

Relationship of immediate and delayed hypersensitivity to nasopharyngeal and intestinal growth of *Candida albicans* in allergic subjects

A. KOIVIKKO,¹ K. KALIMO,² E. NIEMINEN,³ & M. VIANDER³

Departments of ¹Pediatrics, ²Dermatology and ³Medical Microbiology, University of Turku, Finland

The growth of *C. albicans* yeast in the nasopharynx and in the anus as well as allergy symptoms were followed up for 8 months in 67 patients with bronchial asthma, allergic rhinitis and/or atopic eczema. 38 of the patients were skin prick test positive and 29 negative to *C. albicans* allergen extract. 32 of the patients had positive and 19 negative delayed skin reactions. The nasal, bronchial and skin symptoms of the yeast-sensitive allergic patients were not associated with the nasopharyngeal nor anal occurrence of *C. albicans* or other yeasts. The use of nasal or inhaled steroids had no effect on the occurrence of *Candida* in the nasopharynx. It was observed that immediate skin sensitivity had a positive correlation and the delayed sensitivity a negative correlation with the occurrence of *C. albicans* growth in nasopharynx and anus. These findings are in agreement with the concept that impaired cell-mediated immunity to *C. albicans* may lead to increased IgE response. This may explain the increased liability towards *C. albicans* nasopharyngeal and gastrointestinal "saprophytic" growth.

Key words: allergic rhinitis; asthma; atopic eczema; *Candida albicans*; delayed hypersensitivity; immediate hypersensitivity; yeast culture.

Accepted for publication 23 October 1987

There are about 120 species of *Candida* yeasts, which occur globally. *C. albicans* is most often associated with human disease. It is commonly found in cultures from the nasopharynx and the feces of healthy persons and thus considered mainly "saprophytic". In diabetic and immunocompromized patients it can cause local or generalized infections.

In patients with asthma and atopic eczema immediate skin reactivity to *C. albicans* allergen extracts has often been reported. It has also been shown that the immediate skin reactivity is IgE-mediated and correlates with provocation tests and RAST (2, 14).

The clinical significance of immediate skin reaction to *C. albicans* in patients with asthma and other hypersensitivity diseases has remained rather obscure. In European countries *Candida* yeasts occur in only low amounts in

atmospheric air and are thus not likely candidates as important generally distributed aero-allergens. In tropical countries yeasts occur in much larger amounts in the air (17). *Candida* has been associated with chronic vaginitis and urticaria (11, 15, 18). Inhalation of *Candida*-related allergens has been shown to cause respiratory allergy in industrial circumstances (7), and allergic symptoms have been shown to occur also outside the factory (13) due to heavy emission of *C. utilis* dust.

The "saprophytic" nasopharyngeal and intestinal *C. albicans* growth might serve as an allergen source having significance to the allergic symptoms in sensitive patients. As there are no previous studies concerning the possible role of this growth in yeast-sensitive subjects, the present study was designed to evaluate this. The interrelationship of immediate and delayed

skin sensitivity to *Candida* in relationship to the nasopharyngeal and anal occurrence of the yeast gave interesting results, which will also be reported.

MATERIAL AND METHODS

Patient material

Thirty-nine atopic patients with previously observed immediate skin test sensitivity to *C. albicans* were selected for the study. The control group consisted of 28 yeast-negative atopic patients. Both groups had equal skin reactivities to common allergens (pollen or animal dander). Atopic eczema was the main diagnosis in 30, allergic rhinitis in 22 and bronchial asthma in 15 of the patients. The mean age of the patients was 21 years (range 36 to 51 years), 31 were males and 36 females.

Allergen extracts

The *C. albicans* strain 72024 was supplied and typed by the Mycological Laboratory of the Dept. of Dermatology, University of Turku. It was cultured for the study. An isolated colony was transferred from Sabouraud agar plates (Difco 0109-05-5) to 1 l Sabouraud dextrose broth (Difco 0382-05-3) and incubated overnight at 37°C. This culture was then inoculated into 10 l fermentor and incubated for 18-24 h as described in a previous paper (14).

