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IgE, IgA, and IgG responses to common yeasts in atopic patients

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This study was undertaken to analyze the differences in exposure and sensitization to five common environmental yeasts. The responses of IgG, IgA, and IgE to *Candida albicans*, *C. utilis*, *Cryptococcus albidus*, *Rhodotorula rubra*, and *Saccharomyces cerevisiae* and purified *S. cerevisiae* enolase were analyzed by immunoblotting (IgE-IB), and the cross-reactivity of their IgE-binding components by IgE-IB inhibition. Twenty atopic subjects, with asthma, allergic rhinitis, or atopic dermatitis were included. In skin prick tests (SPT), 12 of the patients showed simultaneous reactivity to at least two of the five yeasts, four reacted to one of the yeasts, and four had no responses. Antigens run in SDS–PAGE and transferred to nitrocellulose were probed with enzyme-labeled IgA-, IgG-, and IgE-specific antibodies. The IgE immunoblotting revealed most IgE-binding bands in *C. albicans* (11 bands) followed by *C. utilis* (eight bands), *S. cerevisiae* (five bands), *R. rubra* (five bands), and *Cr. albidus* (four bands). Six of the IgE-binding bands of *C. albicans* and *C. utilis* shared molecular weight, and only two bands shared molecular weight with other yeasts. These were the 46-kDa band, shared by all five yeasts, and a 13-kDa band shared by four yeasts. Prominent IgE binding was seen to a 46-kDa band of *C. albicans* (seven patients), *C. utilis* (five patients), and *S. cerevisiae* (one patient) and to corresponding weak bands of *Cr. albidus* and *R. rubra* (one patient). The possible cross-reactivity of the 46-kDa band was analyzed by IgE-IB inhibition and densitometry, revealing clear *C. albicans* inhibition of *C. utilis* (80%) and enolase (98%) (autoinhibition 100%). The strongest IgG responses were seen against *S. cerevisiae* and *C. albicans*. The responses were mainly against mannans of *C. albicans* and *S. cerevisiae*, suggesting that most of the exposure is to these yeasts. Yeasts with different types of exposure, from saprophytic growth on human mucous membranes to exposure by air and food, were shown to cross-react at the allergenic level. Atopic patients primarily sensitized by *C. albicans* and *S. cerevisiae* may develop allergic symptoms by exposure to other environmental yeasts due to cross-reacting IgE antibodies.

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Several yeasts have been found to play a role in allergic diseases. *Candida albicans*, a common saprophyte of the mucous membranes, has been associated with bronchial asthma, allergic rhinitis, chronic urticaria and atopic dermatitis (1–5). Atopic allergy to a lipophilic yeast, *Pityrosporum ovale*, growing on the skin has been connected with exacerbations of atopic dermatitis (6, 7). *Saccharomyces cerevisiae*, the familiar baker's yeast, is widely used in foods. *S. cerevisiae* may also be important in the pathogenesis of atopic dermatitis as well as chronic urticaria (3, 8).

Despite evident antigenic cross-reactivity, the cross-reacting allergens of yeasts have remained unexamined until recently (9, 10). The allergenic potencies of mannan and several cytoplasmic proteins of *C. albicans*, *P. ovale*, and *S. cerevisiae* have been confirmed (11–18). Immunoblotting and RAST-inhibition studies have revealed cross-reacting allergens in the mannan and protein

allergens of these yeasts. A 46–48-kDa enolase is a main allergen and cross-reacting component of *C. albicans* and *S. cerevisiae* (14, 19–22). Mannan polysaccharide is a cross-reacting allergen of *C. albicans*, *P. ovale*, and *S. cerevisiae* (23–25).

Candida utilis and *Candida tropicalis*, of industrial origin, have been associated with allergic symptoms (26). *Cryptococcus albidus* is the most common airborne yeast in Finland, followed by *Rhodotorula* species (27). There are no previous data on the allergens of *Rhodotorula*, *Cryptococcus*, or *C. utilis*.

This study was undertaken to analyze the differences in exposure and sensitization to five common yeasts by IgE, IgA, and IgG immunoblotting, as well as their allergenic cross-reactivity by immunoblotting inhibition.

Material and methods

Patients

Sixteen atopic patients with varying skin prick test (SPT) responses to the five studied yeasts (Table 1) were included in the study. Four atopic patients not responding to yeasts served as controls. The SPT were performed according to Nordic recommendations, as previously described (28). The serum samples were taken at the time of SPT and stored at –20°C until used. All patients had diagnoses of asthma, allergic rhinitis, or atopic dermatitis mainly in combinations (Table 1). Pooled sera from four nonatopic subjects were used as control in immunoblotting.

