● 原著

気管支喘息における血清 IgE と IgG₄ 値 の関連について

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Correlation between serum levels of IgE and IgG₄ in patients with bronchial asthma

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【Summary】

A correlation between serum levels of IgE and IgG₄ was examined in 79 patients with bronchial asthma. High levels of IgG₄ were not always correlated to high levels of IgE in all subjects with asthma. IgG₄ levels tended to increase as IgE levels increased in patients over age 60, but not in those under age 59. The results show that serum IgG₄ levels are associated with serum IgE levels in patients over age 60 and are related to patient age in all subjects with asthma.

Key words : IgE,
IgG₄,
patient age,
bronchial asthma

【概 要】

血清 IgE および IgG₄ は、気管支喘息における主要な反応系である即時型アレルギー反応に関与する免疫グロブリンと考えられている。このうち、IgE は即時型アレルギー反応をひき起こす抗体として知られているが、IgG₄ については、即時型アレルギー反応に対して遮断抗体として作用するのか、反応の増強因子として作用するのか、なお議論が多い。一方、*in vitro* の系では、両者とも IL-4 刺激により B 細胞での産生亢進が見られる。本論文では、気管支喘息における血清中 IgE および IgG₄ 値の関連について検討を加えた。

対象および方法：対象としては気管支喘息 79 例を選んだ。血清 IgE 値は RIST 法により、また IgG₄ 値は ELISA 法により測定した。

成績：血清 IgE 値は 19 歳までの年齢層で高く、30～69 歳の年齢層に比べ有意の高値を示した。一方、70 歳以上の症例では、血清 IgE 値は増加傾向を示し、50～69 歳の年齢層に比べ有意に高い値を示した。

血清 IgG₄ は、0～9 歳の年齢層で 20～49 歳の年齢層に比べ有意に低く、70 歳以上の年齢層では、60～69 歳の年齢層に比べ有意に高い傾向を示した。しかし、10～69 歳の年齢層では、各年齢層間に有意の差は見られなかった。

血清 IgG₄ と IgE 値は、60 歳以上の年齢層では有意の相関が見られ、これらの症例では、血清 IgE 値が高くなるにつれて、IgG₄ も増加する傾向が見られた。しかし、59 歳以下の症例では IgE と IgG₄ 値の間の関連は見られなかった。

血清 IgG₄ 値が 100 mg/dl 以上を示す 13 症例の検討では、8 例 (61.5%) が 60 歳以上、9 例 (69.2%) が血清 IgE が 400 IU/ml 以上であった。

これらの結果は、血清 IgG₄ は、60 歳以上の症例では血清 IgE 値と、また全症例の検討では年齢と、関連を有していることを示している。

I. Introduction

It is widely accepted that the immediate allergic reaction, in which primarily IgE and IgG₄ antibodies participate, plays an important role in the mechanism of onset of bronchial asthma. The role of IgE antibodies is well defined; They induce the release of chemical mediators from tissue mast cells and blood basophils by bridging IgE receptors on the membranes of these cells¹⁻³⁾. IgG₄ is thought to be involved in atopic disorders; however, whether the action of IgG₄ antibodies in the immediate allergic reaction is protective or anaphylactic is a major point of contention⁴⁻⁸⁾.

Several investigators have found high levels of IgE and IgG₄, but normal levels of IgG₁, IgG₂ and IgG₃ in patients with atopic eczema and bronchial asthma⁹⁻¹¹⁾. Recent studies have demonstrated significantly higher levels of IgG₁ and IgG₄ in infants with elevated IgE levels than in those with lower levels¹²⁾. Furthermore, interleukin 4 (IL-4), which induces human B cells to secrete significant amounts of IgE¹³⁾, also induces IgG₄ production, but has no effect on the production of IgG₁, IgG₂, and IgG₃ by B cells¹⁴⁻¹⁶⁾. To date, however, few studies have addressed correlations between serum levels of IgE and IgG₄ in adult bronchial asthma patients.

