

Allergens of *Pityrosporum ovale* and *Candida albicans*

I. Cross-reactivity of IgE-binding components

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In atopic dermatitis (AD), a high prevalence has been reported of type I reactions and specific IgE to extracts of the commensal lipophilic skin yeast *Pityrosporum ovale*. In the present study, a highly significant correlation ($r = 0.77$) was found between levels of anti-*P. ovale* IgE and of IgE reacting with extracts of *Candida albicans*, both measured by a sensitive ELISA method. In a series of 128 AD sera, 34 sera reacted positively with both yeast extracts, 38 reacted with *P. ovale* but not with *C. albicans*, and only one of the 56 anti-*P. ovale*-negative sera showed a very weak reaction with *C. albicans*. The correlation was due to a marked cross-reactivity, as shown by inhibition ELISA. Fluid-phase preincubation of double-positive sera with either of the two yeast extracts resulted in a dose-dependent, and at high concentrations complete, inhibition of the IgE reactions with both coated *P. ovale* and *C. albicans* allergens. Mutual inhibition of IgE-binding could also be achieved with pools of glycoproteins and/or polysaccharides isolated from the crude extracts by Con A affinity chromatography. *P. ovale* allergens were, however, more potent fluid-phase inhibitors than the corresponding *C. albicans* components. The apparently higher avidity for *P. ovale* allergens suggests that these antiyeast IgE antibodies in AD result from sensitization to *P. ovale* and cross-react with *C. albicans*.

Clinical aspects

Type I allergy to the commensal skin yeast *Pityrosporum ovale* is very common in atopic dermatitis (AD), and practically absent in atopics with respiratory disease but not eczema. IgE-mediated anti-*Candida albicans* reactions have been described mainly in relation to atopic asthma. This study demonstrates that anti-*P. ovale* IgE antibodies in many AD sera cross-react with extracts of *C. albicans*. The demonstration of an apparent anti-*Candida* IgE response in asthma patients with concurrent AD might therefore often reflect sensitization with *P. ovale*, and thus be related to the eczema, and not to the respiratory disease.

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In various recent reports a high prevalence (50–80%) of type I skin reactions to extracts of the lipophilic yeast *Pityrosporum ovale* has been described in patients with atopic dermatitis (AD) (15, 26, 30), while specific anti-*P. ovale* IgE has also been demonstrated in many AD sera (21, 29, 30). Arguments supporting a pathogenic role of anti-*P. ovale* hypersensitivity in AD include 1) the common occurrence of *P. ovale* as a usually nonpathogenic member of the normal skin microflora of healthy adult persons (6, 25); 2) patch test studies with aeroallergens,

showing that AD exacerbations can be induced by IgE-dependent, contact-allergic reactions to macromolecular allergens present on the skin surface (2, 7, 20, 23); and 3) reports of successful antimycotic treatment of AD patients with “head-and-neck” dermatitis (3, 28); that is, with eczema mainly in the sebaceous areas of the skin. As a lipophilic organism, *P. ovale* preferentially colonizes the same skin areas (6, 25).

In two of the studies on anti-*P. ovale* IgE antibodies in AD, a significant correlation was noted with

levels of serum IgE reacting with extracts of another common yeast, *Candida albicans* (21, 29). Anti-*C. albicans* type I allergy has previously been described in many reports and has been mainly associated with respiratory allergy, particularly extrinsic asthma (1, 5, 8, 10, 14, 17, 18, 22). None of these studies has mentioned a possible relation between these two antiyeast IgE-reactions. We have therefore investigated the correlation between anti-*P. ovale* and anti-*C. albicans* IgE in more detail in a large series of AD sera. Inhibition ELISA (enzyme-linked immunosorbent assay) with crude extracts and with semipurified IgE-binding glycoprotein/polysaccharide fractions revealed the presence of markedly cross-reacting IgE-binding structures in the two yeast extracts.

