

Crossreacting IgE antibodies to *Pityrosporum ovale* and *Candida albicans* in atopic children

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Summary

Sera from 13 patients with positive *Pityrosporum ovale* skin prick test were analysed with IgE immunoblotting. Both *P. ovale* and *Candida albicans* antigens were used to reveal possible crossreactivity of these yeasts. Of the 13 sera, eight sera showed IgE binding to *P. ovale* and six sera to *C. albicans*. Simultaneous IgE-binding to both *C. albicans* and *P. ovale* was observed in five of these sera. The most common IgE-binding band pairs were, a 23 kD band of *P. ovale* and a 27 kD band of *C. albicans*, as one pair, and a 26 kD band and a 13 kD band, respectively, as another pair. In addition to these protein bands, simultaneous reactivity was observed to *C. albicans* mannan, a polysaccharide, and to a diffuse high molecular weight component of *P. ovale*, possibly also a polysaccharide. The five sera with simultaneous IgE-binding were analysed with RAST-inhibition, which revealed presence of crossreacting IgE-antibodies to *P. ovale* and *C. albicans* in two of these sera. Crossreacting epitopes were suggested to be located in the mannan polysaccharide of *C. albicans* and high molecular weight diffuse stain of *P. ovale*, based on the immunoblotting and RAST-inhibition patterns of the studied sera. Crossreacting sera were pooled and used as an IgE probe in CRIE and Tandem-CRIE. These experiments revealed a fused precipitin line indicating presence of a common structure of *P. ovale* and *C. albicans*.

This suggested crossreactivity may imply an interesting phenomenon in atopics who are sensitized by *C. albicans* growth in the gastrointestinal tract and get exposed by *P. ovale* growing on the skin. Further studies are thus needed to clarify the clinical significance of these preliminary findings.

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Introduction

The atopic patient has a tendency for acquiring sensitization to different allergens as a consequence of genetic factors, allergen dose and time of exposure [1]. *Pityrosporum ovale* is the oval form of a lipophilic yeast, known also in the round form as *Pityrosporum orbiculare* [2]. It is a part of the normal microflora in adults and to some extent in healthy infants and children [3,4]. It has been shown that *P. ovale*, in both its morphological forms, can act as an allergen in adult patients with atopic dermatitis (AD) with head and neck distribution [5–7]. A strong

relationship between AD and the occurrence of specific IgE antibodies to *P. orbiculare* has been found [8].

Candida albicans is another common commensal yeast in man colonizing, contrary to *P. ovale*, the gastrointestinal tract, not the skin [9,10]. Many allergic phenomena have been connected to *C. albicans*, including asthma, rhinitis and urticaria [11,12]. Our previous studies have shown correlation with the severity of the AD and IgE response to *C. albicans* (Savolainen *et al.*, unpublished results). On the other hand studies with atopic children have revealed association with *P. ovale* and *C. albicans* RAST [8]. This study was undertaken to analyse this simultaneous reactivity to these two yeasts. Immunoblotting, RAST-inhibition and Tandem-CRIE were performed to recognize possible crossreacting IgE antibodies and their target antigens.

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Material and methods

Patient sera

In total 28 patients were selected. The selection of these patients to this study was based on the previously performed skin prick test with *P. ovale* and yeast cultures from the skin (Table 1). Skin prick test (SPT) was performed on the forearm with a water soluble extract of *P. ovale*, protein concentration 5 mg/ml (ALK Laboratories, Horsholm, Denmark). The results were evaluated in relation to histamine reference equivalent to histamine hydrochloride 10 mg/ml and regarded as 3+ positive if the weal was equal to the histamine skin reaction [13]. Qualitative cultures for *P. ovale* were taken with a curette and transferred to glucose neopeptone yeast extract medium with lipid supplements as previously described [14].

