

Allergen cross-reactivity between *Pityrosporum orbiculare* and *Candida albicans*

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Pityrosporum orbiculare and *Candida albicans* extracts were separated by SDS–PAGE, and IgE binding was detected by immunoblotting with 21 patient sera that were RAST positive to both yeasts. Cross-wise inhibition was performed of IgE binding of a serum pool containing IgE antibodies to both yeasts. The pool was mixed with serial dilutions of *P. orbiculare* or *C. albicans* extracts, and incubated with strips containing separated allergen. IgE binding was quantified by densitometric scanning and percent inhibition was calculated as well as the respective ratios between required extract concentration for 50% inhibition in heterologous compared to homologous inhibition for each component (inhibition ratio). Ten components of *P. orbiculare* were detected by more than 60% of the sera. IgE binding to *C. albicans* was weak, and only to four bands was IgE binding detected by more than 30% of the sera. The most important *C. albicans* allergen was a 48-kDa band, to which IgE of half of the patient sera bound. There was little inhibition of IgE binding to *P. orbiculare* with *C. albicans*. Thus, all but three components exhibited an inhibition ratio higher than 100. The inhibition ratio of the 48-kDa *C. albicans* compound was 50, thus indicating some degree of cross-reactivity. Significant cross-reactivity was shown by *C. albicans* compounds of 18, 24, 26, 34, and 38 kDa, the inhibition ratios of which were less than 10. There was some degree of cross-reactivity between apparent protein allergens of the two yeasts, but IgE antibodies to *C. albicans* do not merely reflect sensitization to *P. orbiculare*.

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The lipophilic yeast *Pityrosporum orbiculare* is not only part of the normal human skin flora but also an opportunistic pathogen (6, 8, 26). *P. orbiculare* is probably identical with *P. ovale* although different in morphology (9). Various recent reports have discussed atopic allergy to *P. orbiculare* as a pathogenic factor in atopic dermatitis (1, 13, 19, 23, 24, 27, 34, 35, 37). The concept gains support from results showing a strong relationship between eczema and the occurrence of serum IgE antibodies to *P. orbiculare*, and one study showed clinical improvement with antimycotic treatment in patients with "head-neck" dermatitis and type I hypersensitivity to this fungus (4). *Candida albicans*, unlike *P. orbiculare*, does not normally colonize the skin, but rather the gastrointestinal tract and the nasopharynx (21). A pathogenic role for allergy to *C. albicans* has for long

been proposed, particularly in asthma and rhinitis (11, 12, 18), and a high rate of positive immediate skin test to *C. albicans* has been observed in atopic patients (2, 3, 17).

Thus, the two yeasts have been implicated in different atopic diseases despite their close taxonomic relationship. In previous studies, we found a correlation between IgE antibodies to *P. orbiculare* and *C. albicans* in atopic children (23). Allergenic cross-reactivity between *P. orbiculare* and *C. albicans* has recently been investigated and found to be significant (5, 28). IgE reactivity to *C. albicans* was even suggested mainly to reflect IgE antibodies to *P. orbiculare* (5). The present study investigated in detail a possible allergenic cross-reactivity between *P. orbiculare* and *C. albicans* by an immunoblotting inhibition technique.

Material and methods

Patient and control sera

Among sera from atopic patients sent to the Department of Clinical Immunology at the Karolinska Hospital (Stockholm, Sweden) for routine IgE antibody analysis from September 1989 to March 1992, 120 were selected on the basis of a positive routine analysis for IgE antibodies to both *P. orbiculare* and *C. albicans* with Phadebas RAST. Twenty-one of the sera which were RAST positive to both fungi (RAST class ≥ 2 ; ≥ 0.7 PRU/ml) and of which sufficient volumes were available were chosen for further study. All of the patients who had donated the sera suffered from atopic eczema. Eight of them were males and 13 females, and their age range was 8–38 years. The total IgE range of these sera was 610–30 200 kU/l, and two of them were above 10 000 kU/l.

In a separate set of experiments, the sera from four children with atopic eczema and IgE binding to *C. albicans* restricted to the high molecular weight (MW) diffuse staining characteristic of mannans (30, 31) were pooled and used for inhibition as described below. These sera were obtained from a separate patient material of 114 children whose sera were analyzed for content of IgE antibodies to *C. albicans* by SDS–PAGE and immunoblotting (X. Huang et al., paper in preparation). Three types of control sera were used in the studies. Eight sera, which were also obtained from the routine laboratory analysis (see above) of atopic patients with total IgE > 1000 kU/l, and another three sera from healthy adult blood donors were included after being proven negative in RAST test to both *P. orbiculare* and *C. albicans*. Furthermore, four different concentrations of myeloma IgE, 1000, 5000, 10 000, and 20 000 kU/l, were used.

