

IgE antibodies to protein and mannan antigens of *Pityrosporum ovale* in atopic dermatitis patients

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Summary

Background *Pityrosporum ovale* is a common saprophyte on the skin capable of inducing IgE antibody production in atopic dermatitis (AD) patients. Allergens of *P. ovale* have been examined in several studies, but consensus on them is lacking.

Objective This study was carried out to obtain more information about the IgE antibody response against *P. ovale*, including mannan.

Methods Sera from 64 AD patients and 10 healthy controls were analysed with immunoblotting and the nitrocellulose radio allergosorbent test (RAST) method specifically developed to detect antimannan *P. ovale* IgE antibodies.

Results In immunoblotting a total of 39 different IgE stained protein bands were seen. A high molecular weight staining was also seen especially in patients who displayed elevated mannan *P. ovale* RAST values. The most commonly stained protein bands in immunoblotting were 9 and 96 kD bands with antibodies in 73 and 65% of AD patients who had been positive in commercial *P. orbiculare* RAST with total serum IgE less than 4000 kU/l. Mannan RAST appeared positive in 77% of them. Positive immunoblotting to either of these bands was seen in 90% and, if added with staining with the 20 kD band, in 100% of these AD patients. A combination of 9 kD IgE staining and mannan *P. ovale* RAST was positive in 92% of the patients and 96 kD and mannan *P. ovale* RAST in 85% of the patients.

Conclusion It is evident that *P. ovale* has several allergens, the 9, 96 and 20 kD regions being the most important. According to our results mannan is also an important allergen of *P. ovale*.

Keywords: *Pityrosporum ovale*, atopic dermatitis, allergen, immunoblotting, IgE, mannan

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Introduction

Pityrosporum ovale is the oval form of a dimorphic lipophilic yeast, known in its round yeast form as *P. orbiculare* and in its hyphal form as *Malassezia furfur* [1]. It belongs to the normal microflora of human skin [2,3], but can also act as an allergen for patients with atopic dermatitis (AD) especially with head, neck and shoulder distributions of the dermatitis [4–6].

Allergens of *P. ovale* have been examined in several studies. According to these results approximately 20 different IgE binding bands can be detected on immunoblotting [7–9]. In these reports the molecular weights of the

IgE-stained bands vary and a consensus on them is lacking [6], due to difficulties in interpreting the results, especially when autoradiography has been used. Some bands are close to each other and in one study the bands between 30 and 67 kD were difficult to differentiate [8].

Mannan and its capacity to give rise to an IgE response is poorly characterized, because the methods of detecting *P. ovale* mannan have not been satisfactory. It appears, however, to be an important allergen, since in an IgE-ELISA study with Concanavacin A (ConA)-purified mannans of *Candida albicans* and *P. ovale* as allergens, an IgE response against mannan was detected in more than 50% of AD patients [10,11]. Furthermore there is evident cross-reactivity between *P. ovale* and *C. albicans*. Radio allergosorbent test (RAST)-inhibition and radioimmuno-electrophoretic studies, affinity chromatography and periodate oxidation

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experiments have suggested that the cross-reacting allergenic component could be mannan [9,10]. *Saccharomyces cerevisiae* extract and purified *S. cerevisiae* enolase have also shown allergenic cross-reactivity with a *C. albicans* extract [12]. Mannans purified by ConA affinity chromatography from *C. albicans* and *S. cerevisiae* have been shown to exhibit a typical diffuse high molecular weight stain on IgE immunoblotting [13]. Generally the mannan of *P. ovale* is regarded as difficult to demonstrate in immunoblotting experiments as it has not been detected in the sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) gel even with a low acrylamide concentration (5%) [9]. Therefore, the analysis of mannan has remained unreliable on immunoblotting.

In order to detect simultaneously the occurrence of IgE antibodies against both mannan and IgE-binding proteins of *P. ovale*, a study using IgE immunoblotting and nitrocellulose RAST technique with chemically purified mannan was carried out to analyse serum samples from AD patients and controls.

Material and methods

Patient sera

Serum samples from 74 patients were analysed. All patients were outpatients from the Department of Dermatology, University Hospital of Turku. The selection consisted of 64 AD patients from whom a specific RAST value (Pharmacia, Uppsala, Sweden) for *P. orbiculare*, *C. albicans* and *S. cerevisiae* IgE antibodies had been analysed previously. The diagnosis of AD was based on the criteria of Hanifin & Rajka [14] and was established by a dermatologist. Of these 64 patients there were 41 females and 23 males. Age varied from 4 to 64 years (mean age 31.8 years). In addition, samples from 10 other patients with no history of atopic diseases were run in the experiments. The patients were divided into five groups (groups 1–5) (Table 1): group 1, 40 patients with AD, with serum total IgE value < 4000 kU/l and

P. orbiculare RAST value positive (> 0.35 kU/l); of these 40 patients, 31 also had *S. cerevisiae* RAST value > 0.35 kU/l and 36 had *C. albicans* RAST value > 0.35 kU/l; group 2, six AD patients with negative *P. orbiculare* RAST value but positive *C. albicans* or *S. cerevisiae* RAST value; group 3, eight AD patients, with serum total IgE value > 10 000 kU/l and *P. orbiculare* RAST value > 0.35 kU/l; group 4, 10 AD patients with negative (≤ 0.35 kU/l) RAST value for yeasts; group 5, 10 patients with no history of atopic diseases.

