

IgE antibodies to *Pityrosporum ovale* in atopic dermatitis

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Summary

An enzyme-linked immunosorbent assay (ELISA) was developed to assess serum IgE antibodies directed against *Pityrosporum ovale* in patients with atopic dermatitis (AD), atopic patients with allergic respiratory disease (ARD: rhinitis or asthma) but without eczema, and in healthy controls. IgE binding to *P. ovale* extract was demonstrated in 49% (35/72) of AD patients. In contrast, anti-*P. ovale* IgE was found in only one of 27 atopic controls without eczema; all healthy control sera ($n = 17$) were negative. Of 37 AD patients tested intracutaneously with *P. ovale*, 31 showed immediate-type reactivity, and 20 of these 31 patients had anti-*P. ovale* IgE detectable by ELISA, while sera from the six non-responders were all negative. Levels of anti-*P. ovale* IgE were highest in AD patients aged 20–30 years. No correlation was found with the severity of AD, but there was a non-significant tendency ($P = 0.06$) to higher levels in AD patients with concomitant respiratory allergy. Anti-*P. ovale* IgE was significantly correlated with total serum IgE, with specific IgE against various aeroallergens as measured by RAST, and with levels of anti-*Candida albicans* IgE, measured with a similar ELISA. Thus, production of IgE antibodies against *P. ovale* occurs very frequently in AD, and rarely in patients with atopic disease without skin involvement.

Atopic dermatitis (AD) is associated with high levels of total and allergen-specific serum IgE and immediate-type reactions to a wide variety of allergens.^{1,2} The aetiological role of allergic reactions has been demonstrated by the induction of inflammatory eczematous reactions after epicutaneous application of mite or pollen allergens on the apparently non-involved skin of AD patients.^{3–6} Although the histopathology and kinetics of these lesions and of the 'spontaneously' involved skin in AD indicate a delayed-type reaction, it is probable that allergen-specific IgE is involved.^{7–10} Atopic patients with allergic respiratory disease (ARD) but no eczema do not, or only occasionally, respond to patch tests with aeroallergens, in spite of frequently enhanced levels of anti-mite or anti-pollen IgE.^{3,4} Thus, skin reactivity towards IgE-binding allergens that enter percutaneously may be a characteristic feature of AD.

This proposed pathogenic mechanism points to the potential significance of allergens derived from the normal skin microflora, in addition to airborne mite and pollen allergens. The presence of such allergens might constitute an important permanent stimulus for allergic skin reactions. One source of possibly relevant allergens is the lipophilic yeast *Pityrosporum ovale* which can be isolated from the skin of up to 90% of healthy adults, especially from sebaceous areas of the face, scalp, upper

back and chest.^{11,12} Although normally non-pathogenic, *P. ovale* has been associated with skin diseases such as pityriasis versicolor, folliculitis, seborrhoeic eczema and psoriasis.^{13–15} Clemmensen and Hjorth¹⁶ showed that treatment with oral ketoconazole improved the skin lesions in a subgroup of AD patients with predominant involvement of the head and neck region and type I skin reactions to *P. ovale*. Recently, immediate and delayed-type anti-*P. ovale* skin reactions have been described in large series of AD patients.^{17–20} These reactions might substantially contribute to the pathogenesis of AD lesions, and assessment of anti-*P. ovale* IgE antibodies may be of diagnostic value. We have therefore developed a sensitive enzyme-linked immunosorbent assay (ELISA), which was used to compare levels of anti-*P. ovale* IgE in the sera of AD patients and of atopic controls with ARD but no eczema. The results confirm the frequent and specific occurrence in AD of IgE-mediated hypersensitivity to *P. ovale*.

Methods

Patients

Sera were collected from 103 atopic patients attending the Utrecht University Hospital between September 1988 and June 1990. Seventy-two patients had AD (56

women, 20 men; median age 23.9 years, range 2–52 years) and 27 had atopic respiratory disease (ARD; asthma or rhinitis) without eczema (15 women, 12 men; median age 32.6 years, range 8–63 years). Four sera were included from atopic adults with current ARD, who had suffered from eczema in their childhood. Control sera ($n=17$) were obtained from healthy laboratory and hospital staff. All sera were stored at -70°C until use.

Only patients with atopic disease confirmed by *in-vitro* diagnostic tests (total serum IgE >150 IU/ml and/or a positive RAST to one or more aeroallergens) were included in both the AD and ARD group. All AD patients fulfilled the Hanifin and Rajka criteria for AD.²¹ Fifty-three AD patients had a personal history of associated ARD and in 37 of these a family history of ARD was also noted. In 13 of the other 19 AD patients there was a known family history of atopic disease. Sera from 35 patients was obtained during hospitalization because of an exacerbation of their AD.

Most patients had been tested intracutaneously with several allergens: grass pollen (100 AU/ml), tree pollen (100 AU/ml), mould-mix (0.1%; *Aspergillus fumigatus*, *Penicillium notatum*, *Alternaria alternata*, *Cladosporium cladosporioides*, *Mucor mucedo*), house dust (0.5%), *Dermatophagoides pteronyssinus* (10 AU/ml), *Artemisia* pollen (10 AU/ml), cat and dog dander (0.01%), and human dander (0.1%) (all from HAL Allergen Laboratory, Haarlem, The Netherlands). Thirty-seven AD patients had been tested intracutaneously with a 1:100 diluted *P. ovale* extract prepared for skin prick tests by ALK Laboratories, Copenhagen, Denmark (Soluprick, 5 mg/ml in 50% glycerol, lot no. 015/83115101).¹⁷

