

Identification of allergen components of the opportunistic yeast *Pityrosporum orbiculare* by monoclonal antibodies

Zargari A, Härfast B, Johansson S, Johansson SGO, Scheynius A.
Identification of allergen components of the opportunistic yeast *Pityrosporum orbiculare* by monoclonal antibodies.

Allergy 1994; 49: 50–56. © Munksgaard 1994.

The yeast *Pityrosporum orbiculare* (*P. orbiculare*) is a member of the normal human cutaneous flora, but it is also associated with several clinical manifestations of the skin. We have previously observed IgE-binding components in *P. orbiculare* extracts, using sera from patients with atopic dermatitis. In the present study, we raised several monoclonal antibodies (MoAbs) against *P. orbiculare* to characterize some of its antigens, and used *Candida albicans* (*C. albicans*) as a control. We obtained several IgG1 MoAbs which specifically recognized *P. orbiculare* in ELISA. Two of these were selected for immunoblotting studies on *P. orbiculare*, and two patterns of reactivity emerged. Firstly, one MoAb showed a distinct band at a molecular mass of 67 kDa. In the second pattern, a sharp band at about 37 kDa appeared. In contrast, the IgM antibodies raised reacted with a 14-kDa component; but they reacted with *C. albicans* in addition to *P. orbiculare*. The IgG1 antibodies seemed to react with proteins, as their ability to react in ELISA with extract pretreated with protease was greatly reduced. In contrast, IgM MoAbs were much less affected, suggesting that they recognized nonprotein components. To determine whether these MoAbs-binding components were also recognized by human IgE, we adopted a radioimmunoassay (RIA) using the MoAbs as catcher antibodies. Both the 67-kDa and the 37-kDa components were IgE-binding proteins. *P. orbiculare* RAST positive sera were scored as positive in the RIA, whereas the control serum was not.

A. Zargari¹, B. Härfast¹, S. Johansson²,
S. G. O. Johansson¹, A. Scheynius¹

¹ Department of Clinical Immunology, Karolinska Hospital, Stockholm, and ² Department of Clinical Microbiological Laboratory, County Hospital, Halmstad, Sweden

Key words: allergens; atopic dermatitis; *Malassezia furfur*; monoclonal antibody; *P. orbiculare*; *P. ovale*; yeast.

Arezou Zargari, MSc
Department of Clinical Immunology
Karolinska Hospital
S-104 01 Stockholm
Sweden

Accepted for publication 5 April 1993

Pityrosporum orbiculare is a lipophilic yeast which colonizes the skin of most healthy adults (22). Under the influence of predisposing factors, exogenous and/or endogenous, *P. orbiculare* becomes pathogenic and causes a chronic superficial skin disease, pityriasis (tinea) versicolor (6, 8). Another skin disorder in which this fungus is involved is a special type of folliculitis, *Pityrosporum folliculitis* (3, 21). *P. orbiculare* is also believed to play an important role in seborrheic dermatitis (28). Both local and humoral immune responses have been studied in patients with these diseases. The cellular response in lesions has shown an increased number of infiltrating T lymphocytes, most of which are CD4+ phenotype (2, 7, 26). Low levels of serum IgG antibodies to *P. orbiculare* can be found in healthy persons (1, 5, 12, 18, 29), whereas raised serum IgG titers are present in patients with *Pityrosporum folliculitis* (7).

The importance of *P. orbiculare* in the pathogenesis of atopic dermatitis has been discussed and investigated during the last 10 years. Studies using the skin prick test (16, 23, 30) and the radioallergosorbent test (RAST) (19, 25, 27) indicate that IgE antibodies are involved in the course of atopic dermatitis, and one treatment study (4) agrees with this notion.

Recently, we observed numerous IgE-binding components in *P. orbiculare* extracts, using RAST positive sera from atopic dermatitis patients and an immunoblotting technique (13). Six of the most easily distinguishable IgE-binding compounds were regarded as important allergens, while two of them were less important allergens. In the present investigation, we have produced monoclonal antibodies to *P. orbiculare* to be used in further characterization of these allergens.