Antigens

The *P. ovale* strain (CBS 7854) used in the assays was isolated from a patient by the mycological laboratory of the University Central Hospital of Turku and typed and deposited in The Centraalbureau Voor Schimmelcultuur, (Delft, Netherlands). The yeast was grown on agar plates on medium containing reduced-fat cream supplementum according to Leeming & Notman [15]. The crude cytoplasmic extract was prepared with disruption of the cells in an X-press (AB Biox, Goteborg, Sweden) chamber according to Savolainen *et al* [16]. *Pityrosporum ovale* mannan was purified according to the Cetavlon method of Nakajima & Ballou [17].

SDS-PAGE and electrophoretic blotting

Immunoblotting was performed essentially according to Bengtson *et al.* as previously described [18]. A 5–17.5% gradient gel was used in the assays. The optimal concentration of *P. ovale* crude extract was determined from experiments using different concentrations of antigen (0.8–100 mg/ml). As an example, protein staining with Coomassie brilliant blue is shown in Fig. 1(c) using an antigen load of 12.5 mg/ml. *Pityrosporum ovale* crude extract (12 mg for one 16-cm wide gel) and molecular weight markers (Rainbow coloured protein, molecular weight marker; Amersham International PLC, Amersham, UK)

Table 1. Classification of the five patient groups included in the study

Group	Number	AD	Total IgE (kU/l)	IgE RAST (kU/l)		
				<i>Pityrosporum orbiculare</i>	<i>Candida albicans</i>	<i>Saccharomyces cerevisiae</i>
1	40	Yes	<4000	>0.35 (n = 40)	>0.35 (n = 36)	>0.35 (n = 31)
2	6	Yes	<4000	<0.35 (n = 6)	>0.35 (n = 6)	>0.35 (n = 2)
3	8	Yes	>10 000	>0.35 (n = 8)	>0.35 (n = 8)	>0.35 (n = 8)
4	10	Yes	<4000	<0.35 (n = 10)	<0.35 (n = 10)	<0.35 (n = 10)
5*	10	No	Not known	Not known	Not known	Not known

* Non-atopics.

AD, Atopic dermatitis; RAST, radio allergosorbent test.