Material and methods

Antigens and antibodies

Lyophilized extracts of *P. ovale* (product no. 33.04) and *C. albicans* (33.01) were kindly provided by Laboratorium Diephuis (Groningen, The Netherlands), a subsidiary of ALK (Allergologisk Laboratorier, Copenhagen, Denmark). In a series of control experiments, anti-*Candida* IgE was also assessed with *C. albicans* extract ("Candidin") from HAL (Haarlem, The Netherlands). The two commercial *C. albicans* preparations gave virtually identical results. Standard stock solutions were prepared by dissolving 10 mg of the dry, lyophilized material in 10 ml phosphate-buffered saline (PBS) containing 0.01% sodium azide. After filtration through a 0.22- μ m Millipore, the solutions were stored in small aliquots at -40°C until use. Semipurified pools enriched for IgE-binding glycoproteins and/or polysaccharides were obtained by affinity chromatography on Sepharose-bound concanavalin A (4). After dialysis against PBS-azide and concentration by ultrafiltration, the pools were stored in aliquots at -40°C .

Sera were obtained from AD patients of the Departments of Dermatology and Allergy of University Hospital Utrecht (29).

ELISA

Anti-*P. ovale* and anti-*C. albicans* IgE reactivity was assessed by ELISA, as described previously (29). Briefly, wells of microtiter plates (Nunc maxisorp, Nunc, Roskilde, Denmark) coated with yeast extracts at 0.1 mg/ml in PBS were incubated for 2 h with 1/10 diluted sera in PBS-Tween (0.05%), and bound IgE was measured by incubations with monoclonal mouse antihuman IgE (Central Laboratory of the Red Cross Blood Transfusion Service (CLB), Amsterdam, The Netherlands), biotinylated rabbit antimouse Ig (Dakopatts, Copenhagen, Denmark),

avidin-peroxidase (DAKO), and *o*-phenylenediamine (Sigma Chemicals, St Louis, MO, USA). Anti-house-dust-mite, anti-grass-pollen, and anti-birch-pollen IgE were determined by the same ELISA method, in which microwells were coated with 1/25 diluted allergens from HAL (product nos. 02-00 (*Dermatophagoides pteronyssinus*), 07-00 (grass pollen mix), and 11-02 (pollen mix of *Betula* species); stock solutions 10000 AU/ml).

Inhibition ELISA

For inhibition ELISA studies, serum samples diluted in PBS-Tween were mixed with dilutions of allergen pools or crude extracts, and preincubated for 2 h with continuous shaking at room temperature, and overnight at 4°C . Positive controls were diluted and preincubated in PBS-Tween alone. After preincubation the mixtures were added directly to microwells that had been coated overnight with either the same or another allergen extract or pool, and the binding of IgE was assessed as described. Serum dilutions were chosen such that positive controls gave OD₄₉₂ values of 1.0–1.5. The preincubation overnight was included for practical reasons and was not strictly required to detect significant inhibition. On the other hand, overnight preincubation in PBS-Tween alone did not affect serum antibody activity, since preincubated diluted sera gave virtually the same results as freshly diluted sera.

Results

Correlation of anti-P. ovale and anti-C. albicans IgE levels

Levels of serum IgE reacting with coated extracts of either *P. ovale* or *C. albicans* showed a strong statistical correlation. In both assays, the OD₄₉₂ values obtained with 128 sera of AD patients showed a log-normal distribution (Fig. 1), and linear regression analysis on this double-log plot revealed a correlation coefficient (*r*) of 0.77 (Fig. 1). The presented data are the combined results of series tested on different days; *r* values in the separate series varied from 0.71 to 0.83. The inclusion of negative data in the analysis (OD values ≤ 0.1) had little effect on the result, since a similar analysis of the subpopulations of anti-*P. ovale* positive, anti-*C. albicans* positive, or double-positive sera showed correlation coefficients of 0.71, 0.71, and 0.70, respectively.

A positive IgE anti-*Candida* reaction was very rare in the absence of detectable anti-*P. ovale* IgE; for most double-positive sera, the anti-*P. ovale* reactivity was markedly stronger. With a threshold OD₄₉₂ value of 0.1 (29), 55 and 34 sera were double-negative or -positive, respectively; 38 sera had detectable anti-*P. ovale* IgE but were negative on a coating of

