

Atopic dermatitis of the face, scalp, and neck: Type I reaction to the yeast *Pityrosporum ovale*?

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We have previously reported that a lipophilic yeast, Pityrosporum ovale (P. ovale) produced a high frequency of positive skin prick tests and in vitro histamine-release (HR) tests in patients suffering from atopic dermatitis (AD) of the face, scalp, and neck. In the present study, our aim was to confirm the involvement of P. ovale-specific IgE and to produce a standardized extract for diagnostic tests; 7/20 sera from patients with a positive HR test were positive in RAST. Several IgE-binding proteins could be detected with sodium dodecyl sulfate–polyacrylamide gel electrophoresis, followed by immunoblotting. Comparison of different extraction methods demonstrated that allergens were not released from P. ovale until after mechanical destruction of the yeast cells. Extraction of cultured P. ovale, obtained from the skin of various individuals suffering from AD of the face, scalp, and neck, resulted in very heterogenous patterns of constituents in sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblotting analysis. Nevertheless, all extracts were recognized by patients' IgE. P. ovale may express varying protein patterns, depending on either source or stage of differentiation. Thus, at present, skin and HR tests may be superior to other available assays for diagnosing type I reactions to P. ovale in AD of the face, neck, and scalp. (J ALLERGY CLIN IMMUNOL 1992;89:44-51.)

Key words: Atopic dermatitis, *Pityrosporum ovale*, yeast, IgE, immunoblotting, histamine-release test, RAST

Different attempts have been made to describe mechanisms inducing or propagating AD. Typically, elevated IgE levels have been observed.¹ It has been speculated that increased IgE production was due to defective T-lymphocyte function with subsequent elevated B cell levels.² In contrast, pharmacologic imbalance was observed in AD, for example, elevated histamine levels in the tissue³ and hyporesponsiveness

Abbreviations used

AD:	Atopic dermatitis
HR:	Histamine release
HR ⁺ :	Positive histamine release
<i>P. ovale</i> :	<i>Pityrosporum ovale</i>
RAST ⁺ :	RAST positive
RAST ⁻ :	RAST negative
SDS–PAGE:	Sodium dodecyl sulfate–polyacrylamide gel electrophoresis
SPT:	Skin prick test
cAMP:	Cyclic adenosine monophosphate
MW:	Molecular weight
EACA:	ε-Aminocaproic acid

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of cAMP⁴ associated with increased levels of cAMP phosphodiesterase.⁵ Similar pharmacologic changes also occur in skin diseases and may therefore only be secondary to inflammation.⁶ There are, moreover, certain difficulties in distinguishing between healthy subjects and patients suffering from AD.⁶

A special type of AD, located on the face, scalp, and neck, that is, in seborrheic sites of the skin, is predominantly present in young postpuberty women.⁷ In skin lesions of patients suffering from face, scalp, and neck dermatitis, a lipophilic yeast, *P. ovale*, probably identical with *P. orbiculare*,⁸ was detected.⁷ This yeast, which is part of the normal microflora of the skin of adults,^{9, 10} may sometimes cause tinea versicolor¹¹ and induce specific precipitating antibodies.¹² *P. ovale* also appears to induce type I reactions, revealing positive skin prick reactions in patients suffering from AD of the face, scalp, and neck.^{6, 13} Recently, it was demonstrated that such patients also had positive reactions to *P. ovale* in HR test.¹⁴ This special type of AD may therefore include type I reaction to yeasts in the skin.

In the present study, extracts of *P. ovale* were prepared for characterization of the allergenic, IgE-binding components of the yeast. When sera from patients with AD of the face, scalp, and neck were tested in immunoblotting, only patients with positive reactions to *P. ovale* in RAST also demonstrated strong IgE binding in immunoblotting. *P. ovale* obtained from the skin of nine additional patients suffering from AD of the face, scalp, and neck contained components quite different from components of our standard preparation of *P. ovale*. However, these extracts also were recognized by IgE of patients with AD of the face, scalp, and neck. Extracts of *P. ovale* may therefore hardly be standardized by means of MW analysis. Elucidation of a possible IgE-mediated reactivity to this yeast may be clinically important, since this form of AD will respond relatively easy to antimycotic treatment.