Yeast extracts

Four different yeast strains were cultured for these studies, as previously described (28). *C. albicans* strain 72024, *R. rubra* strain 70765, and *Cr. albidus* strain 71078 were supplied and typed by the Mycological Laboratory of the Department of Dermatology, University of Turku (Finland). *C. utilis*, strain VTT-C-71015 was obtained from the Department of Clinical Microbiology, University of Kuopio (Finland). *S. cerevisiae* cultures were provided by OY Alko AB, Helsinki, Finland (courtesy of Matti Korhola, Microbiological Laboratory of Alko). The extracts used for immunoblotting were prepared from lyophilized yeast cells, as previously described (28). Commercially available purified *S. cerevisiae* enolase was purchased from Sigma Chemicals (Sigma E 6126). Chemically purified *C. albicans* mannan was prepared according to the Peat method, as previously described (24).

SDS–PAGE and immunoblotting

Antigens and molecular weight markers (Electrophoresis Low Molecular Weight Calibration Kit, Pharmacia Fine Chemicals, Uppsala, Sweden) were separated with SDS–PAGE in 5–17.5% gradient gel according to Laemmli (29). Separated antigens were transferred to nitrocellulose sheets (Millipore 0.22 µm, Millipore, Bedford, MA, USA) in Transphor apparatus (LKB, Bromma, Sweden) according to Towbin et al. (30). IgA- and IgG-class antibody detection was performed with horseradish peroxidase (HRP)-conjugated anti-IgA and anti-IgG

Table 1. SPT, IgE-immunoblotting, and mannan-RAST results of study patients

Patient no.	Sex	Age (years)	Diagnosis[a]	*C. albicans*			*C. utilis*		*R. rubra*		*S. cerevisiae*		*Cr. albidus*	
				Man[b]	SPT[c]	IgE[d]	SPT	IgE	SPT	IgE	SPT	IgE	SPT	IgE
1	F	6	A, R, E	**0.65**	+	+	+	+	+	+	+	+	+	+
2	M	9	A, E	0.22	+	+	+	+	+	+	+	+	+	+
3	F	16	R, E	**0.60**	+	+	+	+	+	+	+	+	+	–
4	F	18	E	**1.27**	+	+	+	+	+	+	+	–	+	–
5	M	33	E	**0.64**	+	+	+	+	+	–	+	–	+	–
6	F	17	A, R, E	**9.52**	+	+	+	+	+	–	+	–	+	+
7	M	26	R, E	**0.41**	+	+	+	–	+	+	+	+	+	–
8	F	20	E	n.d.	+	–	+	–	+	–	+	–	+	–
9	F	15	A, R, E	**4.40**	+	+	+	+	+	+	+	–	–	–
10	M	37	E	**0.94**	+	+	–	–	+	–	+	–	–	–
11	M	14	R, E	0.16	+	+	–	–	+	+	–	–	–	–
12	F	17	R	**0.61**	+	+	–	+	–	–	–	–	–	–
13	F	17	E	**0.62**	+	+	–	–	–	–	–	–	–	–
14	F	45	E	0.32	+	+	–	–	–	–	–	–	–	–
15	F	16	R	n.d.	–	+	+	+	+	+	+	–	+	+
16	F	15	A, E	n.d.	–	–	–	–	+	–	–	–	–	–
17	F	35	R	n.d.	–	–	–	–	–	–	–	–	–	–
18	F	16	R	0.18	–	–	–	–	–	–	–	–	–	–
19	M	11	R	n.d.	–	–	–	–	–	–	–	–	–	–
20	M	16	A, E	n.d.	–	–	–	–	–	–	–	–	–	–
Total SPT/IgE[e]					14	14	10	9	13	8	11	4	9	4

[a]Diagnosis: A=asthma; R=rhinitis; E=eczema.
[b]Mannan-RAST index (positive values in bold).
[c]Skin prick test result.
[d]IgE response in immunoblotting.
[e]Total number of patients positive in SPT/IgE.