Yeast extracts

P. orbiculare extract. An isolate of *P. orbiculare* (no. 42132) purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA) was grown for 4 days at 37°C on a special glucose-neopeptone agar containing olive oil (2%), glycerol monostearate (2.5 g/l), and polysorbate 80 (Tween 80) (0.2%) except that no yeast extract was added to the substrate (7). The *P. orbiculare* cells were carefully harvested from the surface of the solid agar medium, and a thick yeast cell suspension was obtained in sterile distilled water. After freeze-drying, the cells were resuspended in 0.05 M phosphate buffered saline (PBS) with 0.14 M NaCl and 0.02% NaN₃ (pH 7.4) to a concentration of 1/10 (w/v). The cell suspension was sonicated for 5 min at amplitude 10 on ice and extracted at 4°C overnight. The extract was

centrifuged at 38 000 g for 10 min. The supernatant was filtered through an 0.8-µm Millex-AA filter (Millipore SA 67 Molsheim, France), desalted on a PD-10 column (Sephadex G-25M) (Pharmacia LKB Biotechnology, Uppsala, Sweden), lyophilized, and stored at –20°C.

C. albicans extract. *C. albicans* (CBS 562) from Centraalbureau voor Schimmelcultures (Delft, The Netherlands) was cultivated at 37°C for 4 days on Saboraud dextrose agar with neither yeast extract nor antibiotics in the substrate. The cells were harvested, extracted, lyophilized, and stored as described above.

The protein concentrations in the extracts were measured with BCA Protein Assay Reagent (Pierce Chemical Company, Rockford, IL, USA) according to the manufacturer's instructions. The amounts of protein in the lyophilized extracts of *P. orbiculare* and *C. albicans* were 0.30 and 0.35 mg/mg dry wt., respectively.

Preparation of RAST disks

Guided by allergen titrations, the *C. albicans* extract was coupled to cyanbromide-activated paper disks with 100 µg dry weight of *C. albicans* extract per disk (16, 36). *P. orbiculare* RAST disks (batch no. 9208) prepared as described earlier (23) were kindly provided by MIAB (Knivsta, Uppsala, Sweden).

Radioallergosorbent test (RAST)

Serum IgE antibodies were assayed by Phadebas RAST (16, 36). A volume of 50 µl of undiluted serum was incubated in duplicate with the respective disks for 3 h, washed three times in PBS containing 0.1% Tween 20, and subsequently incubated overnight with 50 µl of rabbit ¹²⁵I-labeled anti-IgE (Kabi-Pharmacia Diagnostics AB). After washing, the count rates were measured in a gamma-counter. The results were expressed in relation to a reference serum in Phadebas RAST units (PRU/ml).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE)

SDS–PAGE was run on a Mini-Protein Dual Slab cell (Bio-Rad, Richmond, CA, USA), at 200 V with a gradual drop of the electric current from 70 to 40 mA for 50 min, in Tris-glycine buffer containing 25 mM Tris, 192 mM glycine, and 0.1% SDS (pH 8.3). MW standards were run in parallel using low MW proteins (LMW, electrophoresis calibration kits, Pharmacia). The lowest amounts of the *P. orbiculare* and *C. albicans* extracts, which in preliminary titrations had given clear IgE binding, approxi-

mately 25 and 50 µg dry weight/mm gel, respectively, were applied to the gels. The extracts were dissolved in PBS containing 2% (w/v) SDS, 5% β-mercaptoethanol (β-ME), and 8% glycerol, 0.62% (w/v) bromophenol blue as tracking dye. The sample mixtures were boiled at 100°C for 5 min, and applied to an SDS–polyacrylamide (PAA) gradient gel (7.5–20%) in 68-mm wells.

Electrophoretic transfer and immunologic detection

After electrophoresis, the separated yeast components and the standards were transferred electrophoretically to a nitrocellulose (NC) membrane (pore size 0.45 µm, Bio-Rad) in a Mini-Trans-Blot cell (Bio-Rad). Before transfer, the gel was equilibrated for at least 20 min in precooled (4°C) blotting buffer containing 25 mM Tris, 192 mM glycine, and 20% (v/v) methanol (pH 8.6). The transfer was run for 1 h at 100 V with a gradual increase of the electric current from 200 to 210 mA. Protein binding sites still available on the NC membrane after completed transfer were blocked by incubation for 1 h at 37°C with 0.5% Tween 20 in 0.05 M PBS (pH 7.4) containing 0.15 M NaCl, 0.2% bovine serum albumin, and 0.02% NaN₃. The NC membrane with the reference LMW proteins was stained with AuroDye forte (Olen, Janssen, Belgium).

The NC membrane with blotted yeast components was cut into 3-mm-wide strips and incubated overnight at room temperature with 500 µl of the individual patient sera diluted 1/6 in the *P. orbiculare* and 1/3 in the *C. albicans* experiments, respectively. After washing three times, the strips were incubated for 2 h with 500 µl rabbit antihuman IgE (1:2000) (MIAB) and subsequently after washing incubated with a goat antirabbit IgG alkaline phosphatase conjugate (Bio-Rad) for 2 h. The strips were then washed and IgE-binding compounds were visualized with an alkaline phosphatase conjugate substrate kit (Bio-Rad) which contained 5-bromo-4-chloro-3-indolyl phosphate and nitroblue-tetrazolium. The MW of the respective IgE-binding compounds were determined by comparison of their relative mobilities with those of the AuroDye-stained LMW protein.