Material and methods

Organisms, culture conditions, and antigen preparation

P. orbiculare from American Type Culture Collection (No. 42132) was cultured on glycerol monostearate olive oil (Go) media, as previously described (13). As a negative control, *Candida albicans* from CBS, Mold, Yeast Division, Delft, the Netherlands (No. 562) was cultured on Sabouraud dextrose agar containing neopeptone (10 g/l), agar (13.5 g/l), and glucose (40 g/l), pH 5.5. The yeast cultures were incubated at 37°C for 4 d; then the yeast cells were harvested and freeze-dried. Extraction was performed in 0.05 M phosphate-buffered saline (PBS), pH 7.4, containing 0.2% NaN₃ 1/11 (w/v), sonicated for 3 min and incubated at 4°C overnight. The mixture was centrifuged at 8000 g for 10 min, and the supernatant was filtered through an 0.8-µm Millex-HA filter (Millipore, Bedford, MA, USA). Whole cell antigens were prepared by harvesting *P. orbiculare* and *C. albicans* from 4-d-old cultures on Go substrate and Sabouraud dextrose media. The yeast cell suspensions were washed three times in sterile distilled water. The cells were resuspended in distilled water and counted.

Production and initial screening of MoAbs

Five female Balb/c mice aged 6 weeks were immunized three to six times intraperitoneally with a total volume of 0.15 ml of adjuvant and antigen (*P. orbiculare* whole yeast cells 10⁸ cells/ml, or soluble extract, 1 mg protein/ml). In the first three mice, complete Freund's adjuvant (Sigma, St Louis, MO, USA) was used for the first immunization, followed by incomplete Freund's adjuvant in the booster doses. In the last two mice, optiviant adjuvant (TSI, Worcester, MA, USA), according to the instructions of the manufacturer, was used.

Hybridomas were produced by fusion method from spleen cells of immunized mice. The myeloma cell line Sp2/0 was used as a cell fusion partner. After selection of the cultures for 2 weeks in HAT medium (6 mM hypoxanthine, 50 mM aminopterin, and 2 mM thymidine), an ELISA assay was performed as previously described (12) for screening hybridoma supernatants. Briefly, ELISA microtiter plates (A/S Nunc, Roskilde, Denmark) were coated with 200 µl/well of *P. orbiculare* whole cells (10⁷ cells/ml in sodium carbonate buffer, pH 9.3) or with *P. orbiculare* extract. For soluble extract, the concentration of protein was 50 µg/well. *C. albicans* whole yeast cells and extract were used as negative controls. The hybridoma supernatants were diluted 1:2 in PBS buffer containing 0.2% bovine serum albumin and 0.1% Tween 20, pH 7.4 (this buffer is referred to as the PSA-Tween buffer) and added to the

wells. The plates were incubated overnight at room temperature.

Alkaline phosphatase-conjugated rabbit anti-mouse immunoglobulin (Dakopatts, Glostrup, Denmark) was used in the dilution of 1:1000 in the PSA-Tween buffer and incubated for 4 h. Finally, substrate (4-nitrophenyl phosphate 1 mg/ml in 1 M diethanolamine buffer, pH 9.8) was added to the wells, and the absorbance was read after 15 min at 405 nm in a microtiter plate reader (SLT-lab Instruments, Salzburg, Austria). Cells producing antibodies reacting with *P. orbiculare* antigen were selected, and the isotypes of the MoAbs were determined with ELISA (ICN Biomedical Incorporated, Birmingham, IL, USA), according to the manufacturer's instructions.

Protease test

For determination of whether the antigenic determinants recognized by MoAbs were of protein nature, *P. orbiculare* was treated with protease (Proteinase K, type XI, Sigma). For this purpose, a microtiter plate coated with *P. orbiculare* extract (50 µg protein/well) was either treated with different concentrations of protease (300, 30, and 3 µg/ml) in PSA-Tween buffer, or left untreated and incubated at 37°C for 1 h. The ELISA activity of the MoAbs against protease-treated and untreated *P. orbiculare* extract was subsequently assessed.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

P. orbiculare and *C. albicans* extracts were desalted on a Sephadex G-25 column (Pharmacia, Uppsala, Sweden) and lyophilized. The amount of protein in both extracts was 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 crude extracts of *P. orbiculare* and *C. albicans* were 300 µg/mg and 350 µg/mg, respectively. The extracts were treated with SDS-reducing buffer containing 5% 2-β-mercaptoethanol and heated for 5 min at 100°C. The gel electrophoresis was performed essentially as previously described (11, 13). Both extracts were subjected to polyacrylamide gradient gels (7.5–20%) with the stacking polyacrylamide gels (4%) and at an approximate concentration of 7.5 µg protein/mm gel. The separation gel buffer was 1.5 M Tris-HCl, pH 8.8. The stacking gel buffer was 0.5 M Tris-HCl, pH 6.8, and the electrode buffer contained 1.5% Tris base, 7.2% glycerol, and 0.5% SDS, pH 8.3. The electrophoresis was run at 200 V for 45–50 min, current decreasing from 60 to 30 mA. One well was used for a low-molecular-mass protein standard

(LMW, Electrophoresis calibration kit, Pharmacia). The standard was diluted 1:40 in SDS-reducing buffer and heated for 5 min at 100°C.

