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Characteristics of airway response in patients with steroid-dependent intractable asthma (SDIA)

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Abstract : Characteristics of airway response in steroid-dependent intractable asthma (SDIA) were examined in 86 asthma patients (43 with SDIA and 43 with non-SDIA) divided into three age groups : 20−39, 40−59 and 60+ years, by observing cellular composition of bronchoalveolar lavage (BAL) fluid and ventilatory function.

1. The level of serum cortisol was significantly lower in patients with SDIA than in those with non-SDIA in all age groups. 2. The proportion of lymphocytes in BAL fluid was significantly decreased in patients with SDIA compared to results in non-SDIA patients in the age between 40−59, and over 60+ years, While BAL neutrophils were significantly increased in SDIA patients compared to results in non-SDIA patients in the age group over 60 years. 3. Of ventilatory parameters, the values of %FVC, $FEV_{1.0\%}$, %MMF and $\%\dot{V}_{25}$ were significantly lower in SDIA patients over the age of 60 compared with non-SDIA subjects of the same age group.

These results show that in SDIA patients the proportion of BAL lymphocytes decreases and the proportion of BAL neutrophils increases with aging, and that ventilatory function in SDIA patients also decreases with aging compared to non-SDIA patients.

Key words : bronchial asthma, bronchoalveolar cells, ventilatory function, glucocorticoids, serum cortiosol levels

Introduction

In recent years, airway inflammation that occurs after the release of mediators has been identified as a substantial feature of asthma[1-6]. In the inflammatory process of asthma, lymphocytes, neutrophils, and eosinophils which migrate from the peripheral

blood have been studied as effector cells implicated in the pathophysiological changes in the airways[7-13]. Intracellular enzymes, and proteins such as eosinophil cationic protein (ECP) and myeloperoxidase (MPO), which are released from activated cells, have been also studied in relation to airway inflammation[14-16].

Number of patients with bronchial asthma has been increasing in Japan, and the number of patients whose asthma attacks cannot be controlled without resorting to long-term glucocorticoid regimen has been also increasing. In these patients with steroid-dependent intractable asthma (SDIA)[17,18], the glucocorticoids themselves, in addition to manifesting adverse side effects, may affect the airway response of asthma.

This study was undertaken to clarify the characteristics of airway response of patients with SDIA by airway inflammation, particularly as reflected by the effects of glucocorticoids on the proportions of lymphocytes, neutrophils, and eosinophils in the BAL fluid and ventilatory function.

Subjects and Methods

The subjects of this study were 86 patients (47 females and 39 males) with bronchial asthma. Of these, 43 (mean age 51.2 years) had mild or moderate asthma (non-SDIA) ; asthma attacks in many of these patients had been controlled with the usual antiasthmatic drugs, although in some patients an occasional glucocorticoid regimen had been required. The remaining 43 patients (mean age 50.2 years) had steroid-dependent intractable asthma (SDIA), and had been treated with glucocorticoids for more than 2 years. The maintenance dose of prednisolone for SDIA patients was between 5 and 15 *mg* /day. They were all non-smokers, since smokers were excluded before the results were analyzed. The patients were divided into three age groups : 20−39, 40−59 and 60+ years.

Bronchoalveolar lavage (BAL) was performed in all study subjects by a previously reported method[19-21]. 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℃ 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 27.2 ± 11.5% (mean ± SD) and the total cell number was $8.02 \pm 9.3 \times 10^6$.

Ventilatory function tests were carried out in all subjects during an attack-free stage, using a Box Spiror 81−S (Chest Co.).

Serum cortisol levels were measured by radioimmunoassay (RIA) between 7 and 8 o'clock in the morning within a few days after the patients were admitted to our hospital.

The level of serum IgE was determined by radioimmuno-sorbent test (RIST) and IgE antibodies against house duse were estimated by radioallergosorbent test (RAST).

Results

Figure 1 shows the frequency of positive IgE antibodies against house dust in patients who had had long-term glucocorticoid therapy (steroid-dependent intractable asthma ; SDIA) and those who had not (non-SDIA). Number of patients with IgE antibodies against house dust was lower in SDIA than in non-SDIA in all age groups.

Serum cortisol levels were significantly lower in patients with SDIA than in those with non-SDIA in all age groups, as shown in Fig. 2.