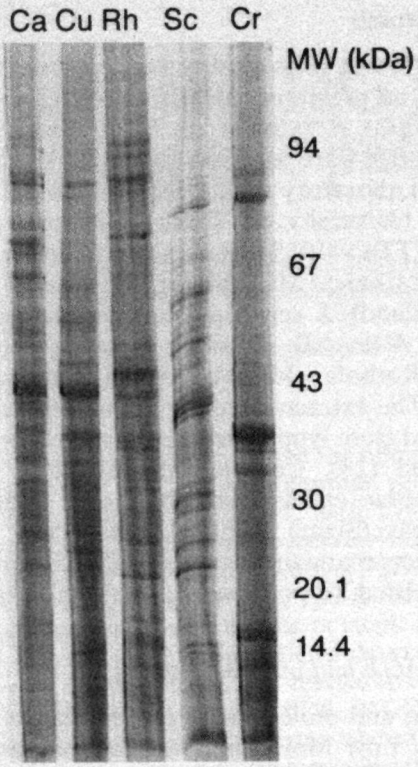


Fig. 1. Extracts of five study yeasts, *Candida albicans* (Ca), *C. utilis* (Cu), *Rhodotorula rubra* (Rh), *Saccharomyces cerevisiae* (Sc), and *Cryptococcus albidus* (Cr), run in 5–17.5% gradient SDS–PAGE gel and stained with Coomassie brilliant blue. Molecular weight markers (MW) are shown on right.

antibodies (DAKO Immunoglobulins, Denmark), as previously described (18). The IgE detection was done according to Bengtson et al. with β-galactosidase-labeled anti-IgE antibodies (Phadezym-RAST, Pharmacia Diagnostics, Uppsala, Sweden), as previously described (31). In IgE-immunoblotting inhibition prior to the incubation with the nitrocellulose strips, the sera were incubated with serial dilutions of the antigens for 30 min at 37°C.

Nitrocellulose RAST

The IgE antibodies against *C. albicans* mannan were analyzed with nitrocellulose RAST, as previously described (24).

Results

The protein profiles of the study extracts of all five yeasts run in a 5–17.5% gradient SDS–PAGE gel stained with Coomassie brilliant blue are presented in Fig. 1. The greatest similarity in the protein profiles was seen between *C. albicans* and *C. utilis*, which both showed a dominant protein staining of 46 kDa. In IgE immunoblotting, prominent IgE binding to the 46-kDa band was seen in one serum (patient 1) not only to *C. albicans* and *C. utilis*, but also to *S. cerevisiae*, as well as to, a lesser extent, to corresponding weak bands of *Cr. albidus* and *R. rubra* (Fig. 2).

Fig. 2 presents the parallel IgE immunoblots of all the five yeasts of five patients, who showed simultaneous strong IgE binding to at least three of the five yeasts. A comparison of the IgE immunoblotting, mannan-RAST, and SPT results of the patients presented, together with the clinical diagnosis, age, and sex, is shown in Table 1, and the summary of all the IgE-binding bands of all the five yeasts in Table 2. The yeast most often positive in IgE immunoblotting was *C. albicans* with 14 positive sera, followed by *C. utilis* (nine sera) and *R. rubra* (eight sera). Among SPT-positive patients, IgE antibodies were most often found against these yeasts, whereas IgE antibodies against both *S. cerevisiae* and *Cr. albidus* were seen in only four sera, in clearly less than 50% of the SPT-positive patients.

The IgE immunoblotting also revealed most IgE-binding bands in *C. albicans* (11 bands), again followed by *C. utilis* (eight bands). Five IgE-binding bands were seen in *R. rubra* and *S. cerevisiae* and four in *Cr. albidus*. Six IgE-binding bands of *C. albicans* and *C. utilis* had the same molecular weight. In general, only two bands shared molecular weight with other yeasts. These bands were the 46-kDa band, shared by all five yeasts, and a 13-kDa band shared by four yeasts.

Since the 46-kDa allergen was a major allergen of both *C. albicans* and *C. utilis* in this population, the possible cross-reactivity of it was analyzed by immunoblotting inhibition. Fig. 3 shows the inhibition of the 46-kDa bands of *C. albicans* and *C. utilis* and purified commercial *S. cerevisiae* enolase with individual serum from patient 1, who had an IgG response to *C. utilis*. For elimination of the effect of background differences, the net integrated intensities of the bands were assessed densitometrically and inhibition percentages were calculated. The *C. albicans* inhibition of *C. utilis* clearly reached 80% inhibition, the corresponding concentration giving a full 100% autoinhibition of *C. albicans*. The enolase staining was almost as completely inhibited (98%), with *C. albicans* as the autoinhibition. *S. cerevisiae* enolase inhibited *C. albicans* staining to 55% and *C. utilis* staining to only 15%, while the same concentration gave an enolase autoinhibition of 98%.