Electrophoretic transfer, quenching, and immunologic detection

The separated proteins and standard proteins were transferred from the SDS separation gel to a nitrocellulose (NC) membrane (Bio-Rad, Richmond, CA, USA) in a Bio-Rad Mini-Trans-Blot cell, as described previously (11). After the electrophoretic transfer, the NC membrane with the Pharmacia standard protein was incubated for 30 min with PBS containing 0.3% Tween 20 at 37°C and washed three times with the same buffer and twice with distilled water. It was then incubated for 10 min in Aurodyne-forte protein stain (Janssen, Olen, Belgium) and finally washed twice in distilled water.

As a means of blocking nonspecific protein present on the NC membrane, the NC membrane was incubated for 1 h at 37°C in PSA-Tween buffer containing 0.5% Tween 20 ("quenching"). After electrophoretic blotting and quenching, the NC membrane was cut into narrow strips and incubated overnight with 500 µl of diluted MoAbs or with sera. After each incubation step, the strips were washed three times with saline containing 0.5% Tween 20 and 0.02% NaN₃. The strips incubated with MoAbs were incubated for 2 h with alkaline phosphatase-conjugated rabbit antimouse immunoglobulin (Dakopatts) diluted 1:4000 in PSA-0.5% Tween buffer. The strips incubated with sera were washed and incubated for 2 h with rabbit antihuman IgE (Pharmacia), followed by incubation for 2 h with alkaline phosphatase-conjugated goat antirabbit IgG (Bio-Rad). Both types of antibody were diluted 1:2000 in PSA-0.5% Tween. The strips were then washed and developed in a chromogenic substrate containing 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium in carbonate buffer, pH 9.8.

Radioimmunoassay for allergen binding of MoAbs

Microtiter plates (Nunc) were coated with an immunosorbent purified goat antimouse antibody (200 µl/well; 160 µg/ml) (Dakopatts). The plates were incubated overnight at room temperature and subsequently washed. MoAb culture supernatants were added and allowed to incubate for 9 h, followed by incubation with 100 µl/well extract of *P. orbiculare* or *C. albicans* (250 µg protein/ml) overnight. Finally, ¹²⁵I-labeled rabbit antihuman IgE antibody (Phadebas RAST anti-IgE tracer, Pharmacia), 50 µl/well, was added and allowed to incubate overnight. The wells were treated with citric buffer, pH 3, for 1 h, and the solution was subsequently transferred to

Table 1. Reactivity of MoAbs against crude extracts of *P. orbiculare* and *C. albicans* in ELISA

MoAbs	ELISA activity* to <i>P. orbiculare</i>	ELISA activity* to <i>C. albicans</i>	Ig isotype
1B6D7	1.610	0.082	IgG1
6F5B9	1.723	0.063	IgG1
2E4F2	1.852	0.096	IgG1
10B3E4	1.786	0.066	IgG1
11E2D9	1.775	0.072	IgG1
5B9D10	1.687	0.056	IgG1
1D5G8	1.623	0.084	IgG1
9G9C8	2.553	0.072	IgG1
7B11B2	0.884	0.592	IgM
5F11B2	2.200	0.956	IgM

Absorbance values were determined at 405 nm after 15 min.

* Values <0.100 were considered to be negative.

tubes and the radioactivity determined by a gamma counter. All samples were run in triplicate, and mean values and standard deviations (SD) were calculated.