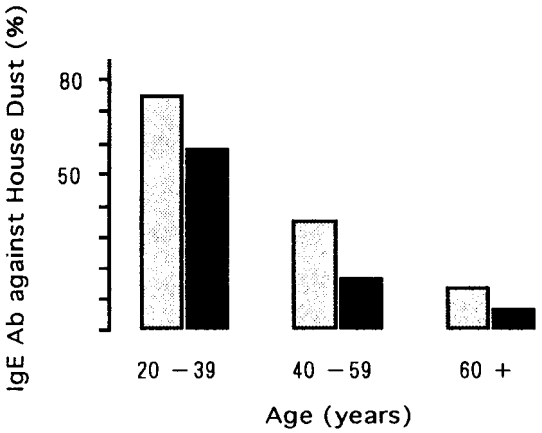

Fig. 1. Frequency of positive IgE Ab against House dust in patients with non-SDIA ( ) and SDIA ( )

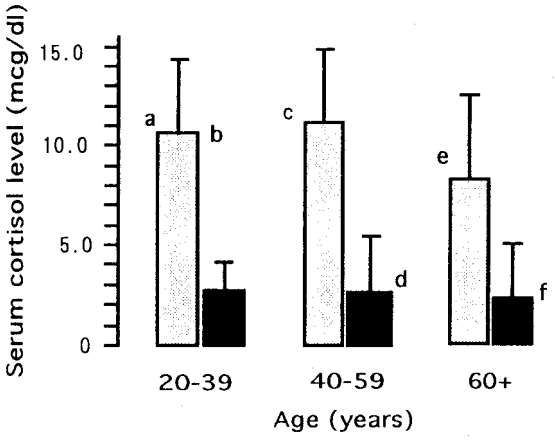

Fig. 2. Serum cortisol level in patients with non-SDIA ( ) and SDIA ( ). a and b, c and d, e and f : $p<0.001$.

The proportion of BAL lymphocytes was lower in patients with SDIA than in those with non-SDIA in all age groups, and these differences were significant, as shown in Fig. 3, in patients between 40 and 59, and over 60 years.

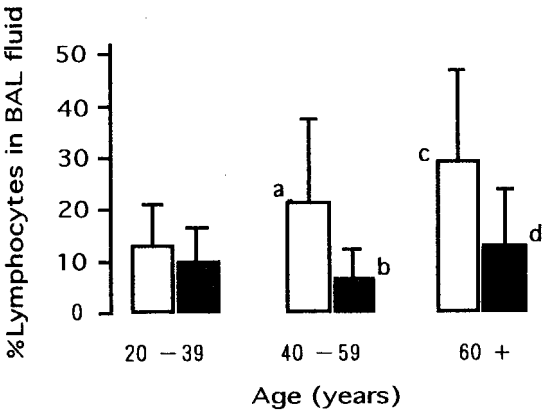

Fig. 3. %Lymphocytes in BAL fluid of patients with non-SDIA ( ) and SDIA ( ). a and b ; $P<0.001$, c and d ; $p<0.01$.

The number of neutrophils in BAL fluid, as shown in Fig. 4, tended to increase in patients with SDIA, compared with non-SDIA. The proportion of BAL neutrophils was significantly higher in SDIA patients over the age of 60 years than in non-SDIA patients of the same age group.

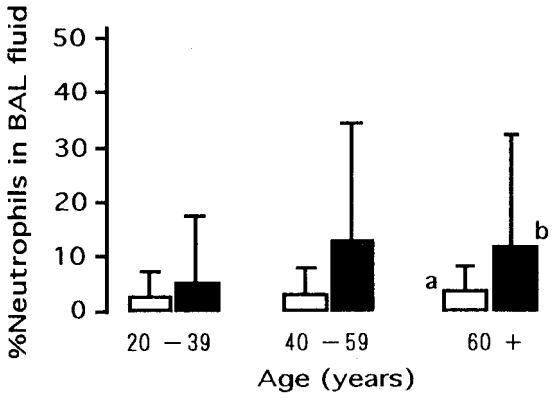

Fig. 4. %Neutrophils in BAL fluid of patients with non-SDIA ( ) and SDIA ( ) a and b ; $p<0.01$.

Figure 5 shows the proportion of eosinophils in BAL fluid in SDIA and non-SDIA patients classified by age. No significant difference between SDIA patients and non-SDIA patients was found in the proportion of BAL eosinophils in all age groups.

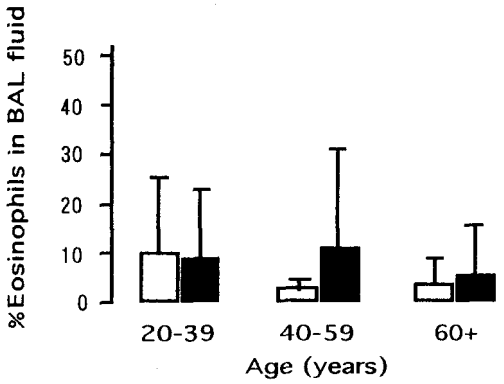

Fig. 5. %Eosinophils in BAL fluid of patients with non-SDIA (▭) and SDIA (▬).