Inhibition experiments

The immunoblot multiallergen inhibition experiments were performed essentially as described by Johansson et al. (14), by incubating strips blotted with *P. orbiculare* and *C. albicans* with a mixture of 250 µl of a diluted pooled serum containing IgE to most of the important allergenic compounds of both yeasts (see below) and 250 µl of different concentrations of yeast extract. The latter were prepared by serial dilution of extract in buffer (PBS with 0.5% Tween

20). The utilized amounts of *P. orbiculare* were 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, and 30 mg (dry wt.); of *C. albicans*, 0.0003, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1, and 3 mg (dry wt.) for the homologous, and 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, and 100 mg (dry wt.) for the heterologous inhibition experiment. The serum pool was prediluted 1/6 for the *P. orbiculare* and 1/1.5 in the *C. albicans* experiments. Reference strips were incubated with the serum pool and buffer solution alone.

Bovine serum albumin, β-lactoglobulin, and egg albumin at concentrations of 7–200 mg/ml were used as controls for nonspecific effects of high protein concentrations in the inhibition experiments.

Densitometric scanning of IgE-binding components and calculation of inhibition results

The intensity of the stained yeast components was scanned with a Video Densitometer Model 620 (Bio-Rad). A diagram with the amplitude of the absorbency versus position was obtained for each strip, subtracting the background obtained with the buffer. A large majority of the stained bands visible on the strips was detectable by the densitometer. Optical densities (OD) ≥ 0.41, 0.40–0.11, 0.10–0.01 and < 0.01 were arbitrarily classified as strong, intermediate, weak, and negative, respectively, after subtraction of nonspecific binding corresponding to that of myeloma IgE. The results of the inhibition experiments were expressed as percent inhibition. For all components, the amount of extract needed for heterologous and homologous 50% inhibition and the respective ratios between the two (inhibition ratios) were calculated.

Statistics

Correlations between antibody levels were calculated with Spearman's rank test and differences between levels with Wilcoxon's matched-pairs signed-rank test.

Results

Eighty-three of 120 sera were positive to both *P. orbiculare* and *C. albicans* (RAST class ≥ 2) (≥ 0.7 PRU/ml), and another 15 sera exhibited IgE binding to *P. orbiculare* corresponding to RAST class ≥ 2, and RAST class 1 (≥ 0.35; < 0.7 PRU/ml) to *C. albicans*. There was a rough correlation between the IgE antibody levels to the two yeasts ($r = 0.52$), and those to *P. orbiculare* were significantly higher ($P < 0.001$) than those to *C. albicans*.

In immunoblotting of *P. orbiculare*, at least 18 IgE-binding components were detected (Fig. 1A). These bands ranged between 14 and 90 kDa. In *C.*

