

A crossreactivity at the immunoglobulin E level of the cell wall mannoproteins of *Candida albicans* with other pathogenic *Candida* and airborne yeast species

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Summary

Background *Candida albicans* crossreacts with *Saccharomyces cerevisiae* or *Pityrosporum ovale* at the IgE level. However, the extent of crossreactivity of *C. albicans* with other yeast species is not known.

Objective The crossreactivity at the immunoglobulin E (IgE) level of *Candida albicans* with other pathogenic *Candida* species and to the airborne yeast species *Cryptococcus* and *Rhodotorula* was studied by immunoblot analysis.

Methods Crude antigens, designated as heat extract, were prepared from 13 different yeast species and a dot blot test was performed to detect IgE antibodies against each of the heat extracts in 349 patients with allergies who were positive for IgE antibodies against *C. albicans* in a CAP system.

Results In the dot blot test, most of the sera reacted with the heat extracts of not only *C. albicans* but also those prepared from the other yeast species. The sera of 41 of the 349 patients (11.7%) reacted with the heat extracts of all 13 yeast species. The extent of the binding of IgE antibodies to multiple yeast species correlated with both the fluorescence intensities measured in the CAP system and the intensities of dots generated by the heat extract of *C. albicans* in the dot blot test. In an inhibition dot blot test, mannoproteins, but not proteins, of *C. albicans* strongly inhibited the subsequent binding of IgE antibodies to all yeast species.

Conclusion Our data suggest that the *C. albicans* mannoproteins are responsible for the crossreactivity among these yeast species at the IgE level.

Keywords: *C. albicans*, mannoproteins, crossreactivity, IgE, airborne yeasts

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Introduction

The opportunistic fungal pathogen *Candida albicans* is a commensal yeast and has been suspected as a fungal species for fungal allergy [1–3]. Immunoglobulin E (IgE) antibodies to this fungus are frequently found in patients with bronchial asthma, rhinitis or atopic dermatitis [4,5]. Studies of the allergenic properties of *C. albicans* have identified

and characterized several possible proteins of this fungus [6–8], and several recent reports concerning polysaccharide antigens of *C. albicans* have focussed on IgE antibodies to *C. albicans* mannans [9–11]. More recently, it has been reported that IgE antibodies against phosphomannoproteins of *C. albicans* are seen most frequently in the sera of patients with allergies who were positive for IgE antibodies to *C. albicans* in a CAP system [12].

Some fungal antigens show crossreactivity [13–15], which prevents the development of standards for species-specific fungal antigen [16]. There are reports that the

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C. albicans crossreacts with *S. cerevisiae* [17,18] and with *Pityrosporum ovale* [19–21], and each of these studies showed that mannans are responsible for the crossreactivity. Recently, Ito *et al.* [22] reported that epitopes of the enolase of *C. albicans* shares partly crossreact with those of *Saccharomyces cerevisiae*. However, we do not know the extent to which the *C. albicans* mannoproteins crossreact with those of other yeast species. Determination of the cross-reactivities among these yeast species may help to standardize yeast allergen and assign allergens in fungal allergies.

In the present study, to determine the extent of cross-reactivity of *C. albicans* antigens with other yeast species, we examined the binding properties of IgE antibodies from allergic patients who were positive for IgE antibodies to *C. albicans* in the CAP system to each of 13 yeast species, including pathogenic *Candida* and airborne yeast species. We show here evidence that mannoproteins of *C. albicans* are responsible for crossreactivity with several yeast species at the IgE level.

Materials and methods

Organisms and culture conditions

Eight species of the pathogenic yeast *Candida*, *Candida albicans* C9 (serotype A), *C. glabrata* TIMM 1063, *C. guilliermondii* NUM 4, *C. kefyr* IFO 0586, *C. krusei* IFO 0584, *C. parapsilosis* IFO 1068, *C. stellatoidea*, *C. albicans* serotype B NUM 692 and *C. tropicalis* NUM 1041, and four species of the airborne yeasts *Cryptococcus albidus* TIMM 0350, *Cr. laurentii* TIMM 0353, *Rhodotorula glutinis* TIMM 1486 and *R. rubra* TIMM 0923, and bakers' yeast *Saccharomyces cerevisiae* X2180 were used in this study. All cultures, which had been stored at -80°C in 50% glycerol, were spread on GYEP (2% glucose, 1% peptone, 0.3% yeast extract) agar plates and cultured for 48 h at 30°C . Yeast cells from a single colony were inoculated into GYEP broth and allowed to grow for 36 h at 30°C with aeration on a reciprocal shaker at 100 strokes per min. For preparation of mannoproteins from *C. albicans*, yeast cells were cultured in GYEP for 24 h at 37°C [12,23,24].

