Humoral and Cellular Immunity to *Candida albicans* in Patients with Bronchial Asthma

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Delayed cutaneous reactivity to *Candida albicans* (*C. albicans*) and PPD (purified protein derivative) was examined in 52 patients with bronchial asthma in relation to the production of specific IgG₄ antibodies against the antigen. 1. The frequency of a positive, immediate skin reaction to *C. albicans* was similar among the five age groups, ranging from 60.0% to 66.7%. 2. The incidence of a positive delayed skin reaction to *C. albicans* was lower in patients between the ages of 10 and 30 and tended to decrease with aging in the patients over the age of 51. 3. A delayed skin reaction to PPD was positive in patients between 31 and 50 with a higher incidence; this incidence decreased in patients over age 51. 4. The level of *C. albicans*-specific IgG₄ antibodies was significantly higher (26.7 u/ml) in patients with a negative delayed skin reaction to the antigen than in those with a positive reaction (5.9 u/ml) ($p < 0.001$). There was no correlation between delayed skin reaction to PPD and production of specific IgG₄ antibodies. (Internal Medicine 31: 766–769, 1992)

Key words: specific IgG₄, delayed cutaneous hypersensitivity, PPD, atopics, aging

Introduction

Candida albicans (*C. albicans*) is a common antigen found in patients with bronchial asthma. The antigen causes IgE-mediated allergic reactions in asthmatics (1–3). It has been shown that IgE and IgG₄ antibodies participate in immediate immune response. The response mediated by IgE plays an important role in the onset mechanism of bronchial asthma (4, 5). The role of IgG₄ antibodies, whether anaphylactic or protective, is however still controversial (6–10). Patients who have IgE-mediated immune responses which cause asthma attacks are evaluated as atopic (11, 12). It has been suggested that delayed immune responses are depressed in these patients (13). An increased production of specific IgG antibodies to *C. albicans* (14) and a diminished delayed skin reaction have been observed in some atopics (13, 15). A depressed delayed immune response leads to saprophytic *C. albicans* growth, which accelerates the production of specific IgG antibodies (16, 17); this is due to the fact that in the cell-mediated immune system plays a major role in the defense against *C. albicans* (18).

In the present study, the delayed cutaneous hypersensitivity to *C. albicans* and PPD (purified protein derivative) was examined in patients with bronchial asthma to evaluate the relationships between IgE-mediated allergic reactions to depressed cell-mediated immune

response and those to the increased production of specific IgG₄ antibodies. IgG₄ antibodies, which may participate in immediate allergic response (6–10), were selected to examine the changes in the IgG response to *C. albicans*.

Subjects and Methods

Fifty-two patients (38 females and 14 males) with bronchial asthma, with a mean age of 48.6 years (range, 11 to 77 years) were divided into five groups according to age: 1) 10–30 years, 2) 31–50 years, 3) 51–60 years, 4) 61–70, and 5) 71 years and over.

An intradermal skin test was performed with 0.02 ml of commercial *Candida* allergen extract (Torii Co, Japan) and by intradermal injection of 0.1 ml PPD (Japan BCG Co). The diameters of flare and wheal at 20 minutes, and flare and induration at 48 hours were measured after the test. A flare diameter of larger than 20 mm or a wheal diameter of larger than 9 mm was regarded as positive in the immediate skin reaction. A flare or induration diameter of larger than 10 mm was regarded as positive in the delayed skin reaction.

C. albicans-specific IgG₄ antibodies were measured by an indirect enzyme-linked immunosorbent assay (ELISA) (19) modified from that described by Engvall and Perlmann (20). Polystyrene microtiter plates were coated with 100 µl of *C. albicans* extract (Torii Co)

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at a 1:20,000 dilution in phosphate-buffered saline (PBS) at 4°C overnight, and then washed three times with PBS-Tween 20. After washing, 200 µl of 1% BSA-PBS was added to the wells, incubated for 1 hour at 37°C, then discarded. Reference serum or serum samples at a 1:1,000 dilution in BSA-PBS were added and incubated at 37°C for 2 hours. Monoclonal anti-human IgG₄ (Yamasa Shoyu Co.) was added to the wells at a 1:1,000 dilution in BSA-PBS and incubated at 4°C overnight. After washing, 100 µl of peroxidase-conjugated anti-mouse IgG (Cappel Lab.) was added to the wells at 1:10,000 dilution in BSA-PBS for 1 hour at 37°C. After another washing, the substrate, O-phenylenediamine, was added to the wells and incubated for 1 hour at room temperature. Color development was stopped by the addition of 100 µl of 4 N H₂SO₄, and the OD was determined at 500 nm (19). Specific IgG₄ levels were expressed as arbitrary units per milliliter against a positive serum pool.