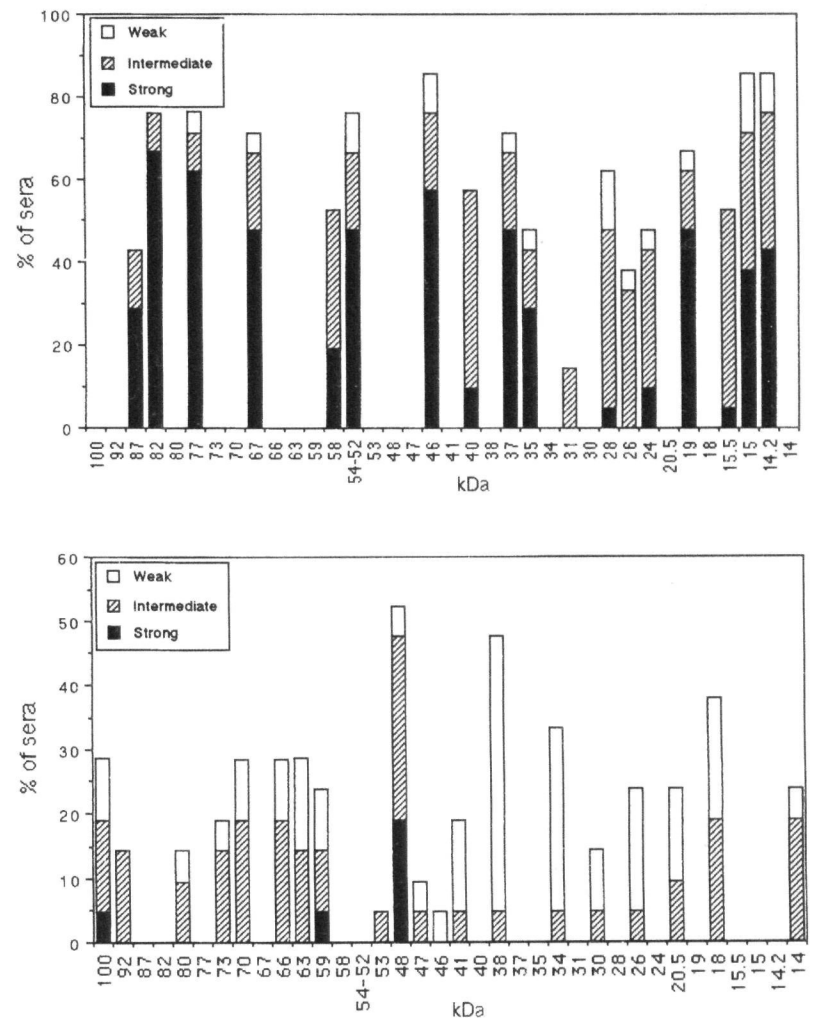
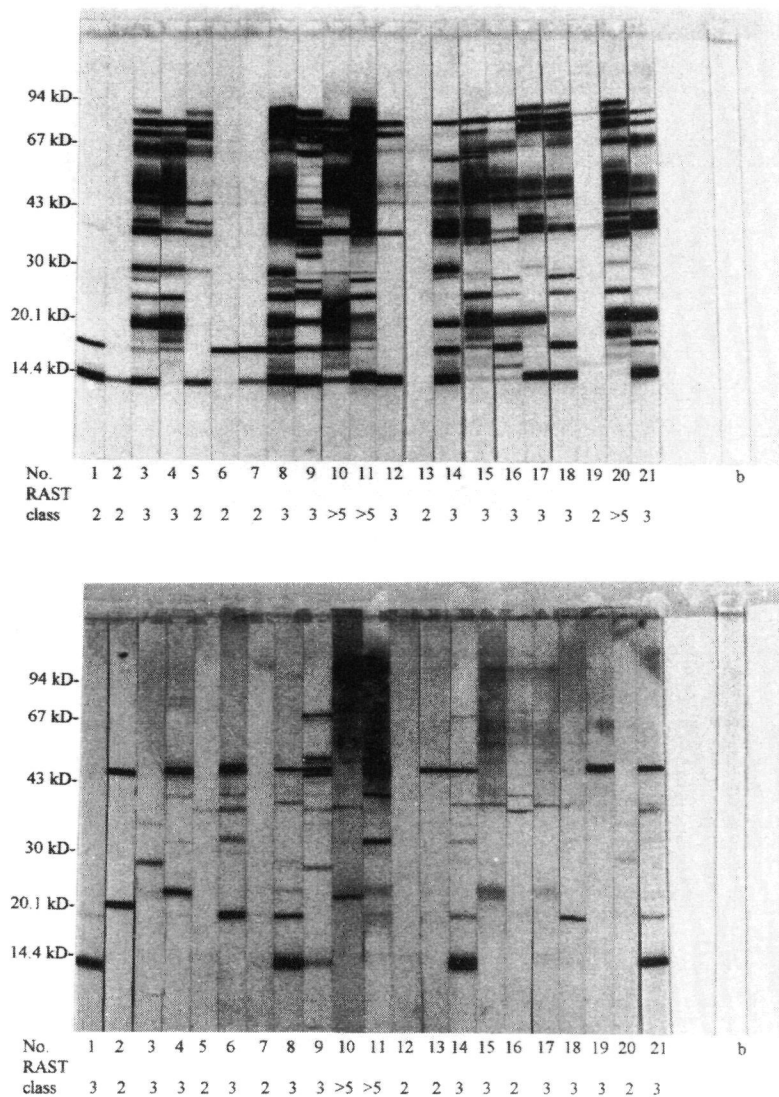


Fig. 2A. Allergograms of IgE binding to *P. orbiculare* and (B) to *C. albicans* with 21 patient sera semiquantified after scanning (for details, see text).

Fig. 1A. IgE binding to *P. orbiculare* and (B) to *C. albicans* components in immunoblotting experiments with 21 patient sera found to be RAST-positive to both *P. orbiculare* and *C. albicans*. b: buffer control.

albicans at least 20 IgE-binding components were found, ranging between 14 and 100 kDa (Fig. 1B). In Fig. 2, the frequency and semiquantified intensity of staining reflecting binding of IgE to the various components of *P. orbiculare* and *C. albicans* are depicted. IgE binding by at least 60% of the 21 analyzed sera were found to 10 of the *P. orbiculare* components. Only one of the *C. albicans* components, the 48-kDa band, was recognized by >50% of the sera and the 38-kDa component was recognized by 10 sera but with low intensity of staining. A diffuse staining was observed in the high MW area corresponding to *Candida* mannans with four of the sera (nos. 10, 11, 15, and 18) in the *C. albicans* experiments, but no corresponding finding was seen in the *P. orbiculare* strips.

Little nonspecific binding was observed with the different concentrations of myeloma protein (data not shown). Only with 20 000 kU/l of myeloma IgE was a low degree of staining, below OD 0.02, observed to the 48-, 38-, and 14-kDa components of *C. albicans*. In *P. orbiculare*, the 46- and 35-kDa bands were observed with an OD of 0.02 with 10 000 kU/l and 0.05 with 20 000 kU/l of myeloma IgE.

