

Atopic asthma caused by *Candida albicans* acid protease: case reports

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Two cases of atopic asthma caused by acid protease produced by *Candida albicans* are reported. Both patients had high levels of serum IgE antibodies against the acid protease and showed positive conjunctival and immediate bronchial responses when challenged with the protease. Significant histamine release was detected in both patients when their peripheral leukocytes were challenged with the protease antigen. These findings clearly showed that *C. albicans* acid protease is the causative allergen of atopic asthma.

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The yeast *Candida albicans* is a common commensal of the human oral and vaginal mucosae and gastrointestinal tract as part of the normal flora. It is also known as the source of one of the important allergens to cause atopic asthma. In Japan, *C. albicans* antigen is widely used in allergy skin tests. *C. albicans* secretes acid protease, which cleaves IgA, its secretory component, and other immunoglobulins, promoting persistence of the yeast on mucous membranes (7). This paper reports two cases of atopic asthma caused by acid protease produced by *C. albicans*.

Material and methods

Antigen preparation

C. albicans strain no. 114 was obtained from the Research Center for Medical Mycology, Teikyo University School of Medicine, Tokyo, Japan. *C. albicans* acid protease (CAAP) was induced with YCB-BSA medium, and incubation was continued until the proteolytic activity of the culture medium reached the highest level. CAAP was purified according to the method of Yamamoto et al. (10). The cell-free broth was loaded onto a DEAE Sephacel column and eluted. Fractions containing proteolytic activity were pooled and further purification was done. Approximately 15 mg of CAAP was recovered from the

2 l of culture. CAAP was stored as lyophilized at -80°C before clinical use.

Skin test

Intradermal skin tests were done on patients' forearms with 35 common allergens such as those of house-dust mite, Japanese cedar, and other pollens and molds. Crude *Candida* antigen was obtained from Torii Pharmaceutical Co (Tokyo, Japan). Immediate reaction was checked 15 min after injection.

Measurement of IgE antibody

Anti-crude *Candida* and anti-CAAP IgE antibodies were measured by radioallergosorbent test (RAST), as described previously (11). Solid-phase antigens were prepared by linking either 50 $\mu\text{g}/\text{ml}$ of purified CAAP or 2 mg/ml of crude *Candida* culture filtrate to 200 pieces of cyanogen bromide-activated paper disks in 10 ml borate-buffered saline (pH 8.5). Radiolabeled anti-IgE was obtained from Pharmacia Diagnostics (Phadebas RAST kit, Uppsala, Sweden). RAST results were expressed as percent total counts (percent binding), which was calculated by dividing the counts bound by the total counts added. The percent binding more than twice the mean values of three healthy controls (1.2% for crude *Candida* and 1.0% for CAAP) was considered to be positive.

Histamine release from peripheral blood leukocytes

Mixed leukocytes were prepared from citrated blood of the patients by the dextran sedimentation procedure, as described previously (6). These mixed leukocytes were challenged with a series of concentrations of crude *Candida*, CAAP, and anti-IgE as positive control *in vitro*. The release of histamine more than 10% of total histamine content after subtraction of spontaneous histamine release was considered to be positive. CAAP and crude *Candida* were found not to release histamine from the peripheral leukocytes of normal control subjects.

Conjunctival test

One hundred micrograms/ml of CAAP antigen and 1:400 w/v crude *Candida* antigen were used for the conjunctival test. One conjunctiva was used as a control, which was challenged by diluent, and the other was challenged by crude *Candida* antigen. After assessing the results of crude *Candida* antigen, the control side was challenged by CAAP. These antigens were tested on several normal controls and elicited no reactions in their conjunctivae. This test was considered to be positive when more than two of the three signs and symptoms, such as conjunctival itching, hyperemia, and edema, appeared within 15 min of the antigen challenge in the conjunctiva. The results of the conjunctival test have been shown to correlate highly with the results of the bronchial provocation test with *Candida* antigens (1, 2).

Bronchial provocation test

Bronchial provocation tests were performed according to the standardized method proposed by the Japanese Society of Allergology (5). Twenty percent or more decrease of FEV₁ from the prechallenge value was considered to be positive. One milliliter of 1 µg/ml CAAP antigen was used as an initial challenge dose, and 10-fold increased doses up to 100 µg/ml were used until 20% decrease of FEV₁ was recorded. A volume of 100 µg/ml of CAAP did not elicit any decrease of FEV₁ in normal controls.