All patients were outpatients from the Department of Dermatology, Sahlgrenska Hospital or Allergy Clinic at the Department of Pediatrics, Eastern Hospital, Gothenburg. The diagnosis AD was based on the criteria of Hanifin and Rajka [15]; children with no history of AD, asthma or rhinoconjunctivitis were regarded as non-atopics. Thirteen children with positive SPT were included, aged 3–21 years (median 14 years). Two were male and 11 were female. Twelve patients had AD, three young adults with typical head and neck distribution and one child was non-atopic. Fifteen children with negative SPT were included, aged 4–20 years (median 13 years). Five were male and 10 female. Of these children seven had AD, four asthma and/or rhinoconjunctivitis and four healthy children had no history of atopy.

Table 1. *P. ovale* skin test results and cultures of the patients in the study

	<i>P. ovale</i> prick	<i>P. ovale</i> culture		Total
		Positive	Negative	
Eczema	Positive	7	5	12
	Negative	3	4	7
Asthma or hay fever	Positive	—	—	—
	Negative	2	2	4
Healthy	Positive	—	1	1
	Negative	2	2	4
Total				28

Antigens

P. ovale antigen was purchased in lyophilized form from ALK, Horsholm, Denmark. Crude cytoplasmic antigen of *C. albicans*, CBS 1894, was prepared as previously described [16].

Sodium dodecyl sulphate—polyacrylamide gel electrophoresis (SDS-PAGE) and IgE-immunoblotting

Immunoblotting was performed essentially according to Bengtson *et al.* as previously described [17,18]. *P. ovale* and *C. albicans* extracts, corresponding to 2 mg protein per large gel, and molecular weight markers (LMW Calibration Kit, Pharmacia LKB Biotechnology, Uppsala, Sweden) were separated with SDS-PAGE and transferred to nitrocellulose sheets. The specific IgE-binding to allergens on nitrocellulose was probed with beta-galactosidase-labelled anti-IgE antibodies (Phadezym RAST, Pharmacia Diagnostics, Uppsala, Sweden). The dilution of individual patient sera as well as the conjugate was 1:4. Two millilitres diluted serum was used for each strip.

RAST-inhibition

RAST-inhibition was performed with selected allergic and non-atopic control sera according to Yman *et al.* except that nitrocellulose discs were used instead of CnBr-activated paper discs [19]. The solid phase allergen was *P. ovale* extract coupled to nitrocellulose discs by the method of Walsh *et al.* [20]. One millilitre of 10 mg/ml dilution of the extract was used per 25 nitrocellulose discs, based on uptake titration curves according to Walsh *et al.* [20]. *C. albicans* extract was used as the inhibiting antigen in tenfold dilutions from 40 mg/ml.

CIE, CRIE and Tandem-CRIE

CRIE and Tandem-CRIE were performed by the basic principles originally presented by Weeke and Loewenstein and Kroell as modified by Uhlin and Einarsson [21–23]. CIE was performed with the gel moulded on a Gel Bond plastic sheet (Pharmacia, Uppsala, Sweden) which could be bent along the inside of a cylinder for roller incubation to reduce the volume of serum and conjugate needed [24]. A 1% agarose gel (Litex, Glostrup, Denmark) and a Tris-Barbital buffer pH 8.6 were used. *P. ovale* and *C. albicans* extracts, corresponding to 20–30 µg protein per hole, were applied for the first dimension electrophoresis in two holes each, one for the single CRIE and one for the Tandem-CRIE. The second dimension run was performed with the anti-*C. albicans* antiserum (DAKO immunoglobulins, Denmark), corresponding to

9 μl per cm^2 , absorbed in a filter paper and laid on the gel according to Kuusi [25]. The first dimension was performed at $40 \text{ V} \times \text{cm}^{-1}$ for 35 min and the second dimension at $10 \text{ V} \times \text{cm}^{-1}$ for 20 hr. The plate was washed, pressed and dried in room temperature. For CRIE the plate was incubated for 16 hr at room temperature in the cylinder with 4 millilitres of pooled allergic sera undiluted (sera H, J and N, Figure 1). Pooled non-atopic sera was used as control. The plate was washed three times and then incubated for 2 hr on a roller at $+37^\circ\text{C}$ with ^{125}I -labelled anti-IgE antibodies (Phadebas RAST, Pharmacia, Uppsala, Sweden) diluted 1:10. After incubation with the conjugate the plate was washed three times with PBS-Tween and twice with aqua. The washed CRIE-plate was dried and laid on an X-ray film (Trimax) with intensifying screens (Trimax) and put in -70°C for autoradiography.

