. All subjects had been treated with conventional antiasthma drugs such as bronchodilators (e.g. β -stimulant and aminophylline), expectorants and antiallergic agents. They were all nonsmokers, since smokers were excluded before the results were analyzed. The subjects were divided into 5 groups according to their age: < 39, 40-49, 50-59, 60-69, and 70+ years.

Bronchoalveolar lavage (BAL) was performed in all subjects when they were free of attacks according to the previous reported method¹⁴⁻¹⁶.

Informed consent for the BAL examination was obtained from all subjects. Aspirate taken with a bronchofiberscope was centrifuged at 1200 rpm for 10 min at 4°C after filtration through sterile steel mesh, and the resultant cell pellet was resuspended in Tris ACM. Smear preparations made with the cell suspension were stained with May Giemsa. BAL cytology was done on 500 cells, excluding epithelial cells. In the present study, the mean recovery rate at BAL was $26.8 \pm 5.3\%$ (mean \pm SD) and the total cell number was $7.3 \pm 2.3 \times 10^6$.

The level of serum IgE was determined by radioimmunosorbent test (RIST), and IgE antibodies against inhalant allergens were estimated by radioallergosorbent test (RAST).

Statistically significant differences of the mean were estimated using the unpaired Student's t-test. A p value of <0.05 was regarded as significant.

Results

The proportion of lymphocytes in BAL fluid tended to increase with aging in patients under age 69. The proportion of BAL lymphocytes in patients between the ages of 60 and 69 was significantly higher than in those under age 39 ($p < 0.01$) and between the ages of 40 and 49

($p < 0.01$).

The proportion of BAL lymphocytes was decreased in patients over age 70 compared to that in those between the ages of 60 and 69, however, this was not significant (Fig. 1, Table 1).

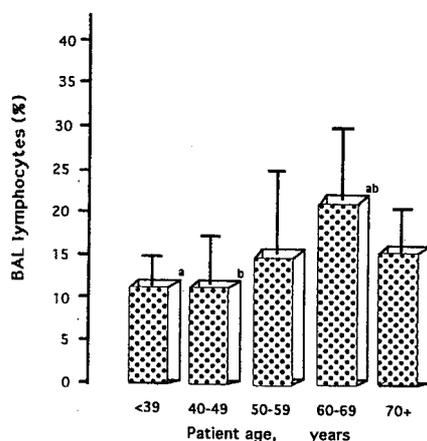


Fig. 1. The proportion of BAL lymphocytes of patients with asthma in relation to age. a and b: $p < 0.01$.

Table 1. Number of lymphocytes and neutrophils in BAL fluid of patients with asthma in relation to age

Age years	No of patients	Recovery rate (%)	No of total cells ($\times 10^6$)	Lymphocytes Total (/ml) ($\times 10^4$)	Neutrophils Total (/ml) ($\times 10^4$)		
<39	11	26.3	6.6	77.7	2.33	24.8	0.8
40-49	14	31.1	7.9	80.4	1.74	49.5	1.8
50-59	24	28.1	7.1	192.0	2.13	44.7	1.7
60-69	20	25.5	9.5	255.2	5.10	30.5	0.7
70+	12	25.2	7.3	112.9	2.11	9.4	0.2

The proportion of BAL neutrophils was not significantly different among five groups classified by age. However, the proportion of BAL neutrophils in patients over age 70 was considerably low compared to the proportion in those of other age groups (Fig. 2, Table 1). The proportion of BAL eosinophils was not related to patient age (Fig. 3).

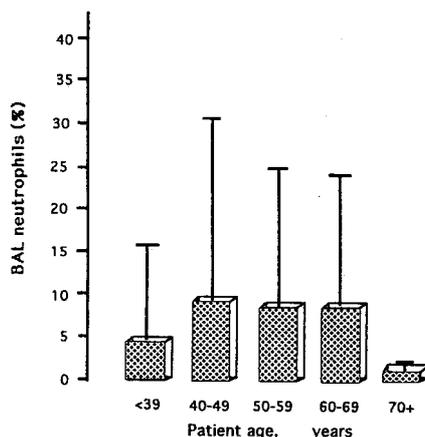


Fig. 2. The proportion of BAL neutrophils of patients with asthma in relation to age

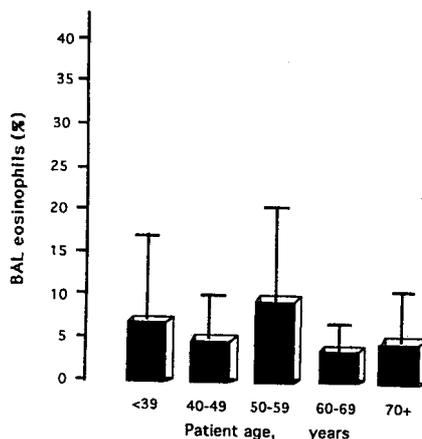


Fig. 3. The proportion of BAL eosinophils of patients with asthma in relation to age

The dose and duration of glucocorticoid regimen was not so remarkably different among the five groups classified by age, although the duration was shorter in patients under age 39 (Table 2).