Ventilatory function was compared between SDIA and non-SDIA patients in each age group. %FVC value was significantly lower in SDIA patients over the age of 60 than non-SDIA patients of the same age group. There was, however, no significant difference between SDIA and non-SDIA patients with age between 20 and 39, and between 40 and 59, shown in Fig. 6.

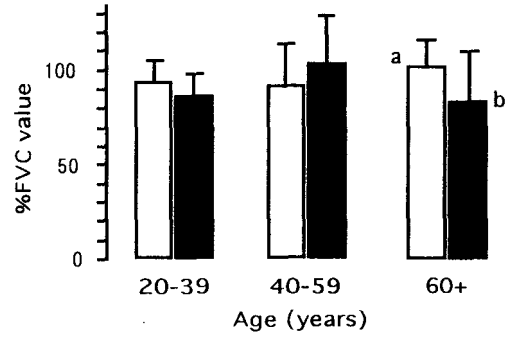

Fig. 6. %FVC value in patients with non-SDIA (▭) and SDIA (▬). a and b ; $p<0.01$.

$FEV_{1.0\%}$ value was also significantly lower in patients with SDIA over 60 years, however, there was no significant difference between SDIA and non-SDIA in three age groups (Fig. 7).

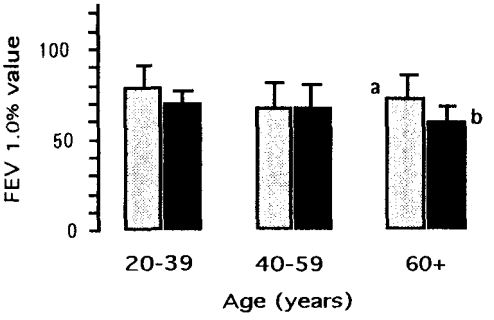

Fig. 7. FEV 1.0% value in patients with non-SDIA (▭) and SDIA (▬). a and b ; $p<0.01$

%PEFR value was not significantly different between patients with SDIA and non-SDIA in all age groups (Fig. 8). The values of %MMF and $\%\dot{V}_{25}$ were significantly lower in patients with SDIA than in those with non-SDIA in the age group over 60, although the difference was not significant in the other age groups (Fig. 9, 10).

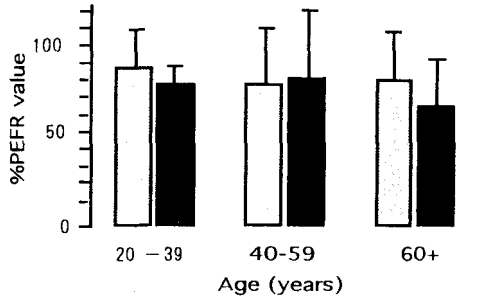

Fig. 8. %PEFR value in patients with non-SDIA (▭) and SDIA (▬)

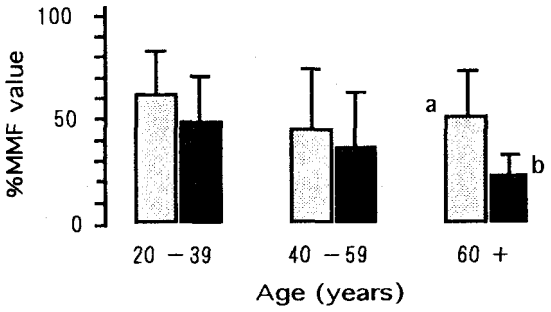

Fig. 9. %MMF value in patients with non-SDIA ( ) and SDIA ( ), a and b ; $p<0.01$.

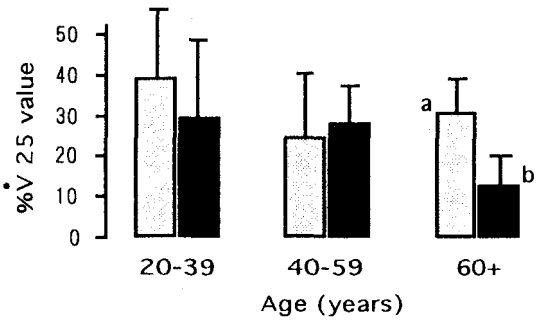

Fig. 10. $\%\dot{V}_{25}$ value in patients with non-SDIA ( ) and SDIA ( ). a and b ; $p<0.001$.