Crude yeast antigens

Yeast cells were grown in GYEP broth for 36 h at 30°C and washed three times with distilled water. The cell pellets were suspended in acetone, left overnight, harvested by centrifugation and air-dried. Samples (50 mg) of each of the dried yeast cells were suspended in 2 mL of distilled water. The cell suspensions were heated for 30 min at 100°C and then centrifuged at $10\,000\times g$ for 10 min. The resultant supernatant containing the crude antigen was designated as the heat extract and used in this study. The heat extracts were kept at -30°C before use.

Mannoproteins of *C. albicans*

Mannoproteins were isolated from *C. albicans* yeast cells as described previously [12,23,24]. In brief, *C. albicans* C9 cells grown in GYEP were treated with 300 mM β -mercaptoethanol in 100 mM EDTA (pH 9.0) for 30 min at 20 to 25°C and the resultant supernatant (2-ME extract) was applied onto a column of concanavalin A (Con-A)-agarose (Honen Co., Tokyo, Japan). Fractions eluted with PBS were pooled, dialysed against distilled water, lyophilized, and designated as the protein complex. The materials remaining bound to the column were eluted with 0.5 M α -methyl-D-mannopyranoside, and the phosphomannoprotein fractions of *C. albicans* serotype A strain were designated as CAMP/A [12,23]. The acid-stable part of the mannoproteins was prepared by HCl treatment (10 mM HCl, 100°C , 60 min) followed gel filtration with a size exclusion column (Toyo-pearl HW 40S, Toyo Soda Ltd, Tokyo, Japan). The fractions eluted in the void volume were pooled. The pooled fraction, which was the acid-stable part of the mannoprotein [24,25], was designated as CAMP-S/A [12].

Sera

Sera used in the present study were collected from 349 patients with allergies who were positive for IgE antibodies against *C. albicans* in a CAP system (Pharmacie Diagnostics, Uppsala, Sweden). Based on the results obtained with the CAP system, the sera were estimated to be in classes 3 to 6, and the values (KU/L) for *C. albicans*, obtained from the fluorescence intensities, ranged from 3.66 to greater than 100 kU/L. All sera were kindly donated by the Special Reference Laboratories (SRL) Ltd, Nagoya, Japan. For control experiments, sera from 20 additional individuals were used. Fifteen of these sera were positive for *Aspergillus* spp, *Alternaria* spp. or *Penicillium* spp. (five each) in the CAP system and five were from healthy females. All sera were stored at -80°C before use.

SDS-PAGE and lectin blot

The procedure of SDS-PAGE was similar to that described by Laemmli [26]. Five microlitre of the heat extracts was loaded on a 10% polyacrylamide gel. After electrophoresis, the gel was fixed with a mixture of acetic acid and methanol, and then stained with Coomassie brilliant blue R (CBB) to detect proteins in the heat extracts. For detection of mannans in each heat extract, lectin blotting was performed with ConA. After SDS-PAGE, the gel was electroblotted onto a polyvinylidene difluoride (PVDF) sheet (Millipore, Bedford, MA, USA) and processed as described previously [12,23]. In brief, the PVDF sheet was incubated with 5 μg per mL of HRP-conjugated ConA (Honen Co.) for 40 min at 20 to 25°C , and the sheet was then incubated with a mixture of 4-chloro-1-naphthol and H_2O_2 .

Dot blot and Western blot

To detect IgE antibodies in the sera against each of the heat extracts, a dot blot test was performed [12]. In the dot blot test, 1 μ L of each heat extract was blotted to a nitrocellulose (NC) sheet (Cellulosenitrat BA85; Schleicher & Schuell, Dassel, Germany) and dried for 30 min at 37°C. This process was repeated for a total of 349 sera. Each sheet was soaked in 20 mM Tris-HCl containing 0.5 M NaCl (TBS, pH 7.5) and 3% skim milk for 30 min at 21 to 25°C to block non-specific binding sites on the sheet. The sheets were then immersed in each serum, diluted to 1:20 with TBS containing 1% skim milk, and incubated overnight at 4 to 6°C with gentle shaking. After several washes in TBS containing 1% skim milk, each sheet was incubated with alkaline phosphatase (AP)-conjugated antibody raised in goat against human IgE (ϵ) (Kirkegaard & Perry Laboratories Inc., MD, USA) diluted 1:2,000 with 1% skim milk in TBS for 3 h at 21 to 25°C. Finally, the sheets were incubated in a mixture of nitroblue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate in 100 mM Tris-HCl buffer (pH 9.5) containing 100 mM NaCl and 5 mM $MgCl_2$ for 30 to 40 min at 21 to 25°C [7,12].

For a Western blot analysis of the serum samples, the heat extracts were subjected to SDS-PAGE and electroblotted to PVDF sheets, and the sheets were then incubated with the patient's sera.