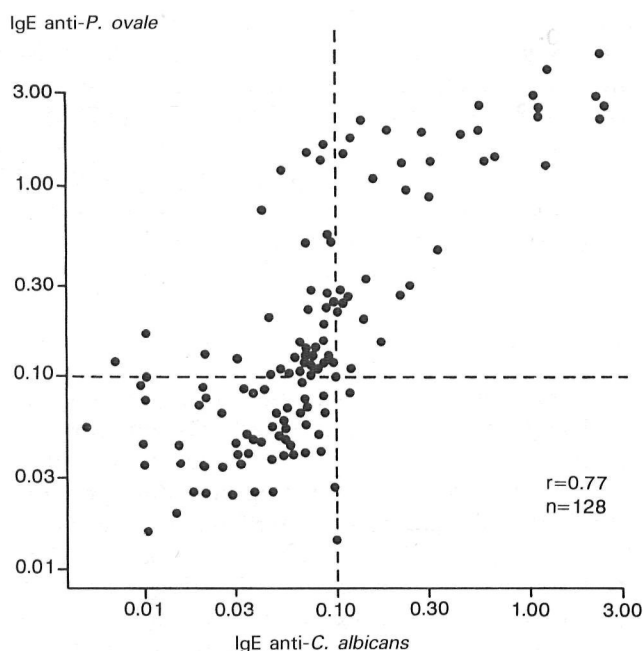


Fig. 1. Correlation between anti-*P. ovale* and anti-*C. albicans* IgE reactivity in atopic dermatitis. OD₄₉₂ values obtained with 1/10 diluted sera in ELISA wells coated with *C. albicans* or *P. ovale* extract (0.1 mg/ml) are plotted on x-axis and y-axis, respectively. Dotted lines mark lower detection limits for positive reaction.

C. albicans; and only one serum showed a positive, although very weak, reaction with *C. albicans* in the absence of detectable anti-*P. ovale* IgE (Fig. 1).

Cross-reactivity of whole extracts

Inhibition ELISA was performed to assess whether the correlation shown in Fig. 1 was due to immunologic cross-reactivity. In sera with a pronounced IgE reaction towards both *P. ovale* and *C. albicans*, complete and dose-dependent inhibition was observed after preincubation with either of the two extracts; indeed, the extracts showed no quantitative differences in their capabilities to inhibit IgE reactions with the same or the other extract (Fig. 2).

In contrast, *C. albicans* extract had either no capacity or a much lower fluid-phase inhibitory capa-

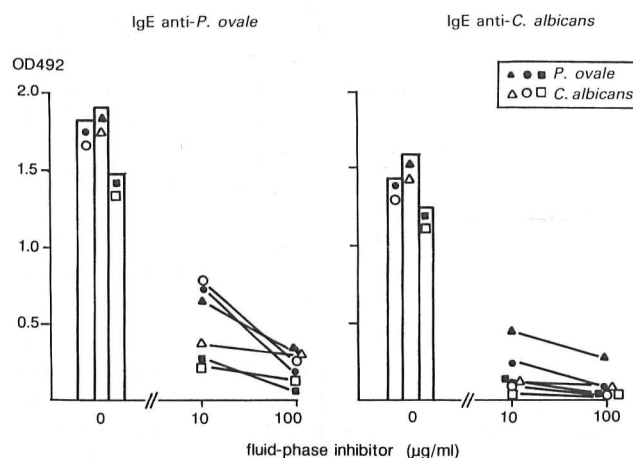


Fig. 2. Inhibition of anti-*P. ovale* and anti-*C. albicans* IgE by fluid-phase incubation with extracts of *P. ovale* (closed symbols) or *C. albicans* (open symbols). Circles, triangles, and squares represent data obtained with three different AD sera. Bars show OD₄₉₂ values obtained after preincubation of sera in buffer alone.

city than *P. ovale* allergens, when inhibition of the anti-*P. ovale* IgE reaction was studied in three sera with a strong (sera 015 and 020) or moderate (013) anti-*P. ovale* reaction, and a low (015) or (practically) absent (013, 020) anti-*C. albicans* titer (Table 1). This indicates that the low anti-*Candida* IgE reactivity of these sera was not due simply to an insufficient coating of the relevant allergens.

To exclude the possibility that the observed cross-inhibition was due to nonspecific binding of IgE, we performed similar experiments with coated extracts of house-dust mites, grass pollen, and birch pollen, using AD sera with a known level of appropriate specific IgE. None of these IgE reactions was affected by preincubation of the sera with *P. ovale* or *C. albicans* extracts (Fig. 3).