Scanty binding was also observed to *C. albicans* of the RAST-negative patient and blood donor controls, two of which exhibited diffuse staining in the high MW range (*Candida* mannans) and one to the 38-kDa component. None of these latter control sera exhibited any visible binding to *P. orbiculare*.

According to the pattern of IgE reactivity, a serum pool that held IgE antibodies to most of the important allergens of both *P. orbiculare* and *C. albicans* was made of equal parts of sera nos. 8 and 14 and used in the inhibition experiments.

In the homologous *P. orbiculare* experiment (Fig. 3A), there was considerable inhibition already with the lowest amount of inhibitor, 0.001 mg dry wt., corresponding to a concentration of only 0.002 mg/ml in the experiment. Fifty percent inhibition was reached with low amounts of inhibitor for all components below 60 kDa. To achieve 50% inhibition of the components above that level, considerably higher amounts of extract were required (Table 1). When *C. albicans* was used as inhibitor of IgE binding to *P. orbiculare*, high extract concentrations were required for 50% inhibition, i.e., there was only a very low degree of inhibition (Fig. 3B and Table 1). The relationships between required extract concentrations for heterologous/homologous inhibition (inhibition ratios) were thus very high. Thus, the

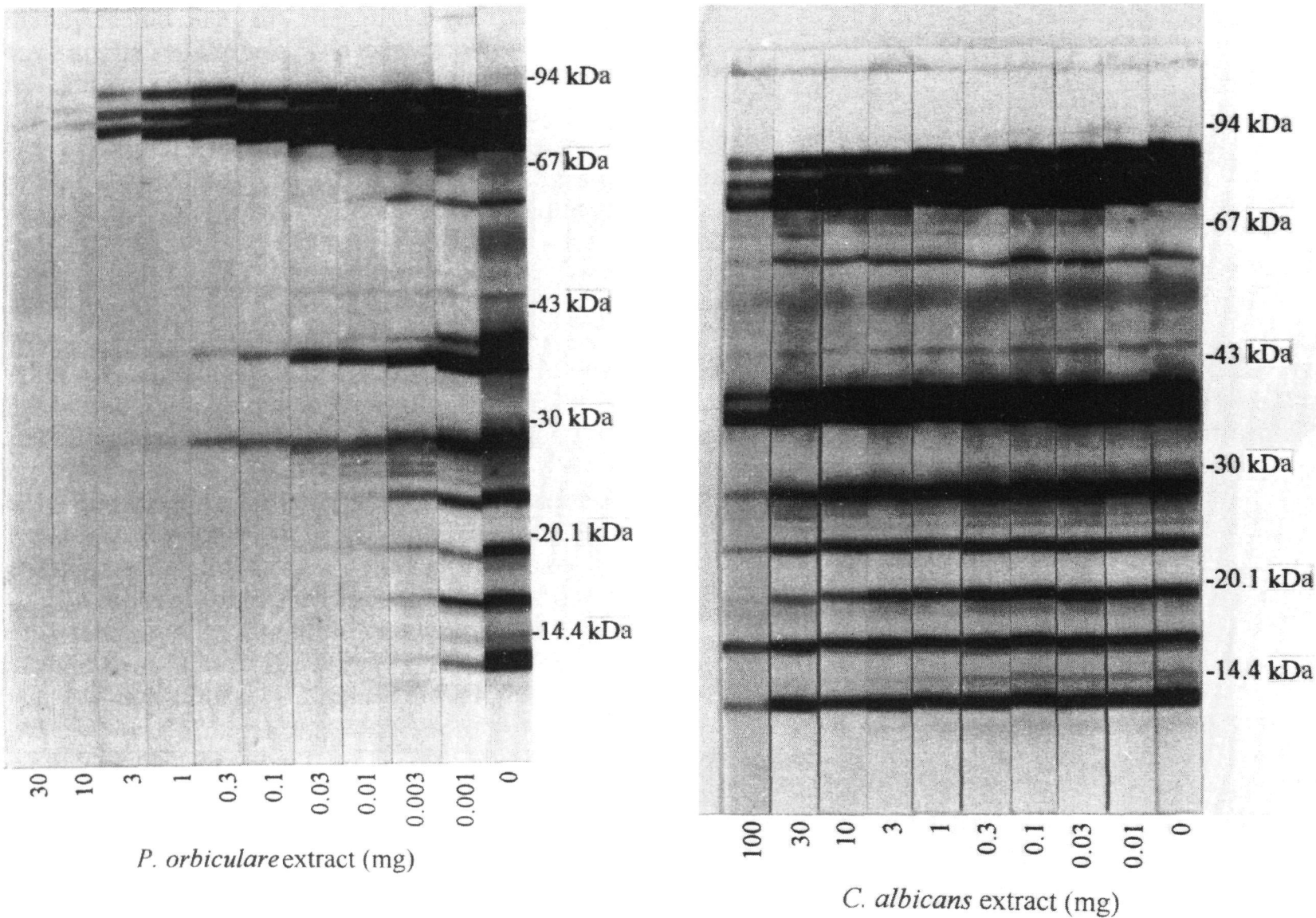


Fig. 3. IgE binding to components of *P. orbiculare* inhibited in A with *P. orbiculare* allergen extract (homologous experiment) and in B with *C. albicans* allergen extract (heterologous experiment).