Discussion

Regarding inflammatory process of asthma, cells, such as lymphocytes, neutrophils, and eosinophils, which are involved in the pathophysiology of the airways, have been extensively studied by analyzing cells in bronchoalveolar lavage (BAL) fluid. It has been reported that an increased number of lymphocytes was observed in the BAL fluid of asthma patients[2,22,23], and that the increase of lymphocytes was confined to the T cell population[9]. Eosinophils have been shown to be increased in the BAL fluid of asthma patients[11,24]. Furthermore, a close correlation has been observed between numbers of BAL CD^{4+} $IL2R^{+}$ T cells and the number of eosinophils[25]. An increase in the number of BAL neutrophils has also been demonstrated in the airways with allergic reaction[13], although the role of neutrophils in BAL fluid is not clear.

There have been, however, few studies of the airway response in patients with steroid-dependent intractable asthma (SDIA). In the present study, the airway response in patients with SDIA was examined by the cellular composition of BAL fluid and ventilatory function.

Regarding the proportion of BAL cells, some differences were observed between patients with SDIA and those with non-SDIA. The proportion of lymphocytes in BAL fluid was in general lower in SDIA patients than in non-SDIA, and there was significant difference between them in the age over 60. The proportion of BAL neutrophils was significantly higher in SDIA patients over 60 years than in non-SDIA patients with the same age group. The proportion of BAL eosinophils was not significantly different between SDIA and non-SDIA patients. These results suggest that the proportions of lymphocytes and neutrophils in BAL fluid are affected by long-term glucocorticoid regimen. This may be related to clinical course of SDIA patients. Thus, it is important for physicians to know how the duration of glucocorticoid regimens affects the cellular composition of the airways, since changes in the proportion of inflammatory cells in the airways may lead to increased severity of asthma. In fact, it has been suggested that increased numbers of activated T cells and eosinophils are related to the severity of asthma[25].

Regarding ventilatory function, the values of ventilatory parameters such as %FVC,

%MMF and $\%\dot{V}_{25}$ were significantly lower in SDIA patients over the age of 60 than in non-SDIA patients with the same age group. There was, however, no significant difference between SDIA and non-SDIA patients in the other age group. The results show that in patients with SDIA the values of ventilatory parameters are more decreased with aging, particularly over the age of 60, compared to patients with non-SDIA.

References

1. Nadel JA : Inflammation and asthma. J Allergy Clin Immunol 73 : 651−653, 1984.
2. Kirby JG, Hargreave FE, Gleich GJ, O'Byrne PM : Bronchoalveolar cell profiles of asthmatics and nonasthmatic subjects. Am Rev Respir Dis 136 : 379−383, 1987.
3. Lozewicz S, Gomez E, Ferguson H, Davies RJ : Inflammatory cells in the airways in mild asthma. Br Med J 297 : 1515−1516, 1988.
4. Pauwels R : The relationship between airway inflammation and bronchial hyperresponsiveness. Clin Exp Allergy 19 : 395−398, 1989.
5. Beasley RM, Roche WR, Roberts A, Holgate ST : Cellular events with bronchi in mild asthma and after bronchial provocation. Am Rev Respir Dis 139 : 806−817, 1989.
6. Holgate ST, Djukanovic R, Wilson J, Roche W, Howarth PH : Inflammatory processes and bronchial hyperresponsiveness. Clin Exp Allergy 21 : 30−36, 1991.
7. Gonzalez MC, Diaz P, Gallenguillos 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.
8. Tanizaki Y, Sudo M, Kitani H, Ochi K, Araki H, Tsuji M, Takahashi K, Kimura I : Eosinophilic leucocytes and arylsulphatase activity in bronchoalveolar lavage fluid of patients with bronchial asthma. Acta Med Okayama 42 : 227−232, 1988.
9. Kelly CA, stenton SC, Ward G, Hendrick DJ, Walters EH : Lymphocyte subsets in bronchoalveolar lavage fluid obtained from stable asthmatics, and their correlations with bronchial responsiveness. Clin Exp Allergy 19 : 169−177, 1989.
10. Wardlaw AJ, Kay AB : The role of the eosinophil in the pathogenesis of asthma. Allergy 42 : 321−335, 1989.
11. deMonchy SGR, Kauffman EF, 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.
12. Wardlaw AJ, Dunnette 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.
13. Fabbri LM, Baschetto P, Zocca E, Milani G, Pivirotto 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.
14. Wardlaw AJ, Hay H, Cromwell O, Collins JV, Kay AB : Leukotrienes, LTC_4 and LTB_4 in bronchoalveolar lavage in bronchial asthma and other respiratory diseases. J Allergy Clin Immunol 84 : 762−772, 1989.
15. Wenzel SE, Westcott JY, Larsen GL : Bronchoalveolar lavage fluid mediator levels 5 minutes after allergen challenge in atopic subjects with asthma : Relationship

to the development of late asthmatic responses. J Allergy Clin Immunol 87 : 540－548, 1991.

