

BASOPHIL HISTAMINE RELEASE INDUCED BY *CANDIDA ALBICANS* Relation to Specific IgE and IgG Antibodies

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INTRODUCTION

Allergens such as ragweed and house dust induce IgE-mediated hypersensitivity in patients with bronchial asthma. In these patients immediate skin and bronchial reactions to allergens can be observed, in which specific IgE antibodies for allergens participate. Basophils from these patients release a significant amount of histamine by stimulation with allergens. On the other hand, *Candida albicans* also participates in the onset mechanism of bronchial asthma as an allergen¹⁾, but the onset mechanism caused by *C. albicans* is somewhat complicated. In bronchial challenge tests, *C. albicans* provokes immediate and late asthmatic reactions and precipitating antibodies against *C. albicans* are frequently found in the serum from the patients with bronchial asthma²⁾. It has been demonstrated that *C. albicans* produces specific IgE^{3,4)} and precipitating antibodies²⁾, and each of these antibodies is capable of mediating hypersensitive reactions in allergic individuals. However, there is little information about histamine release from basophils in relation to IgE- and precipitating antibodies-mediated hypersensitivity induced by *C. albicans*.

In the present study, *C. albicans*-induced histamine release was examined in relation to specific IgE antibodies, and the participation of specific IgG antibodies to the allergen was evaluated in asthmatic patients sensitive to *C. albicans*.

MATERIALS AND METHODS

Subjects

Thirty-four patients (10 females and 24 males) with bronchial asthma were selected to examine *C. albicans*-induced histamine release. Their ages varied from 21 to 72 years with a mean of 47.8 years. All cases showed a positive skin test to *C. albicans*. The IgE-radioallergosorbent test (RAST: Pharmacia) for *C. albicans* was positive (2 +<) in 12 out of the 34 cases (35.3%).

Histamine release

Histamine release from basophils was examined using a whole blood method, as previously described⁵⁾. The challenge of whole blood with various concentrations of *C. albicans* was carried out in a dose-response fashion. After a 15-minute incubation, the histamine content of the cells and of the supernatant fluid was assayed by an automated spectrofluorometric histamine analysis system (Technicon)⁶⁾. The results were expressed as a percentage of the total histamine content.

Serum IgE and allergen-specific IgE

Serum levels of total IgE were estimated by the radioimmunosorbent test (Pharmacia). Specific IgE antibodies to *C. albicans* in serum were measured by the RAST. The results of specific IgE concentrations were expressed as a score 0+ to 3+.

Allergen-specific IgG in serum

C. albicans-specific IgG antibodies in serum were estimated by an enzyme-linked immunosorbent assay (ELISA)⁷⁾. Polystyrene tubes were coated with *C. albicans* extract: 0.5 ml of a solution of *C. albicans* extract (16 mcg/ml, Torii Co,

Abbreviations: *C. albicans* *Candida albicans*; RAST radioallergosorbent test; RIST radioimmunosorbent test

Tokyo) diluted in 2×10^{-5} times with 0.1 M sodium carbonate buffer was added to each test tube. The tubes were incubated at 4°C for 24 hr, and then washed five times with 1 ml of PBS-tween 20 containing 0.05% tween 20 and 0.02% NaN_3 . To the antigen-coated tubes 0.5 ml serum diluted 1:4000 with PBS-tween 20 was added, and the tubes were incubated for 3 hr at room temperature. After washing five times, 0.5 ml alkaline phosphatase conjugated anti-IgG (heavy and light chain specific, Miles-Yeda Ltd. Israel) diluted 1:2000 with PBS-tween 20 was added. The tubes were incubated overnight at room temperature, and then the excess of conjugate was washed out. To each tube 1 ml 0.05 M sodium carbonate buffer (pH 9.8) containing 1 mg/ml p-nitrophenylphosphate (NPP) and 10^{-5} M MgCl_2 was added. After incubation for 1 hr at room temperature, the enzyme reaction in each tube was stopped by the addition of 0.1 ml 1 N NaOH. The absorbance was measured in a spectrophotometer at 405 nm.

Serum-precipitating antibodies to C. albicans

Serum-precipitating antibodies against *C. albicans* were estimated by the double diffusion method described by Pepys et al²⁾.

RESULTS

Relationship between histamine release and serum IgE level

Candida albicans-induced histamine release from whole blood was observed in relation to serum IgE levels. As shown in Table 1, the mean maximum percent histamine release with *C. albicans* was highest in cases where the serum IgE levels were between 501 and 1000 IU/ml and lowest in cases where the levels were between 201 and 300 IU/ml. However, no statistically significant difference was found between the two groups.