Inhibition dot blot test

For the inhibition dot blot test, pooled serum which was prepared from 28 patients, each of whose sera reacted with all heat extracts prepared from 13 different yeast species in the dot blot test, was used. Prior to incubation of the pooled serum with the heat extracts blotted on a NC sheet, the pooled serum was diluted to 1:20 with TBS that contained 1% skim milk and 0 to 500 μ g/mL of protein complex, CAMP/A or CAMP-S/A fractionated from *C. albicans* yeast cells. The mixtures were then incubated for 2 h at 30°C. Each of the pretreated serum samples containing various concentrations of the *C. albicans* antigen fractions was added to a NC sheet blotted with 1 μ L of each heat extract (10 mg/mL each), and the sheets were incubated overnight at 4 to 6°C with gentle shaking. The NC sheets were then processed as in the dot blot test. The intensities of dots generated for each heat extract were measured. The crossreactivity of *C. albicans* to other yeast species was taken as the percentage of the ability of the fractions of *C. albicans* to inhibit the binding of IgE antibodies with each of the heat extracts of the yeast species.

Measurement of the intensities of dots on dot blots

In both the dot blot and inhibition dot blot tests, to determine the degree of reaction of IgE antibodies against the heat

extracts in the patients' sera or the pooled serum, the dot blot intensity (arbitrary unit) of each dot was measured with a Digital Image System IS 1000 instrument (Alpha Innotech Co., San Leandro, USA) [12,27]. Dots with an intensity greater than 100 were considered to be positive for IgE antibodies to each of the yeast species.

Results

CBB staining and ConA blot of the heat extracts

CBB staining of the crude antigen fractions, designated as heat extract, prepared by heat extraction of acetone-dried yeast cells showed a few faint bands in the heat extracts of *C. guilliermondii* (Fig. 1, upper panel), *C. parapsilosis*, *Cr. albidus* and *R. rubra*, and no bands in the heat extracts from the other species. Similar results were obtained with a more sensitive silver stain (data not shown). In contrast, the lectin blot with ConA revealed that all heat extracts

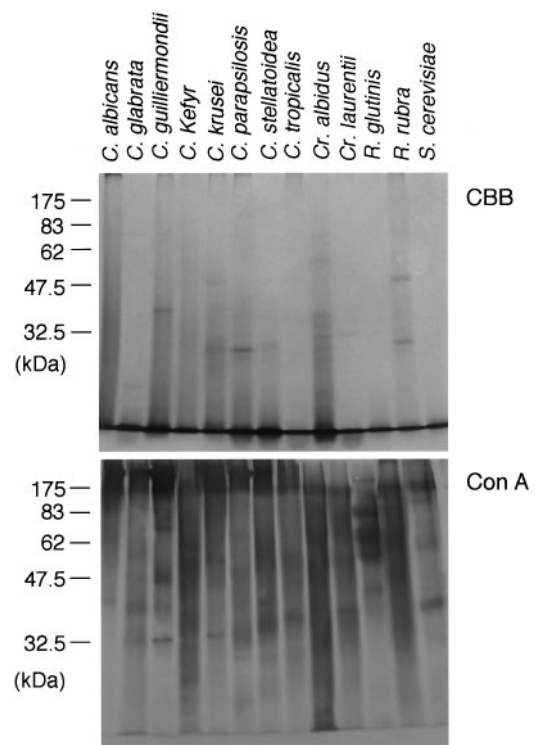


Fig. 1. Proteins and mannoproteins in the heat extracts of 13 yeast species. Each of the heat extracts was subjected to 10% SDS-PAGE. One gel was stained with CBB to monitor proteins (upper panel), and the other gel was subjected to a lectin blot with ConA to detect mannoproteins (lower panel). In the CBB-stained gel, faint bands were observed in the heat extracts of *C. guilliermondii*, *C. krusei*, *C. parapsilosis*, *Cr. albidus* and *R. rubra*, whereas mannoproteins with a variety of molecular weights were found in all the heat extracts. Mobilities of molecular mass markers (kDa) are shown on the left of each part of the figure.

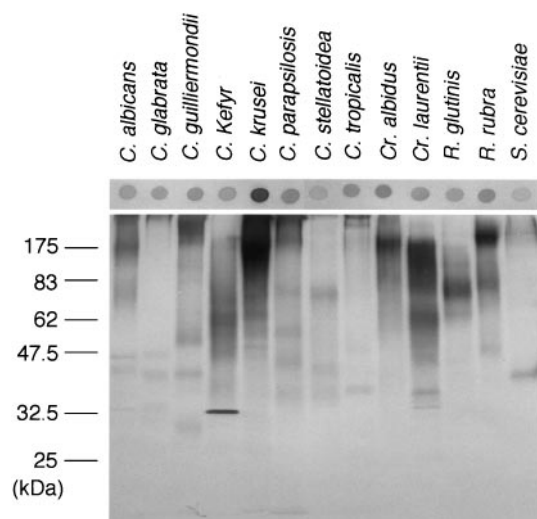


Fig. 2. Patterns of binding of IgE antibodies to the heat extracts of 13 yeast species in the dot blot test (upper panel) and Western blot test (lower panel). The dot blot test showed the presence of IgE antibodies against the yeast species. The serum sample used in the Western blot is the same as that used in the dot blot test and is one of the 41 serum samples out of 349 tested that reacted with all heat extracts. The dot blot intensities corresponded with the average intensities in the lanes of the Western blot. Mobilities of molecular mass markers (kDa) are shown on the left of the figure.