Inhibition-ELISA with isolated glycoprotein/polysaccharides

The cross-reactivity of *P. ovale* and *C. albicans* allergens was investigated in more detail with the glycoprotein/polysaccharide pools eluted from

Table 1. Fluid-phase inhibition of anti-*P. ovale* IgE by *P. ovale*, but not by *C. albicans*, extract, in sera with low anti-*C. albicans* IgE reactivity

Preincubation	Reaction with coating of <i>P. ovale</i>		
	Serum 013	Serum 015	Serum 020
Buffer (PBS-Tween)	0.62	1.40	1.38
<i>P. ovale</i> 0.01 mg/ml	0.27 (56%)	0.41 (71%)	0.29 (79%)
<i>P. ovale</i> 0.1 mg/ml	0.13 (79%)	0.05 (96%)	0.10 (93%)
<i>C. albicans</i> 0.01 mg/ml	0.70 (<0%)	1.38 (1%)	1.58 (<0%)
<i>C. albicans</i> 0.1 mg/ml	0.56 (10%)	1.08 (23%)	1.50 (<0%)

* OD₄₉₂ values (and % inhibition compared with positive control). In ELISA for anti-*C. albicans* IgE, the OD₄₉₂ values with 1/10 diluted sera were 0.069 (serum 013), 0.27 (015), and 0.11 (020).

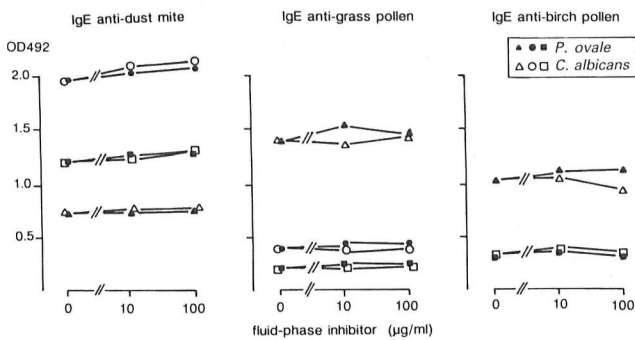


Fig. 3. Specificity of the cross-reactivity of *P. ovale* and *C. albicans*. Binding of IgE to ELISA wells coated with house-dust-mite, grass-pollen, or birch-pollen allergens after preincubation of three AD sera with extracts of *P. ovale* (closed symbols) or *C. albicans* (open symbols).

Sephacrose-concanavalin A columns. As shown elsewhere, more than 90% of the IgE-binding capacity of the crude yeast extracts resides in these pools of lectin-binding components (4). The 0.5 M mannoside eluates were used since these had the highest IgE-binding activity per microgram (4). Inhibition ELISA was performed with four different sera, since preliminary experiments had revealed a marked variation among sera in the avidity of their specific IgE antibodies. After preincubation with three concentrations of the allergen pools, the residual specific IgE antibody activity was assessed in wells coated with either whole extracts or wells coated with the isolated Con A pools.

In 30 of the 32 tested combinations of serum, coated allergen, and fluid-phase inhibitor, a dose-dependent, and sometimes nearly complete inhibition was observed (Fig. 4). In one serum (no. 6380), the Con A eluates of both extracts did not inhibit the binding of IgE to the *Candida* whole extract. The anti-*Candida* IgE of this serum might thus be partially directed against components that do not bind to Con A (4). The other three IgE reactions of this serum – with *P. ovale* whole extract and with the Con A eluates of both yeast extracts – were inhibited for at least 40% at the highest inhibitor concentrations. These observations and the data obtained with the other three sera thus confirmed our conclusion that anti-*P. ovale* and anti-*C. albicans* IgE in most AD sera are mainly directed against the Con A-binding fractions (4).