Results

In Fig. 1 are presented the IgE-immunoblotting results of the *P. ovale* prick positive patients (A–M) and one prick negative patient (N) with both *P. ovale* and *C. albicans* as antigens. Immunoblotting of non-atopic sera revealed no bands. One asthmatic control (strip N) showed strong IgE-response to both yeasts even though she failed to respond in the skin prick test. The immunoblotting responses were in agreement with her RAST-results. All other prick-negative individuals were IgE-negative in the blotting experiments. A detailed analysis of the detected IgE-binding bands is presented in Table 2. The simultaneous IgE-binding to both *P. ovale* and *C. albicans* in most of the IgE-positive sera was prominent. In detailed analysis it became evident that simultaneous binding occurred to the 23 kD band of *P. ovale* and the 27 kD band of *C. albicans* as one pair (strips H, J and N). Another pair consisted of the 26 kD band of *P. ovale* and the 13 kD band of *C. albicans* (strips H, I and J). All these proteins were dominant allergens in this immunoblotting material (Table 2). Four sera showed IgE binding to the diffuse high molecular weight region of *C. albicans* (strips B, H, I and N) and three of these sera gave also stain in the diffuse high molecular weight region of *P. ovale* (strips H, I and N). *P. ovale* cultures in the eight patients showing IgE-response to *P. ovale* were negative in four patients and positive in four patients.

The sera that showed simultaneous IgE-binding to *P. ovale* and *C. albicans* were chosen for RAST-inhibition. The RAST-inhibition curves in Fig. 2 reveal inhibition with sera H and N. Sera C, I and J show inhibition, still remaining below 50%, only at very high concentrations of inhibiting antigen. This inhibition was regarded as non-specific even though serum J shows the same inhibitory