contained mannans whose binding with ConA was variable among the yeast species (Fig. 1, lower panel). These results indicated that each of the heat extracts contained mannan components.

Extent of the binding of IgE antibodies among yeast species

For several patients, the binding patterns of the dot blot test corresponded to those of the Western blot. An example of one serum that reacted with all heat extracts is shown in Fig. 2. In control experiments designed to test the specificities of the positive dots generated with each of the heat extracts, no binding of IgE antibodies to any of the extract was observed with sera from healthy persons or from any of the patients with allergies who were positive for IgE antibodies against *Aspergillus*, *Alternaria* or *Penicillium* in the CAP system (data not shown). Thus, the dot blot was specific for detecting IgE antibodies to the heat extracts. Subsequently, the dot blot test was used to test the reactivity of the IgE antibodies in the sera of all 349 allergy patients with the heat extracts. Each of these patients appeared from the results of the CAP system to be positive for IgE antibodies to *C. albicans* antigen. For the dot blot test, heat extracts prepared from each of the 13 different yeast species were blotted on an NC sheet and incubated with each of the serum samples. The results of the dot blot test showed that almost all sera

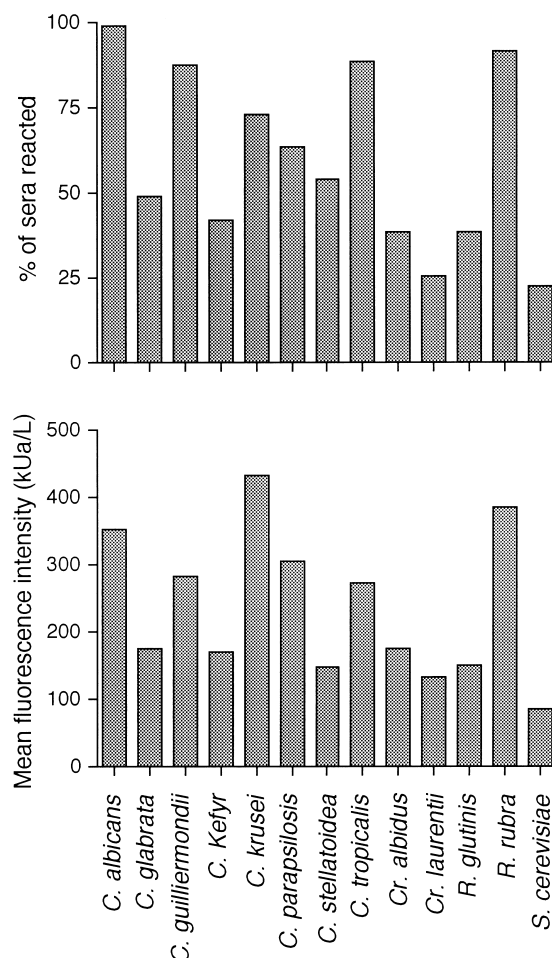
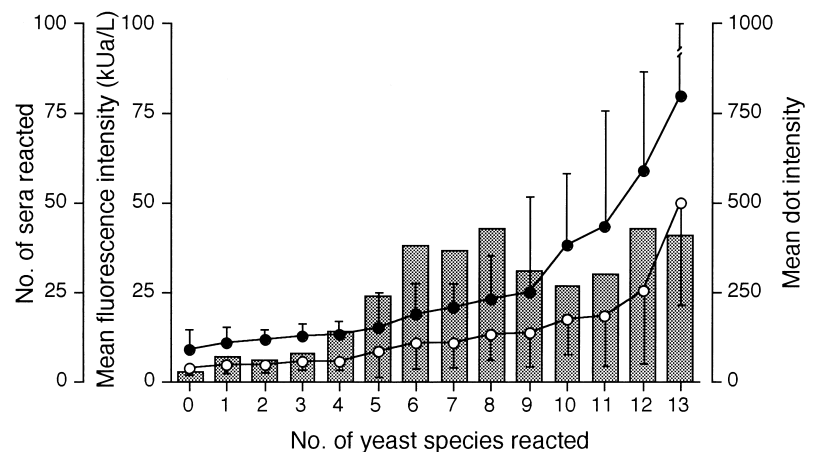