Marked quantitative differences were noted, however, among the various inhibition curves, from which we determined the allergen concentration required for 50% reduction of the OD₄₉₂ value ($C_i(50)$), and calculated $1/C_i(50)$ as a measure of the inhibitory capacity (Table 2). Since fluid-phase reactions are involved, this parameter should depend only on the concentrations of relevant components in the allergen pools, and their avidities for the patients'

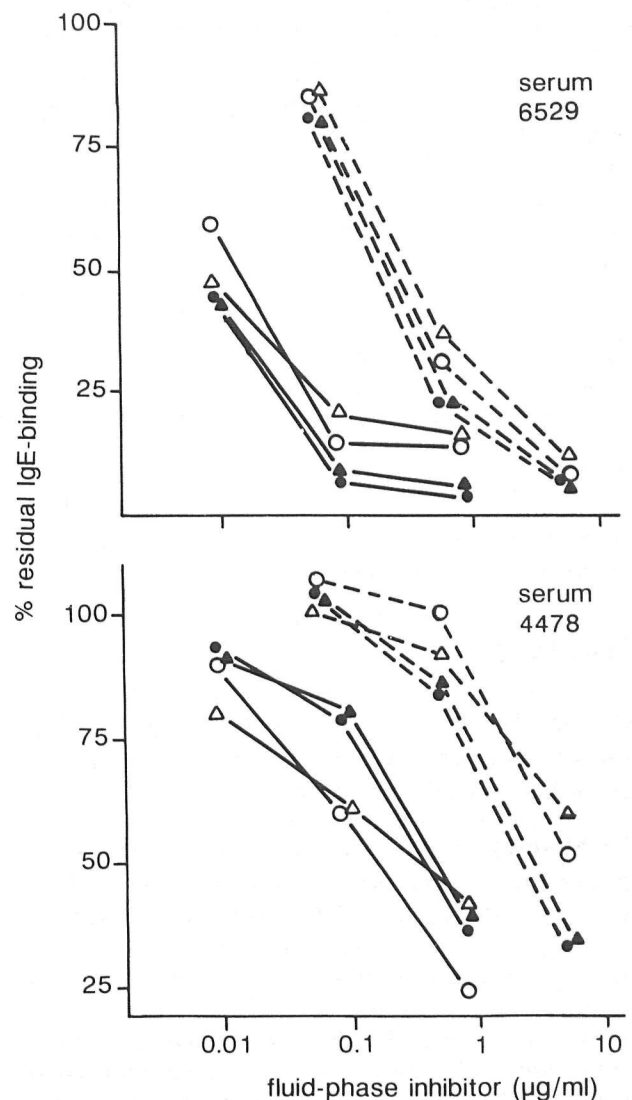


Fig. 4. Cross-reactivity of IgE-binding, Con A binding glycoproteins/polysaccharides isolated from *P. ovale* and *C. albicans* extracts. Diluted AD sera were preincubated with three concentrations of Con A eluates of *P. ovale* (closed lines) and *C. albicans* (dotted lines), and then tested for residual IgE binding to ELISA wells coated with *P. ovale* Con A eluate (●), *C. albicans* Con A eluate (○), *P. ovale* whole extract (▲), or *C. albicans* whole extract (△). Residual IgE-binding is plotted as the relative OD₄₉₂ value compared with that of the corresponding positive controls.

IgE antibodies. The observed values appeared to be determined by the choice of the serum and the fluid-phase inhibitor, while being relatively independent of the coated allergen in the final readout assay (Table 2). This confirms our conclusion that in most sera the same population of IgE antibodies was involved in the reactions with the two crude extracts and the two Con A pools of *P. ovale* and *C. albicans*. The avidity of the IgE antibodies might, however, differ 10–50-fold among different sera. It further appeared that in all cases the *P. ovale* allergens were more potent fluid-phase inhibitors, giving $1/C_i(50)$

Table 2. Fluid-phase binding of IgE by *P. ovale* and *C. albicans* Con A eluates. The figures show $1/C_i(50)$ as a measure of the inhibitory capacity, in which $C_i(50)$ is the concentration (μg protein and carbohydrate per ml) required for 50% reduction of IgE binding in inhibition ELISA

ELISA coating	Fluid-phase inhibitor	Serum no.			
		6529	6380	2212	4478
P (e)	P (e)	151.5	20.0	32.3	2.5
P (w)	P (e)	175.4	20.0	34.5	2.2
C (e)	P (e)	76.9	17.5	52.6	6.3
C (w)	P (e)	153.8	<0.05	38.5	3.4
P (e)	C (e)	5.7	0.5	1.1	0.4
P (w)	C (e)	5.7	0.4	1.2	0.4
C (e)	C (e)	4.4	0.1	3.0	0.2
C (w)	C (e)	4.8	<0.05	1.4	0.1

P (w): *P. ovale* whole extract; P (e): *P. ovale* Con A eluate; C (w): *C. albicans* whole extract; C (e): *C. albicans* Con A eluate.

values that were 5–100-fold higher than those obtained with the corresponding *C. albicans* pools.