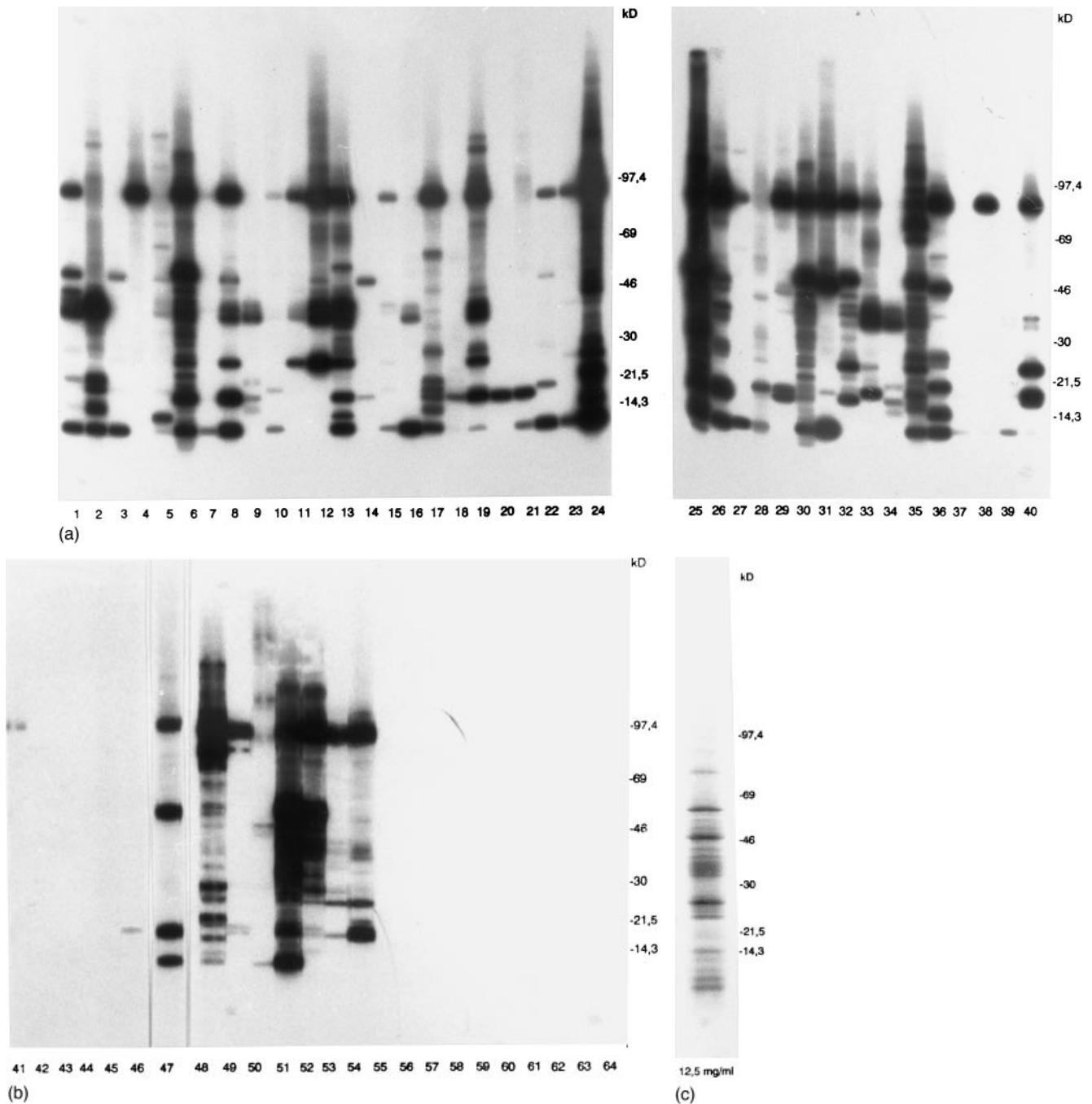


Fig 1. (a) and (b) Immunoblot analysis for specific IgE antibodies against antigens of *Pityrosporum ovale*. Numbers 1–40 are patients in group 1 (atopic dermatitis (AD) patients, with serum total IgE value < 4000 kU/l and *P. orbiculare* radio allergosorbent test (RAST) value positive); numbers 41–46 are patients in group 2 (AD patients with negative *P. orbiculare* RAST value, but positive *Candida albicans* or *Saccharomyces cerevisiae* RAST values); numbers 47–54 are patients in group 3 (AD patients, with serum total IgE value > 10 000 kU/l and positive *P. orbiculare* RAST value) and numbers 55–64 are patients in group 4 (AD patients with negative RAST value for yeasts). There were no bands on immunoblot in group 5 (patients with no history of atopic diseases). (c) Coomassie brilliant blue stained sodium dodecyl sulphate polyacrylamide gel electrophoresis with 12.5 mg/ml *P. ovale* crude extract.

were diluted with water and Tris-buffer, adjusted to pH 6.8, containing 2% (w/v) SDS and 10% (w/v) glycerol. The samples were incubated for 5 min at 100°C without reduction and then separated with SDS-PAGE in a 5–17.5% gradient gel overnight according to Laemmli [19]. Separated antigens were then transferred to nitrocellulose sheets (Schleicher & Schuell, Dassel, Germany) in a Transphor apparatus at 1.0 A for 1.5 h (Hoefer, San Francisco, USA) according to Towbin *et al* [20].

IgE antibody detection

The specific IgE binding of the allergens on nitrocellulose was identified as described [18]. Nitrocellulose strips were first incubated in PBS-Tween 20 0.5% for 1 h and thereafter overnight at 20°C with the patients' sera, diluted to 1:4 in PBS-Tween. After three washes with PBS-Tween, ¹²⁵I-labelled anti-IgE antibodies (Pharmacia CAP Systems, RAST RIA, Kabi Pharmacia Diagnostics, Uppsala, Sweden) diluted 1:10 in PBS-Tween were added and incubated for 4 h at 20°C with the strips. The ¹²⁵I-labelled bands were visualized on a Trimax X-ray film in a cassette with intensifying screens (Trimax XM, 3M, Italy) after storage at –70°C prior to developing.

P. ovale mannan-RAST

A modification of the previously described RAST method used to detect *C. albicans* mannan by Nermes *et al.* [21] was applied. Discs of a diameter of 5.5 mm

were cut from nitrocellulose sheets (pore size 0.22 µm; Schleicher & Schuell). The optimum concentration for mannan absorption to the discs was sought from the concentration series from 0.0039 to 1 mg/ml. According to these experiments 0.15 mg/ml was selected. The discs were incubated for 2 h at 37°C in a shaker. After three washes with PBS-Tween the discs were blocked by incubation for 1 h with PBS-Tween in a shaker at 37°C. The discs were set in RAST tubes, one disc in each tube, and 50 µl of non-diluted serum samples was applied on the discs to be incubated at 4°C overnight. They were then washed three times with 0.9% NaCl Tween 20 and ¹²⁵I-labelled anti-IgE (KabiPharmacia) was added to be incubated for 2 h at 37°C. Finally, the discs were washed three times with NaCl Tween and radioactivity bound to the discs was detected by a gamma-counter (LKB Wallac, Turku, Finland). The results were expressed as a mannan RAST index: [sample (c.p.m.) — buffer (c.p.m.) × 10²] / [total activity (c.p.m.) — buffer (c.p.m.)].

Densitometry

The staining intensity of the high molecular weight region of each individual strip was measured by a densitometer (Bio-Rad, CA, USA). The values obtained were presented in optic density (OD) for statistical analysis.

Statistics

Spearman's rank correlation test was used for statistical analysis of correlations in Figs 4–6.

