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Association of increased number of bronchoalveolar lymphocytes with patient age and IgE-mediated allergic reaction

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**Abstract :** The proportion and number of lymphocytes in the airways were analyzed in 15 patients with a high proportion of lymphocytes in bronchoalveolar lavage (BAL) fluid (more than 30%) and in 17 patients with less than 20% of BAL lymphocytes. 1. Both atopic and nonatopic asthma patients with a high proportion of BAL lymphocytes were aged more than 50 years. In contrast, the age of patients with less than 20% of BAL lymphocytes ranged widely, from 29 to 63 years, in the two asthma types. 2. Age at onset of the disease, serum IgE levels, and ventilatory function test were not related to the proportion of BAL lymphocytes. 3. In patients with a high proportion of BAL lymphocytes, the mean proportion of these cells was 47.3% in atopic patients and 36.4% in non-atopic patients, i. e., there was no significant difference between the two asthma types. The absolute number of BAL lymphocytes in these patients was significantly higher in atopic ( $5.62 \times 10^6$ ) than in nonatopic asthma ( $0.77 \times 10^6$ ) ( $p < 0.01$ ).

These findings show that an increased number of lymphocytes in the airways is clearly related to patient age and IgE-mediated allergic reaction.

**Key words :** BAL lymphocytes, patient age, IgE-mediated allergic reaction, bronchial asthma

### Introduction

IgE-mediated allergy is a major immunological reaction participating in the pathogenesis of bronchial asthma (1-3); asthma induced by IgE-mediated allergy is

known as atopic. Thus, bronchial asthma is usually divided into two types clinically, atopic and nonatopic, on the basis of the presence or absence of IgE-mediated allergic reactions (4), although investigators in several studies have suggested that asthma is

almost always associated with some type of IgE-related reaction (5, 6). In classifying asthma by the presence or absence of IgE-mediated allergic reactions, it has been suggested that there are some differences between the mechanism of onset in atopic and nonatopic types.

In recent years, airway inflammation, in which lymphocytes, neutrophils, eosinophils, and basophils from the bloodstream are involved (7-12), has been noted as a major causative factor in late asthmatic reaction (LAR) (13, 14). In the inflammatory process, blood cells migrate into allergic reaction sites, and these cells play an important role in the pathogenesis of asthma.

In the present study, we evaluated the association between BAL lymphocyte number and the pathogenesis of asthma in patients with atopic and nonatopic asthma.

### Subjects and Methods

To observe the association between lymphocyte number in the airways and the pathogenesis of asthma, we selected 15 asthma patients (5 females and 10 males; mean age 62.0 years, range 51-73 years) with an increased proportion of lymphocytes more than 30% in bronchoalveolar lavage (BAL) fluid. Seventeen asthma patients (7 females and 10 males; mean age 47.8 years, range 29-63 years) with less than 20% of BAL lymphocytes were selected as control subjects. These patients were further divided into two asthma types, atopic and nonatopic, on the basis of IgE-mediated allergic reactions (15): patients with IgE antibodies to inhalant allergens or serum IgE level over 500 IU/ml were evaluated as atopic, and those with negative skin reaction to allergens and serum IgE levels less than 100 IU/ml were

evaluated as nonatopic.

Bronchoalveolar lavage (BAL) was performed after informed consent was obtained from all subjects. The BAL examination was carried out, according to a previously described method (15-18), in all subjects when they were free of attacks. Smear preparations were made with cell suspensions prepared from samples obtained by bronchofiberscope. Slides containing these suspensions were air dried and stained with May Giemsa. BAL cytology was performed by observing 500 cells, excluding epithelial cells, on the smear preparations. In this study, the mean recovery rate at BAL was  $28.2 \pm 15.2\%$  (10.0% - 74.0%) and the total number of cells aspirated in the BAL fluid was  $5.48 \times 10^6$  ( $0.4 \times 10^6$  -  $24.6 \times 10^6$ ). The results were expressed as a percentage of the total cell number and as an absolute count in BAL fluid.