16. Bousquet J, Chanez P, Lascoste JY, Enander I, Venge P, Peterson C, Ahlstedt S, Michel FB, Godard P : Indirect evidence of bronchial inflammation assessed by titration of inflammatory mediators in BAL fluid of patients with asthma. J Allergy Clin Immunol 88 : 649－660, 1991.
17. Tanizaki Y, Sudo M, Kitani H, Araki H, Oki K, Soda R, Tada S, Takahashi K, Kimura I : Clinical studies on steroid-dependent intractable asthma. Comparison between early and late onset asthma. Jpn J Allergol 38 : 68－76, 1989.
18. Tanizaki Y, Kitani H, Mifune T, Mitsunobu F, Kajimoto K, Sugimoto K : Effects of glucocorticoids on humoral and cellular immunity and on airway inflammation in patients with steroid-dependent intractable asthma. J Asthma (USA) 30 : 485－492, 1993.
19. Tanizaki Y, Sudo M, Kitani H, Kawauchi K, Mifune T, Takeyama H, Kohi F, Tada S, Takahashi K, Kimura I : Characteristics of cell components in bronchoalveolar lavage fluid (BALF) in patients with bronchial asthma classified by clinical symptoms. Jpn J Allergol 39 : 75－83, 1990.
20. 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.
21. Tanizaki Y, Kitani H, Okazaki M, Mifune T, Mitsunobu F, Kimura I : Changes in the proportions of bronchoalveolar lymphocytes, neutrophils and basophilic cells and the release of histamine and leukotrienes from bronchoalveolar cells in patients with steroid-dependent intractable asthma. Int Archs Allergy Immunol 101 : 196 － 202, 1993.
22. Tomioka M, Ida S, Shindoh Y, Ishihara T, Takishima T : Mast cecils in bronchoalveolar lavage of patients with bronchial asthma. Am Rev Respir Dis 129 : 1000－1005, 1984.
23. Kelly CA, Ward C, Bird G, Stenton SC, Hendrick DJ, Walters EH : Diffferential cell count in asthma, and their relationship to bronchial hyperrespon-siveness. Thorax 42 : 224－231, 1987.
24. Fint KC, Leung KB, Hudspith BN, Brostoff J, Pearce FL, Johnson NM : Bronchoalveolar mast cells in extrinsic asthma ; a mechanism for the initiation of antigen specific bronchoconstriction. Br Med J 291 : 923－930, 1985.
25. Walker C, Kaegi MK, Braun P, Blaser K : Activated T cells and eosinophils in bronchoalveolar lavages from subjects with asthma correlate with disease severity. J Allergy Clin Immunol 88 : 935－943, 1991.

ステロイド依存性重症難治性喘息における気道反応の特徴

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年齢により３群（20－39才，40－59才，60才以上）に分類された気管支喘息86例（ステロイド依存性重症難治性喘息：SDIA；43例，非SDIA；43例）を対象に，気管支肺胞洗浄液（BALF）中の細胞成分および換気機能よりSDIAにおける気道反応の特徴を検討した。

1．血清コーチゾール値は，SDIA症例において，非SDIA症例に比べいずれの年齢層においても有意の低値を示した。

2．BALF中リンパ球頻度は，40－59才および60才以上のSDIA症例において，同年齢の非SDIA症例に比べ有意の低値を示した。一方，BALF中好中球頻度は，60才以上のSDIA症例で，同年齢層の非SDIA症例に比べ有意に高い値を示した。また，BALF中好酸球頻度は，いずれの年齢層においても，SDIAと非SDIA症例の間に有意の差は見られなかった。

3．換気機能では，%FVC，FEV1.0%，%MMF，$\%\dot{V}_{25}$ などの換気パラメーターは，60才以上のSDIA症例において，同年齢層の非SDIA症例に比べて有意の低値を示した。

これらの結果は，40－59才あるいは60才以上のSDIA症例では，非SDIA症例に比べ，BALF中リンパ球頻度の低下，好中球頻度の増加，換気パラメーター値の低下などがより高度に見られることを示している。

キーワード：気管支喘息，気管支肺胞洗浄液中の細胞，換気機能，グルココーチコイド，血清コーチゾール値