Immunological responses to Candida albicans in patients with bronchial asthma and its relationship to patient age

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[Summary]

The release of histamine induced by C. albicans was significantly higher in asthmatics aged 21-30 yr, 41-50 yr and 61+ years old compared with the age-matched groups of healthy subjects. The level of C. albicans specific IgG was significantly higher in asthmatics aged 41-50 years old compared with age-matched group of healthy subjects. No significant difference was found among any age groups of healthy and asthmatic subjects. The increased level of C. albicans specific IgG seemed not to be of significance in the pathogenesis of asthma.

Key words: Candida albicans specific IgG, basophil histamine release, Bronchial asthma, aging
谷崎・カンジダに対する免疫反応

対象および方法：対象としては、気管支喘息 150 例およびアレルギー疾患を有しない健常人 43 名を選んだ。なお、喘息症例については、カンジダによる喘息かどうかの診断は行われなかった。カンジダ抗原による好塩基球からのヒスタミン遊離は、全塩法により、またカンジダ特異的 IgG 抗体は ELISA 法により測定した。

成績：カンジダによる好塩基球からのヒスタミン遊離は、若年層（21~30 歳）、中年層（41~50 歳）および高年層（61 歳以上）において、それぞれの年齢層の健常人に比べ有意に高い値を示した。

カンジダ特異的 IgG 抗体価は、41~50 歳の年齢層において、同じ年齢層の健常人に比べ有意に高い値を示した。他の年齢層では、気管支喘息症例と健常人との間に有意の差はみられなかった。

ヒスタミン遊離は、気管支喘息症例において、加齢とともに増加する傾向がみられたが、推計学的には有意差はみられなかった。また、カンジダ特異的 IgG 抗体価は、気管支喘息症例および健常人において加齢とともに増加する傾向がみられたが、いずれにおいても各年齢層の間に有意の差はみられなかった。

以上の結果は、カンジダに対する IgE 系の反応は、いずれの年齢層においても観察され、特に 41~50 歳の年齢層で特に高度であることを示すが、IgG 系反応の免疫アレルギー反応への関与は明らかでなかった。

I. Introduction

It has been considered that Candida albicans is sometimes associated with the pathogenesis of bronchial asthma. The antigen causes a release of histamine from basophils through IgE receptors1-4), eliciting an immediate allergic reaction (IAR)5,6). The amount of histamine release induced by C.albicans correlates with the concentration of the specific IgE antibodies in sera1,3,4). On the other hand, some investigators propose that the antigen induces late asthmatic response (LAR) by precipitating antibodies (mainly IgG antibodies)7,8). It is, however, unclear whether late asthmatic response is mediated by IgE or IgG antibodies.

Because of the popularity of C. albicans in the circumstances, the antigen can sensitize even healthy persons for a long term, and the number of persons sensitized by C. albicans, who show an increased level of specific IgG antibodies against the antigen, increases with aging9). It has been suggested that in C. albicans-induced asthma8,9,10,11), specific IgG antibodies, as well specific IgE antibodies, participate in the local allergic reactions7,8). Therefore, when the antigen is evaluated as an allergen of bronchial asthma which is related to specific IgG antibodies, immunological reactions induced by the antigen should be compared between healthy and asthmatic subjects.

In this study, immunological responses to C. albicans were compared between healthy and asthmatic subjects by observing serum levels of specific IgG antibodies against C. albicans, basophil histamine release and relationship to patient age.

II. Subjects and methods

The subjects were 150 patients with bronchial asthma (82 females and 68 males), whose age ranged from 12 to 70 years with a mean of 39.9 years. Forty-three healthy subjects (20 females and 23 males) who had no allergic disease were selected as healthy controls. Their age ranged from 14 to 68 years with a mean of 38.6 years.

The subjects were divided into six groups according to their age; 10~20 yr (group A), 21~30 yr (B), 31~40 yr (C), 41~50 yr (D), 51~60 yr (E) and 61+ yr (F).