Ventilatory function tests, using a Box Spiro 81-S (Chest Co.), was carried out in all subjects when they were asymptomatic.

Immediate skin reaction to allergens was determined following the intradermal injection of 0.02ml of commercial allergen extract (Torii pharmaceutical Co.). The skin reaction was measured in millimeters at 20min after the test. Wheals larger than 9mm or flares larger than 20mm at 20min were regarded as positive.

Serum IgE levels were measured by a radioimmunosorbent test (RIST) and IgE antibodies to inhalant allergens were evaluated by a radioallergosorbent test (RAST).

### Results

Age of all atopic asthma patients with a high proportion of BAL lymphocytes (more than 30%) was more than 50 years (mean

59.8 years). In contrast, the age of atopic asthma patients with less than 20% of BAL lymphocytes ranged widely, from 29 to 59 years (mean 42.7 years). In nonatopic asthma, the age of all patients with a high proportion of BAL lymphocytes was also more than 50 years (mean 65.3 years), while in nonatopic patients with less than 20% of BAL lymphocytes, the age ranged from 50 to 63 years (mean 57.0 years), as shown in Fig. 1. Thus, the age of patients with a high proportion of BAL lymphocytes was, in general, high (over 50 years).

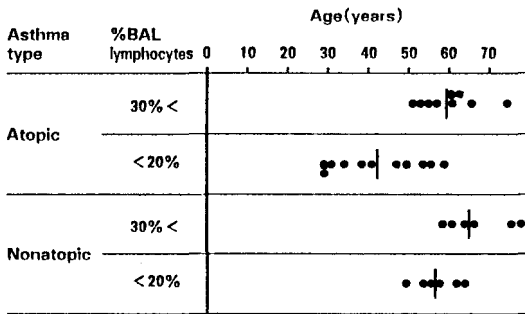


Fig. 1. Age in atopic and nonatopic asthma patients in relation to proportion of BAL lymphocytes.

Figure 2 shows the age at onset of the disease in all subjects. There was no significant difference between atopic asthma patients with more than 30% of BAL lymphocytes and those with less than 20% in age at onset of the disease. There was also no difference in age at onset between nonatopic asthma patients classified by the proportion of BAL lymphocytes. In atopic asthma patients with a high proportion of BAL lymphocytes, the mean level of serum IgE was  $726 \pm 664$  IU/ml, and in those with less than 20% of BAL lymphocytes it was  $947 \pm 808$  IU/ml. There was no significant difference

between the two groups. In nonatopic asthma patients, the serum IgE level was low, and there was no significant difference between patients with a high proportion of BAL lymphocytes ( $51 \pm 42$  IU/ml) and those with less than 20% of BAL lymphocytes ( $71 \pm 28$  IU/ml).

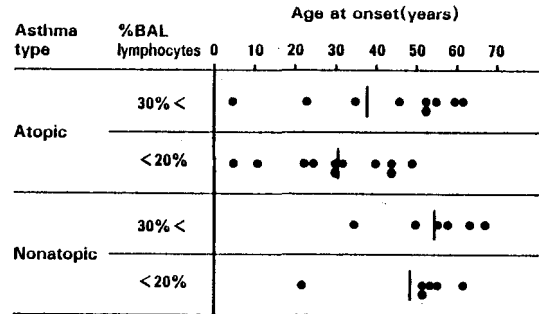


Fig. 2. Age at onset of the disease in atopic and nonatopic asthma patients in relation to proportion of BAL lymphocytes.

Table 1 shows the ventilatory function in patients classified by proportion of BAL lymphocytes. FEV<sub>1.0%</sub> value was lowest in nonatopic asthma patients with a high proportion of BAL lymphocytes and highest in those with less than 20% of BAL lymphocytes. The other ventilatory parameters, %PEFR, %MMF, % $\dot{V}_{50}$ , and % $\dot{V}_{25}$  were also lowest in nonatopic asthmatics with a high proportion of BAL lymphocytes. In patients classified by the proportion of BAL lymphocytes and by IgE-mediated allergic reaction, however, there were no significant differences in the values of these ventilatory parameters.