Examples of the IgG immunoblotting with the five yeasts are presented in Fig. 4, and the frequency of IgA and IgG antibodies against the yeasts in total and against mannan is shown in Table 3. The strongest IgG responses were seen against *S. cerevisiae* and

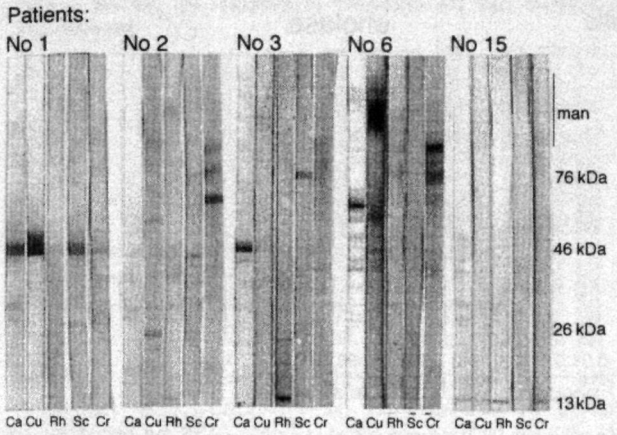


Fig. 2. IgE immunoblotting strips of all five study yeasts incubated in sera of five study patients. Lyophilized antigen load per strip was 0.6 mg for *Candida albicans* (Ca), 0.45 mg for *C. utilis* (Cu), 0.35 mg for *Rhodotorula rubra* (Rh), 0.9 mg for *Saccharomyces cerevisiae* (Sc), and 0.5 mg for *Cryptococcus albidus* (Cr).

C. albicans, against which 19 (95%) and 18 (90%) patients, respectively, had a response. The corresponding patient numbers for IgA class antibodies were 15 (75%) and 17 (85%). Eleven (55%) patients had both IgG and IgA responses to *Cr. albidus*, whereas seven patients (35%) had IgG and five patients (25%) IgA against *R. rubra*. None of the patients had IgA against *C. utilis*, and only four patients (20%) had an IgG response.

Important individual antigens were the high-molecular-weight, mannan-like components, against which 17 (*C. albicans*), 14 (*S. cerevisiae*), 10 (*Cr. albidus*), and seven (*R. rubra*) patients had an IgG response. The corresponding patient numbers in IgA immunoblotting were 17 (*C. albicans*), seven (*S. cerevisiae*), two (*Cr. albidus*), and none (*R. rubra*). Neither IgG nor IgA responses against *C. utilis* mannan were seen in this group. Only occasional IgG responses were seen against yeast proteins except for the 20-kDa (18 patients) and 25-kDa (14 patients) bands of *S. cerevisiae*, a 32-kDa (seven patients) band of *Cr. albidus*, and a 21-kDa (five patients) band of *C. albicans*. An IgA response to the 20-kDa band of *S. cerevisiae* was seen in 12 patients.

Discussion

Although different yeasts have been long known to share common antigens, their significance as allergens has, until recently, been evaluated only by SPT (9, 10, 28). In SPT, the patients react to several yeast extracts simultaneously, a fact which suggests possible allergenic cross-reactivity among the yeast species studied, or that patients have been exposed to all these yeasts. Despite abundant simultaneous skin reactivity, specific IgE antibodies in immunoblotting to the same extent were seen only against *C. albicans* and *C. utilis*. Natural exposure to *C. albicans* occurs, as indicated by the strong IgG response, which correlates with the extent of saprophytic growth; in other words, exposure (32).

The skin reactivity, as well as IgE antibodies, to other yeasts, except *C. albicans*, is probably due to cross-reacting antibodies. This is especially striking for *C. utilis*, which, according to the IgA and IgG

Table 2. IgE binding of 20 studied sera to allergenic bands of five studied yeasts

Mol. wt. (kDa)	*C. albicans*		*C. utilis*		*R. rubra*		*S. cerevisiae*		*Cr. albidus*	
	n[a]	%[b]	*n*	%	*n*	%	*n*	%	*n*	%
150	2	14	–		–		–		–	
95	1	7	–		–		–		1	25
76	–		–		–		2	50	–	
65	–		–		–		–		1	25
63	3	21	–		–		–		–	
57	1	7	1	11	–		–		–	
50	1	7	–		–		–		–	
46	7	50	5	56	1	13	1	25	1	25
43	1	7	1	11	–		1	25	–	
39	1	7	1	11	–		–		–	
37	2	14	2	22	–		–		–	
32	–		2	22	–		–		–	
29	9	64	–		–		–		–	
28	–		–		1	13	1	25	–	
27	–		1	11	–		–		–	
26	–		–		4	50	–		–	
20	–		–		1	13	1	25	–	
13	2	14	2	22	4	50	–		1	25
Total[c]	14		9		8		4		4	

[a]No. of patients with IgE response to band.
[b]Percentage of all patients with IgE response to yeast.
[c]Total number of patients with IgE response to each yeast.