Specific IgE antibodies against house dust and *C. albicans* were examined by a radioallergosorbent test (RAST). The results were expressed by a score from 0+ to 4+ and a score of 2+ or more was evaluated as positive, meaning sensitization by the allergen. The level of total serum IgE was measured by a radioimmunosorbent test (RIST).

Results

1. Characteristics of the patients

The mean level of serum total IgE was the highest in the patients between the ages of 10 and 30, and the level was significantly higher than that in those between the ages of 31 and 50 ($p < 0.001$). The frequency of a positive RAST score to house dust and/or *C. albicans* was the highest in the patients between the ages of 10 and 30, in which 11 (84.6%) of the 13 patients showed a positive RAST. The frequency of a positive RAST score was the lowest (11.1%) in patients over the age of 71. The patients with steroid-dependent intractable asthma (SDIA) was most frequently observed in those between 51 and 60. The frequency of SDIA was the lowest in patients aged between 10 and 30. The results indicate that many of the patients aged between 10 and 30 were

atopic asthmatics, while many of those between the ages of 51 and 60 had SDIA (Table 1).

2. Delayed skin reaction and patient age

The incidence of patients with a positive immediate skin response to *C. albicans* was from 60.0 to 66.7%. Among the five age groups, no differences were found. A delayed skin reaction to *C. albicans* was positive in 23 cases (44.2%) and that to PPD was positive in 21 (40.4%) of the 52 subjects. Compared with the other groups, the frequency of a positive delayed skin reaction to *C. albicans* was high in patients aged between 31 and 50 (63.6%) and slightly high in those between 51 and 60 (55.6%). The lowest frequency (22.2%) was observed in patients aged over 71. A delayed skin reaction to PPD was positive in patients aged between 31 and 50 with the highest frequency. The incidence of patients positive to PPD was lower in those between 10 and 30 years and showed a decreasing tendency in those over 51 (Fig. 1).

3. Serum levels of *C. albicans*-specific IgG₄ antibodies in each age group

C. albicans-specific IgG₄ antibody levels were the highest in patients over age 71 and the lowest in those between 31 and 50. A significant difference was present between the two groups ($p < 0.05$). The patients aged between 10 and 30 and between 61 and 70 had considerably high concentrations of *C. albicans*-specific IgG₄ antibodies. The level of *C. albicans*-specific IgG₄ in patients between 51 and 60, who showed a slightly suppressed delayed skin reaction to *C. albicans*, was similar to the level in those between 61 and 70 (Table 2).

4. Delayed skin reactivity to *C. albicans* and serum levels of specific IgG₄ antibodies

The mean level of *C. albicans*-specific IgG₄ antibodies in the patients with a negative reaction to the

Table 1. Characteristics of Patients Classified by Age

Age years	No. of cases	Serum IgE (IU/ml)	RAST+ to HD or Ca	No. of cases with SDIA
10-30	13	849 ± 620 ^a	11 (84.6%)	1 (7.7%)
31-50	11	228 ± 234 ^a	3 (27.3%)	3 (27.3%)
51-60	9	469 ± 441	3 (33.3%)	6 (66.7%)
61-70	10	442 ± 497	3 (30.0%)	4 (40.0%)
70+	9	634 ± 852	1 (11.1%)	2 (22.2%)

* Mean ± sd, HD: house dust, Ca: *Candida albicans*, SDIA: steroid-dependent intractable asthma. ^a: $p < 0.001$. Number in parentheses represents the percentage of all subjects in each age group

Fig. 1. Immediate (□) and delayed cutaneous reaction to *C. albicans* (■), and delayed reaction to PPD (purified protein derivative) (▨) in patients with bronchial asthma.

Table 2. Serum Levels of *C. albicans*-specific IgG₄ of Asthmatics of Various Age Groups

Age years	No. of cases	Specific IgG ₄ antibodies (u/ml) (mean $\pm$ sd)
10–30	13	19.6 $\pm$ 22.4
31–50	11	7.1 $\pm$ 7.4 ^a
51–60	9	14.5 $\pm$ 13.9
61–70	10	15.7 $\pm$ 10.8
71+	9	25.0 $\pm$ 10.8 ^a

^a: $p < 0.05$

Fig. 2. Serum levels of *C. albicans*-specific IgG₄ antibodies in patients with bronchial asthma in relation to a delayed cutaneous reaction to *C. albicans* and PPD (purified protein derivative). *Significant difference from the level of negative patients at $p < 0.001$.

antigen was 25.8 ± 21.4 u/ml and the level in those that were positive, was 6.0 ± 6.3 u/ml. A significant difference in the level of specific IgG₄ antibodies against *C. albicans* was found between the two groups ($p < 0.001$). On the contrary, the mean level of the patients with a positive delayed skin reaction to PPD was 16.5 ± 19.8 u/ml and the level in those with a negative reaction was 17.5 ± 19.0 u/ml. No significant difference was observed between the patients with positive and negative reactions to PPD (Fig. 2).