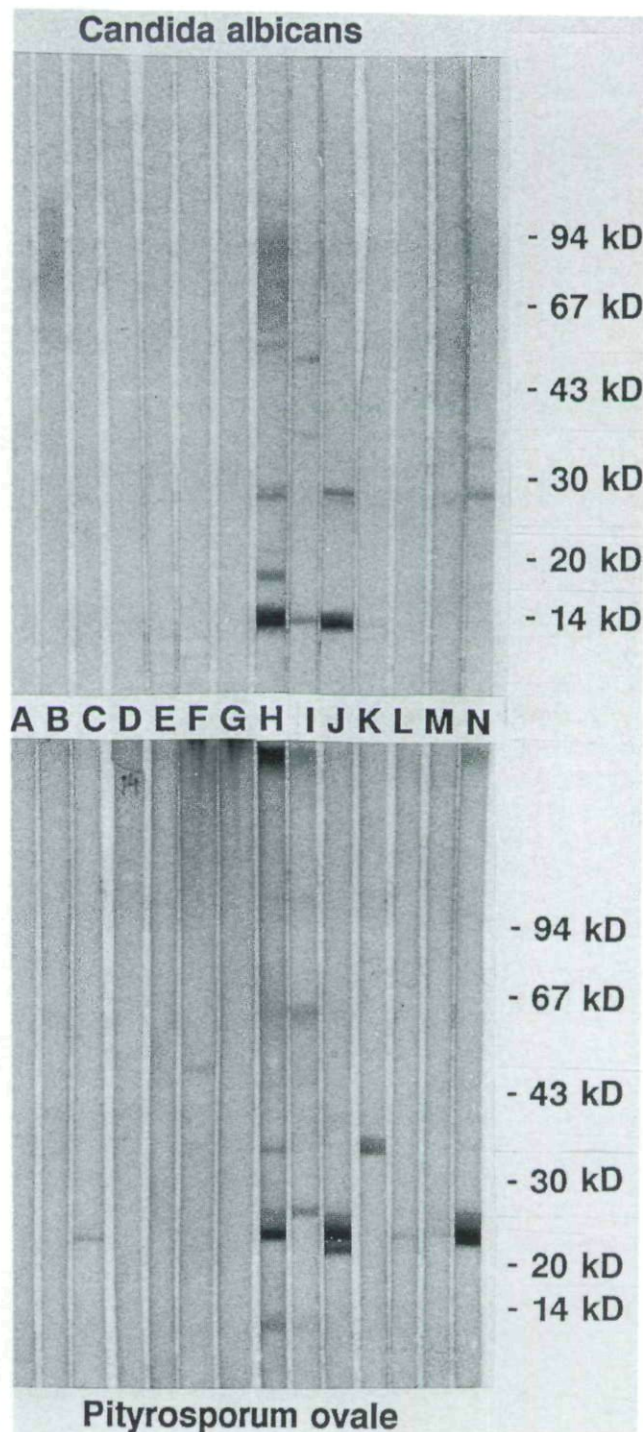


Fig. 1. IgE-immunoblotting with *P. ovale* skin prick test positive sera (2 ml 1:4 diluted sera per strip, 16 hr incubation) (strip N, SPT-negative sera) using *P. ovale* (200 μg per strip) and *C. albicans* (300 μg per strip) as antigens. IgE-immunodetection was performed with beta-galactosidase-labelled rabbit anti-IgE-antibodies. Molecular weight markers are shown on the right.

pattern as serum H, only with 100 times higher concentrations of inhibiting antigen. Serum N reached over 50% steady state inhibition with a low concentration ($< 50 \mu\text{g/ml}$) of inhibiting antigen suggesting one major epitope to remain totally uninhibited. The inhibition curve of serum H reaches 50% at 0.4 mg/ml and is nearly linear.

Taken together with the immunoblotting results, the RAST-inhibition results suggest one major crossreacting epitope and one major uninhibited epitope of *P. ovale* recognized by serum N. The crossreacting epitope is located either in the mannan polysaccharide of *C. albicans* and within the high molecular weight stain of *P. ovale* or, alternatively, in the 27 kD band of *C. albicans* and in the 23 kD band of *P. ovale*. The major uninhibited epitope of *P. ovale* is likely to be located in the 23 kD band, as it is a strong allergenic band. Sera H and J have similar immunoblotting patterns with the major exception that with serum H there are strong stainings of the high molecular weight region of *P. ovale* and the mannan of *C. albicans*. It seems therefore that the observed inhibition is due to crossreacting epitopes in *C. albicans* mannan and the high molecular weight strain of *P. ovale*. If the 23–27 kD band pair would crossreact a strong inhibition would be expected with serum J. Crossreactivity of mannan of *C. albicans* and high molecular weight region of *P. ovale* explains all the observed RAST-inhibition and immunoblotting patterns.

An attempt to identify the crossreacting allergens was performed utilizing a Tandem-CRIE using anti-*C. albicans* rabbit antisera. Figure 3 shows the CRIE-patterns of this experiment. The combination of *C. albicans*-specific rabbit antibodies and pooled IgE-positive sera (sera H, J

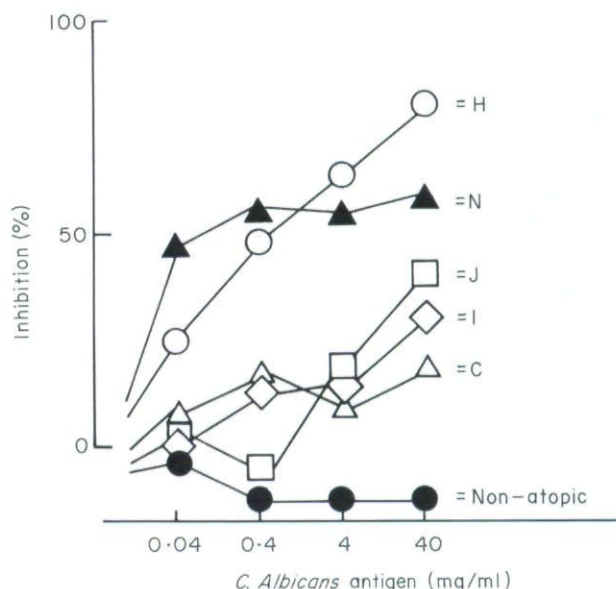


Fig. 2. RAST-inhibition with *P. ovale* is a solid phase antigen coupled to nitrocellulose discs and *C. albicans* as an inhibiting antigen in serial dilutions incubated with the individual sera. Letters for sera are as in Fig. 1. The measurements were performed as duplicate and the mean of individual values was used.