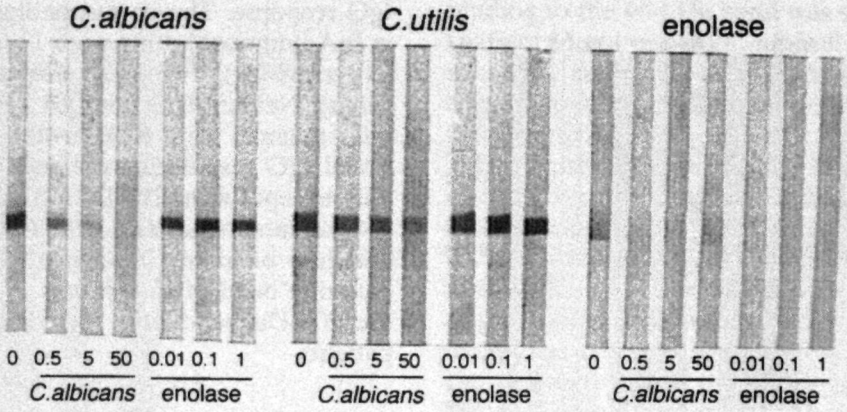


Fig. 3. IgG immunoblotting inhibition of blotted strips of *Candida albicans* (0.3 mg per strip), *C. utilis* (0.38 mg per strip), and purified *Saccharomyces cerevisiae* enolase (0.08 mg per strip), with individual serum from patient 1. Concentrations of liquid phase inhibiting antigens of *C. albicans* and *S. cerevisiae* enolase were mg/ml.

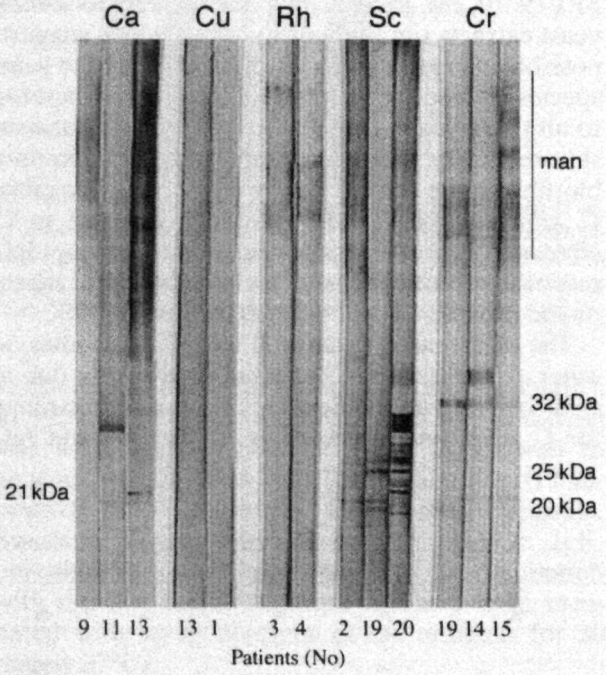


Fig. 4. Examples of IgG immunoblotting with five study yeasts, *Candida albicans* (Ca), *C. utilis* (Cu), *Rhodotorula rubra* (Rh), *Saccharomyces cerevisiae* (Sc), and *Cryptococcus albidus* (Cr). Antigen concentrations of strips were as in Fig. 2. Most important IgG-binding antigens are indicated on left and right.

responses, causes very little exposure, at least as compared to patients exposed to industrial *C. utilis* dust. In the 1970s, emission of *C. utilis* yeast dust of industrial origin caused allergic symptoms in Äänekoski (Finland). In a population of 60 patients from the factory environment, 30% were positive in *C. utilis* IgG immunoblotting and 42% in IgA immunoblotting (results not shown). As exposure to *C. utilis* causes IgG and IgA responses, most of the patients of this study may not have been exposed to *C. utilis*. The patients did not report any history of industrial yeast exposure.