Case reports (Table 1)

Case 1

A 58-year-old housewife, whose asthmatic symptoms started when she was 40 years old, usually suffered from asthmatic attacks in late spring and early autumn. Her father also had asthma. She had no other allergic complications. Her bronchial sensitivity as tested by inhalation of acetylcholine (ACh) was moderately positive (the threshold dose of ACh was 1000 µg/ml). She was tested for intradermal skin reactions with 35 common allergens including *C. albicans* antigen. She showed positive immediate skin reactions against house-dust mite, orchard grass pollen, Japanese cedar pollen, ragweed pollen, *C. albicans*, and other pollens. *C. albicans* was the only antigen to which she showed positive immediate skin reaction among those of other fungi, including *Cladosporium*, *Alternaria*, *Aspergillus*, *Penicillium*,

Table 1. Details of two atopic asthma patients reported

	Case 1	Case 2
Onset age	Female, 58 years old 40 years	Female, 49 years old 23 years
Family history	Father: bronchial asthma	Mother, brother: bronchial asthma
Bronchial sensitivity (acetylcholine inhalation test threshold dose)	1000 µg/ml	313 µg/ml
Serum IgE level	730 IU/ml	1324 IU/ml
Immediate intradermal skin test (threshold dose: dilution)	Crude <i>Candida</i> 10 ⁻⁷ , mite 10 ⁻⁴ , HD 10 ⁻³ , Japanese cedar 10 ⁻³	Crude <i>Candida</i> 10 ⁻⁶ , mite 10 ⁻⁵ , HD 10 ⁻⁵ , Japanese cedar 10 ⁻³
Serum IgE antibody (Pharmacia RAST score)	Crude <i>Candida</i> 3, mite 3, HD 2, Japanese cedar 2	Crude <i>Candida</i> 2, mite 4, HD 3, Japanese cedar 1
Serum IgE antibody (% total counts; normal control 0.5–0.6)		
Antiacid protease	7.5	2.7
Anticrude <i>Candida</i>	26.5	6.9
Conjunctival provocation test		
Acid protease	+	+
Crude <i>Candida</i>	+	–
Bronchial provocation test (threshold dose)		
Acid protease	100 µg/ml IAR(+) LAR(–)	10 µg/ml IAR(+) LAR(–)

IAR: immediate asthmatic response; LAR: late asthmatic response; HD: house dust extract.

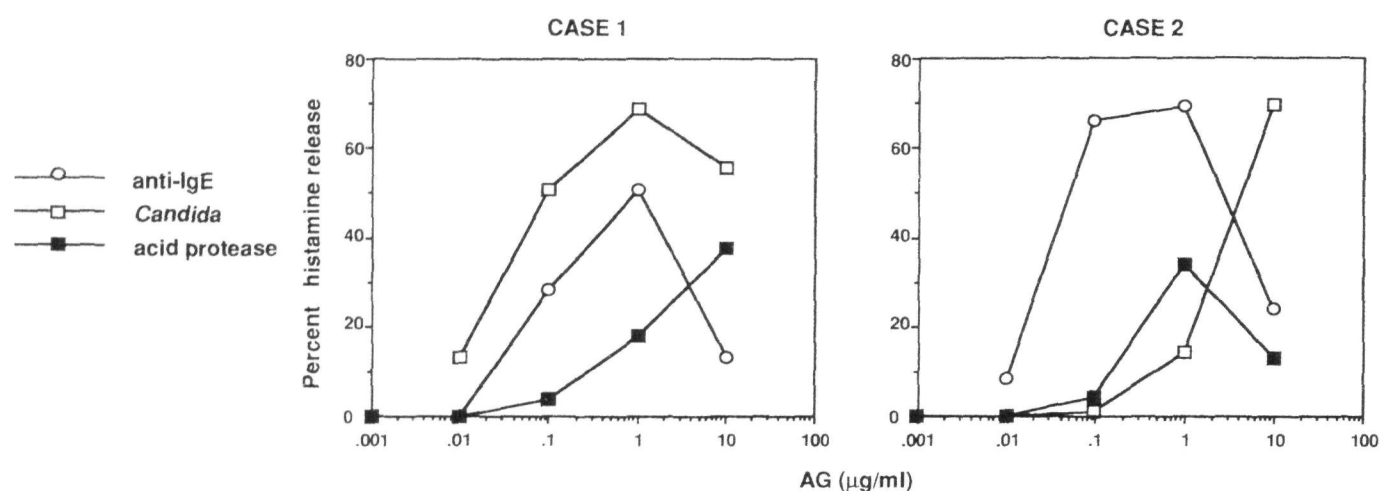


Fig. 1. Histamine release from peripheral leukocytes. AG: antigen concentration.