MATERIAL AND METHODS

Patients and sera

Twenty patients suffering from AD (i.e., fulfilling the criteria of Hannifin and Rajka¹⁵) of the face, scalp, and neck¹⁴ were selected based on a positive SPT and a positive HR test with extracts of *P. ovale*. SPT was performed as previously described,^{14, 16} and all patients demonstrated a positive reaction (wheal diameter at least corresponding to wheal diameter of histamine dihydrochloride, 1 mg/ml). Blood and sera from these patients were further exposed to *P. ovale* in HR test and RAST (tests described below). All patients demonstrated positive reactions to *P. ovale* in HR test (inclusion criteria), whereas only 7/20 were RAST⁺ to *P. ovale*. Twelve nonallergic volunteers served as control subjects. They did not react with extracts of *P. ovale* in SPT, HR test, or RAST. Aliquots of sera of the two patient groups (HR⁺/RAST⁺, seven patients; HR⁺/RAST⁻, 13 patients) and the control group were pooled. Individuals from patient and control groups were examined for their reactivity to a number of other potential allergens with SPT and HR.

These allergens included different *Saccharomyces* sp. *Candida albicans*, and inhalant allergens.

Chemicals and general reagents

Tris, NaCl, HCl, glycine, acetic acid, methanol, ethanol, phenol, 2-propanol, NH₄HCO₃, Na₂HPO₄, NaH₂PO₄, bromophenolblue, ethylenediaminetetraacetic acid, ϵ -aminocaproic acid, phenylmethylsulfonyl fluoride, Tween 20, 2- β -mercaptoethanol, and *N,N,N,N*-tetramethylethylenediamine were obtained from Merck (Darmstadt, Germany). Acrylamide was obtained from Serva Feinbiochemicals (Heidelberg, Germany). Ammonium peroxy disulfate was obtained from Bio-Rad Laboratories (Richmond, Calif.). Sodium dodecyl sulfate and glycerol were obtained from BDH Chemicals (Poole, England), and bis-acrylamide and Coomassie brilliant blue R250 from Sigma Chemical Co. (St. Louis, Mo.).

Culture of *P. ovale*

P. ovale was collected from forehead or chest of patients with AD and was grown at 37° C on a selective, fatty culture medium, Dixon's agar modified according to the method of Randjandiche,¹⁷ containing malt extract, 5%; a mixture of bile salts, 0.05% (Merck); Tween 40, 1%; and agar, 2%. The yeasts were identified microscopically. Plates were stored at 4° C for 1 week, and subsequently, the cream-yellow colored colonies were harvested.

Extracts of *P. ovale*

A commercial extract of *P. ovale* (5 mg/ml) was obtained from ALK (Hoersholm, Denmark). This extract was used as antigen for skin tests, RAST, and HR test. The strain of *P. ovale* used by this company was similar to the strain used in our laboratory for in-house extractions A to D (see below).

In-house extracts, *P. ovale* colonies, were carefully scraped from agar plates; 5 gm of colony material from each plate was prepared with 10 ml of extraction buffer. Extraction was performed according to the method of Axelsen¹⁸ with microglass beads (diameter, 0.5 mm) on a vortex mixer (5 minutes) (extracts A, B, C, and D; see below). Samples were kept on ice during extraction. Extraction buffers (pH 7.4), A, Coca's fluid: 0.4% (wt/vol) phenol; 0.275% (wt/vol) NaHCO₃; and 0.5% (wt/vol) NaCl¹⁹; B, Dulbecco's phosphate-buffered saline; C, 10 mmol/L of Na-phosphate buffer; and D, 125 mmol/L of NH₄HCO₃ buffer.

Extracts of medium constituents were prepared by extracting 10 gm of agar in 10 ml of buffer D. Alternatively, ultrasonication (5 minutes) was used instead of glass beads (extracts a, b, c, and d). Protease inhibitors (20 mmol/L of EACA, 5 mmol/L of ethylenediaminetetraacetic acid, and 1 mmol/L of phenylmethylsulfonyl fluoride) were added to all extraction buffers. After extraction, samples were centrifuged for 5 minutes at 800 g at 4° C; supernatants were collected and centrifuged once more (5 minutes at 800 g at 4° C). Supernatants were dialyzed at 4° C (dialysis membranes with a cutoff of 10 kd) against 0.9% saline (48 hours) and distilled H₂O (24 hours). Finally, extracts were

lyophilized and stored at -20°C . Extract D was chosen as standard preparation throughout the study. In addition to the above extraction of *P. ovale*, yeasts from nine additional patients suffering from AD of the head, scalp, and neck were isolated and cultured under the same conditions as the standard strain. Colonies were extracted with glass beads according to the described standard procedure D.

RAST

RAST disks were coupled with extracts of *P. ovale*. Disks were then incubated with patients' sera and ^{125}I -labeled polyclonal anti-IgE antibody according to the manufacturer's instructions (Pharmacia, Uppsala, Sweden). RAST reactivity of the patients was compared with reaction of the IgE standard (Pharmacia), and RAST classes of 3 and 4 were considered as positive.