Table 1. Ventilatory function in patients with atopic and nonatopic asthma in relation to proportion of BAL lymphocytes.

Asthma type	%BAL lymphocytes	Ventilatory parameters					
		%FVC	FEV <sub>1.0</sub>	%PEFR	%MMF	%V <sub>50</sub>	%V <sub>25</sub>
Atopic	30% <	98.6 <sup>†</sup> ±17.2	68.1 ± 9.5	72.1 ±22.1	43.2 ±22.8	32.4 ±20.0	28.0 ±14.9
	<20%	88.4 ±11.4	69.7 ±13.6	68.7 ±25.1	40.8 ±24.6	34.8 ±19.9	26.1 ±18.2
Nonatopic	30% <	79.2 ±17.8	58.8 ±16.8	53.1 ±18.3	34.0 ±40.5	23.4 ±30.9	24.7 ±31.2
	<20%	102.9 ± 11.1	76.6 ±18.1	79.3 ±24.3	36.8 ±14.1	29.9 ±13.9	23.2 ± 9.1

<sup>†</sup> Mean ± SD

In both atopic and nonatopic asthma, the proportions of macrophages, neutrophils and eosinophil in BAL fluid were higher in patients with less than 20% of BAL lymphocytes than in those with a high proportion of BAL lymphocytes. However, this difference was not significant (Table 2).

Table 2. Cellular composition in BAL fluid of patients with atopic and nonatopic asthma in relation to proportion of BAL lymphocytes.

Asthma type	%BAL lymphocytes	Mac	Lym	Neut	Eos
Atopic	30% <	49.6 <sup>†</sup> ±11.6	47.3 ±11.8	1.6 ± 1.6	1.5 ±0.8
	<20%	75.5 ±20.1	11.5 ± 5.4	8.6 ±21.6	7.1 ±9.8
Nonatopic	30% <	56.0 ± 6.4	36.4 ± 3.6	5.0 ± 4.0	2.4 ±2.3
	<20%	74.3 ±26.3	5.3 ± 4.8	13.2 ±24.9	7.1 ±9.8

<sup>†</sup> Mean ± SD. Mac, macrophages; Lym, lymphocytes; Neut, neutrophils; Eos, eosinophils.

In patients with a high proportion of BAL lymphocytes (more than 30%), the mean value was 47.3 ± 11.8% (range 30.4%–73.7%) in atopic asthma and 36.4 ± 3.6% (range 31.4%–42.1%) in nonatopic asthma. Although

the mean value was higher in atopic than in nonatopic asthma, the difference was not significant. In patients with less than 20% of BAL lymphocytes, the mean value was higher in atopic asthma (11.5 ± 5.4%) than in nonatopic asthma (5.3 ± 4.8%), as shown in Fig. 3. However, this difference was not significant.

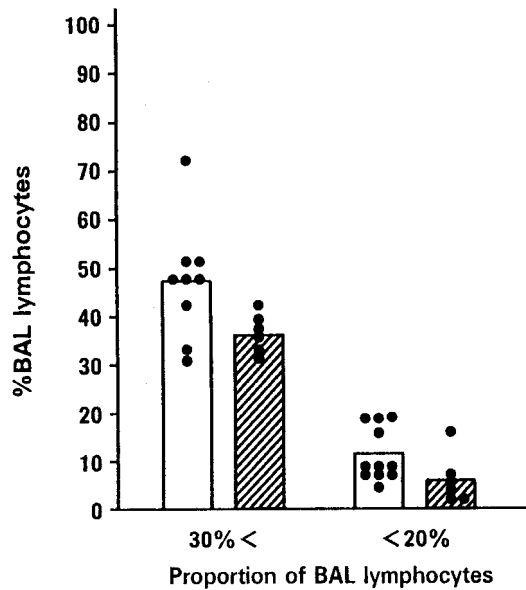


Fig. 3. Proportion of BAL lymphocytes in patients with atopic (□) and nonatopic asthma (▨).