In the present study, we examined the correlation between levels of serum IgE and IgG₄ in bronchial

asthma patients of various ages to compare differences in these levels.

II. Subjects and Methods

The subjects were 79 bronchial asthma patients (47 females and 32 males; mean age 47.4 years, range 6 to 80). The mean level of serum IgE was 456 IU/ml (range, 8~2,626 IU/ml). Specific IgE antibodies to inhalant allergens were found in 33 of the 79 patients (41.8%). The subjects were divided into eight groups according to age: 0~9, 10~19, 20~29, 30~39, 40~49, 50~59, 60~69 and 70+ years. They were also divided into five groups according to serum IgE level: 0~100, 101~200, 201~500, 501~1,000, and 1,001+ IU/ml. Bronchial asthma was clinically diagnosed according to previously defined criteria¹⁷⁾.

Serum IgG₄ levels were measured by an enzyme-linked immunosorbent assay (ELISA), using a modified method¹⁸⁻²⁰⁾ based on that described by Engvall et al.²¹⁾. The wells of polystyrene microtiter plates were coated overnight with 200- μ l samples of monoclonal anti-IgG₄ (Yamasa Shoyu Co), at a 1:1,000 dilution in 0.05 M phosphate buffer solution (pH 7.4). After three washes with 0.05 M phosphate buffered saline (PBS), 250 μ l of PBS containing 0.5% bovine serum albumin (BSA) was added to the wells and they were incubated for 2 hr at room temperature. After three washes with PBS

containing Tween 20 (PBS-TW 20), 200 μ l serum samples, at a 1:20,000 dilution in PBS-TW 20 containing 0.1% bovine γ -globulin, were added to the wells, which were then incubated at 4°C overnight. After the wells were washed with PBS-TW 20, 200 μ l of peroxidase-conjugated anti-mouse IgE (Cappel Lab.), at a 1:10,000 dilution in PBS-TW 20 containing 1% γ -globulin, was added to the wells, and they were incubated for 2 hr at room temperature. After a further washing, 200 μ l of the substrate, o-phenylenediamine, was added to the wells and they were incubated for 30 min at room

temperature. Color development was stopped by adding 50 μ l of 4 N H₂SO₄, and the OD was determined at 490 nm. These assays were carried out in duplicate. Serum IgG₄ was expressed as mg/dl.

Serum IgE levels were determined by a radioimmunosorbent test (RIST), and the results were expressed as IU/ml. The levels of specific IgE antibodies to inhalant allergens were evaluated by a radioallergosorbent test (RAST). The results were expressed as a score, a score of 2+ or more being regarded as positive.

Statistically significant differences of the mean were assessed using Student's unpaired t test. The levels of significance were expressed as p values.

Table 1 Differences in serum levels of IgE and IgG₄ in subjects classified by age

Age, years	No of patients	Serum levels	
		IgE (IU/ml)	IgG ₄ (mg/dl)
0~9	5	1,298 $\pm$ 642 ^{aabcd}	16.2 $\pm$ 4.2 ^{jk1}
10~19	8	1,341 $\pm$ 819 ^{efg}	62.3 $\pm$ 45.2
20~29	5	1,026 $\pm$ 787	57.4 $\pm$ 28.9 ^j
30~39	8	545 $\pm$ 571 ^a	60.5 $\pm$ 42.7 ^k
40~49	8	343 $\pm$ 370 ^{be}	56.5 $\pm$ 28.3 ⁱ
50~59	14	285 $\pm$ 391 ^{cfh}	57.4 $\pm$ 55.4
60~69	17	312 $\pm$ 255 ^{dgi}	54.2 $\pm$ 52.9 ^m
70+	14	836 $\pm$ 787 ^{hi}	98.4 $\pm$ 66.9 ^m

*Mean $\pm$ SD. a, h, k and m, $p<0.05$; b, f and l, $p<0.01$; c, d, e, f, g and j, $p<0.001$; i, $p<0.02$.