Table 1. Amounts of inhibiting extract required for 50% inhibition of binding to *P. orbiculare* in homologous and heterologous experiments and respective ratios between them (inhibition ratio)

P.o** MW (kDa)	Homologous extract P.o** (d.w. #) (mg)	Heterologous extract C.a* (d.w. #) (mg)	Heterol/homol (inhibition ratio)
87	0.92	72	80
82	0.81	67	80
77	2.1	83	40
67	0.017	> 100	> 6000
58	0.003	81	30 000
54–52	< 0.001	79	> 80 000
46	< 0.001	55	> 50 000
40	< 0.001	79	> 80 000
37	0.005	84	20 000
35	0.001	No	
28	0.035	30	1000
26	0.003	3	1000
24	0.002	34	20 000
19	< 0.001	33	> 30 000
15.5	< 0.001	30	> 30 000
15	0.0012	86	70 000
Between	< 0.001	1	> 1000
14.2	< 0.001	0.25	> 250

* C.a: *Candida albicans*; ** P.o: *Pityrosporum orbiculare*; # d.w.: dry weight.

inhibition ratio for the important 46-kDa *P. orbiculare* allergen was 50 000. However, in the case of the high MW *P. orbiculare* components, there was detectable heterologous inhibition only with 40–80 times higher extract concentration than with the homologous inhibitor.

The homologous *C. albicans* inhibition experiment gave results similar to that with *P. orbiculare* (Fig. 4 and Table 2), and inhibition was readily obtained at low concentrations of the *C. albicans* extract. In the heterologous experiment, considerable inhibition was obtained with *P. orbiculare* extract to some of the *C. albicans* components. Thus, binding to the 38-kDa component and an adjacent component of 37 kDa, which was detected only in the experiments with the serum pool, was even more readily inhibited by *P. orbiculare* than with *C. albicans*. Fifty percent inhibition was obtained with the same extract concentration of the heterologous as of the homologous inhibitor to the 26- and 34-kDa *C. albicans* components, and 50 percent inhibition to the 18- and 24-kDa allergen required only six times more of the homologous inhibitor (Table 2). However, heterologous inhibition of IgE binding to the important