Fig. 3. Percentages of serum samples that showed IgE antibody reactions with heat extracts and degree of the binding of IgE antibodies to the heat extracts of 13 yeast species. The heat extracts were blotted onto NC sheets and the sheets were incubated with each serum of 349 patients with allergies who were positive for IgE antibodies to the *C. albicans* antigen in the CAP system. Many sera, in addition to the heat extracts of *C. albicans*, reacted with the other yeast species, especially, with *C. guilliermondii*, *C. tropicalis* and *R. rubra*. The mean intensity of dots generated for each of the yeast species was compared. The mean intensity for *C. krusei* was rather higher than those of *C. albicans* or *R. rubra*, in spite of the fact that the number of sera that reacted with *C. krusei* was lower than the number of sera that reacted with either of the other species.

reacted with more than two yeast species, even though the sera were positive for IgE antibodies to *C. albicans*. Surprisingly, of the 349 patients, 41 (11.7%) had sera that reacted with all yeast species (an example is shown in Fig. 2). The binding patterns of IgE antibodies to heat extracts shown in the Western blot were variable among the yeast species (Fig. 2, lower panel), and were more similar to those shown in the lectin blot with ConA (Fig. 1, lower panel), than to the CBB staining patterns (Fig. 1, upper panel).

Fig. 4. Correlations between the number of yeast species reacting with the serum samples (obtained from the dot blot test) and the fluorescence and dot intensities for the *C. albicans* antigens. Only seven serum samples (2%) reacted with only *C. albicans*, most sera reacted with more than two yeast species (■). The mean fluorescence intensities of the sera distributed in each group correlated with the number of yeast species that reacted with that group, that is, sera with higher fluorescence intensities reacted with a greater number of yeast species (○). Similar results were found when the number of yeast species was compared with the mean intensities of dots generated with the heat extract of *C. albicans* (●). Bars indicated standard deviation.



Using the results of the dot blot test, the percentage of the serum samples that reacted with each yeast species was tabulated (Fig. 3, upper panel). Almost all the serum samples (99%) react with the heat extract from *C. albicans*. This was expected because the serum samples were selected on the basis of their positive reaction for IgE antibodies against *C. albicans* in the CAP system. Interestingly, only 2% of the serum samples reacted with only the *C. albicans* heat extract, and high percentages of them reacted with the heat extracts prepared from *C. guilliermondii* (87.3%), *C. tropicalis* (85.0%) and *R. rubra* (91.5%) (Fig. 3, upper panel, C, H, and L, respectively). These percentages were close to that for *C. albicans*. The binding of IgE antibodies with the heat extracts of *C. glabrata*, *C. kefyr*, *Cr. albidus*, *Cr. laurentii*, *R. glutinis* and *S. cerevisiae* were also found in 49.0, 42.0, 38.7, 25.4, 38.6 and 22.6% of 349 patients, respectively.

The intensity (arbitrary units) of dots generated for each of the heat extracts were measured by a Digital Image System IS1000 and the mean intensities of dots for yeast species were compared with the degree of the reaction of IgE antibodies with each of the yeast species. The mean intensities of dots are shown in Fig. 3 (lower panel). The mean intensity of *C. krusei* (432.4 ± 578.3) was rather higher than those of *C. albicans* (353.4 ± 326.3) or *R. rubra* (384.4 ± 601.4), in spite of the fact that the number of sera that reacted with *C. krusei* was lower than the number of sera that reacted with either of the other species. The mean intensities of the other yeast species are 175.6 ± 243.1 (*C. glabrata*), 282.6 ± 401.8 (*C. guilliermondii*), 170.0 ± 248.2 (*C. kefyr*), 305.5 ± 310.3 (*C. parapsilosis*), 148.4 ± 195.1 (*C. stellatoidea*), 272.0 ± 299.7 (*C. tropicalis*), 175.2 ± 411.4 (*Cr. albidus*), 133.4 ± 344.9 (*Cr. laurentii*), 149.2 ± 248.1

(*R. glutinis*) and 84.2 ± 118.0 (*S. cerevisiae*) (Fig. 3, lower panel).

