
◎原 著

Serum immunoglobulin levels and peripheral lymphocyte count related to changes in cellular composition of bronchoalveolar lavage fluid in patients with bronchial asthma

Fumihiro Mitsunobu, Hikaru Kitani, Takashi Mifune, Kazuhiro Kajimoto, Satoshi Yokota, Ichiro Takata, Yoshiro Tanizaki, Koji Ochi¹⁾, Hideo Harada¹⁾, Shinya Tada²⁾ and Mine Harada²⁾

Division of Medicine, Misasa Medical Branch, ¹⁾ Department of Laboratory Medicine, ²⁾ 2nd Department of Medicine, Okayama University Medical School

Abstract : Changes in airway inflammation by glucocorticoids were examined in 26 patients with bronchial asthma, divided into two age groups ; 20–59 and 60+ years, in relation to serum immunoglobulin levels and peripheral lymphocyte count.

1. The levels of IgG, IgA and IgM were significantly lower in patients with steroid-dependent intractable asthma (SDIA) of age over 60 years than in those with non-SDIA of the same age group. In patients between the ages of 20 and 59, only serum IgG level was significantly lower in SDIA than in non-SDIA patients.

2. Peripheral lymphocyte count was significantly decreased in SDIA patients than in non-SDIA patients in age group over 60, but not in age group between 20 and 59.

3. Number of lymphocytes in bronchoalveolar lavage (BAL) fluid was significantly lower and number of BAL neutrophils was significantly higher in SDIA patients than in non-SDIA patients in age group over 60, but not in age group between 20 and 59.

These results suggest that levels of IgG, IgA and IgM, and peripheral lymphocyte count were significantly suppressed in SDIA patients of age over 60, accompanied with decrease of BAL lymphocytes and increase of BAL neutrophils.

Key words : serum immunoglobulin levels, lymphocytes, neutrophils, bronchial asthma, glucocorticoids

Introduction

In recent years, it has been shown that airway inflammation is a common feature of bronchial asthma (1–4), and that the inflammation is clearly related to late asthmatic reaction (LAR) (5, 6). In the process of airway inflammation, lymphocytes, neutrophils, eosinophils and basophils migrate into local allergic reaction sites of airways (7–12). Of these cells, lymphocytes and eosinophils have been noted as being main cells participating in inflammatory process.

Our previous studies have shown that asthma is classified into three clinical types according to symptoms and signs: Ia. simple bronchoconstriction type, Ib. bronchoconstriction + hypersecretion type, and II bronchiolar obstruction type (13–15). The clinical asthma types are also related to cellular composition of bronchoalveolar lavage (BAL) fluid. In type Ib asthma, an increase in number of BAL eosinophils is often observed. Type II asthma is characterized by an increased number of BAL neutrophils, accompanied with marked decrease in the values of ventilatory function. An increased number of BAL neutrophils, accompanied with a decrease in number of BAL lymphocytes, is sometimes observed in asthmatic patients with long-term glucocorticoid therapy (16–18). However, an increase in number of BAL neutrophils is not always found in patients with steroid-dependent intractable asthma (SDIA).

In the present study, to analyze the mechanism of an increase in BAL neutrophil count, serum immunoglobulin levels, peripheral lymphocyte count, and cellular composition of BAL fluid were examined in patients with SDIA in relation to patient age.

Subjects and Methods

The subjects of this study were 26 patients with bronchial asthma (12 females and 14 males, mean 53.2 years, range 21–71 years). The mean level of serum IgE was 492 IU/ml (range 11–3058 IU/ml). All the subjects were non-smokers. Of these subjects 13 were regarded as having steroid-dependent intractable asthma (SDIA), since they had been treated with glucocorticoids for more than 2 years. The subjects were divided into two groups according to age: 20–59 and 60+ years.