and N) visualized four IgE-stained precipitates of *C. albicans* and one IgE-stained precipitate of *P. ovale*. In Tandem-CRIE the fuzzy *P. ovale*-precipitate is fused with the fuzzy precipitate of *C. albicans* which rises from the application hole. The antiserum used did not precipitate any clear protein precipitates of *P. ovale*. Coomassie-stained CIE-plate revealed only *C. albicans*-precipitates, *P. ovale* precipitates were seen only in CRIE. Non-atopic control CRIE-plate revealed no precipitates.

Discussion

Crossreacting epitopes of *P. ovale* and *C. albicans* were studied with the combination of immunoblotting and RAST-inhibition in sensitized atopics. This methodology utilizing crude allergen mixtures revealed simultaneous IgE-binding to both studied yeasts, evident crossreactivity in RAST-inhibition as well as antigenical identity in Tandem-CRIE. Even though the crossreacting epitopes and crossreactivity on individual allergen level remain presumed, these data suggest the presence of certain crossreacting allergen pairs, most likely the polysaccharides.

Most allergic individuals were sensitized to both yeasts as shown by immunoblotting. One patient (N) was IgE-positive in RAST and immunoblotting even though she showed negative skin prick test with *P. ovale*. The

Table 2. The IgE binding bands of *P. ovale* and *C. albicans*

<i>Pityrosporum ovale</i>			<i>Candida albicans</i>		
MW*	n	%†	MW	n	%
dhm‡	3	38	man§	3	50
34	3	38	55	1	17
26	3	38	50	1	17
23	5	63	37	1	17
21	1	13	34	1	17
10	2	25	27	4	67
			21	1	17
			18	1	17
			13	3	50

* Molecular weight in kilodaltons.

† Percentual outcome of all IgE-positive.

‡ Diffuse high molecular weight stain.

§ Mannan.