The sera that reacted with only the *C. albicans* heat extract were found in the sera of only seven out of sera of 349 patients (2.0%), and the other sera reacted with more than two yeast species. The mean number of the yeast species that serum samples reacted with was 8.53. Seventy-two per cent of the sera tested reacted with more than eight yeast species, and 11.7% (41 sera) of the sera reacted with all 13 species (Fig. 4). To determine whether there was a correlation between the number of yeast species that a serum sample reacted with and the serum sample's



Fig. 5. Inhibition dot blot test to test crossreactivity of *C. albicans* with other yeast species. Pooled serum, which was prepared from 28 patients whose sera reacted with all yeast species in the dot blot test, was pretreated with 250 µg/mL of the mannoprotein fraction (CAMP/A) of *C. albicans* and then reacted with each of the yeast heat extracts. As compared with the control group in which the pooled serum was not pretreated with CAMP/A (buffer only) (upper), the bindings of IgE antibodies with each heat extract were strongly inhibited after pretreatment with CAMP/A (lower). Slight reactions of IgE antibodies were noted in the heat extracts of *C. krusei*, *C. parapsilosis*, *C. tropicalis*, *Cr. albidus*, *Cr. laurentii* and *R. rubra* at this concentration of CAMP/A. The protein complex (see Materials and methods) had no effect at the same concentration (data not shown).