III. Results

IgE levels were higher in patients younger than 29, and the levels tended to decrease in patients between the ages of 30 and 69. These levels were significantly higher in patients between the ages of 10 and 19 than in those between 30 and 39 ($p<0.05$) and those between 40 and 69. A significantly higher level of IgE was found in patients over the age of 70 compared with levels in patients between 60 and 69 ($p<0.02$), between 50 and 59, ($p<0.05$) and between 40 and 49 ($p<0.001$). IgG₄ levels, in con-

Fig. 1 Serum Levels of IgE (□) and IgG₄ (■) in bronchial asthma patients in relation to age. Vertical bars represent mean $\pm$ SD.

Table 2 Correlation between serum levels of IgE and IgG₄ in bronchial asthma patients

Serum IgE(IU/ml)	No of patients	Serum IgG ₄ (mg/dl)	
		Mean±SD	Range
0~ 100	18	40.6±26.6 a	6~94
101~ 200	13	62.9±48.3	8~150
201~ 500	17	71.7±50.8 a	20~210
501~1,000	15	64.7±60.8	11~200
1,001+	16	70.4±68.1	14~280

a, $p < 0.05$. **Fig. 2** Correlation between serum levels of IgE and IgG₄ in patients between the ages of 10 and 59. Vertical columns represent the mean of each group classified by serum IgE levels.

trast, were significantly lower in patients younger than 9 compared with the levels in patients between 20 and 29 ($p < 0.01$), between 30 and 39 ($p < 0.05$) and between 40 and 49 ($p < 0.01$). No significant difference in serum IgG₄ levels was found among patients aged from 10 to 69. Elevated levels of IgG₄ were found in patients over age 70, in whom these levels were significantly higher than those in

Fig. 3 Correlation between serum levels of IgE and IgG₄ in patients over the age of 60. Vertical columns represent the mean of each group classified by serum IgE levels. a, $p < 0.01$; b, $p < 0.05$; c, $p < 0.02$.

patients between 60 and 69 ($p < 0.05$) (Table 1, Fig. 1).

Levels of IgG₄ were not always more elevated in patients with high levels of serum IgE in the subjects overall, although there was a significant difference between IgG₄ levels in patients with serum IgE levels of 0~100 IU/ml and 201~500 IU/ml ($p < 0.05$) (Table 2). We examined the IgE and IgG₄ levels in patients between the ages of 10 and 59 to determine whether these levels were correlated. No significant correlation was observed (Fig. 2).

IgG₄ levels in patients over the age of 60 tended to increase as levels of IgE increased. Significantly higher IgG₄ levels were found in patients with high serum IgE levels (between 201 and 500 IU/ml ($p < 0.01$), between 501 and 1,000 IU/ml ($p < 0.05$), and over 1,001 IU/ml ($p < 0.02$), than in those with low

Table 3 Correlation between serum levels of IgE and IgG₄ in patients with high IgG₄ level

Case no	Age, years	Sex	IgG ₄ (mg/dl)	IgE (IU/ml)	Inhalant allergens
1	71	F	280	1,152	—
2	52	F	210	578	—
3	65	M	200	790	—
4	70	M	170	485	—
5	64	F	160	724	—
6	72	M	160	673	HD 2+
7	17	F	160	1,930	HD 2+
8	50	F	150	195	HD 2+
9	34	F	140	182	—
10	77	F	128	2,007	JC 2+
11	80	M	120	177	—
12	42	M	100	445	JC 2+
13	62	F	100	292	—

HD, house dust; JC, Japanese cedar.

serum IgE levels (between 0 and 100 IU/ml) (Fig. 3).

Characteristics of patients with high serum IgG₄ (over 100 mg/dl) were analyzed in relation to their age and serum IgE levels. Of the 13 patients with high IgG₄, eight (61.5%) were over the age of 60, and nine (69.2%) had over 400 IU/ml of serum IgE. These results revealed that serum IgG₄ levels tended to be increased in relation to high serum IgE levels and increasing patient age (Table 3).