Relationship between histamine release and specific IgE to C. albicans

Table 2 reveals a close correlation of basophil histamine release by *C. albicans* with a RAST score. The mean maximum percent release by *C. albicans* increased as the IgE RAST score was higher. The cases with a RAST score of 2+ and 3+ showed as significantly increased release of histamine by stimulation with *C. albicans*, while histamine release from basophils of the cases with a RAST score of 0+ and 1+ was very low. A statistically significant difference was found between the cases with a RAST score 1+ and 2+ ($p < 0.01$).

Relationship between precipitins and specific IgG to C. albicans

Figure 1 shows a positive correlation of specific IgG antibodies against *C. albicans* with precipitating antibodies. The mean allergen specific IgG concentration (A_{405}) was 0.45 ± 0.05 ($\pm$ SE) in the cases without precipitins and 1.08 ± 0.1 in the cases with precipitins. A statistically significant difference was found between the cases with and without precipitins ($p < 0.001$). Eleven out of 12 cases without precipitins (91.7%) showed low serum levels (less than 0.5) of allergen specific IgG.

Relationship between histamine release and specific IgG to C. albicans

Table 3 shows the correlation of basophil histamine release with serum levels of specific IgG to *C. albicans*. The maximum percent release was highest in cases where the serum specific IgG levels were between 0.51 and 1.0, and lowest in cases where the levels were more than 1.5. There was no statistically significant difference between the two groups.

Inhibition of histamine release by allergen specific IgG

The results described above demonstrate that *C. albicans*-induced release of histamine is one of the IgE-mediated reactions. In order to clarify the participation of specific IgG antibodies in IgE-mediated basophil reactions, *C. albicans*-induced release of histamine from basophils of the cases with a RAST score of 2+ or higher was examined in relation to

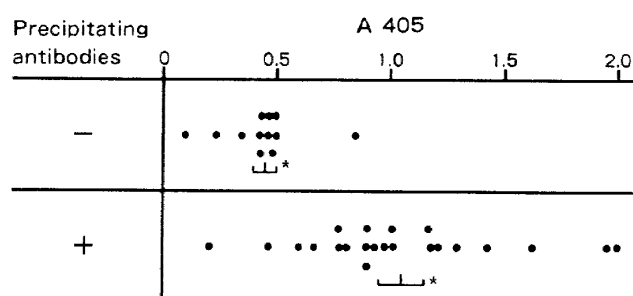
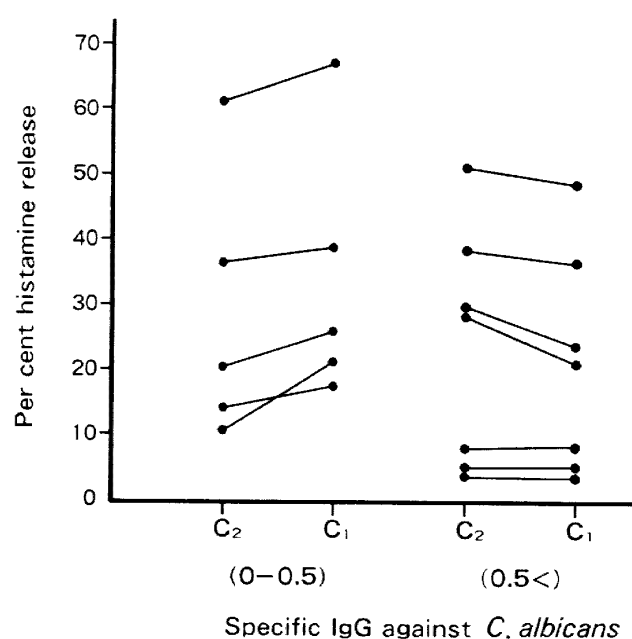
Table 1 Correlation between *C. albicans*-induced histamine release and serum IgE levels

Serum IgE level, IU/ml	Number of cases	Maximum per cent histamine release	
		Mean $\pm$ SEM	Range
0 - 200	10	13.0 $\pm$ 4.3	1.1-37.8
201 - 300	5	9.4 $\pm$ 2.7*	4.3-20.0
301 - 500	4	11.9 $\pm$ 5.3	5.1-27.7
501 - 1000	10	22.7 $\pm$ 6.6*	2.3-66.0
>1000	5	11.4 $\pm$ 6.6	3.5-37.8

*Not significant.