Results

Production and characterization of MoAbs

Cell fusion between the Sp2/0 cell line and spleen cells of Balb/c mice immunized with whole cell and/or crude extracts of *P. orbiculare* resulted in a series of hybridomas secreting MoAbs against antigens of *P. orbiculare* (Table 1). The reactivity of these MoAbs against antigens of *P. orbiculare* and *C. albicans* was screened with ELISA. The isotype of the MoAbs was IgG1 or IgM with κ light chain. The IgG1 MoAbs were negative to *C. albicans* antigen, whereas a strong cross-reactivity to the whole cells or extract was observed with the IgM MoAbs. From all five mice which were immunized three to six times with *P. orbiculare* antigen, IgM antibodies were obtained. IgG1 antibodies were obtained from those mice which were immunized six times with *P. orbiculare* antigen. Among the IgM antibodies, two were chosen to be shown in Table 1. No significant difference between *P. orbiculare* whole cell and extract was observed when the MoAbs were used in ELISA.

Table 2. Percent diminished ELISA response for MoAbs against *P. orbiculare* antigens treated with different concentrations of protease

MoAb*	Ig isotype	Protease concentration		
		300 µg/ml	30 µg/ml	3 µg/ml
1D5G8	IgG1	99	95	88
9G9C8	IgG1	98	93	83
5F11B2	IgM	15	9	2

* The MoAbs were diluted 1:3.

Table 3. Total IgE levels and RAST class values of sera from patients with atopic dermatitis

Sera*	Age (years)	Total IgE (kU/l)**	RAST class value**	
			<i>P. orbiculare</i>	<i>C. albicans</i>
1	23	4100	4	3
2	41	4600	3	2
3	26	4400	3	3
4	44	19	0	0

* 1–3, sera from patients with atopic dermatitis; 4, serum from a healthy control.

** Values were obtained from a previous study (13).

For determination of whether the epitopes recognized by the MoAbs were associated with proteins, binding activities of the MoAbs to protease-treated antigens were examined with ELISA. The activity of the IgG1 MoAbs was greatly reduced after treatment with different concentrations of protease, thus indicating the protein nature of the antigens (Table 2). In contrast, IgM-binding antigens showed resistance to treatment with protease in concentrations of up to 300 µg/ml (Table 2).

Identification of *P. orbiculare* allergens by immunoblotting analysis and RIA

Several MoAbs in different concentrations were run in SDS-PAGE and immunoblotting. Two different IgG1 MoAbs (1D5G8 and 9G9C8) and one IgM MoAb (5F11B2) which exhibited the best reaction with the *P. orbiculare* extract were selected for im-

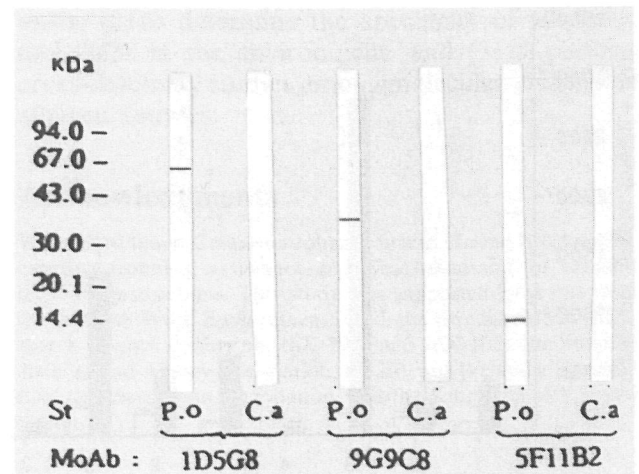


Fig. 1. Antigen components in extracts of *P. orbiculare* (P.o) and *C. albicans* (C.a) on NC membrane detected by MoAbs 1D5G8, 9G9C8, and 5F11B2 in immunoblotting analysis. Molecular mass in kDa was compared with low-molecular-mass standard proteins (Pharmacia).

munoblotting studies. Three *P. orbiculare* RAST-positive sera from patients with atopic dermatitis and one serum from a healthy control used in a previous study (13) were chosen for further investigation. Total serum IgE levels and *P. orbiculare* RAST class values are given in Table 3. After analysis with SDS-PAGE and immunoblotting, both *P. orbiculare* and *C. albicans* extracts were probed with the hybridoma supernatant of the MoAb 1D5G8 (dilution 1:40 000), MoAb 9G9C8 (dilution 1:10 000), and MoAb 5F11B2 (dilution 1:2). The molecular