Discussion

Interest in *P. ovale* as a source of type I allergens is of recent origin. To our knowledge, no reports on the subject were published before 1980, and most have appeared during the last 5 years (3, 12, 13, 15, 21, 26, 28, 30). Their main conclusions are 1) a high (50–80%) prevalence in AD of type I skin reactions and specific IgE in response to *P. ovale* extracts, and 2) a complete or nearly complete absence of this reactivity in either controls with nonatopic skin disease (15, 26) or atopic controls with rhinitis and/or asthma, but no eczema (21, 29, 30). In contrast, anti-*Candida* allergy has been described in many reports, some of which were published more than 50 years ago (1, 5, 10, 14, 18; reviewed in ref. 22), and has been associated with a wide range of diseases. These include skin diseases such as urticaria, cutaneous candidiasis, and psoriasis (11, 22), and – in at least one report – AD (19). Most of the recent studies, however, have focused on the possible role of *Candida* type I allergy in respiratory disease, particularly bronchial asthma (8, 17, 18).

In our previous study (29) and in one other report (21), highly significant correlations were found between anti-*P. ovale* and anti-*C. albicans* IgE reactivity. Although the levels of anti-*P. ovale* IgE also significantly correlated with those of other allergen-specific IgE antibodies, the correlation with anti-*C. albicans* IgE was most pronounced, and was the only one with $r > 0.70$ (29). Because of the taxonomic relationship of the two allergen sources – both being yeasts – we expected this correlation to be due to cross-reactivity of IgE-binding components, and this was confirmed in the present study. Moreover, we demonstrated that the cross-reactive components belong to the pools of Con A-binding

glycoproteins and/or polysaccharides, which in our accompanying study appeared to contain most of the allergenic activity (4).

The avidity of the cross-reacting IgE antibodies in AD sera, however, may be markedly higher for *P. ovale* than for *C. albicans* (Fig. 4; Table 2), and this would explain the generally lower reactivity in direct ELISA with a coating of *C. albicans* extract. Alternatively, the concentration of relevant allergenic components might be higher in the *P. ovale* extracts, but this seems improbable, since in dose-response experiments the regularly used coating concentration (0.1 mg/ml) for most sera appeared to be excessive (not shown). Indeed, for a great number of sera, the difference in reactivity with the two extracts is such that a moderate or strong anti-*P. ovale* IgE reaction is found together with a low or completely absent anti-*C. albicans* IgE titer (Fig. 1), and these apparently *P. ovale*-specific IgE antibodies could be only marginally inhibited by preincubation with *C. albicans* extract (Table 1).

Recently, Savolainen & Broberg have also reported the presence of cross-reacting anti-*P. ovale*/*C. albicans* IgE antibodies in a small number of atopic children (27). They concluded that the cross-reactive components are probably high-mol.-wt. mannan polysaccharides, in agreement with our own findings (4). Further confirmation comes from the observation of a high prevalence in adult AD patients of anti-*C. albicans* mannan IgE, which cross-reacts with mannan pools of several yeast species, including *P. ovale* (Nermes, Savolainen et al., personal communication). In contrast, Koivikko et al. (16) have reported a low level of cross-reactivity (6%) of sheep and rabbit anti-*C. albicans* IgG antibodies with *P. ovale* extract. Similarly, we have found only $\pm 10\%$ cross-reactivity with a commercial (DAKO) rabbit anti-*C. albicans* IgG preparation (not shown). In our opinion, this mainly indicates that the component- and epitope-specificity of IgG

antibodies raised by active immunization completely differs from that of the human IgE response. On the other hand, the sets of potentially IgE-binding epitopes in the two yeast extracts are certainly not identical, as shown by the presence of apparently species-specific anti-*P. ovale* IgE, and the marked quantitative differences in reactivity of cross-reacting antibodies.