HR test

HR test, which is based on the reversible and specific binding of histamine to glass fiber-coated microtiter plates, was performed as previously described.^{20,21} In short, 10 ml of heparinized blood samples was collected in plastic tubes. Fifty microliters of piperazine-*N,N'*-bis-2-ethanesulfonic acid-AMC buffer²⁰ per well were added to glass fiber-prepared microtiter plates from Reference Laboratory, Copenhagen, Denmark (U.S. patent No. 4,550,085). Fifty microliters of the heparinized whole blood was then incubated for 60 minutes at 37°C with 20 μl of *P. ovale* extract (ALK) in concentrations of 0.5, 0.05, 0.005, and 0.0005 ng/ml. After the blood had been washed away, the glass fiber-bound histamine was released and determined in a fluorometer after coupling to a fluorophore (o-phthalaldehyde). The HR was considered significant if histamine content of >0.3 ng per sample could be detected.

SDS-PAGE and immunoblotting

SDS-PAGE was performed in a vertical slab gel apparatus (Hoefer Scientific Instruments, San Francisco, Calif.), according to the method of Laemmli²²; 16% separation gels (180 by 150 by 1.5 mm) and 5% stacking gels were used in a discontinuous buffer system (0.025 mol/L of Tris, 0.2 mol/L of glycine, and 0.1% of SDS (wt/vol)).

Sample preparation. Lyophilized extracts of *P. ovale* were dissolved in distilled water and boiled for 5 minutes at 95°C in fourfold sample buffer (Laemmli²²) containing 2- β -mercaptoethanol. For immunoblotting, 1 mg (dry weight) of extract per 0.5 cm of gel was loaded; for silver staining, 100 μg per 0.5 cm of gel. Silver staining of gels was made with a kit (Bio-Rad Laboratories) according to the manufacturer's instructions. MWs were determined with prestained protein standards (Amersham International, Buckinghamshire, U.K.).

Blotting was performed with a semidry electroblotter (JKA-Biotech, Copenhagen, Denmark), according to the manufacturer's instructions. In short, proteins were transferred to nitrocellulose, pore size, 0.2 mm (Schleicher & Schuell, Kassel, Germany), in a discontinuous buffer system (anodic buffer 1: 0.3 mol/L of Tris and 20% (vol/vol)

of methanol, pH 10.4; and anodic buffer 2: 25 mmol/L of Tris and 20% (vol/vol) of methanol, pH 10.4; cathodic buffer: 25 mmol/L of Tris, 40 mmol/L of EACA, and 20% (vol/vol) of methanol, pH 9.4) for 90 minutes at 200 mA. Proteins transferred to nitrocellulose were stained with India ink.²³ For IgE immunoblotting, nitrocellulose strips or sheets were blocked for 30 minutes with TBS (50 mmol/L of Tris and 150 mmol/L of NaCl, pH 7.4) containing 0.5% of Tween 20 and 3% of powdered milk. Sera were diluted 1:10 in blocking solution, and strips were incubated overnight. Washing was made with TBS/Tween 20 for 30 minutes. Bound IgE was detected with ^{125}I -labeled rabbit anti-human IgE (Pharmacia); 10^5 cpm/ml of blocking solution was applied overnight. Blots were rewashed in TBS/Tween 20 for 30 minutes, dried, and exposed to x-ray films (Hyperfilm MP, Amersham International) for 24 hours at -80°C , in NETO exposure cassettes (Nielsen & Olsen, Copenhagen, Denmark) containing intensifying screens.

RESULTS

Extracts of *P. ovale* analyzed in SDS-PAGE

Our aim was to produce an extract of *P. ovale* suitable for diagnosis of AD of the head, neck, and scalp. *P. ovale*, isolated from skin lesions of a patient suffering from this disease, was cultured on a selective medium. *P. ovale* colonies were collected and extracted with four different buffers and with or without the use of microglass balls. Extracts were subsequently analyzed in silver-stained SDS-PAGE (results not presented). More protein was evidently present in extracts obtained with glass beads. Extracts produced by means of ultrasonication exhibited only faintly stained protein bands when equal amounts of dry weight were loaded to SDS-PAGE gels. Furthermore, background staining similar to the medium control was observed in these samples. This observation indicated that extracting yeast cells "softly" by means of ultrasonication predominantly produced components of *P. ovale*, which were only weakly protein stained. They may, therefore, be heavily glycosylated. Thus, extraction by means of glass beads appeared to be favorable because of the numerous protein bands extracted. However, at this experimental stage, it had not been demonstrated which of the extracts was principally recognized by patients' IgE.