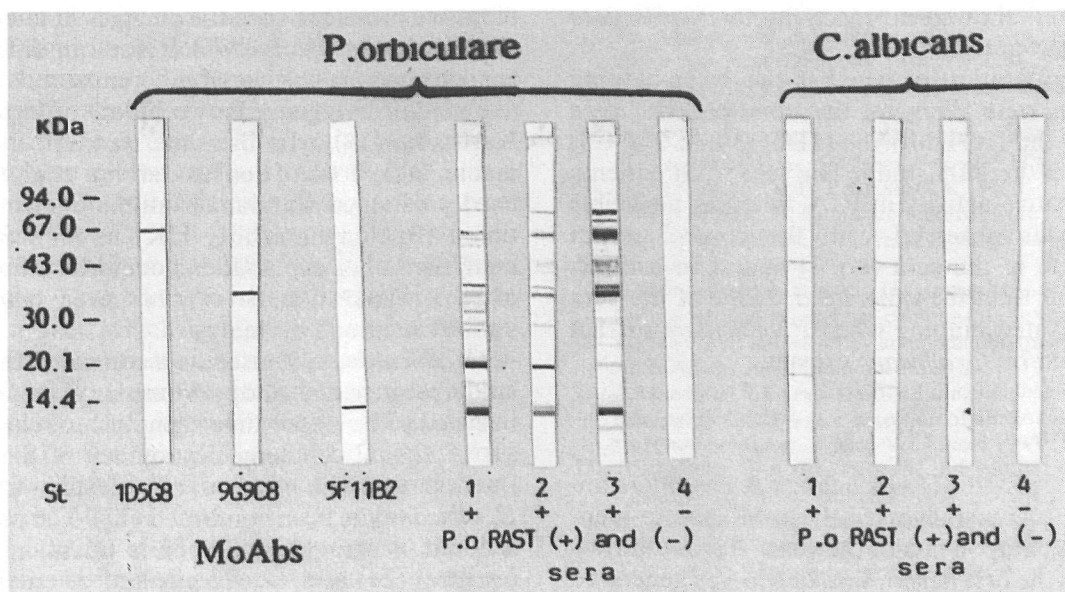


Fig. 2. IgE-binding components in extracts of *P. orbiculare* and *C. albicans* detected by sera from three patients with atopic dermatitis (1–3) and normal control serum (4) and compared with antigenic components detected by MoAbs in Fig. 1. Molecular mass in kDa was compared with low-molecular-mass standard proteins (Pharmacia).

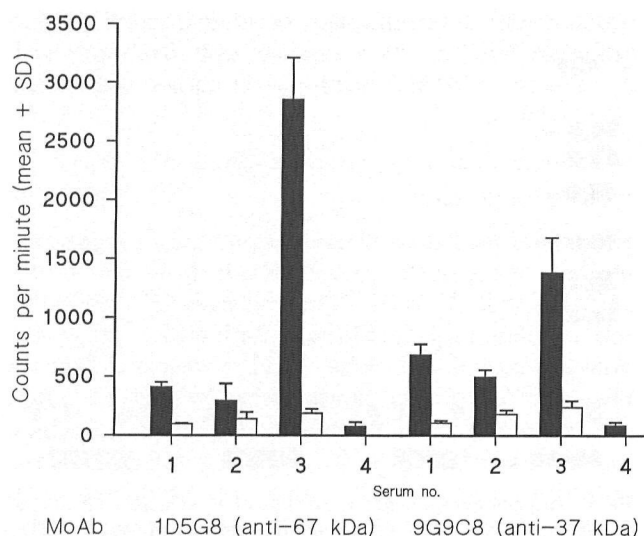


Fig. 3. Radioimmunoassay using sera from three patients with atopic dermatitis (1–3) and one normal control serum (4) together with MoAb 1D5G8 and MoAb 9G9C8. Filled bars give response in counts/min with mean value and standard deviation (SD) of triplicates to *P. orbiculare* and open bars to control antigen *C. albicans*.

mass of each band was determined by comparing the relative mobility of the standard proteins. As shown in Fig. 1, a single sharp band was visualized at about 67 kDa with 1D5G8, 37 kDa for 9G9C8, and 14 kDa for 5F11B2, as compared with the standard. These MoAbs showed no reactivity to *C. albicans* in control immunoblot analyses. The sera were also tested in the immunoblotting (dilution 1:6) and compared with MoAbs (Fig. 2). The patients with high total serum IgE and positive RAST to *P. orbiculare* showed bands at about 67, 37, and 14 kDa. Normal control serum showed no reactivity to *P. orbiculare* or *C. albicans* antigens.