The extracts used for skin prick testing were prepared from the lyophilized yeast cells as described earlier (14). For the preparation of extract the cells were reconstituted with 0.125 M NH_4HCO_3 (Merck 1131) to 20% (w/v) solution and shaken 18 h at 4°C. The supernatant was separated by centrifugation (50,000 g, 30 min) and dialysed 3 × 1 day against 0.005 M NH_4HCO_3 at 4°C. Finally the supernatant was lyophilized and stored at -20°C. The final skin prick test extract was prepared by reconstituting the lyophilized extract with NSA-diluent (Pharmacia Ab, Uppsala, Sweden) to 11 mg/ml solution (corresponding to 10% (w/v) original yeast), which was filtered through 0.22 µ filter

(Millipore, Millex -GS, SLGS 025 B5). Before use the sterility control for bacterial and fungal growth was performed. The extract was stored at 4°C.

Investigations on patients

The patients were invited to the clinic once a month from September to April the following year to have skin, upper airway and lung examination. Yeast cultures were taken from the nasopharynx and the anus.

The patients kept a daily symptom diary on skin, upper and lower airway symptoms and all medicines taken.

Skin prick tests were performed onto the skin of the forearm with the extracts produced. The results were read and transferred onto a transparent tape after 15 min. They were compared with 1 mg/ml Histamine-HCl skin reaction according to the Nordic recommendations (1). The delayed skin reaction was read and measured 72 h after the test.

RESULTS

There was no correlation between the skin and airway symptoms and nasopharyngeal and/or anal growth of *C. albicans* at the moment of examination. Neither was there a correlation between the symptoms (as reported in the diaries) of the observed month and the occurrence of *C. albicans* in the nasopharynx and the anal region. The only symptom which possibly could be connected with yeast growth was an itchy feeling and soreness of the throat at the time of positive yeast culture in some patients. The occurrence of positive *C. albicans* nasopharyngeal cultures had maxima in November, March and April, but this had no effect on the symptoms of the yeast-sensitive patients.

The occurrence of *C. albicans* in the nasopharynx correlated positively with the immediate skin reactivity and negatively with the delayed skin reactivity (Tables 1 and 2).

Nasal or inhaled beclomethasone was used by six patients. Only one of them had *C. albicans* growth in almost all nasopharyngeal cultures the others were negative on all occasions.

Anal growth shows the same positive correlation to the immediate skin reactivity as the nasopharyngeal growth (Table 3). Nasopharyngeal and anal growth also occur simultaneously (Table 4).

Yeasts other than *C. albicans* occurred only seldom in the nasopharyngeal cultures. The species observed were *C. glabrata* and *C. rugosa*, *Debaryomyces hansenii*, *Nocardia* sp., *Pityrospora ovale* and *Yarrowia lipolytica*.

DISCUSSION

It was not possible to demonstrate an effect of the *C. albicans* nasopharyngeal growth on aller-

gy symptoms in yeast-sensitive subjects compared with non yeast-sensitive subjects. However, in most of the *C. albicans* skin and RAST test-positive patients we have shown that they react to nasal and inhalation provocations with *C. albicans* allergenic extracts (14). Thus, the amount and type of exposure may determine the appearance of symptoms.

The observed relationship of immediate and delayed type skin reactivities to the nasopharyngeal growth of *Candida* are interesting from a theoretical point of view. The adherence of microorganisms to epithelial cell surface is the first step in infection. For *C. albicans* this adherence seems to be very specific, because other *Candida* yeasts seldom colonize the pharyngeal epithelium. Sandin et al. (19) have suggested that mannose-containing moieties on the surface of *C. albicans* could mediate the adherence to human epithelium. Also the com-

Table 1

Nasopharyngeal growth of *C. albicans* compared with immediate skin prick test reactivity to *C. albicans* allergenic extract. 482 cultures were taken from 67 patients. The figures in parentheses are number of patients. The number of positive skin prick tests was significantly higher in subjects with a positive nasopharyngeal growth ($P < 0.0005$, Chi-squared test)

Nasopharyngeal growth	Immediate skin prick test reaction			Total
	≥ + + +	+ + or +	-	
Positive	94	42	41	177
Negative	95	56	154	305
Total	189 (n = 23)	98 (n = 15)	195 (n = 29)	482 (n = 67)

Table 2

Nasopharyngeal growth of *C. albicans* compared with delayed skin reactivity to *C. albicans* extract. 361 samples from 51 patients tested for delayed hypersensitivity. The figures in parentheses are number of patients. A significantly higher number of positive delayed skin reactions in subjects with negative nasopharyngeal growth ($P < 0.005$, Chi-squared test)