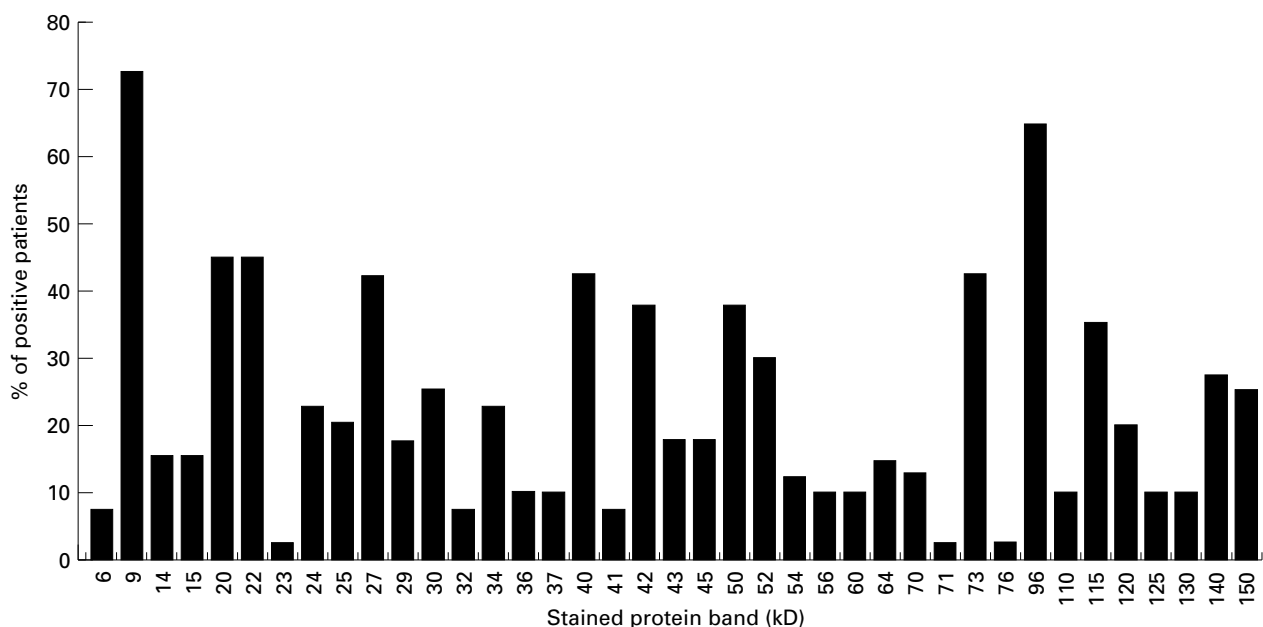


Fig 2. Summarized histogram of the IgE-binding protein bands from 40 atopic dermatitis patients in group 1. Molecular weight (kD) of protein bands stained on immunoblotting against the percentage of patients who displayed positive IgE staining.

Table 2. IgE response against different *Pityrosporum ovale* antigens.

IgE-binding allergens of <i>P. ovale</i>	Patients positive (%)
9 kD	73
96 kD	65
9 kD and/or 96 kD	90
9, 96 and/or 20 kD	100
<i>P. ovale</i> mannan	77
Mannan and/or 9 kD	92
Mannan and/or 96 kD	85
Mannan and/or 20 kD	85

Atopic dermatitis patients with positive commercial *P. orbiculare* radio allergosorbent test and total serum IgE < 4000 kU/l included.

Results

Immunoblotting

IgE immunoblots of the atopic patients (groups 1–4) are presented in Fig. 1(a) and 1(b) and summarized according to the molecular weights in Fig. 2. Each of the 40 patients with AD and positive *P. orbiculare* RAST values and total IgE under 4000 kU/l (group 1) was positive in immunoblots usually staining several bands. The AD patients who had negative *P. orbiculare* RAST values, but positive *C. albicans* and *S. cerevisiae* RAST values (group 2), showed some single positive IgE-binding bands. Four of the six patients

only had positive *C. albicans* RAST values. Three of these patients showed one weak band each. Two of these six patients also had positive *S. cerevisiae* RAST values. Both of them stained *P. ovale* strongly on immunoblots, one in the 96 kD and the other in the 22 kD region. In the group including patients who had positive *P. orbiculare* RAST values and total IgE value > 10 000 kU/l (group 3), dark, rather homogeneous staining and polyspecific response against several bands, difficult to interpret, were seen. The immunoblots of the yeast RAST negative atopics (group 4) and non-atopic controls (group 5) were completely negative in staining.

The IgE response was directed against 39 different protein bands in group 1. Two major allergens (over 50% of patients reacting) were found, 9 kD (73%) and 96 kD (65%). When samples which displayed IgE staining to 9 and 96 kD bands were counted together it appeared that 90% of the patients in group 1 were positive (Table 2). If the positive staining to the 20 kD band was added, each patient in group 1 could be traced. The most important intermediate allergens (10–50% of patients reacting) were the 20 kD (45%), 22 kD (45%), 27 kD (42%), 40 kD (42%), 42 kD (38%), 50 kD (38%), 52 kD (30%), 73 kD (42%), 115 kD (35%), 140 kD (27%) and 150 kD (25%) bands.