Fusarium, *Botrytus*, *Epicoccum*, *Cephalosporium*, *Cephalosecium*, and *Tricophyton*. Her serum IgE level was 720 IU/ml. She had positive serum IgE antibodies against crude *C. albicans* antigen with 26.5% binding of total counts (healthy controls' mean binding was 0.6%) and also positive serum IgE antibodies against CAAP with 7.5% total counts (healthy controls' mean binding was 0.5%). She showed positive conjunctival reaction against CAAP as well as crude *C. albicans* antigen. The histamine release test from the patient's peripheral leukocytes challenged with CAAP showed a positive reaction, as shown in Fig. 1. Her peripheral leukocytes also released histamine with crude *C. albicans* antigen and anti-IgE. The bronchial provocation test was performed with CAAP. An immediate asthmatic response, but not a late asthmatic response, to 100 μg/ml of CAAP was shown. She was diagnosed as having atopic asthma caused by CAAP.

Case 2

A 49-year-old woman, whose asthmatic symptoms had started when she was 23 years old, suffered from asthmatic attack perennially. Her brother and grandmother had asthma. She had eczema and sometimes urticaria as allergic manifestations in the past. Her bronchial sensitivity tested by ACh inhalation was highly positive (the threshold dose of ACh was 313 μg/ml). She was tested for intradermal skin reactions with 35 common allergens, as described above. Positive immediate responses to house-dust mite, orchard grass pollen, Japanese cedar pollen, *C. albicans*, and some other pollens were shown. However, *C. albicans* was the only positive antigen among the fungal antigens. Her serum IgE level was 1324 IU/ml. She had positive IgE antibodies against crude *C. albicans* antigen, with 6.9% binding of total counts, and also against CAAP, with 2.7% total counts. She showed positive conjunctival reaction to CAAP, but not to crude *C. albicans*. Her peripheral leukocytes also released histamine when exposed to

CAAP, crude *C. albicans* antigen, and anti-IgE, as shown in Fig. 1. Positive immediate airway reaction was also shown with 10 μg/ml of CAAP challenge. However, late response was not detectable. She was diagnosed as having atopic asthma caused by CAAP.

Discussion

C. albicans is present as part of the endogenous flora of mucocutaneous regions in human and animal hosts. It usually colonizes the digestive tract, and may involve other anatomic regions, such as the larynx, upper airways, or vagina. Depending upon host immune condition, the fungus may behave as an opportunistic pathogen (8). *C. albicans* is known to produce cytolytic enzymes such as proteases (4, 7) and phospholipases (3). The acid protease of *C. albicans* cleaves IgA1, IgA2, and secretory IgA, all of which play a role in mucosal immunity (7). It is possible that the CAAP produced by *C. albicans* destroys immunoglobulins by cleavage of the molecule and hence promotes localized colonization of the organisms or eventually systemic infection (7). These *C. albicans*-related enzymes have been widely investigated for their pathogenic role in fungal infections (3, 4, 7). However, we could not find any published report describing the relationship of these enzymes and allergy.

The acid protease used in this study was secreted by the 140/140 strains of *C. albicans* tested. Similar acid proteases were secreted by 13/18 strains of *C. tropicalis*, 11/18 strains of *C. parapsilosis*, 1/3 strains of *C. zelandoides*, 1/5 strains of *C. lisitaniae*, and 1/12 strains of *C. guilliermondii*. But these acid proteases are known to differ from CAAP in mol. mass and amino acid sequences (9). No other *Candida* species or yeast secretes the acid protease (10). The mol. mass of CAAP is approximately 44 kDa. Its isoelectric point was pH 4.2, and its optimal pH was 3.2. It was stable below pH 7.0 and lost its activity above pH 8.0 after incubation at 37°C for 60 min. (10).

In this paper, two cases of atopic asthma caused by CAAP were reported. Both had high levels of serum IgE and specific IgE antibodies against both CAAP and crude *C. albicans*. Both patients showed positive conjunctival reactions to CAAP and also showed positive peripheral leukocyte histamine release when challenged with CAAP and *C. albicans*. Since both of them showed positive immediate bronchial response to CAAP, we diagnosed these patients as having atopic asthma caused by CAAP.

Further work on the allergenicity of CAAP is in progress.

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