Histamine release from basophils was carried out using a whole blood method, as previously described12,13). To 4 ml of heparinized venous blood, 0.2 ml of a tenfold dilution of C. albicans (0.16 mcgPN/ml, Torii Co) was added1). After the mixed solution was incubated at 37°C for 15 min, the reaction was stopped by transferring the test tube into an ice bath. The histamine content of the cells and supernatant fluids was measured by an automated spectrofluorometric histamine analysis system (Technicon)14). In this experiment system, spontaneous histamine release after incubation with physiological saline was under 3 mcg/ml. The results were expressed as the percentage of total histamine content.
Specific IgG antibodies against Candida albicans were measured by an enzyme-linked immunosorbent assay by the method\(^{11,15-17}\) modified from that described by Engvall, et al\(^{18}\). ELISA plates were coated with 100 \(\mu\)l of C. albicans extract at a 1:20,000 dilution in phosphate buffered saline (0.1 M sodium phosphate, 0.15 M sodium chloride, pH 7.2) (PBS) at 4°C overnight and then washed three times with PBS-T 20 containing 0.05% tween and 0.02% NaN\(_3\). After washing, 200 \(\mu\)l of 1% BSA-PBS-T 20 were added to the wells with incubation for 1 hr at 37°C and then discarded. Reference serum or serum sample at a 1:4,000 dilution in PBS-T 20 was added and incubated at 37°C for 2 hr. After washing, 100 \(\mu\)l of alkaline phosphatase conjugated anti-human IgG (Miles Co. U.S.A.) was added to the wells at a 1:2,000 dilution in PBS-T 20 and then incubated at 37°C for 1 hr. After another washing, conventional substrate, p-nitrophenyl phosphate (NPP) was added to the wells with incubation for 1 hr at 37°C. The reaction was stopped by addition of 0.1 ml 1 N NaOH. Absorbance was measured in a spectrophotometer at 405 nm. The background value was under 0.01.

Total IgE in sera was measured by radioimmunosorbent test (RIST).

In this study, the statistically significant difference of the mean was estimated using the unpaired Student t test. The levels of significance were expressed as a p value.

### III. Results

1. **Histamine release by Candida albicans**

   The release of histamine from basophils induced by C. albicans was not different among six groups of healthy subjects. The value of histamine release ranged from 1.7% to 4.5% in healthy subjects. The histamine release by C. albicans was the highest in group D (41~50 yr) and the lowest in group A (10~20 yr) of patients with bronchial asthma, although any significant difference was not observed among their age groups.

   A significant difference was found in the histamine release of group B (21~30 yr), D and F

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<th>Table 1 Candida albicans-induced histamine release from basophils of patients with asthma divided into subgroups based on their ages</th>
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* Mean±sd. a, b, and c: p<0.05. Number in parenthesis represents number of cases with steroid-dependent intractable asthma.

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<th>Table 2 Candida-induced histamine release from basophils of patients with bronchial asthma and serum IgE level</th>
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<td>Age, years</td>
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* Mean±sd (61+yr) between healthy and asthmatic subjects (B,D and F : p<0.05) (Table 1).

Histamine release by C. albicans in patients with bronchial asthma was discussed in relation to patient age and serum levels of total IgE. In this section, the subjects were divided into three age groups: 10~40 yr, 41~60 yr and 61+ yr. Histamine release by C. albicans in each age group was 11.9% in cases aged 10~40 years, 14.2% in cases aged 41~60 years and 18.9% in cases aged 61+ years. The release of histamine by C. albicans increases with aging in all groups classified by serum levels of total IgE, as shown in Table 2. The increase in histamine release with aging was the highest in subjects with 0~200 IU/ml serum IgE, from 5.3% in cases 10 to 40 years old to 19.6% in cases aged 61+ years old. The difference in the release of histamine was, however, not significant among three age groups (Table 2).
Fig. 1 Candida albicans specific IgG antibodies in healthy (□) and asthmatic subjects (■) divided into subgroups based on their ages.

* Significant difference from healthy subjects at p<0.05.

2. Serum levels of specific IgG antibodies against Candida albicans

The level of specific IgG antibodies against C. albicans was from 0.459 to 1.082 as OD405 in serum of healthy subjects. Any significant difference was not found in the specific IgG antibody level among five groups of healthy subjects, whose age was under 60 years old. The specific IgG antibody level of healthy subjects was significantly higher in cases aged 61+ years old compared with group D (41-50 yr) (p<0.02) and group E (51-60 yr) (p<0.05). The level of C. albicans specific IgG antibodies was between 0.568 to 1.029 in patients with bronchial asthma. No significant difference was present in the level of specific IgG among six age groups of asthmatics. A significant difference was observed in the level of specific IgG between healthy and asthmatic subjects in group D (41-50 yr) (p<0.05). In the other age groups, no difference was found between healthy and asthmatic subjects (Fig. 1).