The percent recovery at BAL was significantly higher in atopic than in nonatopic asthma patients with a high proportion of BAL lymphocytes (p < 0.02). The total cell number in BAL fluid was greatest in atopic patients with a high proportion of BAL lymphocytes; this value was significantly higher than that in atopic patients with less than 20% of BAL lymphocytes (p < 0.01), in nonatopic patients with more than 30% of BAL lymphocytes (p < 0.01), and in nonatopic

patients with less than 20% of BAL lymphocytes ( $p < 0.01$ ). The number of lymphocytes in the BAL fluid was significantly higher in atopic than in nonatopic asthma patients with a high proportion of BAL lymphocytes ( $P < 0.01$ ; as shown in Table 3 and Fig. 4), although there was no significant difference in the proportion of BAL lymphocytes in these atopic and nonatopic asthma patients. The numbers of total cells and lymphocytes per ml of BAL fluid were also significantly higher in atopic asthma patients with a high proportion of BAL lymphocytes compared with those in the other three groups, as shown in Table 4.

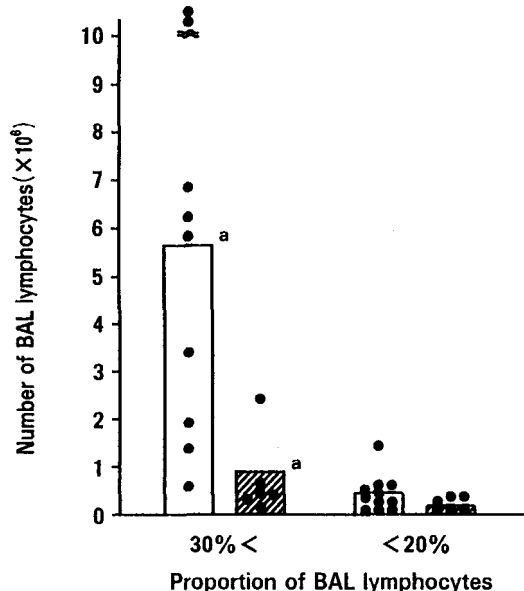


Fig. 4. Number of lymphocytes in BAL fluid of patients with atopic ( $\square$ ) and nonatopic asthma ( $\text{hatched}$ ). a,  $p < 0.02$ .

Table 3. BAL findings in patients with atopic and nonatopic asthma (1)

Asthma type	%BAL lymphocytes	Percent recovery	Total cells ( $\times 10^6$ )	No of lymphocytes ( $\times 10^6$ )
Atopic	30% <	36.2 <sup>a*</sup> $\pm 17.8$	11.2 <sup>bcd</sup> $\pm 6.7$	5.62 <sup>efg</sup> $\pm 4.10$
	<20%	26.2 $\pm 12.5$	3.4 <sup>b</sup> $\pm 2.4$	0.40 <sup>e</sup> $\pm 0.37$
Nonatopic	30% <	19.8 <sup>e</sup> $\pm 7.8$	2.1 <sup>c</sup> $\pm 2.1$	0.77 <sup>f</sup> $\pm 0.77$
	<20%	28.1 $\pm 15.5$	3.9 <sup>d</sup> $\pm 3.1$	0.15 <sup>g</sup> $\pm 0.14$

a and b,  $p < 0.02$ ; c, d, e, f, and g,  $p < 0.01$

\*Mean  $\pm$  SD

Table 4. BAL findings in patients with atopic and nonatopic asthma (2)