Bronchoalveolar lavage (BAL) was performed after informed consent was obtained from all the subjects. The BAL was carried out in all study subjects by a previously reported method (14–18) when their attacks were stable with prednisolone of 5 mg/day or less. Briefly, the aspirates were centrifuged at 1200 rpm for 10 min at 4 °C after filtration through a sterile steel mesh, and the resultant cell pellet was resuspended in Tris ACM. After total cell number was counted, smear preparations made with the cell suspensions were stained with May Giemsa. A differential cell count was performed on 500 cells, excluding epithelial cells. In the present study, the mean recovery rate at BAL was $28.3 \pm 12.4\%$ ($\pm$ SD), and the total cell number was $6.5 \pm 3.1 \times 10^6$. The results were expressed as /ml.

The levels of serum IgG, IgA, and IgM were measured by turbidometric immunoassay. Serum IgE level was estimated by radioimmunosorbent test (RIST).

Results

Table 1 shows characteristics of patients with bronchial asthma divided by age and

asthma severity. The subjects between the ages of 20 and 59 years consisted of 8 patients with SDIA and 8 with non-SDIA. While the subjects over age 60 years comprised 5 SDIA patients and 5 non-SDIA.

Table 1. Characteristics of patients with bronchial asthma studied

Asthma	No of subjects	Age (years)	Sex	Serum IgE (IU/ml)
SDIA	13	43.4	F 4	430
			M 4	± 451
Non-SDIA	13	46.2	F 3	823
			M 5	± 979

Regarding serum levels of immunoglobulins, the levels of IgG, IgA and IgM were significantly lower in SDIA patients over age 60 than in non-SDIA patients of the same age group (IgG and IgM ; $p < 0.001$, IgA ; $p < 0.05$)(Fig. 1 a). In contrast, only serum IgG level was significantly lower in patients with SDIA than in those with non-SDIA in the age group between 20 and 59 ($p < 0.001$). There was no significant difference in serum levels of IgA and IgM between them, as shown in Fig. 1 b. Peripheral neutrophil count was not significantly different between SDIA and non-SDIA patients over age 60, as shown in Fig. 2 a. While peripheral lymphocyte count was significantly more decreased in patients with SDIA over age 60 than in those with non-SDIA of the same age group. In contrast, in age group between 20 and 59 years, numbers of peripheral neutrophils and lymphocytes were not significantly different between SDIA and non-SDIA patients, as shown in Fig. 2 b.

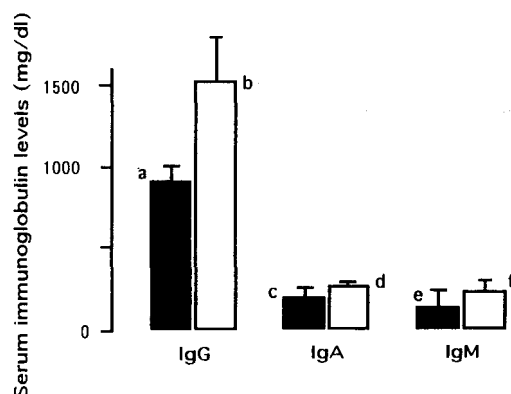


Fig. 1 a. Serum immunoglobulin levels in patients with SDIA (■) and non-SDIA (□) over the age of 60 years. a and b ; $p < 0.001$, c and d ; $p < 0.05$, e and f ; $p < 0.001$.

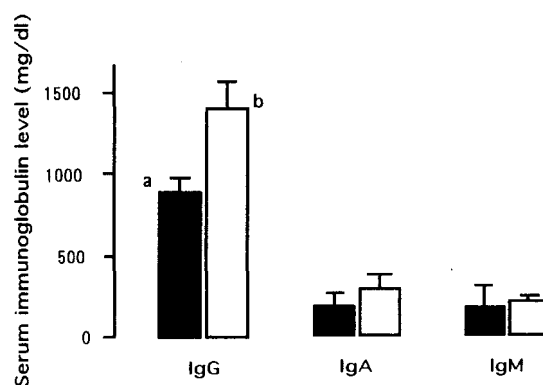


Fig. 1 b. Serum immunoglobulin levels in patients with SDIA (■) and non-SDIA (□) between the ages of 20 and 59 years. a and b ; $p < 0.001$.