Extracts of *P. ovale* in IgE immunoblotting

The four different extracts obtained with or without glass beads were transferred to nitrocellulose. India ink staining of the blot again revealed more protein bands in extracts obtained with beads (Fig. 1). Three different serum pools were used for immunoblotting of corresponding membranes. The most pronounced IgE binding to *P. ovale* was observed in the serum pool from patients who were positive for *P. ovale* in

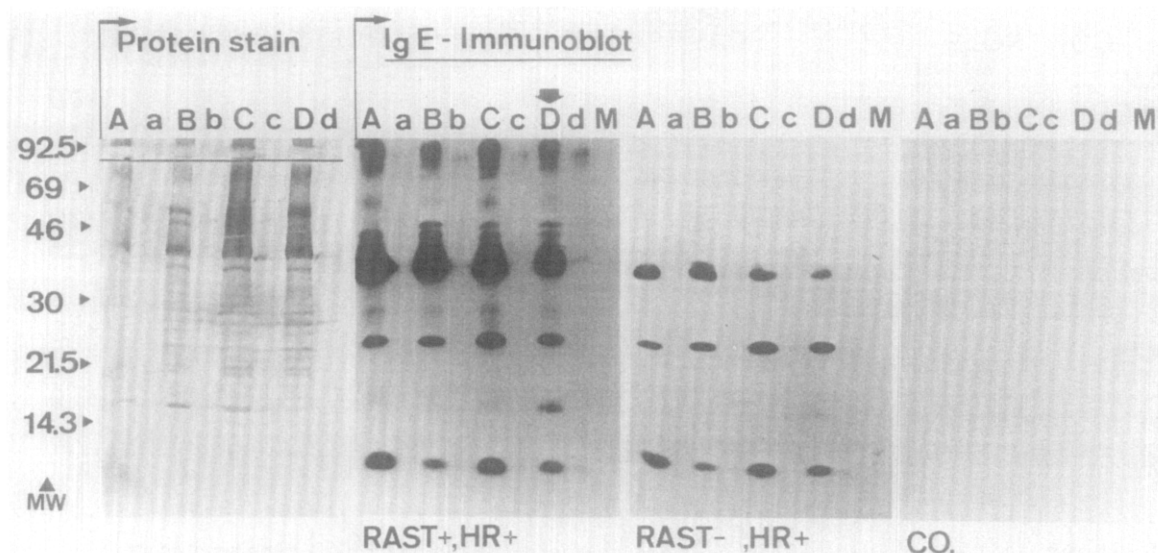


FIG. 1. Different extracts of *P. ovale* in immunoblotting. Extracts A to d, prepared as described in MATERIAL AND METHODS, were separated in 16% SDS-PAGE (1 mg per lane) and transferred to nitrocellulose. Blot membranes were protein stained with India ink or incubated with serum pools from patients suffering from AD of the head, scalp, and neck who reacted positively to extracts of *P. ovale* in RAST and/or HR test. Corresponding membrane was tested with control serum pool from healthy volunteers. Bound IgE was detected by ^{125}I -labeled rabbit antihuman IgE, 10^6 cpm per membrane. Blots were exposed to x-ray film for 24 hours; MWs in kilodaltons; black arrow, *P. ovale* extract selected as standard preparation.

HR test and RAST. Major IgE-binding components were detected at MWs of 9, 15, 25, 45, and 83 kd in extracts obtained with glass beads. The 9 kd allergen in all extracts and the 15 kd allergen in extract D were detected by IgE but remained unstained by India ink. Thus, the 9 and 15 kd allergens may comprise a large nonprotein moiety. The testing of the blots against a serum pool from patients who were RAST negative but HR test positive to *P. ovale* demonstrated IgE binding directed against the 9, 25, and 45 kd allergens.

Components in extracts obtained without glass beads were either not, or only barely recognized, by patients' IgE. Thus, allergens of *P. ovale* are substantially proteins (except the 9 and 15 kd allergens), which are not readily dissolved from the cell surface but may be extracted from the yeast cells with glass beads. In extract D, an additional allergen of 15 kd was detected, which was present only in small amounts in the other extracts. We therefore selected extract D (produced with NH_4HCO_3 and glass beads) as standard preparation for additional experiments.