Asthma type	%BAL lymphocytes	Recovery volume (ml)	Total cells ( $\times 10^4$ /ml)	No of lymphocytes ( $\times 10^4$ /ml)
Atopic	30% <	72.3 <sup>a*</sup> $\pm 36.1$	15.5 <sup>bcd</sup> $\pm 8.0$	7.82 <sup>efg</sup> $\pm 5.12$
	<20%	53.0 $\pm 26.1$	6.0 <sup>b</sup> $\pm 3.3$	0.71 <sup>e</sup> $\pm 0.57$
Nonatopic	30% <	37.7 <sup>a</sup> $\pm 15.5$	4.5 <sup>c</sup> $\pm 2.7$	1.63 <sup>f</sup> $\pm 0.92$
	<20%	56.0 $\pm 34.1$	6.0 <sup>d</sup> $\pm 2.8$	0.44 <sup>g</sup> $\pm 0.45$

a, e, and g,  $p < 0.01$ ; b,  $p < 0.05$ ; c, and f,  $p < 0.001$ ; d,  $p < 0.02$ .

\*Mean  $\pm$  SD

## Discussion

It has been suggested that lymphocytes play an important role in the pathogenesis of asthma: increased numbers of activated blood T cells are found during asthma attacks (19, 20), and CD4<sup>+</sup> T-lymphocytes are reduced in peripheral blood and sequestered in the lung (21). Thus, the activation of T-lymphocytes and the production of lymphokines appear to be involved in the pathogenesis of asthma (22, 23). It has been shown, also, that the number of lymphocytes in BAL fluid is increased in patients with asthma (24, 25), with the median percentage

lymphocyte count in asthmatic patients being 14%, while that of controls was 8% (25): lymphocyte counts of more than 14% are unusual in normal subjects (26). It has been demonstrated that the increase of BAL lymphocytes in asthmatics is due to the increase of T cell subsets (27). Furthermore, it has been shown that there is a close correlation between numbers of BAL CD4<sup>+</sup>, IL2R<sup>+</sup> T cells and numbers of eosinophils, and that the numbers of activated T cells and eosinophils are related to the severity of asthma, as determined by impairment of FEV 1.0% and increased methacholine bronchial responsiveness (28).

In the present study, the clinical features of asthma patients with a high proportion of BAL lymphocytes (more than 30%) were observed in comparison with those with less than 20% of BAL lymphocytes. The age of both atopic and nonatopic asthma patients with a high proportion of BAL lymphocytes was over 50 years. These results show that age is one of the factors related to the high proportion of BAL lymphocytes in the two asthma types. However, age at onset of the disease, serum IgE levels, and ventilatory test results were not related to the proportion of BAL lymphocytes.

In this study, we selected both atopic and nonatopic asthma patients with high proportion of BAL lymphocytes (more than 30%). Although the proportion of BAL lymphocytes was not significantly different in atopic (47.3%) and nonatopic asthma (36.4%), the mean value was higher in the former. In contrast, the absolute number of BAL lymphocytes was significantly higher in atopic ( $5.62 \times 10^6$ ) than in nonatopic asthma ( $0.77 \times 10^6$ ). These results demonstrate that the number of lymphocytes in the airways is

related to IgE-mediated allergic reaction.

It was unclear from the present study which factors, except for age, are implicated in the differences of BAL lymphocyte proportions in atopic asthma. Five of the 9 atopic patients (55.6%) with a high proportion of BAL lymphocytes had asthma attacks within one month after BAL examination. In contrast, 2 of the 11 atopic patients (18.2%) with less than 20% of BAL lymphocytes had asthma attacks during this period. The number of patients whose asthma attacks required glucocorticoid therapy was greater in atopic patients with a high proportion of BAL lymphocytes (5/9; 55.6%) than in those with less than 20% of BAL lymphocytes (3/11; 27.3%). These findings suggest that an increased number of lymphocytes in the airways is associated with both the acute exacerbation and the severity of asthma. Further studies, including analysis of lymphocyte subsets and cell surface markers, are necessary to clarify the mechanisms responsible for this phenomenon.