The IgE response to *C. utilis* is probably due to cross-reacting IgE antibodies, since only one out of nine *C. utilis* IgE-positive subjects showed an IgG response to *C. utilis*. This patient also showed the strongest IgE response to *C. utilis*. By using the serum of this patient, the cross-reactivity of the most important antigens, the 46-kDa proteins of *C. albicans* and *C. utilis*, was confirmed. The cross-reactivity appeared to be partial, suggesting that *C. utilis* has its own epitopes for IgE. This finding suggests true exposure to *C. utilis* in this patient in agreement with the *C. utilis*-specific IgG response.

A recent paper showed partial cross-reactivity between purified *C. albicans* and *S. cerevisae* enolases, both with a molecular weight of 46-kDa (21). It was shown that *C. albicans* enolase could inhibit *S. cerevisiae* enolase completely, whereas *S. cerevisiae* enolase could only partially inhibit IgE binding to *C. albicans* enolase. This implies that, in addition to common epitopes, *C. albicans* enolase also has its own unique epitopes. We found the same phenomenon, suggesting the presence not only of cross-reacting epitopes in *C. utilis* and *S. cerevisiae* enolase but also of unique epitopes of the *C. albicans* 46-kDa band. On the other hand, the 46-kDa band could be a complex of several bands with the same molecular weight. However, our results suggest that the 46-kDa bands of *C. albicans* and *C. utilis* are also enolases.

In our SPT survey of the urban population of Äänekoski, the Finnish industrial town exposed to emission of *C. utilis*, skin reactivity to *C. utilis* was found to be equal to that of another industrial urban population in Varkaus, Finland, a town without yeast dust exposure (results not shown). In a

Table 3. IgA and IgG responses to five yeasts and their mannans in immunoblotting

	IgA		IgG	
	Total[a]	Mannan	Total[b]	Mannan
C. albicans	17 (85%)[c]	17 (100%)[d]	18 (90%)	17 (94%)
C. utilis	0 (0%)	0 (0%)	4 (20%)	0 (0%)
R. rubra	5 (25%)	0 (0%)	7 (35%)	7 (100%)
S. cerevisiae	15 (75%)	7 (47%)	19 (95%)	14 (74%)
Cr. albidus	11 (55%)	2 (18%)	11 (55%)	10 (91%)

[a]Total number of IgA- and [b]IgG-positive patients for each yeast.
[c]Percentage of all studied patients.
[d]Percentage of patients with positive yeast-specific response.

further SPT analysis, these populations also revealed skin reactivity to *C. albicans* in equal proportions. These observations also are well explained by the clear cross-reactivity of the 46-kDa main allergens of both *C. albicans* and *C. utilis* and primary sensitization by *C. albicans.*

More frequent IgE responses, as well as IgG and IgA responses, were observed to *C. albicans* than *S. cerevisiae* and the airborne yeasts, except *C. utilis*, suggesting sensitization by saprophytic growth. Having been primarily sensitized by saprophytic exposure, these patients may suffer symptoms when they come into contact with cross-reacting yeasts, such as *Rhodotorula* and *Cryptococcus*, in food or elsewhere in nature. The cross-reactivity of these yeasts may thus explain some of the symptoms found in patients with respiratory, cutaneous, or gastrointestinal allergy.

S. cerevisiae has earlier been connected with urticaria and atopic dermatitis (3, 8). The present study has shown that although frequent exposure to *S. cerevisiae* occurs, according to IgA and IgG antibodies, atopic sensitization (IgE antibodies) is not as frequent as to *C. albicans.* As the 46-kDa enolase allergen of *S. cerevisiae* is labile in solutions, at high temperatures and in the gastrointestinal tract, it is likely that many of the anti-*S. cerevisiae* enolase antibodies are due to primary sensitization by *C. albicans* enolase (33, 34).

The previously reported cross-reactivities of the antimannan IgE antibodies to *C. albicans*, *S. cerevisiae*, and *P. ovale* were not apparent in this population by immunoblotting analysis (23–25). This is due to the low sensitivity of immunoblotting to detect the antimannan antibodies, although slightly elevated mannan RAST values were found. As regards *P. ovale*, it has been shown that mannan-specific IgE antibodies are difficult to distinguish by immunoblotting, because mannan, as a large molecule, hardly enters the SDS–PAGE gel (35).

The cross-reactivity of polysaccharide as well as protein components of various yeasts is becoming better understood. Exposure to yeasts completely different biologically takes very different forms, ranging from saprophytic growth on skin and mucous membranes to airborne exposure and ingested food. The finding that these different yeast species cross-react on an antigenic and allergenic level will help us to understand and explore their sensitizing properties in the future.

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