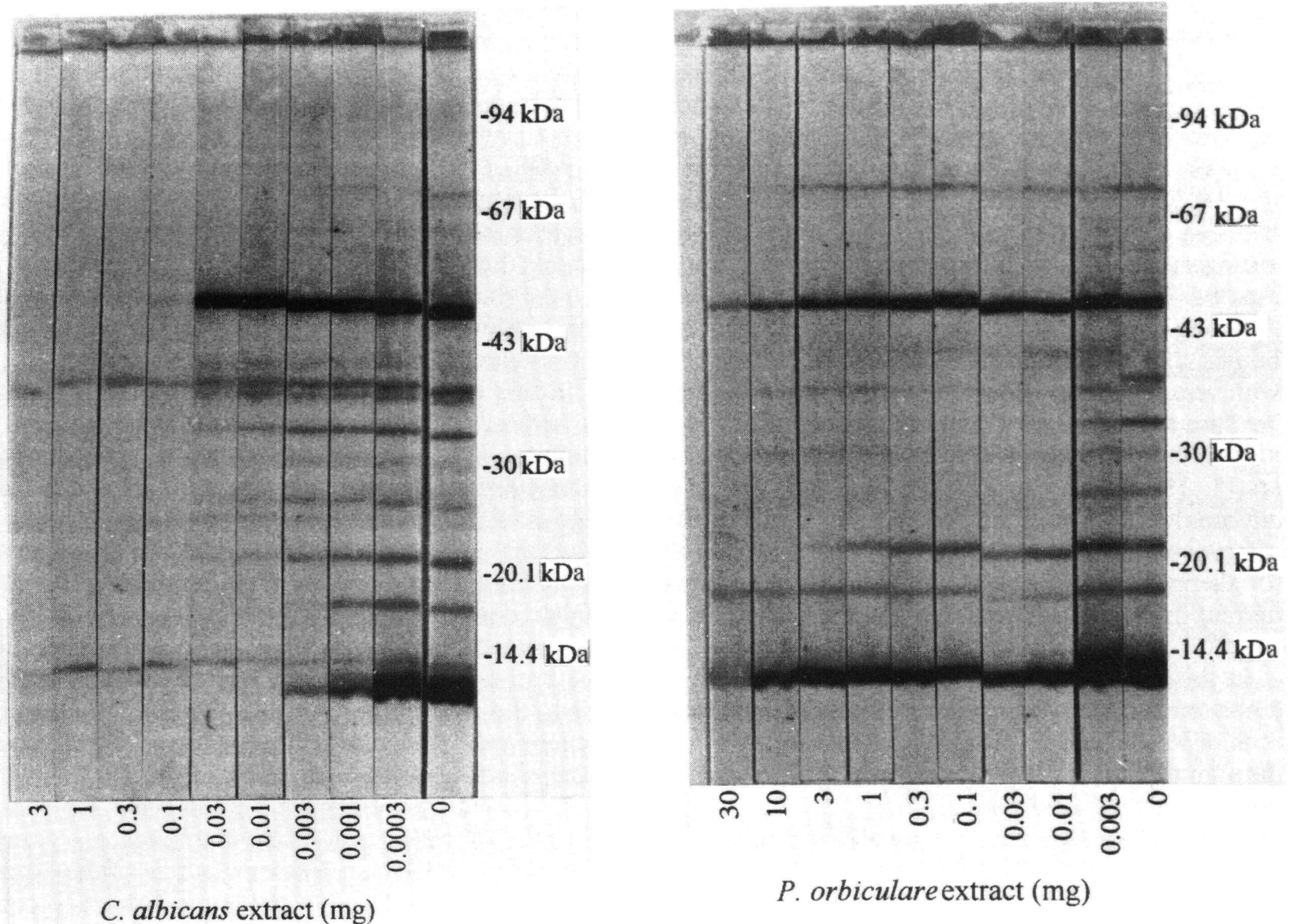


Fig. 4. Inhibition of IgE binding to components of *C. albicans* inhibited in A with *C. albicans* allergen extract (homologous experiment) and in B with *P. orbiculare* allergen extract (heterologous experiment).

Table 2. Amounts of inhibiting extract required for 50% inhibition of binding to *C. albicans* in homologous and heterologous experiments and respective ratios between them (inhibition ratio)

C.a* MW (kDa)	Homologous extract C.a* (d.w. #) (mg)	Heterologous extract P.o.** (d.w. #) (mg)	Heterol/homol (inhibition ratio)
70	0.00065	0.55	1000
48	0.054	2.60	50
38	0.025	<0.003	<0.1
37	0.015	0.0074	0.5
34	0.004	<0.003	<1
30	0.0006	1	2000
26	0.0065	0.006	1
24	0.001	0.006	6
20.5	0.0008	0.77	1000
18	0.0013	0.0074	6
14.2	0.0001	0.0088	90
14	0.0006	0.023	40

* C.a: *Candida albicans*; ** P.o: *Pityrosporum orbiculare*; # d.w.: dry weight.

48-kDa component of *C. albicans* required as much as 50 times more of the heterologous inhibitor.

Separate experiments were performed with another pool of four children's sera (see Material and methods), which exhibited IgE binding only to a

diffuse allergen in the high MW region of *C. albicans* (*Candida* mannans). In these experiments, there was but little inhibition by the *P. orbiculare* extract of the IgE binding to *Candida* mannans, whereas the binding was inhibited by *C. albicans* extract at low concentration. The inhibition ratio of these experiments was 1000.

The control experiments with bovine serum albumin, β -lactoglobulin, and egg albumin exhibited little inhibition at protein concentrations of 20 mg/ml. In none of these control experiments was 50% inhibition reached even at a very high protein concentration (200 mg/ml).

Discussion

Atopic sensitization to fungi is not uncommon among children with atopic diseases and this is true also of atopic adults (10, 22, 25). Patients with sensitivity to fungi are frequently highly atopic, both in the sense that their disease manifestations are severe and that their pattern of IgE reactivity is broad and may include a multitude of different fungal species, inhalant molds in particular. Thus, in a previous study of

sensitization to fungi, we found that of 47 children with IgE antibodies to one or several of 16 fungal species, 15 exhibited IgE antibodies to 10 different species or more (22). In an extension of the study, we found IgE antibodies to *P. orbiculare* almost exclusively in children with some other fungal sensitivity (23). There was also a very strong relationship between sensitization to fungi and eczema, and the strongest relationship to eczema among IgE positivites was afforded by positive RAST to *P. orbiculare*. A strong relationship between eczema and IgE antibodies to *P. orbiculare* was found also in patients with very high total IgE and severe atopy (24). Similar data suggestive of a pathogenic role of *P. orbiculare* in atopic eczema have been published by others (1, 19, 27, 34, 35, 37). On the other hand, *C. albicans* has long been implicated in the pathogenesis of bronchial asthma (11, 12, 17, 18). Recently, a role for allergy to *C. albicans* in adult patients with atopic dermatitis has been discussed by Savolainen et al. (29).

In this study, IgE binding was generally considerably stronger to *P. orbiculare* than to *C. albicans*, both in RAST and to the different components with the immunoblotting technique. A heterogeneous multitude of different components was seen, in the case of *C. albicans* exhibiting intermediate or weak IgE binding, while IgE binding to *P. orbiculare* was mainly strong or intermediate. In the case of *C. albicans*, the most important allergen was a 48-kDa protein, which is likely to correspond to an important 46-kDa allergen (31). Another *C. albicans* component which bound IgE of 10 of the 21 sera, albeit weakly, was the 38-kDa band, which may correspond to a 40-kDa *C. albicans* allergen recently shown to be an alcohol dehydrogenase (32). We also found binding to a diffuse high MW component, most likely corresponding to *Candida* mannans (30, 31). In the case of *P. orbiculare*, there was no distinct single allergen that could be described as more important than the other. Instead there was a multitude of IgE-binding components covering the molecular weight range from 14 to 90 kDa, but we observed no diffuse high MW staining corresponding in appearance to the *Candida* mannans (30, 31).