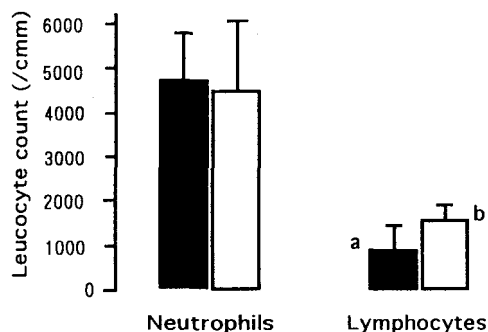


Fig. 2 a. Peripheral leucocyte count in patients with SDIA (■) and non-SDIA (□) over the age of 60 years. a and b; $p < 0.01$.

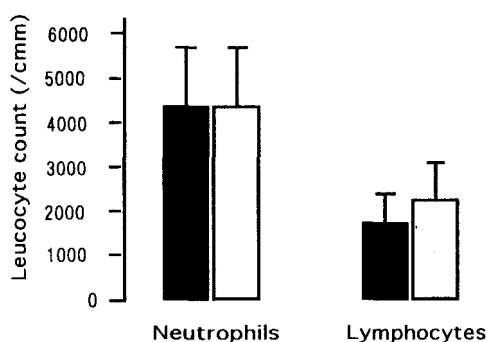


Fig. 2 b. Peripheral leucocyte count in patients with SDIA (■) and non-SDIA (□) between the ages of 20 and 59 years.

Table 2 shows the results of cellular composition of bronchoalveolar lavage (BAL) fluid in study patients. Number of BAL lymphocytes was significantly lower in patients with SDIA over age 60 than in those with non-SDIA of the same age group ($p < 0.01$). In these patients with decreased number of BAL lymphocytes, number of BAL neutrophils was significantly larger in SDIA patients than in non-SDIA patients ($p < 0.05$).

Number of BAL eosinophils was not significantly different between two asthma groups over age 60. In contrast, in age group between 20 and 59, number of BAL lymphocytes and neutrophils were not significantly different between SDIA and non-SDIA patients. BAL eosinophil count was not significantly different between them (Table 2).

Table 2. Cellular composition in BAL fluid of patients with SDIA and non-SDIA

Asthma	Recovery rate (%)	No of total cells ($\times 10^6$)	No of BAL cells ($\times 10^4$ /ml)			
			Mac	Lym	Neut	Eos
Ages between 20 and 59						
SDIA	31.0 ± 11.9	6.3 ± 1.8	14.8 ± 16.9	1.4 ± 1.5	0.41 ± 0.29	2.15 ± 2.3
Non-SDIA	35.0 ± 14.7	7.8 ± 3.6	12.0 ± 6.9	2.7 ± 1.2	0.15 ± 0.1	1.59 ± 3.2
Ages over 60						
SDIA	19.3 ± 6.0	4.5 ± 3.6	6.8 ± 7.5	0.8 ^a ± 0.7	5.1 ^b ± 5.8	2.27 ± 4.8
NON-SDIA	22.8 ± 3.4	6.9 ± 3.4	14.5 ± 5.2	3.7 ^a ± 2.6	0.56 ^b ± 0.37	1.57 ± 2.1

SDIA;steroid-dependent intractable asthma, Mac;macrophages, Lym;lymphocytes, Neut;neutrophils, Eos;eosinophils. a; $p < 0.01$, b; $p < 0.05$.

Discussion

There are increasing evidences that asthma has inflammatory process in the airways as a main pathogenesis of the disease (1–4). The role of inflammatory cells such as lymphocytes (7–9), neutrophils (16–18), eosinophils (10,11), and basophils, which migrate from bloodstream, have been extensively studied in patients with bronchial asthma. It has been reported that numbers of activated T-lymphocytes and eosinophils in BAL fluid are increased in asthma attacks compared to healthy subjects (12). Long-term glucocorticoid therapy may affect these inflammatory cells in patients with asthma.