Discussion

Various allergic and immunological reactions are common in patients with bronchial asthma. The main immunoglobulin produced by exposure to an allergen is IgE antibody in atopic asthmatics. IgG antibodies and the subclasses, IgG₁ and IgG₄ are also produced by

stimulation with an allergen. In addition to these humoral immunological reactions, cell-mediated immune reaction to an allergen is sometimes observed in asthmatics. *C. albicans* has been considered the most common allergen in bronchial asthma (1); this allergen induces production of IgE, IgA and IgG antibodies (17), and further elicits cell-mediated immune response (13).

Asthma induced by *C. albicans* may be complex and different from that elicited by the house dust mite (1–3). Upon bronchial challenge with *C. albicans*, an immediate asthmatic response (IAR), often a late asthmatic response (LAR) and sometimes a delayed response can be observed. *C. albicans*-induced asthma has been often observed in patients over the age of 40 (3, 21, 22). The level of specific IgG to *C. albicans* increases with aging (22). Regarding humoral immune response, an increased level of specific IgG antibodies including IgG₁ and IgG₄ has been observed in some atopic subjects (16, 17). Two mechanisms have been proposed for the increased production of IgG antibodies associated with IgE-mediated immune response: 1) increased production of IgG and IgE antibodies is due to a generally increased permeability of mucosal membranes in atopic subjects with inhalant allergens (23, 24), in whom similar synthesis system between IgE and IgG₄ has been shown by several investigators (25, 26), and 2) increased IgG levels might result from depressed cell-mediated immunity in the *C. albicans*-induced reaction in atopic subjects (16, 17).

In this study, cell-mediated immune response to *C. albicans* and PPD was observed in patients with bronchial asthma in relation to the production of specific IgG₄ antibodies. In *C. albicans*-induced immune response, cell-mediated immunity acts to defend against *C. albicans* invasion (18). A defect in cell-mediated immunity primarily causes saprophytic *C. albicans* growth. Therefore, it has been proposed that the increased production of specific IgG antibodies against *C. albicans* is due to a depressed cell-mediated immune response (16, 17).

This study showed that a depressed delayed cutaneous reaction to *C. albicans* often occurred in younger (10–30 years) and older patients (61–70 and 71+ years), whereas increased *C. albicans* specific-IgG₄ levels was found in the younger patients and those over 71. These results demonstrate that depressed cell-mediated immunity to *C. albicans*, but not to PPD, is associated with the rise of specific IgG₄ antibodies against the antigen. It has been reported that a depressed cell-mediated immune response often occurs in some atopic subjects (13, 15). In the present study, delayed skin reactivity to *C. albicans* was suppressed in patients aged between 10 and 30, the majority (84.6%) of whom were atopics showing a positive RAST. We speculate that the depressed cell-mediated immune response in the patients between 10 and 30 is associated with the state of being atopic. Among the patients over 61, an increase in specific IgG₄

levels relating to diminished cell-mediated immunity, was also found, suggesting that the cell-mediated immune system might be depressed with aging.

Among the patients between 51 and 60, the incidence of a negative delayed skin reaction to *C. albicans* was slightly increased and higher specific IgG₄ levels were observed compared with those between ages 31 and 50, although no significant difference was present in the levels of IgG₄ between the two groups. The depressed delayed skin reaction may have been caused by long-term corticosteroid regimens, since this group included many patients (66.7%) with steroid-dependent intractable asthma (SDIA). An increase in specific IgG₄ antibodies against *C. albicans* has been observed in patients with SDIA (27).

These findings indicate that suppression of cell-mediated immune response can be observed in atopic and elderly patients, as well as in those receiving long-term corticosteroid therapy. Also under these conditions, the production of specific IgG antibodies is predominantly increased, leading to a complex onset mechanism of bronchial asthma. It can be speculated from our data that a depressed cell-mediated immunity and an elevation of IgG antibodies to *C. albicans* is observable in elderly healthy subjects. Further studies are necessary to clarify the correlation of increased *C. albicans* specific-IgG₄ antibodies and the pathogenesis of bronchial asthma.

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