Since many asthma patients have a history of AD or current eczema (9, 24), our data suggest that most of the anti-*C. albicans* IgE reactions found in asthmatic patients with associated AD might be due to the presence of cross-reacting anti-*P. ovale* IgE, thus being primarily related to the patients' eczema, and not to the respiratory disease. In none of the studies on anti-*Candida* type I allergy could we find evidence of different anti-*C. albicans* IgE levels in asthmatic patients with or without a history of eczema. In the present study, AD sera were studied retrospectively, and reliable clinical data on the presence of respiratory allergy were available only in a subgroup of all patients. Analysis of those data and additional preliminary results obtained with sera of patients with AD, asthma, or both atopic diseases suggest that the presence of anti-*C. albicans* IgE in AD patients – like the anti-*P. ovale* IgE reactivity (29) – is only weakly associated with a history of respiratory allergy. On the other hand, until now we have found few examples of sera with apparently species-specific anti-*C. albicans* IgE, and no anti-*P. ovale* IgE, from patients with respiratory allergy but no eczema (not shown). Obviously, larger numbers of patients with AD, asthma, or both diseases have to be studied to assess the contribution of cross-reactive IgE in both types of antiyeast type I allergy. At present, we conclude that in AD, anti-*Candida* IgE can be frequently found, but usually reflects the presence of cross-reacting anti-*P. ovale* IgE.

The clinical significance of antiyeast IgE reactions remains debatable. Since *P. ovale* is one of many allergens to which AD patients may be sensitized, we presume that anti-*P. ovale* IgE is not a single and primary pathogenic factor in AD. On the other hand, IgE-mediated reactions to *P. ovale* might contribute significantly to the inflammatory process, particularly because there is permanent allergen production on the skin by this member of the skin microflora. In this respect, *P. ovale* clearly differs from *C. albicans*, which may also be frequently found on the human skin, but not as a member of the resident microflora. Therefore, if there is a role for antiyeast IgE in the pathogenesis of AD, involvement of anti-*P. ovale* IgE reactions appears to be much more probable than anti-*C. albicans* allergy. In contrast, anti-*C. albicans* IgE reactivity would be more relevant in asthma, where allergen exposure of the air-

ways might result from saprophytic *Candida* growth on the mouth and throat mucosae (16), while the presence of the lipophilic *P. ovale* at these sites is improbable. The cross-reactivity described here, however, suggests that, in patients with both asthma and AD, possibly pathogenic anti-*C. albicans* IgE antibodies result from AD-related sensitization to *P. ovale*.

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References

- AKIYAMA K, YUI Y, SHIDA T, MIYAMOTO T. Relationship between the results of skin, conjunctival and bronchial tests and RAST with *Candida albicans* in patients with asthma. Clin Allergy 1981; 11: 343–51.
- BRUIJNZEEL-KOOMEN CAFM, BRUIJNZEEL PLB. A role for IgE in patch test reactions to inhalant allergens in atopic dermatitis. Allergy 1988; 43 (Suppl. 5): 15–21.
- CLEMMENSEN OJ, HJORTH N. Treatment of dermatitis of the head and neck with ketoconazole in patients with type 1 sensitivity to *Pityrosporum orbiculare*. Semin Dermatol 1983; 2: 26–9.
- DOEKES G, KAAL MJH, VAN IEPEREN-VAN DIJK AG. Allergens of *Pityrosporum ovale* and *Candida albicans*. II. Physicochemical characterization. Allergy 1993; (in this issue).
- EDGE G, PEPYS J. Antibodies in different immunoglobulin classes to *Candida albicans* in allergic respiratory disease. Clin Allergy 1980; 10: 47–58.
- FAERGEMANN J, ALY R, MAIBACH HI. Quantitative variations in distribution of *Pityrosporum orbiculare* on clinically normal skin. Acta Derm Venereol (Stockh) 1983; 63: 346–8.
- GONDO A, SEAKI N, TOKUDO Y. Challenge reactions in atopic dermatitis after percutaneous entry of mite antigen. Br J Dermatol 1986; 115: 485–92.
- GUMOWSKI P, LECH B, CHAVES I, GIRARD J-P. Chronic asthma and rhinitis due to *Candida albicans*, epidermophyton, and trichophyton. Ann Allergy 1987; 59: 48–51.
- HANIFIN JM, RAJKA G. Diagnostic features of atopic eczema. Acta Derm Venereol (Stockh) 1980; Suppl. 92: 44–7.
- ITKIN IH, DENNIS M. Bronchial hypersensitivity to extract of *Candida albicans*. J Allergy 1966; 37: 187–94.
- JAMES J, WARIN RP. An assessment of the role of *Candida albicans* and food yeasts in chronic urticaria. Br J Dermatol 1971; 84: 227–37.
- JENSEN-JAROLIM E, POULSEN LK, WITH H, KIEFFER M, OTTEVANGER V, STAHL SKOV P. Atopic dermatitis of the face, scalp, and neck: type I reaction to the yeast *Pityrosporum ovale*? J Allergy Clin Immunol 1992; 89: 44–51.
- JOHANSSON S, KARLSTROEM K. IgE-binding components in *Pityrosporum orbiculare* identified by immunoblotting technique. Acta Derm Venereol (Stockh) 1991; 71: 11–16.
- KEENEY EL. *Candida* asthma. Ann Intern Med 1951; 34: 223–6.
- KIEFFER M, BERGBRANT I-M, FEARGEMANN J, et al. Immune reactions to *Pityrosporum ovale* in adult patients with atopic and seborrheic dermatitis. J Am Acad Dermatol 1990; 22: 739–42.