Correlation with *P. orbiculare* RAST and low molecular weight immunoblotting was not significant ($r = 0.26$, $P = 0.1$). However, for example four patients (nos 3, 11, 16 and 22) who were positive with *P. orbiculare* RAST stained the 9 and 96 regions without evidence of mannan staining.

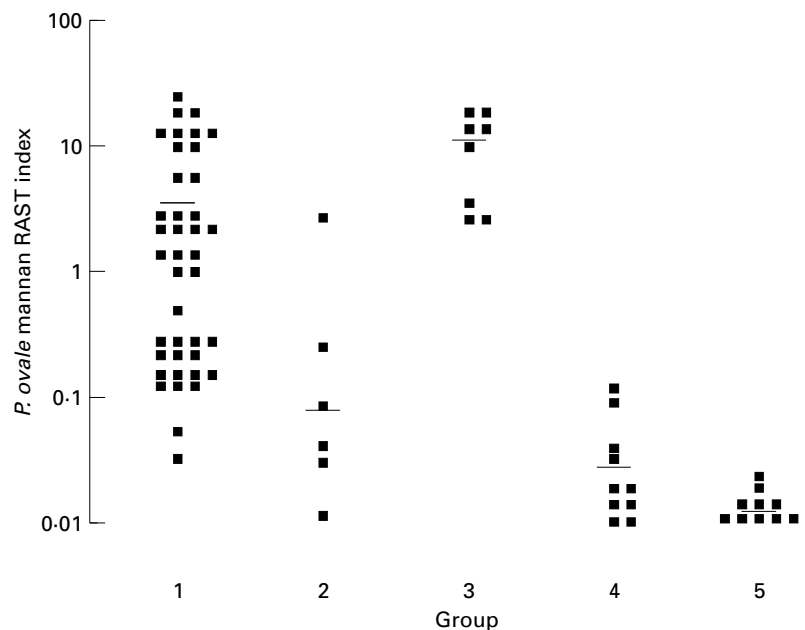


Fig. 3. *Pityrosporum ovale* mannan radio allergosorbent test (RAST) index values of different study groups. The short lines represent mean values. Group 1, mean 6.0, SD $\pm$ 8.9; group 2, mean 0.97, SD $\pm$ 2.1; group 3, mean 17.8, SD $\pm$ 11.7; group 4, mean 0.060, SD $\pm$ 0.046 and group 5, mean 0.013, SD $\pm$ 0.014.

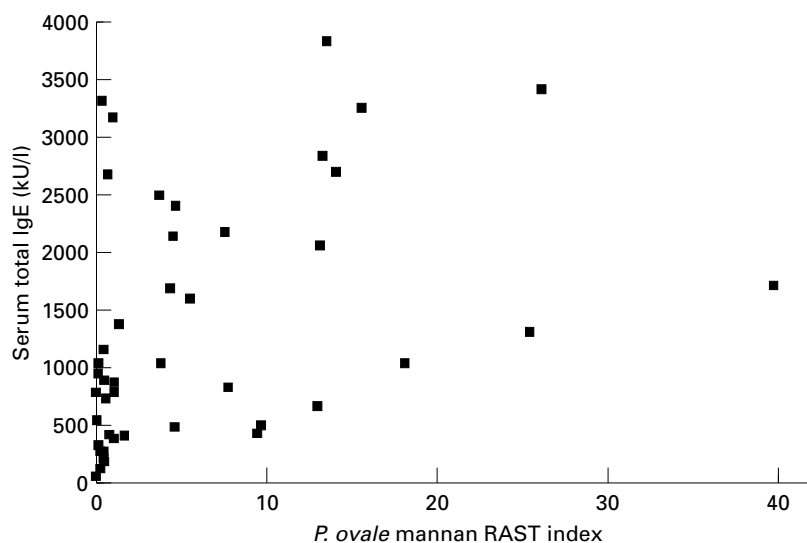


Fig. 4. The correlation between serum total IgE level (kU/l) and *Pityrosporum ovale* mannan radio allergosorbent test (RAST) indices of the 40 atopic dermatitis patients in group 1 ($r = 0.42$, $P < 0.01$).

Mannan RAST values for *P. ovale*

The mannan RAST results in the study groups are presented in Fig. 3. The *P. ovale* mannan RAST indices were clearly elevated in *P. orbiculare* RAST positive patient groups with both moderate (group 1) as well as high (group 3) serum total IgE values. Patients with very high total serum IgE level showed the highest mannan RAST values. A significant correlation ($r = 0.42$, $P < 0.01$) between serum total IgE and *P. ovale* mannan RAST index was seen (Fig. 4). However, occasionally high mannan RAST index (> 5) values were found with serum IgE below 1000 kU/l and also patients with negative mannan RAST had elevated total IgE (> 2500).