Table 2. Dose and duration of glucocorticoid regimen in patients with steroid-dependent intractable asthma (SDIA)

Age years	Steroid therapy	No of patients	glucocorticoids mg/day	duration, (years)
<39	+	5	9.0	3.5
	-	6	-	-
40-49	+	7	6.8	6.8
	-	7	-	-
50-59	+	12	7.1	5.2
	-	12	-	-
60-69	+	10	6.3	6.8
	-	10	-	-
70+	+	6	7.1	8.4
	-	6	-	-

Regarding the effects of glucocorticoids on cellular composition of BAL fluid relating to age, the proportion of BAL lymphocytes was generally lower in patients with SDIA compared to the proportion in those without glucocorticoid therapy, and the proportion of BAL lymphocytes in patients between the ages of 50 and 59 was significantly lower in SDIA than in non-SDIA patients ($p < 0.01$). There was no difference in the proportion of BAL lymphocytes of patients over age 70 between SDIA and non-SDIA (Fig. 4).

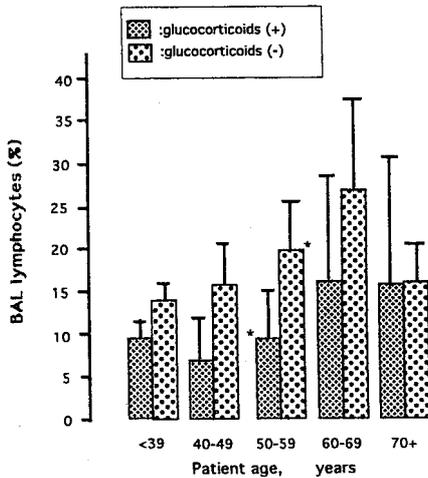


Fig.4. The proportion of BAL lymphocytes of patients with asthma in relation to glucocorticoid therapy. * $p < 0.01$.

In contrast, the proportion of BAL neutrophils was in general higher in patients with SDIA than in those without glucocorticoid therapy, and in patients between the ages of 50 and 59, the proportion of BAL neutrophils was significantly larger in SDIA than in non-SDIA patients (Fig. 5, Table 3). The proportion of BAL eosinophils was not significantly different between SDIA and non-SDIA patients in all age groups (Fig. 6).

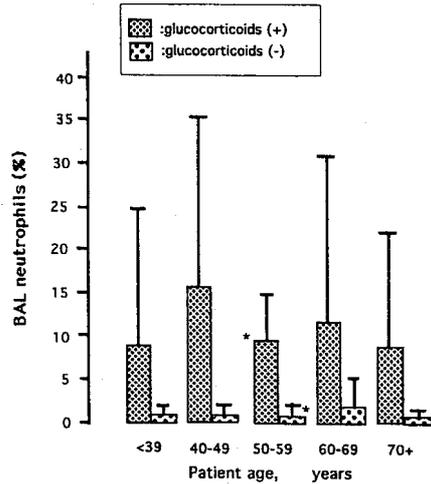


Fig.5. The proportion of BAL neutrophils with asthma in relation to glucocorticoid therapy. * $p < 0.001$.

Table 3. Number of lymphocytes and neutrophils in BAL fluid of patients with asthma in relation to steroid therapy

Age years	Steroid therapy	No of patients	No of total cells ($\times 10^6$)	Lymphocytes Total (/ml) ($\times 10^4$)	Neutrophils Total (/ml) ($\times 10^4$)		
<39	+	5	5.1	68.4	1.33	46.7	1.5
	-	6	6.3	85.4	3.17	6.5	0.3
40-49	+	7	9.3	64.7	1.28	103.5	3.3
	-	7	6.4	96.1	2.19	9.7	0.2
50-59	+	12	6.8	69.5	1.6	81.5	3.2
	-	12	7.5	126.6	2.6	7.9	0.2
60-69	+	10	6.1	119.8	2.8	27.2	0.6
	-	10	13.0	390.7	7.5	33.9	0.6
70+	+	6	5.4	100.0	2.5	69.4	1.4
	-	6	7.3	112.9	2.1	9.1	0.2