Immunoblotting with individual sera

So far, we have demonstrated that a serum pool from patients with positive reactions to *P. ovale* in RAST exhibited enhanced IgE binding in the immunoblots, as compared with sera negative to *P. ovale* in RAST. Next, we intended to examine whether pa-

tients' sera, tested individually, exhibited IgE-binding patterns similar to patterns of the corresponding serum pool.

RAST-positive sera again demonstrated extensive IgE binding (Fig. 2). However, now the IgE binding was typically not directed toward the five allergens that were predominantly recognized by the corresponding serum pool, consisting of the same sera. In contrast, sera negative in *P. ovale* RAST demonstrated IgE binding predominantly directed toward allergens that were recognized by the pool consisting of the same sera. However, the observed IgE binding was weak, and surprisingly, four of the 13 sera in this group did not recognize blotted *P. ovale*, even though the same patients demonstrated positive reactions to *P. ovale* in HR test.

This finding demonstrated that the 9, 15, 25, and 72 kd *P. ovale* allergens were recognized by >50% of the patients reacting positively to *P. ovale* in RAST. Only the 9 and 25 kd allergens were recognized by >50% of patients negative in RAST but positive in HR test to *P. ovale* (Fig. 3). Individual testing of the sera from healthy subjects, used for the control serum pool, demonstrated that most sera did not, in fact, bind to *P. ovale* in immunoblotting. However, three of these 12 control sera, actually negative to *P. ovale* in skin tests, HR test, and RAST, demonstrated "false positive" IgE binding to components of *P. ovale* in

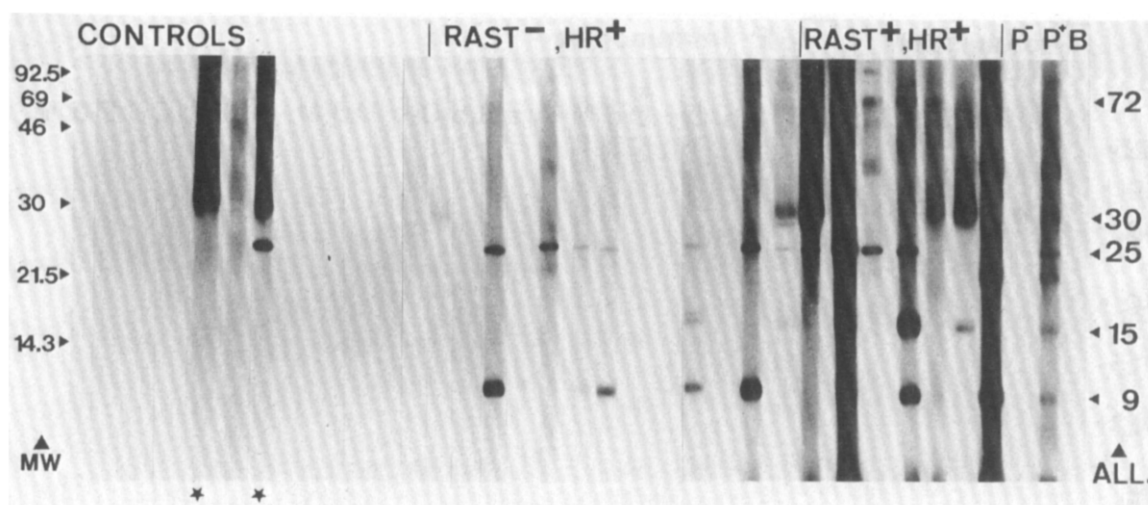


FIG. 2. IgE immunoblotting of sera from individual patients suffering from AD of the head, scalp, and neck and from healthy volunteers. Patients' sera were divided into two groups, RAST⁻/HR⁺ and RAST⁺/HR⁺. Pool of sera from the latter group was used as positive control pool (P⁺). Strips tested with control sera (*controls*) illustrating "false positive" reactions to blotted *P. ovale* extract were marked with *asterisks*. These nonallergic subjects demonstrated positive reactions to other yeasts and house dust-mite extracts in skin tests. Pooled control sera were used as negative control serum pool (P⁻); B, buffer control. Bound IgE was detected with ¹²⁵I-labeled rabbit anti-human IgE, 10⁵ cpm per strip. Blot strips were exposed to x-ray film for 24 hours.