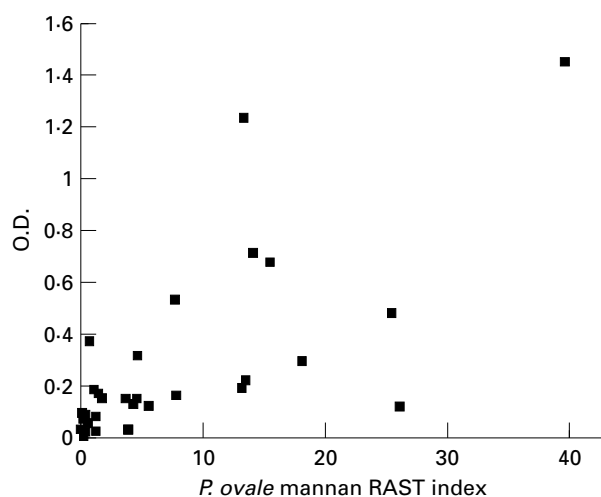


Fig. 5. The correlation between densitometer results (O.D.) and *Pityrosporum ovale* mannan radio allergosorbent test (RAST) indices of the 40 atopic dermatitis patients in group 1 ($r = 0.73$, $P < 0.001$).

In group 1, 31 of 40 (77%) patients displayed *P. ovale* mannan RAST > 0.35 kU/l. If this mannan *P. ovale* RAST result was combined with the positive 9 kD immunoblot staining, 92% of patients in group 1 were positive. If the combination of positive results with the 96 kD band was compared, 85% in group 1 were positive (Table 2).

When mannan RAST values were compared with the high molecular weight diffuse stain of immunoblotting assessed by densitometer, a highly significant correlation ($r = 0.73$, $P < 0.001$) between them was found (Fig. 5). Serum total IgE also correlated with the high molecular weight staining, but to a lesser degree ($r = 0.43$, $P < 0.01$).

The patients with negative *P. orbiculare* RAST but positive RAST for *C. albicans* or *S. cerevisiae* had low *P. ovale* mannan RAST indices. The relationship of commercial *C. albicans*, *S. cerevisiae* and *P. orbiculare* RAST were further compared with *P. ovale* mannan RAST (Fig. 6). Poor correlation ($r = 0.14$, $P = 0.39$) was seen between commercial *P. orbiculare* RAST and mannan RAST (Fig. 6a). Several patients who had negative mannan RAST displayed high value of commercial RAST due to *P. orbiculare* protein specific antibodies. Such a phenomenon is not seen with other yeasts and the correlations between *P. ovale* mannan RAST and commercial *C. albicans* ($r = 0.22$, $P = 0.17$) (Fig. 6b) and *S. cerevisiae* ($r = 0.27$, $P = 0.09$) RAST values (Fig. 6c) were better but still poor.

Discussion

Pityrosporum ovale is a common saprophyte of man colonizing primarily the sebaceous areas of the skin [2,3]. In AD patients it has been detected in 40–70% of patients. Atopic dermatitis in the head, neck and shoulder region has been

connected with immediate hypersensitivity to yeasts, especially to *P. ovale*, *S. cerevisiae* and *C. albicans* [4–6]. Atopic dermatitis patients suffering from severe, widespread dermatitis also commonly have elevated total IgE levels and specific IgE antibodies to yeasts.

According to earlier studies focusing on the IgE-binding allergens of *P. ovale*, in total 20 bands have been detected. In our study we found 39 different IgE-binding bands. One major allergen appeared in the 9 kD band, which is in agreement with the results presented by Jensen-Jarolim *et al.* [7]. They also found the 9 kD band against which

50% of the IgE-positive patients had a response. In two other studies the low molecular weight allergens were estimated to be in the 10 or 13 kD bands [8,9]. The 73 kD intermediate allergen band (42%) was also quite close to the 72 kD band (40%) found by Jensen-Jarolim *et al.* [7]. The 20–27 kD region is more complex. In our study the most important allergenic bands binding IgE (> 40%) were 20, 22 and 27 kD bands. Johansson & Karlström [8] found 21 kD (43%) and 28 kD (57%) bands [8] and, according to Savolainen & Broberg [9], IgE staining was seen against 23 and 26 kD bands. In this region the main band in Jensen-Jarolim's