IV. Discussion

Recently investigators have been much interested in participation of C. albicans in pathogenesis of bronchial asthma. The antigen induces IgE-mediated allergic reactions in asthmatics. The participation of C. albicans specific IgG antibodies in allergic reactions found in asthmatics still remains obscure. Relating to bronchial challenge, C. albicans sometimes elicits immediate asthmatic reaction (IAR) and often late asthmatic reaction (LAR). The reason, why C. albicans often causes LAR is not known. Pepys et al. suggested participation of IgG antibodies in LAR induced by C. albicans. Tsukioka also reported that IgE antibody against C. albicans could not be detected in 61.1% of LAR-positive patients, in whom highly significant increase of antibody against C. albicans was detected by passive hemagglutination reaction. It has been suggested that specific IgG antibodies and the subclass participate in allergic reactions initiated by specific IgE antibodies.

Our previous studies have shown that C. albicans-induced histamine release is one of the IgE-mediated reactions and the release correlates with the RAST score, and that specific IgG levels affect the release of histamine induced by C. albicans-IgE binding; specific IgG inhibits the release of histamine in some cases, and enhances the release in other cases. In the present study, participation of IgE antibodies was observed by IgE-mediated histamine release from basophils and participation of IgG antibodies by measuring serum levels of specific IgG antibodies.

In the experiment of histamine release, a significant difference was found between healthy and asthmatic subjects in group B (21-30 yr), D (41-50 yr) and F (61+ yr). These findings agree with the previous results showing that the incidence of positive RAST score for C. albicans is the highest in cases aged 41-50 years old, and relatively high in cases aged 0-30 years and 61+ years.

Our previous studies demonstrated that histamine release from basophils induced by C. albicans was generally higher in cases under 40 years and in cases with high serum IgE levels compared to cases over 41 years and cases with low serum IgE levels. Basophils of these cases were, however, more reactive to C. albicans and release more histamine by the antigen compared to house dust and anti-
IgE. In this study, histamine release by C. albicans increased with aging in cases whose serum IgE level was between 0 and 200 IU/ml, although no significant difference was present among three age groups. These results suggest that histamine release is high in elderly cases even if their serum IgE level is low. Increased levels of specific IgE, IgA and IgG antibodies against C. albicans have been observed in atopic subjects, in whom cell-mediated immunity was speculated to be suppressed. In this study for the level of C. albicans specific IgG antibodies, there was a significant difference in the level of specific IgG between healthy and asthmatic subjects in group D (41~50 yr). The elevated level of specific IgG antibodies was not concluded to be of significance in allergic reactions, because cases aged 41~50 years old included many steroid-dependent intractable asthmatics, which stimulate generation of specific IgG antibodies. In the other age groups, no difference was found in the IgG level between healthy and asthmatic subjects.

In elderly subjects aged 61+ years old, increased levels of specific IgG antibodies were observed in both healthy and asthmatic subjects. Therefore, increased levels of specific IgG in elderly subjects have no significance in pathogenesis of asthma. The results from the present study could not show that C. albicans specific IgG antibodies participate in allergic or immunological reactions in patients with bronchial asthma.

V. Conclusion

IgE-mediated histamine release from basophils by Candida albicans and serum levels of specific IgG antibodies against C. albicans were examined in 150 patients with bronchial asthma and 43 healthy subjects.

1. The release of histamine induced by C. albicans was significantly higher in asthmatics aged 21~30 yr, 41~50 yr and 61+ years old compared with the age-matched groups of healthy subjects.

2. The level of specific IgG antibodies against C. albicans in sera was significantly higher in asthmatics aged 41~50 years old compared with the age-matched group of healthy subjects. The increased level of specific IgG antibodies in these asthmatics was assumed not to be of significance in the pathogenesis of bronchial asthma.

3. Basophil histamine release and the level of specific IgG antibodies showed a tendency to increase with aging, although the difference was not significant among any age groups of healthy and asthmatic subjects.

References


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