IV. Discussion

IgE is well defined as the antibody, which induces the immediate allergic reaction¹⁻³⁾ that plays the major role in the pathology of bronchial asthma. In accordance with such generally accepted views of the allergic reaction, some attempts have been made to distinguish allergic (extrinsic) from nonallergic asthma (intrinsic)²²⁾. IgG₄, in contrast to IgE, was at first identified as a blocking antibody observed after immunotherapy with offending allergens^{4,5)}. At present, however, there are some differences of opinion as to whether the role of IgG₄ in the immediate type of immune response is anaphylactic or protective^{6-8,23)}.

Some correlations between the serum levels of

IgE and IgG₄ have been demonstrated by several investigators⁹⁻¹²⁾. Lilja et al. showed a weak positive correlation between IgE and IgG₄ in infants at 18 months of age¹²⁾. This parallelism in IgE and IgG response has also been found in non-atopics and in patients with helminthiasis^{24,25)}. It has been suggested that interleukin 4 (IL-4) derived from T cells stimulates B cells to secrete increased amounts of IgE^{13,26-28)}. IL-4 also induces the production of IgG₄, but not that of IgG₁, IgG₂ or IgG₃^{16,29)}. Moreover, It has been demonstrated that there is a clear difference in the capacity of structurally related allergens to stimulate the IgE and IgG₄ response^{30,31)}. It has been revealed that IgE is synthesized predominantly in the lymphoid tissue of the respiratory tract, while IgG₄ is synthesized predominantly in the intestinal lymphoid tissue^{8,32)}.

In this study, elevated levels of IgE and IgG₄ were observed in patients over age 70, and a rise of IgG₄ associated with elevated levels of IgE was found in those over age 60. There was, however, no relationship between IgE and IgG₄ levels in young children under age 9. In these subjects (mean age, 7.6 years), IgG₄ levels were rather lower than those in subjects over age 10. In patients between ages of 10 and 19, serum IgG₄ levels tended to increase in asthma patients with atopic dermatitis. Raised serum IgG₄ levels were not always associated with the rise of IgE in patients between 10 and 59. Our findings from this study show that there was a relationship between the serum levels of IgE and IgG₄ in asthma patients over age 60, but not in those under age 59. Analysis of the findings in patients with high IgG₄ levels (over 100 mg/dl) showed that patients over age 60 often had raised serum levels of both IgE and IgG₄, suggesting that the synthesis of both these immunoglobulins is related to age in asthmatic patients. It was not clear from this study why raised serum levels of both IgE and IgG₄ were found only in asthma patients over age 60. Furthermore, the clinical significance of serum IgG₄ in asthma patients was not known. Further studies are needed to clarify the correlation between serum levels of IgE and IgG₄, including clinical significance of

serum IgG₄ in asthma patients.

V. Conclusion

The possible correlation between serum levels of IgE and IgG₄ was examined in 79 bronchial asthma patients. The levels of IgE were significantly higher in patients younger than 29 compared with levels in patients aged between 30 and 69, in whom the IgE levels was not so increased. The serum IgE levels was significantly elevated in patients over the age of 70 compared with the level in those between 60 and 69 ($p < 0.02$). The level of IgG₄, in contrast, was significantly lower in patients younger than 9 compared with the level in those aged between 20

and 29 ($p < 0.01$). Significantly higher levels of IgG₄ were found in patients over age 70 compared with the levels in those between 60 and 69 ($p < 0.05$). High levels of IgG₄ were not always correlated to high levels of IgE. IgG₄ levels tended to increase as IgE levels increased in patients over age 60, but not in those under age 59. Of the 13 patients with high IgG₄ (over 100 mg/dl), 8 (61.5%) were over age 60 and 9 (69.2%) had over 400 IU/ml of serum IgE. These results demonstrate that serum IgG₄ levels are associated with serum IgE levels in asthma patients over age 60 and are associated with patient age in all asthmatic subjects.

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