Long-term glucocorticoid regimen brings about many side effects such as hypertension, diabetes mellitus, osteoporosis, muscle atrophy, suppression of adrenocortical glands and suppression of humoral and cellular immunity. Of these side effects, immunosuppression by glucocorticoids should be noted, since it may affect inflammatory process in the airways of asthma, and be related to asthma severity. In the present study, changes in cellular composition of BAL fluid by glucocorticoids were examined in patients with bronchial asthma in relation to serum immunoglobulin levels and peripheral lymphocyte count.

The data obtained here showed that serum immunoglobulin levels, IgG, IgA and IgM, and peripheral lymphocyte count were more decreased in patients with steroid-dependent intractable asthma (SDIA) over age of 60 years than in those with non-SDIA of the same age group. In these SDIA patients with a decrease in serum immunoglobulin levels and peripheral lymphocyte count, a decrease in number of BAL lymphocytes and an increase in BAL neutrophil count were observed. These results suggest that immunosuppression (expressed by decreased levels of serum immunoglobulins and decreased number of peripheral lymphocytes) by glucocorticoids leads to changes in numbers of BAL cells, particularly lymphocytes and neutrophils in asthma patients over age 60. In contrast, the numbers of lymphocytes and neutrophils in BAL fluid were not significantly different between SDIA and non-SDIA patients between 20 and 59.

It is suggested from these results that glucocorticoids should be carefully used not to induce immunosuppression in elderly patients with bronchial asthma.

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気管支喘息における血清免疫グロブリン値、末梢血リンパ球数と気管支肺胞洗浄液中の細胞成分との関連

光延文裕，貴谷 光，御船尚志，梶本和宏，横田聡，高田一郎，谷崎勝朗，越智浩二¹⁾，原田英雄¹⁾，多田慎也²⁾，原田実根²⁾

岡山大学医学部附属病院三朝分院，¹⁾岡山大学医学部臨床検査医学，²⁾岡山大学医学部第2内科

気管支喘息26例（ステロイド依存性重症難治性喘息，SDIA 13例，非SDIA 13例）を対象に，血清免疫グロブリン，IgG，IgAおよびIgM値，および末梢血リンパ球数との関連のもとに，副腎皮質ホルモン長期投与による気道炎症細胞の出現頻度の変化について検討を加えた。なお，年齢による影響を観察する目的で，SDIAおよび非SDIA各13例を，20-59才と60才以上の2群に分けて，それぞれのグループの特徴について検討した。

1. 血清IgG，IgAおよびIgM値は，60才以上の症例において，いずれも非SDIA症例に比べ，SDIA症例で有意に低い値を示した。一方，20-

59才の症例群においては，IgG値のみSDIA症例で有意の低下傾向が見られた。

2. 末梢血リンパ球数は，60才以上の症例では，SDIA症例で，非SDIA症例に比べ有意の低値を示したが，20-59才の症例では両群間に有意の差は見られなかった。

3. 気管支肺胞洗浄（BAL）液中のリンパ球数は，60才以上のSDIA症例で，非SDIA症例に比べ有意に少なく，また同時にこれらの症例ではBAL液中好中球が有意に多い傾向が観察された。しかし，20-59才の症例群では，SDIAと非SDIAの間にいずれも有意の差は見られなかった。

これらの結果より，60才以上の症例では，20-59才の症例群に比べ，副腎皮質ホルモンの長期投与により，血清IgG，IgAおよびIgM値，末梢血リンパ球数の減少とともに，BAL液中のリンパ球減少，好中球増加をきたしやすいことが明らかにされた。

キーワード：血清免疫グロブリン値，リンパ球，好中球，気管支喘息，副腎皮質ホルモン