Table 2 Correlation between *C. albicans*-induced histamine release and RAST score to *C. albicans*

RAST score	Number of cases	Maximum per cent histamine release	
		Mean $\pm$ SEM	Range
0 +	17	8.5 $\pm$ 2.1	1.1-37.8
1 +	5	6.8 $\pm$ 1.6*	2.3- 9.9
2 +	9	23.1 $\pm$ 5.1*	4.6-50.1
3 +	3	40.2 $\pm$ 14.2	16.8-66.0

* $p < 0.01$.Fig. 1. Correlation between precipitating antibodies and specific IgG antibodies against *C. albicans*. * $p < 0.001$.Fig. 2. Dose-response slopes of *C. albicans*-induced histamine release and serum levels of specific IgG antibodies against *C. albicans* in cases with a RAST score of 2+ or higher. C₂: $\times 10^3$, C₁: $\times 10^2$ dilutions of *C. albicans* extract.Table 3 Correlation between *C. albicans*-induced histamine release and specific IgG against *C. albicans*

Specific IgG (A ₄₀₅)	Number of cases	Maximum per cent histamine release	
		Mean $\pm$ SEM	Range
0 -0.5	12	15.1 $\pm$ 5.6	1.1-66.0
0.51-1.0	12	17.2 $\pm$ 3.7*	3.3-37.8
1.01-1.5	6	13.8 $\pm$ 7.3	4.6-50.1
>1.5	4	9.2 $\pm$ 3.6*	5.0-20.0

*Not significant.

serum levels of specific IgG to the allergen. As shown in Fig. 2, a significant amount of histamine release was elicited by *C. albicans* in all cases with serum specific IgG levels between 0 and 0.5. On the other hand, no significant release was elicited in 3 out of 7 cases with the levels of more than 0.5 (42.8%).

Increased histamine release by the two different concentrations, $\times 10^3$ (C₂) and $\times 10^2$ (C₁), of *C. albicans* extract was compared in relation to serum levels of specific IgG. The dose-response slopes of histamine release were different between cases with low (0-0.5) and high (more than 0.5) levels of specific IgG. The slope from C₂ to C₁ was positive in all cases with

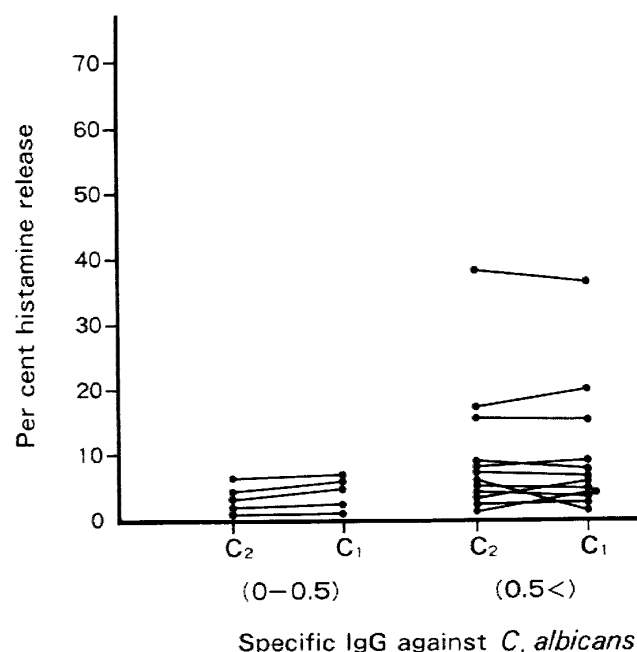


Fig. 3. Dose-response slopes of *C. albicans*-induced histamine release and serum levels of specific IgG antibodies against *C. albicans* in cases with a RAST score of 0+. C₂: $\times 10^3$, C₁: $\times 10^2$ dilutions of *C. albicans* extract.

low serum levels of specific IgG, while negative slopes were shown in all cases with high levels of specific IgG. The negative slopes in these cases may show that basophil reactivity was suppressed by specific IgG (Fig. 2).

These results may suggest that specific IgG inhibits histamine release from basophils by *C. albicans*-IgE reactions.

Histamine release induced by allergen specific IgG

Figure 3 shows the results of *C. albicans*-induced histamine release from basophils of the cases with a RAST score of 0+. No significant release of histamine was elicited by stimulation with *C. albicans* in any of the cases with low levels of specific IgG (0-0.5). On the other hand, a significantly increased release of histamine was induced in 3 out of 12 cases with high levels of allergen specific IgG (more than 0.5). The release of histamine might be elicited by allergen-IgG interaction, but not by allergen-IgE binding.