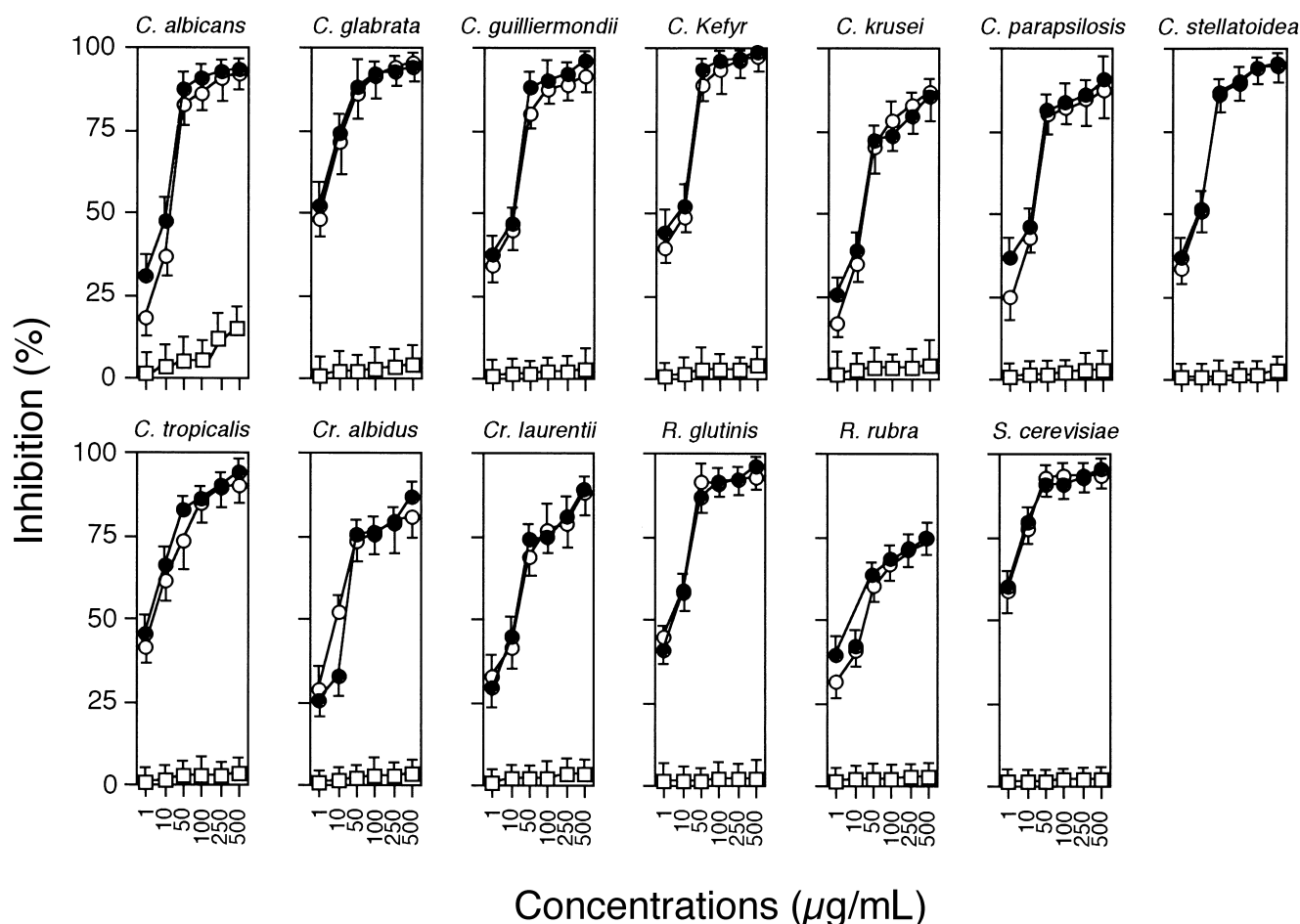


Fig. 6. The ability of protein and mannoprotein fractions of *C. albicans* to inhibit the subsequent binding of IgE antibodies of the pooled serum with each heat extract. Each heat extract was blotted on a NC sheet and the sheet was incubated with pooled serum that had been pretreated with various concentrations (1–500 μg/mL) of the *C. albicans* antigens. The binding of IgE antibodies to each of the heat extracts was inhibited dose-dependently after pretreatment with mannoprotein fractions (● for CAMP/A, ○ for CAMP-SA, respectively) but binding was not inhibited after pretreatment with protein complex (□). The degrees of inhibition by the mannoprotein fractions on the binding of IgE antibodies to heat extracts were lower for the heat extracts of *C. krusei*, *Cr. albidus*, *Cr. laurentii* and *R. rubra* than for the heat extracts of the other species. Bars indicate standard deviation.

fluorescence intensity, the mean fluorescence intensities of all serum samples reacting with a given number of yeast species was determined and plotted (as an IgE concentration) in Fig. 4. The results showed that sera with higher fluorescence intensities reacted with more yeast species. For example, for sera reacting with seven yeast species, the mean IgE concentration was 11.00 ± 7.96 kUa/L and for sera reacting with 13 species, it was 50.23 ± 29.80 kUa/L. Results similar to the fluorescence intensities were obtained when the mean intensities of dots generated for the *C. albicans* heat extract in the dot blot test were similarly plotted (Fig. 4). That is, sera that reacted with more yeast species had higher dot intensities. These results indicate that sera which reacted strongly with the *C.*

albicans antigens tended to react with a wider number of other yeast species.

Evidence that C. albicans mannoproteins are responsible for crossreactivity among the yeast species

To determine whether the results of the dot blot test were due to IgE antibodies that are specific to each species or to IgE antibodies that bind to common antigenic substances among the yeast species tested, we performed an inhibition dot blot test. Twenty-eight serum samples that reacted with all heat extracts in the dot blot test were pooled and pretreated with 0 to 500 μg/mL CAMP/A, CAMP-S/A or

protein complex. These serum samples were then allowed to react with each of the heat extracts. If the binding of IgE antibodies with multiple yeast species was generated antigenic components that shared by *C. albicans*, subsequent IgE binding of the pooled serum to the heat extracts should be inhibited by pretreatment with any fraction of the *C. albicans* antigens. The results of the inhibition dot blot test showed that binding of IgE antibodies to each of the heat extracts was strongly inhibited after pretreatment with the mannoprotein fraction (CAMP/A) and that slight reactions of IgE antibodies were shown in the heat extracts of *C. krusei*, *Cr. albidus*, *Cr. laurentii*, *R. rubra*. (Fig. 5). The intensities of dots generated for each heat extract in the inhibition dot blot test were measured to compare the degree of inhibition to IgE-binding (Fig. 6). The binding of IgE antibodies to heat extracts of *C. albicans* was dose-dependently inhibited by pretreatment with CAMP/A and CAMP-S/A (Fig. 6). Results similar to the inhibition of the heat extract of *C. albicans* were observed in the other yeast species when we tested the ability of the *C. albicans* mannoproteins to inhibit the binding with other yeast species. It was noted that the degree by which both mannoprotein fractions inhibited the reaction with *C. krusei*, *Cr. albidus*, *Cr. laurentii* and *R. rubra* was slightly lower as compared with those to the other species. No differences in the ability of CAMP/A and CAMP-S/A to inhibit binding of IgE antibodies to the heat extracts was found. In contrast, although the protein complex fractionated from *C. albicans* slightly inhibited the binding of IgE antibodies to the heat extract of *C. albicans*, it did not clearly inhibit IgE binding to the other yeast species at any concentration (Fig. 6).

Discussion

The present study demonstrated evidence that the *C. albicans* mannoproteins crossreact with the other *Candida* species and with the airborne yeast species *Cryptococcus* and *Rhodotorula*, at the IgE level.

The dot blot test, which was used to determine IgE antibodies in the serum samples against the various yeast antigens, showed that the sera of most of the patients with allergies, who appeared from the results of the CAP system to be positive for IgE antibodies to *C. albicans*, also reacted with more than two yeast species (Fig. 4). This was greater than we expected. Of 349 serum samples obtained from allergy patients, 11.7% reacted with the heat extracts prepared from 13 yeast species. The number of yeast species to which the IgE antibodies bound was correlated with the fluorescence intensities and with the dot intensities generated for the *C. albicans* heat extract (Fig. 4). The sera that we used in this study ranged from class 3 to class 6 for *C. albicans* in the CAP system. When we used sera of allergy patients whose class were less than 2, no binding IgE

antibodies in the dot blot test was observed either in *C. albicans* or in other yeast species (data not shown). Therefore, the degree of the reaction of IgE antibodies with the *C. albicans* antigen is positively correlated with the number of other yeast species that the IgE antibodies bind to. The kinds of allergic disease and the ages of patients may affect the number of other yeast species that the IgE antibodies will bind to. It has been reported that IgE antibodies are more frequently found in atopic disease than they are in other allergies [9]. Recently, Nermes *et al.* [11] reported that production of IgE antibodies to the *C. albicans* mannans were similar in atopic and healthy infants and children. Our study was done on a limited number of patients with allergies who were judged to be positive for IgE antibodies against *C. albicans* from the CAP system, and our information on the patients' sera was, unfortunately, limited to the values of fluorescence intensities to the *C. albicans* antigen. To obtain more details of the extent of cross-reaction of *C. albicans* antigen with other yeast species, the kinds of allergies and the ages of patients should be controlled.