For determination of whether the IgG1-binding components were allergens, the same patients' sera and two of the IgG1 MoAbs (1D5G8 and 9G9C8) were used in the RIA study. The sera from patients showed positive activity in RIA, whereas negligible reactivity was observed with the control serum (Fig. 3). One of the sera (no. 3) bound to a much higher extent than the others did. None of the sera showed elevated binding when *P. orbiculare* extract was replaced by *C. albicans* extract.

Discussion

We have generated MoAbs against *P. orbiculare* antigens, and successfully used these MoAbs to identify allergen components of the yeast. To our knowledge, this is the first description of MoAbs generated against *P. orbiculare*. The isotypes of the MoAbs were determined as IgG1 and IgM. Allergen components which bound to the IgG1 MoAbs were of

protein nature, and further characterization with immunoblotting analysis showed that at least two different proteins were recognized by these IgG1 MoAbs. The molecular masses of the protein components, distinguished by MoAb 1D5G8 and MoAb 9G9C8, were about 67 and 37 kDa, respectively. The IgM MoAb (5F11B2) recognized a component at about 14 kDa. According to our study, this 14-kDa component was not affected by pretreatment with protease. Therefore, this IgM antibody probably reacted with carbohydrate moieties which also could exist in extracts of other yeasts such as *C. albicans*. The reactivity of this IgM antibody to *C. albicans* in ELISA could explain this finding. Possibly, as the yeast cell wall consists mostly of carbohydrates, the 14-kDa component may be located in the cell wall. Our results confirm the data from Jensen-Jarolim et al. which suggested that the 9-kDa and 15-kDa allergens of *P. orbiculare* extract comprise a large nonprotein moiety which can exist in the cell wall (10). A heterologous immunoblotting inhibition test performed by using other yeasts as an inhibitor or cross-reactivity test could be useful for determining the existence of this component in the cell walls of the other yeasts.

In this study, we observed that different standards may result in different molecular masses for one component. Thus, a biotinylated SDS-PAGE low-molecular-mass standard (Bio-Rad, Richmond, CA, USA) gave the molecular masses of the bands in the position as about 83, 45, and 13 kDa (data not shown) instead of 67, 37, and 14 kDa according to the standard of Pharmacia. One reason for the different result could be that as a result of adding biotin the mobility of modified protein may differ from that of native protein: hence the changes in linear regression of standard curve which result in different molecular masses. The use of different standards might explain the divergence between data of Johansson & Karlström (13), who used the standard from Pharmacia, and those of Jensen-Jarolim et al. (10), who used prestained standard from Amersham International (Buckinghamshire, UK) in immunoblotting analysis. Other explanations could be differences in extract preparation, strains, or even batches and various methods of analysis.

C. albicans was chosen as a control in the present study because it is an opportunistic yeast and is also believed to be a causative agent of bronchial asthma (9, 15, 20). The allergenic potency of the mannan fraction and the purified cytoplasmic fraction of *C. albicans* has been confirmed (17). The presence of a common structure of *P. ovale* (identical to *P. orbiculare*) (24) and *C. albicans* and of cross-reactive IgE antibodies against these antigens in atopic children has been shown (25). However, our IgG1 MoAbs were directed against allergen components

of *P. orbiculare*, and the fact that they did not show any cross-reactivity to *C. albicans* antigen components confirms their specificity against *P. orbiculare* allergens.

Atopic dermatitis is a chronic inflammatory skin disorder that frequently occurs in patients with personal or family history of allergic disease (14). It is known that IgE-mediated hypersensitivity to *P. orbiculare* as measured by RAST and ELISA (19, 27, 31) is common in atopic dermatitis patients and that serum IgE levels are elevated in most of these patients (16, 32). For the immunoblotting analysis, we chose sera from three patients with atopic dermatitis from a previous study (13) with high RAST values to *P. orbiculare* and high total IgE levels for immunoblotting analysis. Our data showed that the components at about 67, 37, and 14 kDa recognized by the patients' sera also could be detected by MoAbs 1D5G8, 9G9C8, and 5F11B2. Johansson & Karlström (13) showed that allergens of about 67 and 14 kDa were frequent and detected by 86% and 73% of the sera from patients with atopic dermatitis, respectively; i.e., major allergens. The 37-kDa component was not designated as a major allergen in that study because of difficulty in distinguishing the bands in the region of 30–67 kDa.