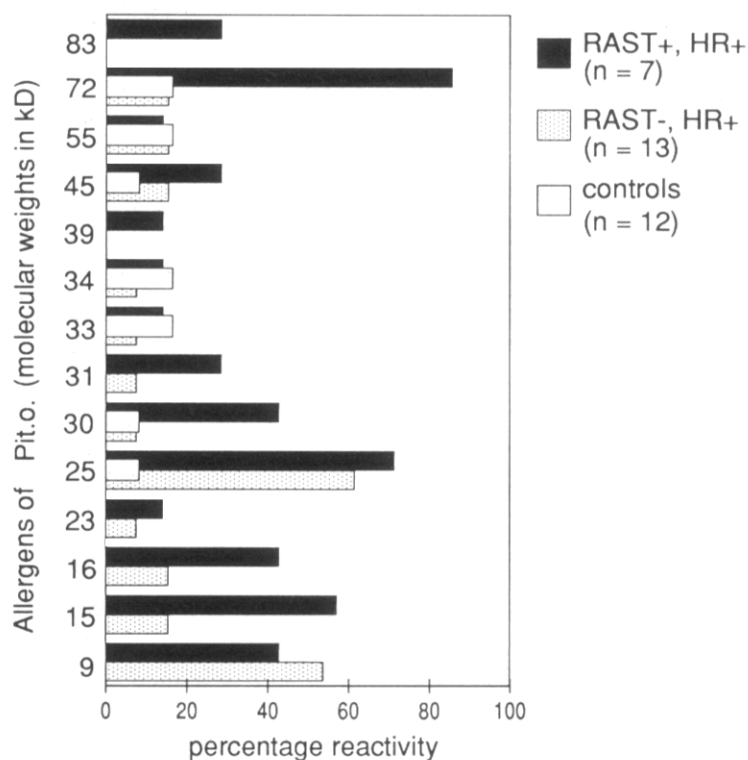


FIG. 3. IgE binding to allergens of *P. ovale* in immunoblotting of patients suffering from AD of the head, neck, and scalp and healthy control subjects; *black columns*, RAST⁺/HR⁺ patients (N = 7); *shaded columns*, RAST⁻/HR⁺ patients (N = 13); *open columns*, control subjects (N = 12); Y axis, MWs of *P. ovale* allergens in SDS-PAGE; X axis, percentage of patients and control subjects reacting to individual allergens in IgE immunoblotting.

the immunoblots. Reexamination of the test protocols revealed that these three individuals were the only individuals in the control group reacting with allergens other than *P. ovale* in HR test (*Saccharomyces brugeri*, *Candida albicans*, house dust mite, *Cladosporium*, and *Alternaria*). In consequence, we reexamined the case reports of the two groups of patients. In skin tests, both groups demonstrated higher incidences of positive reactions to other yeasts and to extracts of house dust mite as compared with the control group (Fig. 4). This finding was most pronounced in the patients reacting with *P. ovale*, not only in skin tests and HR test but also in RAST.

Individual testing of the patients' sera in immunoblotting demonstrated very heterogeneous IgE-binding patterns to *P. ovale*. The most intense IgE binding corresponded to positive reactivity in RAST. However, patients reacting with *P. ovale* in SPT and HR tests did not necessarily demonstrate IgE binding in RAST and immunoblot. Positive reactivity in the latter tests may require higher titer or affinity of specific IgE in the patients' sera.

Different sources of *P. ovale* in immunoblotting

Four of 20 patients, reacting positively to *P. ovale* in HR test, demonstrated no IgE binding in the immunoblots. This finding might be due to lack of allergenic compounds in the in-house standard preparation of *P. ovale*. We therefore sampled *P. ovale* from skin lesions of nine additional patients suffering from AD of the head, scalp, and neck. The yeasts were extracted according to the standard protocol and compared with the standard extract in immunoblotting. Again, the pools of sera reacting either positively or negatively to *P. ovale* in RAST were used.

As illustrated in Fig. 5, the IgE-binding patterns of the RAST-positive serum pool to the nine additional *P. ovale* extracts were heterogeneous and different from the IgE-binding pattern to the standard preparation. A comparison of protein staining and immunoblot confirmed that protein compounds were not necessarily recognized by IgE and that, in contrast, IgE did also bind to bands that were not protein stained.