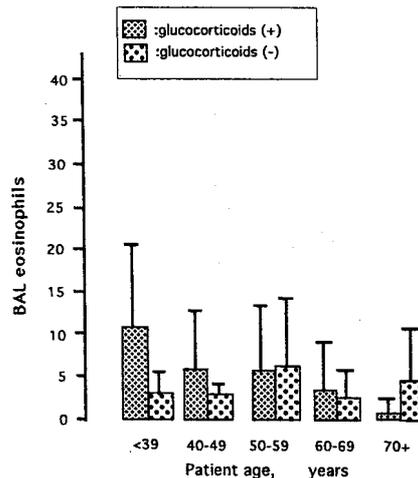


Fig.6. The proportion of BAL eosinophils of patients with asthma in relation to glucocorticoid therapy

Discussion

Airway inflammation that occurs after the release of mediators has been identified as a substantial feature of asthma. In the inflammatory process, lymphocytes^{2,9)}, neutrophils^{10,11)}, and eosinophils¹³⁾ that migrate from the peripheral blood have been studied as effector cells implicated in the pathophysiological changes in the airways¹⁻⁸⁾.

In recent years, the number of elderly patients with asthma has been increasing. The characteristic of asthma in the elderly is different from that in younger patients, suggesting the possibility that aging affects the proportion of inflammatory cells in the airways. In contrast, there are some asthma patients whose attacks cannot be controlled without resorting to long-term glucocorticoid regimen, despite the availability of many kinds of newly developed antiasthmatic drugs. In these patients with steroid-dependent intractable asthma (SDIA)¹⁷⁾, the glucocorticoids themselves, in addition to manifesting adverse side effects, may affect the pathophysiology of asthma.

Our previous studies have shown that the proportion of lymphocytes in bronchoalveolar lavage (BAL) fluid was significantly decreased in patients with steroid-dependent intractable asthma (SDIA) compared to results in non-SDIA patients, while BAL neutrophils were significantly increased in SDIA patients compared to results in non-SDIA patients^{14,15)}.

In the present study, effects of aging on cellular composition of BAL fluid were examined in patients with asthma in relation to glucocorticoid therapy. The results obtained here show that the proportion of BAL lymphocytes is affected by aging: the proportion was significantly higher in patients between the ages of 60 and

69 compared to the proportion of those under age 39 and between the ages of 40 and 49. The proportion of other inflammatory cells such as neutrophils and eosinophils was not affected by aging.

Regarding the effects of glucocorticoids on cellular composition of BAL fluid, a decreased proportion of BAL lymphocytes and an increased proportion of BAL neutrophils were observed in SDIA patients between the ages of 50 and 59, but not the proportion of BAL eosinophils. These results suggest that cellular composition of BAL fluid is affected by aging and glucocorticoids regimen. As for elderly patients over age 70, the proportion of inflammatory cells such as lymphocytes, neutrophils, and eosinophils was considerably lower compared to the proportion in patients between the ages of 60 and 69.

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気管支喘息における気管支肺胞洗浄液中の細胞成分に対する加齢および副腎皮質ホルモンの影響

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気管支喘息81例を対象に, 気管支肺胞洗浄(BAL)液中の細胞成分, 特にリンパ球, 好中球および好酸球に対する加齢および副腎皮質ホルモンの影響について検討を加えた。1. BAL液中のリンパ球頻度は, 69才以下の症例では加齢とともに増加する傾向を示し, 60-69才の年齢層では,

39才以下および40-49才の年齢層の症例に比べ有意に高い値を示した。2. BAL液中の好中球および好酸球頻度と加齢の間には関連が見られなかった。3. BAL液中のリンパ球頻度は, 69才以下の症例では, ステロイド依存性重症難治性喘息(SDIA)に比べ, ステロイド非使用例で有意に高い値を示し, 50-59才の年齢層ではその差は有意であった。4. BAL液中好中球頻度は, ステロイド非使用例に比べSDIA症例において高い値を示し, 60-69才の年齢層ではその差は有意であった。5. BAL液中好酸球頻度は, ステロイド使用の有無との関連は見られなかった。これらの結果は, BAL液中リンパ球および好中球頻度は加齢や副腎皮質ホルモン投与の影響を受けるが, BAL液中好酸球頻度には影響しないことを示唆している。