The sera from patients used in the present study were positive against *C. albicans* in RAST, whereas the immunoblotting analysis showed a few weak bands with *C. albicans* antigens. With more concentrated sera, the bands became stronger, but no additional bands appeared (data not shown). One explanation for this finding could be that *C. albicans* allergens are weaker than those of *P. orbiculare*. Alternatively, lack of binding could be due to partial denaturation of *C. albicans* antigens caused by boiling before separation in SDS-PAGE. A third possibility is that excess amounts of carbohydrate prevent enough protein from being inserted into the gel. Yet another, trivial, explanation could be that preparing extracts by the method used results in less allergen components from *C. albicans* than from *P. orbiculare*.

Positive activity in the RIA study showed that IgE-binding components were recognized by MoAbs, a fact which confirms that the MoAb-binding components are indeed allergens. The *P. orbiculare* RAST values for patient sera showed accordance with reactivity in immunoblotting analysis and RIA. RIA screening of sera from a large group of patients with atopic dermatitis is now underway, using MoAbs.

The application of MoAb technology in the field of atopic dermatitis will provide facilities to characterize and distinguish the allergens in crude extracts of *P. orbiculare*. Suitable MoAbs against allergens may also be used (1) to establish the clinical diag-

nosis, (2) to determine the specificity of allergenic molecules in the environment, and (3) to perform cross-reactivity studies among molecules in different allergen sources.

Acknowledgments

We wish to thank Catharina Johansson and Helene Bröchner for excellent technical assistance and Eva Johansson for valuable technical instructions. This study was supported by grants from the Swedish Work Environment Fund, the Swedish Medical Research Council (grants no. 16X-7924 and 16X-105), the Swedish Asthma and Allergy Association, the Edvard Welander Foundation, the Hesselman Foundation, and the King Gustaf V Foundation.

References

1. BERGBRANT I-M, FAERGEMANN J. Variations in *Pityrosporum orbiculare* in middle-aged and elderly individuals. *Acta Derm Venereol* (Stockh) 1988; 68: 537–40.
2. BERGBRANT I-M, JOHANSSON S, ROBBINS D, SCHEYNIUS A, FAERGEMANN J, SÖDERSTRÖM T. An immunological study in patients with seborrhoeic dermatitis. *Clin Exp Dermatol* 1991; 16: 331–8.
3. BÄCK O, FAERGEMANN J, HÖRNQVIST R. *Pityrosporum* folliculitis: a common disease of the young and middle-aged. *J Am Acad Dermatol* 1985; 12: 56–61.
4. CLEMMENSEN OJ, HJORTH N. Treatment of dermatitis of the head and neck with ketoconazole in patients with type I hypersensitivity for *Pityrosporum orbiculare*. *Semin Dermatol* 1983; 2: 26–9.
5. DAMERT GJ, KIRKPATRICK CH, SOHNLE PG. Comparison of antibody responses in chronic mucocutaneous candidiasis and tinea versicolor. *Int Arch Allergy Appl Immunol* 1980; 63: 97–104.
6. FAERGEMANN J. Tinea versicolor and *Pityrosporum orbiculare*: mycological investigations, experimental infections and epidemiological surveys. *Acta Derm Venereol* (Stockh) 1979; Suppl. 86: 1–23.
7. FAERGEMANN J, JOHANSSON S, BÄCK O, SCHEYNIUS A. An immunologic and cultural study of *Pityrosporum* folliculitis. *J Am Acad Dermatol* 1986; 14: 429–33.
8. GORDON MA. Lipophilic yeastlike organisms associated with tinea versicolor. *J Invest Dermatol* 1951; 17: 267–72.
9. ITKIN IH, DENNIS M. Bronchial hypersensitivity to extract of *Candida albicans*. *J Allergy* 1966; 37: 187–94.
10. JENSEN-JAROLIM E, POULSEN LK, WITH H, KIEFFER M, OTTEVANGER V. Atopic dermatitis of the face, scalp, and neck: type I reaction to the yeast *Pityrosporum ovale*? *J Allergy Clin Immunol* 1992; 89: 44–51.
11. JOHANSSON E, VAN HAGE-HAMSTEN M, JOHANSSON SGO. Demonstration of allergen components in the storage mite *Lepidoglyphus destructor* by an immunoblotting technique. *Int Arch Allergy Appl Immunol* 1988; 85: 8–13.
12. JOHANSSON S, FAERGEMANN J. Enzyme-linked immunosorbent assay (ELISA) for detection of antibodies against *Pityrosporum orbiculare*. *J Med Vet Mycol* 1990; 28: 257–60.
13. JOHANSSON S, KARLSTRÖM K. IgE-binding components in *Pityrosporum orbiculare* identified by an immunoblotting technique. *Acta Derm Venereol* (Stockh) 1991; 71: 11–16.
14. JUHLIN L, JOHANSSON SGO, BENNICHT H, HÖGMAN C, THYRESSON N. Immunoglobulin E levels in atopic dermatitis, urticaria, and various dermatoses. *Arch Dermatol* 1969; 100: 12–16.
15. KEENY EL. *Candida* asthma. *Ann Intern Med* 1951; 34: 223–6.