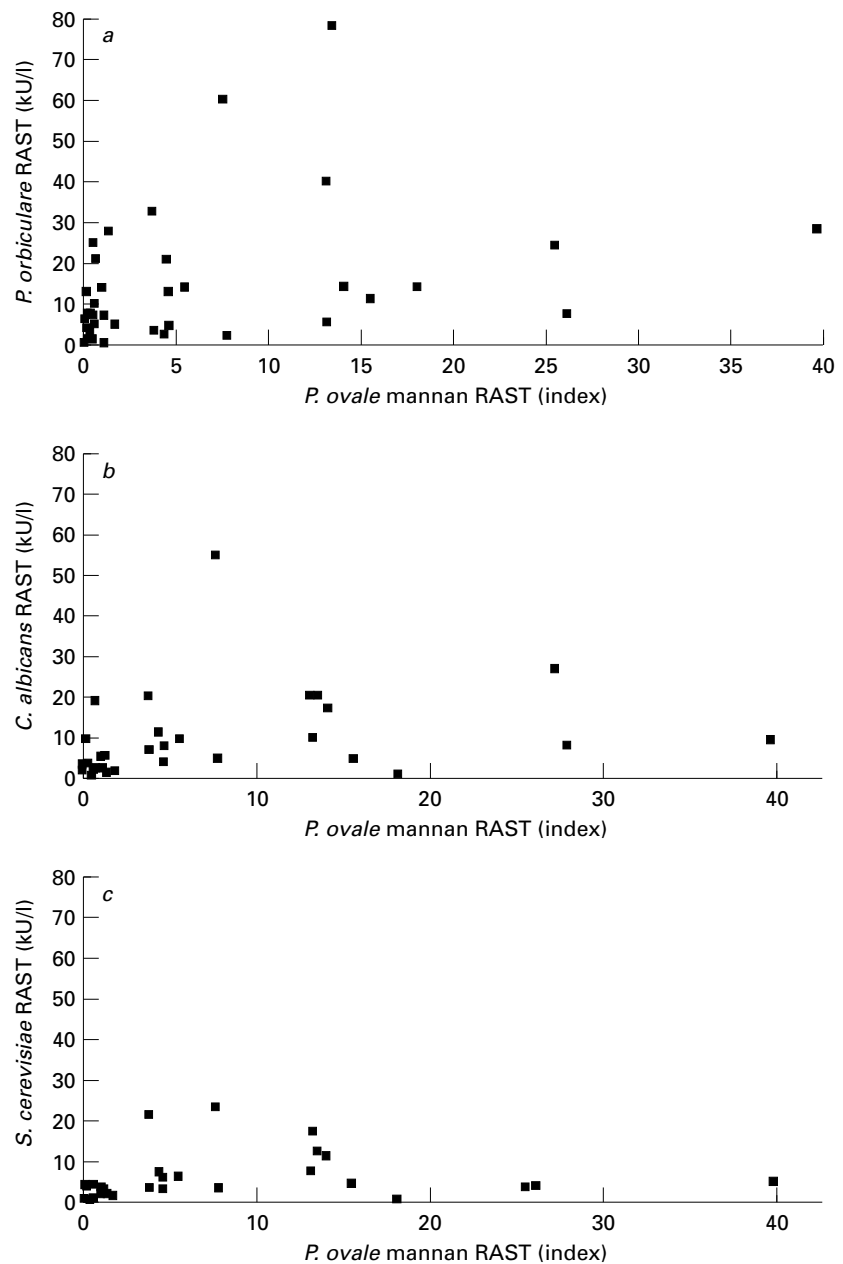


Fig. 6. The correlation between commercial radio allergosorbent test (RAST) results (kU/l) with *Pityrosporum ovale* mannan RAST index (a) with *P. orbiculare*, (b) with *Candida albicans* and (c) with *Saccharomyces cerevisiae*.

study was 25 kD. It is likely that at least some of these bands represent the same allergens. The main allergen reported by Johansson was a 67 kD band, the closest corresponding band in our study was 73 kD intermediate allergen.

The total number of allergens in earlier immunoblotting studies has been amazingly low. Figure 1 clearly shows that the resolution was adequate to distinguish bands with 1 kD intervals, especially below 50 kD. On the other hand, the cut-off level for allergen bands in SDS-PAGE in the two studies mentioned earlier seemed to be the 90 kD band [7,8]. This would have excluded the 96 kD major allergen, four intermediate allergens (115, 120, 140 and 150 kD) and three minor allergens found in our analysis. This may also be the explanation why mannan was not visualized in these studies.

A high molecular weight staining was visible on immunoblotting, as the acrylamide concentration used in the gradient gel was low. With a suitable acrylamide concentration *P. ovale* mannan was visualized in SDS-PAGE in the same way as has been reported before with *C. albicans* and *S. cerevisiae* [13,16]. Patients who had high molecular staining on immunoblotting also had an elevated mannan RAST level against *P. ovale*.

The IgE response against protein allergens of yeasts seems to be specific. In this study patients who were *P. orbiculare* RAST-negative, but positive to *C. albicans* or *S. cerevisiae* RAST, showed only faint IgE staining to single bands on immunoblotting. In contrast, some patients with negative *P. ovale* mannan RAST were highly positive in commercial *P. orbiculare* RAST. These patients evidently had specific IgE antibodies to *P. ovale* proteins but not to *P. ovale* mannan. The explanation for that might be that carbohydrates cannot be coupled covalently to cyanogen bromide activated paper discs. When the results were compared between *P. ovale* mannan RAST and commercial *C. albicans* and *S. cerevisiae* RAST, only those who had elevated mannan RAST values also displayed positive results in *C. albicans* or *S. cerevisiae* RAST. This could be due to the cross-reacting anti-mannan IgE antibodies, as previously shown with both *S. cerevisiae* and *C. albicans* [22]. In these patients *P. ovale* may be the primary sensitizer, indicated by protein specific antibodies, allergy to other yeasts occurring due to cross-reacting anti-mannan IgE antibodies as suggested by Doekes *et al.* [10,11].

Atopic dermatitis patients suffering from severe, widespread dermatitis also commonly have elevated total serum IgE level and specific IgE antibodies to yeasts in addition to several other allergens. The specificity and clinical significance of these IgE antibodies are not clear. On immunoblotting the patients with very high total serum IgE raised a polyspecific response with laborious interpretation of the bands. Therefore, the interpretation of our results was done without these patients, which should be common practice in allergen characterization.