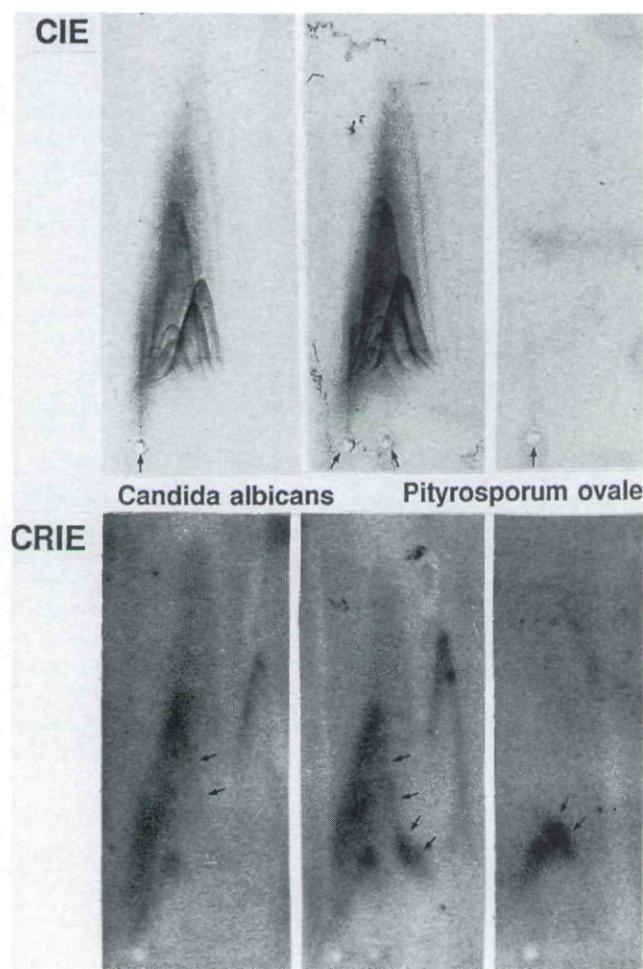


Fig. 3. CIE, CRIE and Tandem-CRIE with *P. ovale* (on the right) and *C. albicans* (on the left) antigens. Tandem-CIE and -CRIE are in the middle with *C. albicans* (60 μ g) applied on the first hole and *P. ovale* (40 μ g) on the second. The fusing precipitin lines, indicating antigenic and allergenic identity, are marked with arrows. The antibody concentration in gel corresponds to 90 μ l/cm². The Gel-Bond plates were incubated 16 hr in a rolled cylinder in 4 ml of undiluted pooled sera (sera H, J, N). The film was exposed with the plate for 72 hr.

reproducibility of skin prick tests is worse than that of IgE-assays. Groups of patients were negative in IgE-immunoblotting even though they were skin prick test-positive, which is a phenomenon seen also with *C. albicans* [26]. This may be due to allergen degradation in SDS-PAGE or false positive in skin prick tests due to high extract concentrations.

The molecular weights of the bands recognized by allergic sera were different in *P. ovale* and *C. albicans* but clear allergen pairs with simultaneous reactivity could be found. In addition to protein band pairs (27 kD and 23 kD and 13 kD and 21 kD), diffuse high molecular weight

stains, typical of IgE-binding to *C. albicans* mannan, a polysaccharide and a diffuse high molecular weight stain of *P. ovale* were seen simultaneously [18]. The *C. albicans* mannan is a polysaccharide consisting of branching mannose with IgE-binding capacity [18]. The crossreacting structure was recognized also in CRIE and most likely as a fuzzy polysaccharide precipitate could be due to mannan. It is possible that *P. ovale* has polysaccharide components in the cell wall with structural similarity to *C. albicans* mannan.

Mannan and the 27 kD protein band are important allergens of *C. albicans* especially in patients with severe atopic dermatitis [10, Savolainen *et al.*, unpublished results]. The 23 kD band and the diffuse high molecular weight stain of *P. ovale* are the two most important allergenic components in this little population of children with atopic dermatitis. This observed crossreactivity may thus be of importance in these patients. Small populations may however reveal different dominant allergens as has been shown with *C. albicans* [10,26,27]. A different panel of allergens, compared to ours, was detected with *P. orbiculare* in a recent immunoblotting study [28]. This might also be due to analysis of small populations, use of different growth phase of *P. ovale* or due to technical interlaboratory variation in SDS-PAGE.

Both *C. albicans* and *P. ovale* can act as environmental allergens inducing production of specific IgE-antibodies [8,10,26,27]. *C. albicans* sensitization is due to colonization of bowel and nasopharynx and *P. ovale* sensitization due to colonization of the skin [5–10]. Among young adults with head and neck distribution of AD this sensitization seems to be of importance and recent studies indicate that healthy infants and children are also exposed to *P. ovale* [5,6]. Nordvall and Johansson found a strong relationship between AD and the occurrence of IgE-antibodies to *P. ovale* and a correlation between this antibody level and IgE antibodies to *C. albicans* [8]. Immediate type hypersensitivity to *C. albicans* favours colonization by this yeast [10]. As another saprophytic yeast, this may be true also for *P. ovale*. This suggested crossreactivity between these yeasts is therefore of interest. The role of IgE antibodies in AD is not clarified [29]. Specific IgE-antibodies to food antigens can have clinical relevance but not always [30]. Specific crossreacting IgE antibodies to *P. ovale* and/or to *C. albicans* does not necessarily mean clinically important allergy, but reports in the literature support the clinical relevance in groups of patients with AD [5,31].

Efficacy of the combination of RAST-inhibition and immunoblotting could not fully recognize the crossreacting epitopes of the studied yeasts even though they strongly suggested the presence of them. Only RAST-inhibition studies with purified allergens would finally

give the answer. Further studies with RAST-inhibition utilizing both purified protein allergens and as well as purified oligosaccharides are needed to demonstrate the individual crossreacting allergens and epitopes. Well-controlled clinical studies are needed to evaluate the clinical significance of the suggested allergenic crossreactivity of *P. ovale* and *C. albicans*.

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