#### References

1. Ishizaka, T: Analysis of triggering events in mast cells for immunoglobulin E-mediated histamine release. *J Allergy Clin Immunol* 67: 90-96, 1981.
2. Tanizaki Y, Komagoe H, Sudo M, Morinaga H, Kitani H, Goda Y, Tada S, Takahashi K, Kimura I: IgE-mediated histamine release from whole blood in atopic asthmatics. *Jpn J Allergol* 32: 1079-1083, 1983.
3. Tanizaki Y, Komagoe H, Morinaga H, Kitani H, Goda Y, Kimura I: Allergen- and anti-IgE-induced histamine release from whole blood. *Int Arch Allergy Appl Immunol* 73: 141-145, 1984.

4. Ostergaard PA : Non-IgE-mediated asthma in children. *Acta Paediatr Scand* 74 : 713–719, 1985.
5. Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG : Association of asthma with serum IgE levels and skin-test reactivity to allergen. *New Engl J Med* 320 : 271–277, 1989.
6. Tollerud DJ, O'Conner GT, Sparrow D, Weiss ST : Asthma, hay fever, and phlegm production associated with distinct patterns of allergy skin reactivity, eosinophilia and serum IgE levels. The normative aging study. *Am. Rev. Respir. Dis.* 144 : 776–781, 1991.
7. Nadel JA : Inflammation and asthma. *J Allergy Clin Immunol* 73 : 651–653, 1984.
8. Pauwels R : The relationship between airway inflammation and bronchial hyperresponsiveness. *Clin Exp Allergy* 19 : 395–398, 1989.
9. Kirby JG, Hargreave FE, Gleich GJ, O'Byrne PM : Bronchoalveolar cell profiles of asthmatic and nonasthmatic subjects. *Am Rev Respir Dis* 136 : 379–383, 1987.
10. Wardlaw AJ, Dunnetts S, Gleich GJ, Collins JV, Kay AB : Eosinophils and mast cells in bronchoalveolar lavage in subjects with mild asthma. *Am Rev Respir Dis* 177 : 62–69, 1988.
11. Boichot E, Lagente V, Carre C, Waltman P, Mencia-Huerta JM, Braquet P : Bronchial hyperresponsiveness and cellular infiltration in the lung of guinea-pigs sensitized and challenged by aerosol. *Clin Exp Allergy* 21 : 67–76, 1991.
12. Holgate ST, Djukanovic R, Wilson J, Roche W, Howarth PH : Inflammatory process and bronchial hyperresponsiveness. *Clin Exp Allergy* 21 : 30–36, 1991.
13. deMonchy SGR, Kauffman HF, Venge P, Koefer K, Sluiter HJ, Jansen HM, deVries K : Bronchoalveolar eosinophilia during allergen-induced late asthmatic reaction. *Am Rev Respir Dis* 131 : 373–376, 1985.
14. Fabbri LM, Baschetto P, Zocca E, Milani G, Piviratto F, Piebani M, Burlina A, Licata B, Mapp C : Bronchoalveolar neutrophilia during late asthmatic reactions induced by toluene di-isocyanate. *Am Rev Respir Dis* 136 : 36–42, 1987.
15. Tanizaki Y, Kitani H, Okazaki M, Mifune T, Mtsunobu F, Okano T, Tada S, Takahashi K, Kimura I : Airway inflammation and bronchial hyperresponsiveness in patients with asthma. Comparison between atopic and nonatopic asthma. *Jpn J Allergol.* 42 : 26–33, 1993.
16. Tanizaki Y, Sudo M, Kitani H, Kawauchi K, Mifune T, Takeyama H, Kohi F, Tada S, Takahashi K, Kimura I : Characteristic of cell components in bronchoalveolar lavage fluid (BALF) in patients with bronchial asthma classified by clinical symptoms. *Jpn J Allergol* 39 : 75–81, 1990.
17. Tanizaki Y, Kitani H, Okazaki M, Mifune T, Mitsunobu F, Ochi K, Harada H : Cellular composition of fluid in the airways of patients with house dust sensitive asthma, classified by clinical symptoms. *Internal Medicine* 31 : 333–338, 1992.
18. Tanizaki Y, Kitani H, Okazaki M, Mifune T, Mitsunobu F, Kotoh N, Tada S, Takahashi K, Kimura I : Asthma classification by score calculated from clinical findings and examinations. Comparison between clinical diagnosis and score diagnosis. *Jpn J Allergol* 41 : 489–496, 1992.
19. Corrigan CJ, Hartnell A, Kay AB : T-lymphocyte activation in acute severe asthma. *Lancet* 1 : 1129–1135, 1988.
20. Corrigan CJ, Kay AB : CD4 T-lymphocyte