The immunopathogenetic importance of *P. ovale* mannan in AD is not clearly known. Clinical studies to confirm its significance are needed. The cross-reactivity of anti-mannan IgE antibodies should also be examined with *P. ovale* mannan and other yeasts. The stability of *P. ovale* allergens during storage should be analysed before starting clinical research.

Acknowledgements

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References

- 1 Faergeman J, Tjernlund U, Scheynius A, Bernander S. Antigenic similarities and differences in genus *Pityrosporum*. *J Invest Dermatol* 1982; 78:28–31.
- 2 Roberts SOB. *Pityrosporum orbiculare*: incidence and distribution on clinically normal skin. *Br J Derm* 1969; 81:264–9.
- 3 Ruiz-Maldonado R, Lopez-Matinez R, Perez Chavarria E, Castañón L, Tamayo L. *Pityrosporum ovale* in infantile seborrheic dermatitis. *Pediatric Dermatol* 1989; 6:16–20.
- 4 Clemmensen OJ, Hjort N. Treatment of dermatitis of the head and neck with ketokonazole in patients with type I sensitivity to *Pityrosporum orbiculare*. *Semin Dermatol* 1983; 2:26–9.
- 5 Woerstedt A, Hjort N. *Pityrosporum orbiculare*—a pathogenic factor in atopic dermatitis of the face, scalp and neck? *Acta Derm Venereol (Stockh)* 1985; Suppl 114:146–8.
- 6 Kieffer M, Bergbrandt I-M, Faergeman J *et al.* Immune reactions to *Pityrosporum ovale* in adult patients with atopic dermatitis and seborrheic dermatitis. *JAAD* 1990; 22:739–49.
- 7 Jensen-Jarolim E, Poulsen LK, With H, Kieffer M, Ottevanger V, Skov PS. Atopic dermatitis of the face, scalp and neck: type I reaction to the yeast *Pityrosporum ovale*? *J Allergy Clin Immunol* 1992; 89:44–51.
- 8 Johansson S, Karlström K. IgE-binding components in *Pityrosporum orbiculare* identified by an immunoblotting technique. *Acta Derm Venereol (Stockh)* 1991; 71:11–6.
- 9 Savolainen J, Broberg A. Allergenic crossreactivity of *Pityrosporum ovale* and *Candida albicans*. *Clin Exp Allergy* 1992; 22:469–74.
- 10 Doekes G, van Ieperen-van Dijk AG. Allergens of *Pityrosporum ovale* and *Candida albicans* I. Cross-reactivity of IgE-binding components. *Allergy* 1993; 48:394–400.
- 11 Doekes G, Kaal MJH, van Ieperen-van Dijk AG. Allergens of *Pityrosporum ovale* and *Candida albicans* II. Physicochemical characterization. *Allergy* 1993; 48:401–8.
- 12 Baldo BA, Baker RS. Inhalant allergies to fungi: reactions to bakers' yeast (*Saccharomyces cerevisiae*) and identification of bakers' yeast enolase as an important allergen. *Int Arch Allergy Appl Immunol* 1988; 86:201–8.
- 13 Kortekangas-Savolainen O, Kalimo K, Lammintausta K, Savolainen J. IgE-binding components of baker's yeast recognized

- by immunoblotting analysis. Simultaneous IgE binding to mannan and 46–48 kD allergens of *S. cerevisiae* and *C. albicans*. Clin Exp Allergy 1993; 23:179–84.
- 14 Hanifin J, Rajka G. Diagnostic features of atopic dermatitis. Acta Derm Venereol (Stockh) 1980; Suppl 92:42–7.
- 15 Leeming J, Notman F. Improved methods for isolation and enumeration of *Malassezia furfur* for human skin. J Clin Microbiol 1987; 25:2017–9.
- 16 Savolainen J, Viander M, Einarsson R, Koivikko A. Immunoblotting analysis of concanavalin A-isolated allergens of *Candida albicans*. Allergy 1990; 45:40–6.
- 17 Nakajima T, Ballou CE. Characterization of the carbohydrate fragments obtained from *Saccharomyces cerevisiae* mannan by alkaline deacration. J Biol Chem 1974; 23:7679–84.
- 18 Bengtson A, Rolfsen W, Einarsson R. Characterization of allergens and patient sera by a nitrocellulose immunoprint technique. Int Archs Allergy Appl Immunol 1985; 78:139.
- 19 Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 227:680–5.
- 20 Towbin HT, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 1979; 76:4350–4.
- 21 Nermes M, Savolainen J, Kalimo K, Lammintausta K, Viander M. Determination of IgE antibodies to *Candida albicans* mannan with nitrocellulose-RAST in patients with atopic diseases. Clin Exp Allergy 1994; 24:318–23.
- 22 Nermes M, Savolainen J, Kortekangas-Savolainen O. Nitrocellulose-RAST analysis of allergenic crossreactivity of *Candida albicans* and *Saccharomyces cerevisiae* mannans. Int Arch Allergy Immunol 1995; 106:118–23.