The binding of the serum samples to multiple yeast species as shown by the dot blot test leads to three possibilities. The first possibility is that sera of these patients contain IgE antibodies specific for each of the yeast species used. This means, for example, if we accept this possibility, it shows the result that the 11.7% of the patients whose sera reacted with all the yeast species had been exposed to all species tested and had IgE antibodies specific for each species that should react independently with the heat extract of *C. albicans*. The second possibility is that the patients' sera contain IgE antibodies produced against component(s) that are common to each of the yeast species. The last possibility is that both types of IgE antibodies are present in the patients' sera.

The results of the inhibition dot blot test ruled out the first possibility and strongly suggested the second possibility since the binding of IgE antibodies with yeast species was inhibited by the *C. albicans* antigens (Fig. 6). Furthermore, the inhibition dot blot test indicated that the *C. albicans* mannoproteins are responsible for the crossreactivity among yeast species as they are common antigenic components. The ability of CAMP-S/A to inhibit binding of IgE antibodies to each heat extract was similar to that of CAMP/A, suggesting that crossreacting site(s) that are responsible for the IgE antibodies are located in the acid-stable part of the *C. albicans* mannoproteins. A crossreactivity at the IgE level was also found between *C. albicans* and *Pityrosporum ovale* and was shown to be to mannans [20,21]. Furthermore, in the crossreaction of *C. albicans* with *S. cerevisiae*, mannan is known to be a crossreacting component [17,18]. In our study, 22.6% of the sera tested reacted with *S. cerevisiae* and the binding of IgE antibodies to the heat extract of *S.*

cerevisiae was completely inhibited by mannoproteins of *C. albicans*. This indicates that mannoproteins of *C. albicans* crossreact with those of *S. cerevisiae*. Recently, Ito *et al.* reported that *C. albicans* enolase, a major protein allergen of *C. albicans*, also shares crossreacting epitopes of *S. cerevisiae* enolase [22].

As described above, most of the sera which reacted strongly with the *C. albicans* antigens tended to react with a wider number of other yeast species, depending on titre of IgE antibodies for *C. albicans* antigen (Fig. 4). However, in the inhibition dot blot test, a slight binding of IgE antibodies with the heat extracts of *C. krusei*, *Cr. albidus* and *R. rubra* was found and was remained after pretreatment of the pooled serum with higher concentrations of both CAMP/A and CAMP-S/A. Furthermore, the comparative study of the mean intensities of the dots showed that the mean intensities for *C. krusei* and *R. rubra* were higher than that of *C. albicans* (Fig. 3), indicating that the reaction of IgE antibodies with *C. krusei* and *R. rubra* was stronger than that with *C. albicans*. These results may suggest the third possibility concerning the multiple binding among yeast species in which both species-specific and common IgE antibodies are present in the patients' sera, rather than the extent of crossreactivity of *C. albicans* with other yeast species depend simply on the titre of IgE antibodies for *C. albicans*. We have shown that IgE antibodies specific for *R. rubra*, which were not inhibited completely by any *C. albicans* antigens, were present in some patients with allergies who were positive for IgE antibodies against the *C. albicans* antigen (manuscript in preparation).

We showed that the sera of most allergic patients who are positive for IgE antibodies to *C. albicans* react with a variety of yeast species. This result makes it difficult to understand the actual antigen(s) in the allergic patients if the multiple binding is due to common epitopes. What is the actual yeast species that produces IgE antibodies in each patient? *C. albicans* and *R. rubra* are the most likely candidates for the actual antigenic species among the yeast species tested in this study, because *C. albicans* is a commensal yeast in humans, and *R. rubra* is the most frequently isolated fungus in our environment [28,29]. Therefore, we have been most frequently exposed to these fungus. The study has demonstrated the crossreactivity among several yeast species in *in vitro* immunoblot tests. However, we have not tested the extent of the crossreactivity of the mannoproteins *in vivo* among these yeast species. The ability of mannoproteins of these yeast species to induce a skin reaction and the ability of *C. albicans* to inhibit the skin reaction should be tested as the next step for studying common fungal allergens.

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