16. KOIVIKKO A, KALIMO K, NIEMINEN E, SAVOLAINEN J, VILJANEN M, VIANDER M. Allergenic cross-reactivity of yeasts. *Allergy* 1988; 43: 192–200.
17. KOIVIKKO A, KALIMO K, NIEMINEN E, VIANDER M. Relationship of immediate and delayed hypersensitivity to nasopharyngeal and intestinal growth of *Candida albicans* in allergic subjects. *Allergy* 1988; 43: 201–5.
18. KURIMOTO Y. Relationship among skin tests, bronchial challenge and serology in house dust and *Candida albicans* allergic asthma. *Ann Allergy* 1975; 35: 131–41.
19. MENZEL I. Zur Provokation der Dermatitis atopica durch intestinale Candidamykose. *Z Hautkr* 1984; 59: 1463–8.
20. MITCHELL E, CHAPMAN M, POPE F, CROW S, JOUHAL S, PLATTS-MILLS T. Basophils in allergen-induced patch test sites in atopic dermatitis. *Lancet* 1982; i: 127–31.
21. NORDVALL SL, JOHANSSON S. IgE antibodies to *Pityrosporum ovale* in children with atopic diseases. *Acta Paediatr Scand* 1990; 79: 343–8.
22. ODDS FC. *Candida* allergy. In: ODDS FC. *Candida* and candidosis – a review and bibliography (ch. 24). 2nd ed. London: Baillière Tindall, 1988: 231–4.
23. REITAMO S, VISA K, KAHONEN K, KAYHKO K, STUBB S, SALO OP. Eczematous reactions in atopic patients caused by epicutaneous testing with inhalant allergens. *Br J Dermatol* 1986; 114: 303–9.
24. RING J. Atopy: condition, disease, or syndrome? In: RUZICKA T, RING J, PRZYBILLA B, eds. *Handbook of atopic eczema*. Berlin: Springer-Verlag, 1991: 3–8.
25. ROBERTS SOB. *Pityrosporum orbiculare*: incidence and distribution on clinically normal skin. *Br J Dermatol* 1969; 81: 264–9.
26. ROKUGO M, TAGAMI H, USUBA Y, TOMITA Y. Contact sensitivity to *Pityrosporum ovale* in patients with atopic dermatitis. *Arch Dermatol* 1990; 126: 627–32.
27. SAVOLAINEN J, BROBERG A. Crossreacting IgE antibodies to *Pityrosporum ovale* and *Candida albicans* in atopic children. *Clin Exp Allergy* 1992; 22: 469–74.
28. WEARSTEDT 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.
29. WESSELS MW, DOEKES G, VAN IEPEREN-VAN DIJK AG, KOERS WJ, YOUNG E. IgE antibodies to *Pityrosporum ovale* in atopic dermatitis. *Br J Dermatol* 1991; 125: 227–32.
30. YOUNG E, KOERS WJ, BERRENS L. Intracutaneous tests with *Pityrosporum* extract in atopic dermatitis. *Acta Derm Venereol* (Stockh) 1989; Suppl. 144: 122–4.

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