The test with the serum pool of patients positive to *P. ovale* in HR test, but negative in RAST, demonstrated that four of these nine *P. ovale* extracts were not recognized at all by IgE. This result was unexpected, since all nine extracts contained at least one of the 9, 15, 25, or 44 kd allergens supposed to be a prerequisite for IgE binding of this serum pool (Fig. 1). This lack of binding may be due to relatively lower amounts of the above allergens in these preparations, since IgE binding could be detected after exposure of

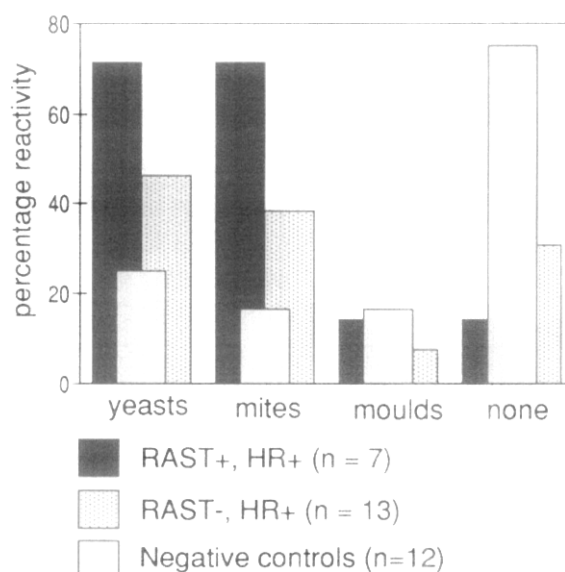


FIG. 4. SPTs in patients suffering from AD of the head, neck, and scalp and in control subjects with allergens other than *P. ovale*. Patients who were SPT positive to *P. ovale* were divided into two groups, HR⁺/RAST⁺ (black columns; N = 7) and HR⁺/RAST⁻ to *P. ovale* (shaded column; N = 13); open columns, healthy control subjects who were negative to *P. ovale* in SPT, HR test, and RAST (open column); Y axis, percentage of patients from these different groups also demonstrating positive skin tests to allergens other than *P. ovale* (other yeasts, house dust mite, and molds) or to none.

the autoradiographs for 1 week (data not presented).

Our results indicate that extracts of *P. ovale*, obtained from various patients suffering from AD of the head, scalp, and neck, may demonstrate very different MW patterns in immunoblotting. Nevertheless, all extracts were recognized by the IgE of other patients suffering from AD of the head, scalp, and neck. This finding implies that it may be impossible to standardize extracts of *P. ovale* with MW analysis. However, the above selected "standard preparation" contains a large spectrum of IgE-binding components that makes it suitable for diagnosis; it may, however, still be improved, for example, by adding extracts of *P. ovale* obtained from several different subjects.

DISCUSSION

In the present study, our aim was to produce extracts of *P. ovale* to obtain a stable and well-characterized reagent for diagnosis of AD of the head, scalp, and neck. Comparison of different extracts of *P. ovale* demonstrated that without preceding mechanical destruction of the yeast cells, only minute amounts of proteins were solubilized from the cell surface. These compounds may therefore mainly be carbohydrates or

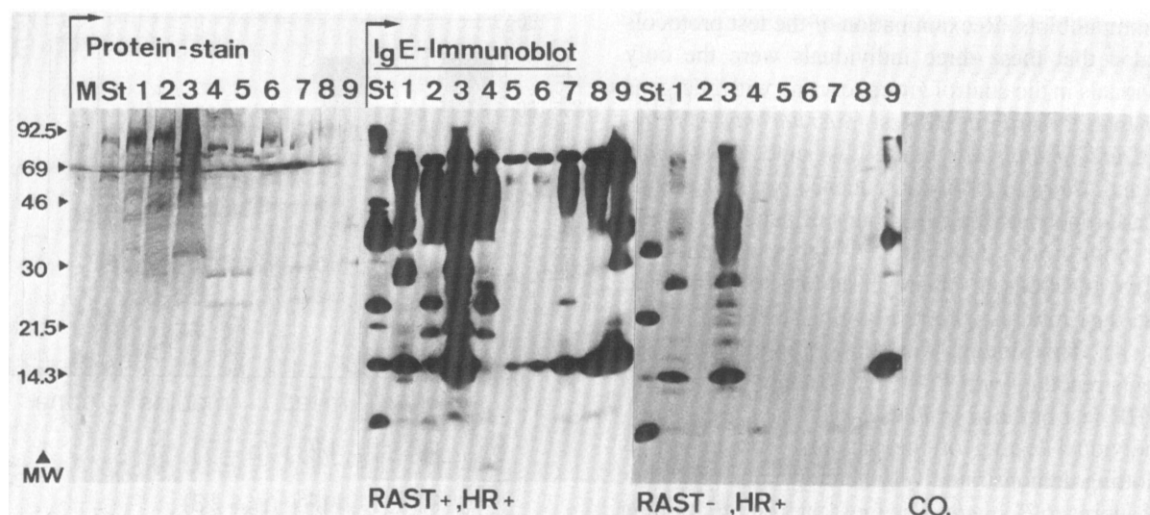


FIG. 5. Immunoblot analyses of standard extract (*St*) and extracts of *P. ovale* obtained from nine additional patients suffering from AD of the head, scalp, and neck. Membranes were protein stained with India ink or incubated with the serum pools from patients either HR⁺/RAST⁺ or HR⁺/RAST⁻ to *P. ovale*. One membrane was incubated with the control serum pool. Bound IgE was detected with ¹²⁵I-labeled rabbit antihuman IgE, 10⁶ cpm per membrane. Blots were exposed to x-ray film for 24 hours; MWs in kilodaltons.