DISCUSSION

Allergen-induced histamine release from leucocytes has been extensively studied in patients with hay fever and bronchial asthma. The release of histamine induced by allergen correlates with skin sensitivity^{8,9)} and with the degree of severity of clinical symptoms¹⁰⁾. Anti-IgE-induced histamine release correlates to a certain extent with serum IgE levels¹¹⁾. Our previous studies⁹⁾ demonstrated that anti-IgE-induced release of histamine paralleled the release induced by allergen. These investigations have shown the presence of specific IgE against allergens in hypersensitive individuals. In fact, allergens such as ragweed and house dust frequently cause increased production of specific IgE in these subjects.

On the other hand, *C. albicans* induced immediate hypersensitivity in patients with bronchial asthma, as demonstrated by bronchial challenge tests¹⁾. However, there are some differences between *C. albicans* and allergens such as ragweed and house dust. Firstly, it has been suggested that production of specific IgE is less frequently increased with *C. albicans* than with ragweed and house dust⁹⁾. In the present study, 12 out of 34 cases with positive skin reaction to *C. albicans* (35.3%) showed a positive IgE-RAST score (2+ or higher) to *C. albicans*, while 20 out of 30 cases with positive skin reaction to house dust (66.7%) showed a positive RAST score to house dust (data not shown). Secondly, as Pepys et al.²⁾ suggested, precipitins against *C. albicans* play an important role in the pathogenesis of bronchial asthma. A good correlation is found between the precipitin reactions and the production of type III skin and inhalation test reactions.

There is little information about histamine release from basophils induced by *C. albicans*. The results obtained here revealed that *C. albicans* induced release of histamine is one of the IgE-mediated reactions, and that the release correlates

with the RAST score. It has been suggested that some type of IgG has characteristics of IgE antibodies and participates in immediate hypersensitivity. Parish, et al.¹²⁾¹³⁾ have reported the presence of IgG antibodies sensitizing the skin for a short-term, and they named the antibodies as IgG ST-S, and later Bryant, et al.¹⁴⁾ reported the participation of IgG "reaginic" antibody in bronchial asthma. Now IgG ST-S and IgG "reaginic" antibody are thought to be IgG₄, a subclass of IgG. There are some other reports suggesting that IgG₄ binds membrane receptors of mast cells and basophils and induces histamine release from these cells¹⁵⁾¹⁶⁾. On the other hand, some reports have demonstrated that IgG₄ acts as blocking antibodies and inhibits the release of histamine from mast cells and basophils¹⁷⁾¹⁸⁾. In the present study, it was found that serum specific IgG levels, which correlated with serum levels of precipitins, affected the release of histamine induced by *C. albicans*-IgE binding. Specific IgG to *C. albicans* inhibited the release of histamine from basophils in some cases, and enhanced the release in other cases. These results suggest that specific IgG antibodies act in two different ways. The first action of specific IgG is the inhibition of IgE-mediated reactions like blocking antibodies, and the second action is the enhancement of basophil histamine release. If the second action of specific IgG participates in the immunological reactions of bronchial asthma, there is some possibility that IgG-mediated reactions are present in patients with bronchial asthma, who have significant amounts of specific IgG to *C. albicans*.

When whole blood is used in the experiments for basophil histamine release, several factors in serum may participate in the release mechanisms. We have studied on the factors affecting basophil histamine release in a whole blood method. Our results have shown that IgE antibodies contained in serum affect basophil histamine release when serum IgE levels are high, and that allergen (house dust)-induced histamine release from basophils is not affected by serum factors¹⁹⁾.

Histamine is also released from basophils by activated complement²⁰⁾. Since whole blood was used for the experiments of histamine release here, the effect of complement activated by *C. albicans* on the release mechanism can not be completely neglected. However, the amount of histamine release induced by *C. albicans* correlated with serum specific IgE levels, and the inhibition and enhancement by specific IgG of basophil histamine release were related to serum levels of specific IgG. In addition of the effect of activated complement, a factor derived from lymphocytes incubated with *C. albicans* has been reported to enhance basophil histamine release²¹⁾. Further studies are needed to clarify *C. albicans*-induced histamine release from basophils.

SUMMARY

Candida albicans-induced histamine release from basophils was examined in 34 patients with bronchial asthma. All patients showed positive skin reaction of *C. albicans*. 1. Histamine release induced by *C. albicans* correlates with serum levels of specific IgE, and a significant amount of histamine release was elicited by *C. albicans* in cases where the RAST score was 2+ or higher. 2. *C. albicans*-induced, IgE-mediated histamine release was inhibited in some cases with high serum levels of specific IgG antibodies. On the other hand, the release of histamine induced by *C. albicans* seemed to be enhanced by specific IgG antibodies in some cases with a RAST score of 0+. These results suggest that specific IgG antibodies act in two different ways: inhibition and enhancement of histamine release induced by *C. albicans*.

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