Sensitization to several fungi in atopic patients may be a consequence of multiple independent sensitization, cross-reactivity of fungal allergens, or both. Significant cross-reactivity has been demonstrated between clinically important inhalant molds (33). The possibility that *P. orbiculare* and *C. albicans* share allergenic epitopes has been investigated by Savolainen & Broberg (28), who found that a *C. albicans* extract could partly inhibit the IgE binding to *P. orbiculare* of two among five sera. Their interpretation of the data, based on the immunoblotting and RAST-inhibition patterns, was that

cross-reactive epitopes reside mainly in the mannan polysaccharide-rich part of the *C. albicans* extract, and in a corresponding possibly mannans-rich high MW diffuse band of *P. orbiculare*. Recently, similar data have been published by Doekes & van Ieperen-van Dijk (5). The mutual interpretation of the works of these groups is that there is considerable allergenic cross-reactivity between the two yeast species, among the mannans-rich high MW components. The data of the latter group even indicate that most of the IgE-binding capacity of both yeasts resides within those allergen fractions.

In this study, we found inhibition of IgE binding to several *C. albicans* bands, using *P. orbiculare* as inhibitor. When we arbitrarily denote the need of less than 100 times higher concentration of the heterologous (*P. orbiculare*) than the homologous (*C. albicans*) inhibitor to obtain 50% inhibition as evidence of inhibition, and the need of less than 10 times higher concentration as significant inhibition, it is recognized that most of the *C. albicans* components are not, or are only partly, cross-reactive. Among them, the most important 48-kDa *C. albicans* component exhibited but little cross-reactivity. Significant cross-reactivity was observed to the 34-, 37-, and 38-kDa components of *C. albicans*, which exhibited 50% inhibition of binding even at a lower concentration of the heterologous than of the homologous inhibitor. None of these cross-reactive components, however, were found to be of major importance as *C. albicans* allergens. Inhibition of IgE binding to *P. orbiculare* with the *C. albicans* extract was sparse; only a few of the *P. orbiculare* components in the highest molecular range exhibited a little inhibition, and no inhibition of the high MW *Candida* mannans was observed with the *P. orbiculare* extract.

Our results thus differ from those of the previously mentioned works on the topic (5, 28). Most importantly, our *P. orbiculare* extract, as previously demonstrated (15), contained a multitude of different components, probably of protein nature, with ample binding of IgE antibodies in the immunoblotting experiments. The two commercially available *P. orbiculare* extracts investigated by Savolainen & Broberg (28) and Doekes & van Ieperen-van Dijk (5), respectively, seem to have contained little of such protein components; in the latter case, even SDS-PAGE was unsuccessful. In *C. albicans*, IgE-binding corresponding closely to that described for *Candida* mannans (30, 31) was observed in the high MW range. In the immunoblotting experiments of our *P. orbiculare* extract, we observed no IgE-binding counterpart of *Candida* mannans, and our *P. orbiculare* extract in a separate experiment failed to inhibit IgE binding to this component. Probably, our *P. orbiculare* preparation, albeit rich in protein allergens,

does not contain these carbohydrate-rich allergens.

The present study does not support the notion that IgE antibodies to *C. albicans* merely reflect those to *P. orbiculare*, even though it appears that *P. orbiculare* is the more important of the two. The degree of allergenic cross-reactivity between the protein components of the two yeasts was limited. This conclusion is further supported by recent observations in RAST and immunoblotting experiments of sera of children with atopic eczema, in which we found IgE reactivity to protein components of *C. albicans* in the total absence of IgE antibodies to *P. orbiculare* (X. Huang et al., paper in preparation).

In the interpretation of these data, just as those of any other study of cross-reactivity using human sera, it must be appreciated that the results depend on which of the antigens under study induced an IgE antibody response in the serum donors. Sensitization to the two yeasts here studied is complex and must be looked upon in view of the process of colonization. Already in the earliest infancy, colonization of *C. albicans* occurs in the gastrointestinal tract and the upper airways (21). In that age group and all through childhood before puberty, skin colonization by *P. orbiculare* is scarce, but increases with the oily skin at puberty (8, 26). Sensitization to *C. albicans* allergens may be important already in infancy, and possibly the degree of cross-reactivity is such that an immune response elicited in early life to this yeast may be of importance for sensitization later in life to *P. orbiculare*. In the context, it is noteworthy that there are indications of a relationship between colonization and sensitization to *C. albicans*, but not of a corresponding relationship for *P. orbiculare* (1, 20). However, since IgE antibodies to *P. orbiculare* may be found also in the very young, antigens of this yeast probably also contribute to the process, just as do environmental yeasts and possibly other cross-reactive fungi.

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