Nasopharyngeal growth	Diameter of delayed skin reaction		Total
	≤ 3 mm	> 3mm	
Positive	67	56	123
Negative	72	166	238
Total	139 (n = 19)	222 (n = 32)	361 (n = 51)

Table 3

Anal growth of *C. albicans* compared with immediate skin prick test sensitivity to *C. albicans* allergen extract. 421 cultures were taken from 67 patients. The figures in parentheses are number of patients. A positive correlation between anal growth and skin prick reaction ($P < 0.0005$, Chi-squared test).

Anal growth	Immediate skin prick reaction			Total
	≥ + + +	+ + or +	-	
Positive	51	21	12	84
Negative	106	64	167	337
Total	157 (n = 23)	85 (n = 15)	179 (n = 29)	421 (n = 67)

Table 4

Interrelationship of nasopharyngeal and anal growth of *C. albicans* in simultaneously taken cultures. A positive correlation was found between the anal and nasopharyngeal growth of *C. albicans*. ($P < 0.0005$, Chi-squared test).

Nasopharyngeal	Anal growth		Total
	positive	negative	
Positive	51	80	131
Negative	14	267	281
Total	65	347	412

plement system and the complement binding sites on various human cells may be responsible for the specificity to *C. albicans*. Unlike many other species, *C. albicans* and *C. stellatoidea* bind to C3 fragments (10).

Cell mediated immunity is usually considered important in chronic mucocutaneous candidiasis (16) and hyper-IgE-syndrome (4), but its role is not well known in oral candidiasis (8). According to our results cell-mediated immunity to *Candida* may be important already in the adherence stage.

It is not known whether the defect of cell-mediated immunity to *Candida* is an innate phenomenon or induced by the yeast itself. Tsuji et al. have presented evidence that cell-mediated immunity to *Candida* could be associated with a HLA-linked Ir gene in man (22). There is also evidence that *Candida* infection may induce immunosuppression (16). Recently, Carrow & Domer (6) have found that a *C. albicans* cell wall glycoprotein containing mannan and a heat-labile moiety causes specific suppression of the cell-mediated responses in a murine model.

Anti-*Candida*-IgE may also be the channel of the specific immune suppression to *Candida albicans*. The anti-*Candida*-IgE may alter the host resistance to *C. albicans* by coating the yeast cells with non-opsonizing antibodies and inducing immediate hypersensitivity reactions in the tissues. Defective yeast opsonization may also have connection with the complement system. This is suggested by the findings of defective yeast opsonization and C2 deficiency in atopic patients by Turner et al. (23). The complement cascade has also been considered to be important by Hanson et al. (9). It is likewise possible that release of mediators from basophil or mast cell granules can inhibit, non-specifically, a subsequent delayed type of hyperreactivity reaction leading to defective cell-mediated immune defence (5, 12).

The high anti-*Candida*-IgE may result from IgE-specific immunoregulatory disturbances. It is known from animal experiments that defective T-cell immunity leads to high IgE-response in normally unresponsive mice (21). An analogous situation has been observed also in man. In immunodeficient children with the hyper-

IgE-syndrome the IgG antibodies to *C. albicans* and Tetanus toxoid were comparable with those of control persons, whereas IgE antibodies to these antigens were high (20). Also patients with dermatophytosis have a negative correlation between S-IgE and blood T-cell counts (3).

Our results suggest that impaired cell-mediated immunity to *C. albicans* may lead to increased IgE response. This might explain the increased liability to *C. albicans* growth in the nasopharynx and the gastrointestinal tract of persons with positive immediate skin reactivity to this yeast. These observations also point out that *C. albicans* may be a suitable tool in the investigation of the interrelationship of allergy and infection.

ACKNOWLEDGEMENTS

The authors wish to thank Mrs. Maija Salonen, Mrs. Seija Laanti, and Mrs. Anja Aaltojärvi for their skillful technical assistance. The support of the Finnish Academy, the Yrjö Jahnsson Foundation and the Allergy Research Foundation is gratefully acknowledged.

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Address:

Antti Koivikko

Department of Pediatrics

University of Turku

SF-20520 Turku

Finland

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