16. KIEFFER M, BERGBRANT I-M, FAERGEMANN J, et al. Immune reactions to *Pityrosporum ovale* in adult patients with atopic and seborrhoeic dermatitis. *J Am Acad Dermatol* 1990; 22: 739-49.
17. LONGBOTTOM JL, BRIGHTON WD, EDGE G, PEPYS J. Antibodies mediating type I skin test reactions to polysaccharide and protein antigens of *Candida albicans*. *Clin Allergy* 1967; 6: 41-9.
18. MIDGLEY G, HAY RJ. Serological responses to *Pityrosporum* (*Malassezia*) in seborrhoeic dermatitis demonstrated by ELISA and Western blotting. *Bull Soc Fr Mycol Med* 1988; 17: 267-76.
19. NORDVALL L, JOHANSSON S. IgE antibodies to *Pityrosporum orbiculare* in children with atopic diseases. *Acta Paediatr Scand* 1990; 79: 343-8.
20. PEPYS J, FAUX JA, LONGBOTTOM JL, MCCARTHY DS, HARGREAVE FE. *Candida* precipitins in respiratory disease in man. *J Allergy* 1968; 41: 305-18.
21. POTTER BS, BURGOON CF, JOHNSON WC. *Pityrosporum* folliculitis. *Arch Dermatol* 1973; 107: 388-91.
22. ROBERTS SOB. *Pityrosporum orbiculare*: incidence and distribution on clinically normal skin. *Br J Dermatol* 1969; 81: 264-9.
23. RUKUGO M, TAGAMI H, USUBA Y, TOMITA Y. Contact sensitivity to *Pityrosporum ovale* in patients with atopic dermatitis. *Arch Dermatol* 1990; 126: 627-32.
24. SALKIN IF, GORDON MA. Polymorphism of *Malassezia furfur*. *Can J Microbiol* 1977; 23: 471-5.
25. SAVOLAINEN J, BROBERG A. Crossreacting IgE antibodies to *Pityrosporum ovale* and *C. albicans* in atopic children. *Clin Exp Allergy* 1992; 22: 469-74.
26. SCHEYNIUS A, FAERGEMANN J, FORSUM U, SJÖBERG O. Phenotypic characterization *in situ* of inflammatory cells in pityriasis (tinea) versicolor. *Acta Derm Venereol (Stockh)* 1984; 64: 473-9.
27. SCHRENKER T, KALVERAM KJ, HORNSTEIN OP, HAUCK HD, BÄURLE G. Specific IgE antibodies to *P. orbiculare* in patients with atopic dermatitis. *Z Hautkr* 1989; 64: 478-9.
28. SHUSTER S. The aetiology of dandruff and the mode of action of therapeutic agents. *Br J Dermatol* 1984; 111: 235-42.
29. SOHNLE PG, COLLINS-LECH C, HUHTA KE. Class-specific antibodies in young and aged humans against organisms producing superficial fungal infections. *Br J Dermatol* 1983; 108: 69-76.
30. WAERSTED A, HJORTH N. *Pityrosporum orbiculare* a pathogenic factor in atopic dermatitis of the face, scalp and neck? *Acta Derm Venereol (Stockh)* 1985; Suppl. 114: 146-8.
31. WESSELS MW, DOEKES G, VAN IEPEREN-VAN KUIK AG, KOERS WJ, YOUNG E. IgE antibodies to *P. ovale* in atopic dermatitis. *Br J Dermatol* 1991; 125: 227-32.
32. ÖHMAN S, JOHANSSON SGO. Immunoglobulins in atopic dermatitis. *Acta Derm Venereol (Stockh)* 1974; 54: 193-202.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.