- activation in acute severe asthma : relationship to disease severity and atopic status. *Am Rev Respir Dis* 141 : 970, 1990.
21. Gerblin A, Campbell AE, Schyler MR : Changes in T-lymphocytes subpopulations after antigenic bronchial provocation in asthmatics. *N Engl J Med* 310 : 1349-1454, 1984.
22. Leung DJH, Geha RS : Regulation of the human IgE antibody response. *Intern Rev Immunol* 2 : 75-81, 1987.
23. Walker C, Virchow JC, Bruijnzeel PLB, Blaser K : T cell subsets and their soluble products regulate eosinophilia in allergic and nonallergic asthma. *J Immunol* 146 : 1829-1835, 1991.
24. Kelly CA, Ward C, Stenton CS, Bird G, Hendrick DJ, Walters EM : Numbers and activity by inflammatory cell in bronchoalveolar lavage fluid in asthma, and their relationships to airway responsiveness. *Thorax* 43 : 684-692, 1988.
25. Kelly CA, Stenton SC, Ward C, Hendrick DJ, Walters EH : Lymphocyte subsets in bronchoalveolar lavage fluid obtained from stable asthmatics, and their correlations with bronchial responsiveness. *Clin Exp Allergy* 19 : 169-175, 1989.
26. Laviolette M : Lymphocyte fluctuation in bronchoalveolar lavage fluid in normal volunteers. *Thorax* 40 : 651-655, 1985.
27. Gonzalez MC, Diaz P, Galleguillos FR, Ancic P, Cromwell O, Kay AB : Allergen-induced recruitment of bronchoalveolar helper (OKT4) and suppressor (OKT8) T cells in asthma. *Am Rev Respir Dis* 136 : 600-604, 1987.
28. Walker C, Kaegi MK, Braun P, Blaser K : Activated T cells and eosinophilia in bronchoalveolar lavages from subjects with asthma correlated with disease severity. *J Allergy Clin Immunol* 88 : 935-942, 1991.

#### 気管支肺胞洗浄液中リンパ球増多と患者年齢およびIgE系アレルギー反応との関連について

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気管支肺胞洗浄 (BAL) 液中のリンパ球頻度が30%以上の15例および20%以下の17例の気管支喘息患者を対象に, BAL液中リンパ球頻度と年齢および IgE 系反応との関連について検討を加えた。1. BALリンパ球が高頻度 (30%以上) を示す症例は, アトピー型, 非アトピー型を問わず, 50才以上の年齢層に多い傾向が見られた。一

方, BALリンパ球20%以下の症例の年齢は, 29-63才まで幅広く分布していた。2. 発症年齢, 血清 IgE 値, 換気機能と BAL 液中リンパ球頻度との間には関連は見られなかった。3. BAL液中リンパ球頻度が高い症例では, アトピー型では平均リンパ球頻度は47.3%, 非アトピー型では36.4%であったが, 両群間には有意の差は見られなかった。しかし, BAL液中のリンパ球の絶対数は, アトピー型 ( $5.62 \times 10^6$ ) において, 非アトピー型 ( $0.77 \times 10^6$ ) に比べ有意に高い値を示した ( $p < 0.01$ )。

これらの結果は, 気道内リンパ球の増加は, 患者年齢と IgE 系反応と密接に関連していることを示唆している。