glycoproteins, easily dissolved from the cell surface, as has been demonstrated to be the case with other yeasts.²⁴ This finding may also apply to *P. ovale*, since it was also demonstrated that the cell walls of different yeasts are similarly composed.²⁵ After destruction of the yeast cells with microglass beads, two nonprotein-stained allergens (9 and 15 kd) were observed. These allergens may comprise a large carbohydrate moiety. Carbohydrate antigens were recently described to be of importance also in extract of another yeast, *C. albicans*.²⁶

Most allergenic molecules could only be extracted from *P. ovale* after mechanical destruction of the cells. This finding may indicate that allergens of *P. ovale* are either intracellular components or tightly connected to the cell wall. In AD of the head, neck, and scalp, it remains to be explained whether *P. ovale* is, in fact, the causative agent or whether it is secondarily pathogenic only. On normal skin, *P. ovale* is present as saprophyte.²⁷ In AD, typically starting with erythema and itching,²⁸ the subsequent scratching might then transfer *P. ovale* to preexisting skin lesions and expose it to digestion by enzymes released by inflammatory cells. This hypothesis fits well with our data demonstrating that allergens are released only after disruption of the yeast cell walls. *P. ovale* might then, as a secondary pathogenic factor, accelerate the ongoing AD.

When the reactivity of patients suffering from AD of the head, scalp, and neck in skin test, HR test, RAST, and IgE immunoblotting was compared, it was

observed that all patients positive in RAST were also positive in IgE immunoblotting. Surprisingly, patients who were positive in skin and HR tests did not necessarily exhibit positive reactions to *P. ovale* in RAST, and four of 20 patients did not at all recognize *P. ovale* in immunoblot. This may be due to lack of binding to partially denatured allergens, which were boiled before separation in SDS-PAGE.

We compared extracts of *P. ovale*, obtained from skin lesions of various patients suffering from AD of the head, scalp, and neck. Unexpectedly, all extracts contained quite different components, as estimated from MWs in SDS-PAGE. Recently, extracts of *P. orbiculare*, identical to *P. ovale*,⁸ have been analyzed in an immunoblotting study, demonstrating MWs of constituents differing from our data.²⁹ The observed heterogeneity of different *P. ovale* extracts may be due to extraction of *P. ovale* from different sources or extraction of *P. ovale* during various stages of differentiation. However, all these extracts of *P. ovale* contained allergens recognized by the IgE of patients suffering from head, scalp, and neck AD.

Furthermore, patients with the most intense IgE binding to *P. ovale* in immunoblotting exhibited the highest incidence of positive reactivity to other yeasts in the skin tests. Cross-reactivity between different types of yeasts has previously been observed,³⁰ but surprisingly, the patients investigated in the present study often demonstrated positive reactions in skin and HR tests to extracts of both yeasts and of house dust mite. In three of the 12 healthy control subjects,

"false positive" reactions with *P. ovale* allergens in immunoblot were observed, although other tests did not indicate reactivity to this yeast (skin, HR tests, and RAST). These reactions were observed in individuals with positive skin test to yeasts other than *P. ovale*. This finding may indicate that cross-reactive determinants on allergens of *P. ovale* and other yeasts may exist. In fact, both yeasts, *Saccharomyces cerevisiae* (baker's yeast) and *S. carlsbergensis* (brewer's yeast), and a house dust mite (*Dermatophagoides pteronyssinus*) proved to have a certain cross-reactivity with *P. ovale* when they were used in RAST-inhibition studies with *P. ovale* and the pool of HR⁺/RAST⁺ patient sera (results not presented). The specificity of the RAST test was confirmed, since timothy-grass pollen (*Phleum pratense*) did not demonstrate any inhibition at all.

In the present study, a complex heterogeneity of *P. ovale* allergens was observed. However, the selected standard extraction procedure appears to be suitable for preparing this yeast, since a large spectrum of allergens was detected in extracts prepared according to this protocol. Our data suggest that at present, skin test, as well as HR test is superior to the RAST assay for diagnosing head, scalp, and neck AD.

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