IgE determinations

Total serum IgE was measured by PRIST (Pharmacia) and specific IgE levels to atopic allergens such as house dust, house dust mite, several pollen extracts, and cat and dog dander by RAST (Pharmacia). Twenty-nine of the AD sera had also been tested by a RAST for anti-*P. ovale* IgE.¹⁷

ELISA for anti-*P. ovale* IgE

The *P. ovale* extract from ALK (Soluprick, 5 mg/ml; lot no. 015/83115101) was also used in the ELISA for anti-*P. ovale* IgE. The extract was dialysed overnight at 4°C against phosphate-buffered saline (PBS, pH 7.4) to remove the glycerol, and stored in aliquots at -40°C . Microtitre plates (Maxisorp, no. 4-39454A; Nunc, Roskilde, Denmark) were coated with a 1:50 dilution of the

extract in 0.1 M carbonate/bicarbonate buffer, pH 9.5. In each step of the assay (except the last) 0.1 ml portions were added to the wells. Coating was performed overnight at 37°C . The wells were rinsed extensively with PBS containing 0.05% Tween-20 (PBS-Tween). Serum samples diluted 1/10 in PBS-Tween were added, and incubated with shaking for 2 h at room temperature. Subsequent incubations took place for 1 h at 37°C or (with continuous shaking) at room temperature, and were followed by extensive washing with PBS-Tween. Binding of IgE was assessed by incubation with mouse monoclonal anti-human IgE (Central Laboratory of the Blood Transfusion Service, Amsterdam, The Netherlands, batch no. MH25-01-MO2), diluted 1/3000 in PBS-Tween containing 1% gelatin and 0.1 mg/ml azide, followed by incubation with biotinylated rabbit anti-mouse Ig (Dakopatts, Copenhagen, Denmark), diluted 1/1000 in PBS-Tween, and thereafter with avidin-peroxidase (Dakopatts) at 1/2000 in PBS-Tween. Finally, each well received 0.15 ml of a solution of 2.1 mM *o*-phenylenediamine (OPD; Sigma Chemicals, St Louis, MO, U.S.A.) and 4.4 mM H_2O_2 in 33 mM phosphate-citrate buffer, pH 4.9. After 30 min at room temperature, the reaction was stopped with 0.05 ml 2.5 N H_2SO_4 , and the absorption was read at 490 nm. Each sample was tested in duplicate, in both *P. ovale*-coated and in non-coated control wells. All data were corrected for OD(490) values in non-coated wells, which were in the range of 0.05–0.20. In each microtitre plate at least two PBS controls in coated and non-coated wells were tested to exclude any anti-*P. ovale* reactivity of the various other reagents. OD(490) values in such PBS-treated wells were low (<0.1) and did not differ between coated and non-coated wells. A positive AD serum giving at a 1/10 dilution a corrected OD(490) value of 0.8–1.0, was included in each plate as a positive control.

Anti-*Candida* IgE was determined in an identical ELISA procedure, with microtitre plates coated with *Candida albicans* extract (Laboratorium Diephuis, Groningen, The Netherlands) at 0.1 mg/ml.

Results

Presence of anti-*P. ovale* serum IgE

The ELISA results of patients and controls are presented in Figure 1. Positivity was defined as an OD(490) exceeding the mean OD(490) + 3 SD of the healthy control group, which meant a value >0.11 . Thus, 35 of the 72 AD patients (49%) had detectable anti-*P. ovale* IgE antibodies. In contrast, only one of the sera of 27

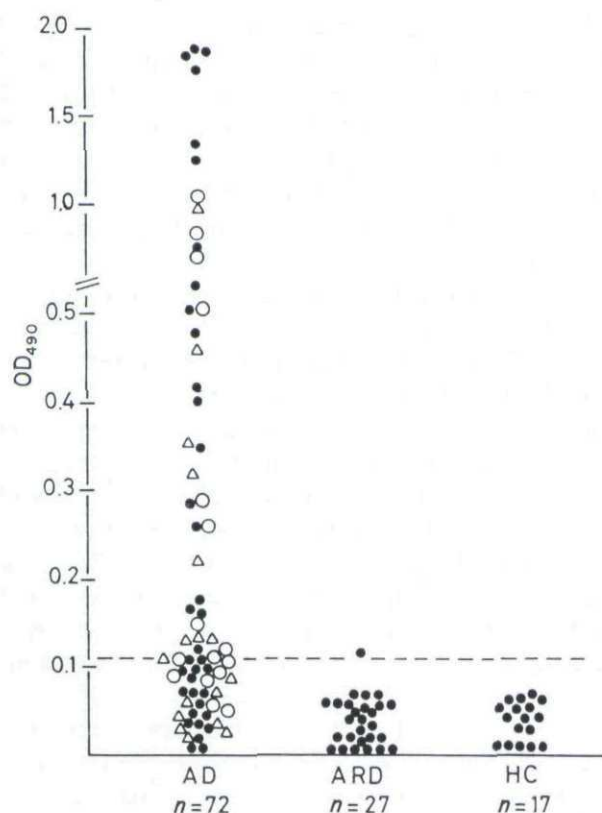


Figure 1. Anti-*P. ovale* IgE antibodies determined by ELISA in 1/10 diluted sera from 72 patients with atopic dermatitis (AD), 27 patients with atopic rhinitis or asthma, but no eczema (ARD), and 17 healthy controls (HC). AD patients had either an individual history of associated ARD (○), only a family history of atopic disease (△), or both (●). OD(490) values were all corrected for non-specific binding of serum IgE in non-coated and of test reagents in coated wells.

patients with ARD and no skin involvement was found to be positive. The sera of four atopic controls with present ARD and previous eczema in their childhood were all negative (data not shown). When the mean OD(490) + 2 SD of healthy control sera was used as the cut-off value (0.85), the sensitivity of the assay for AD increased to 68% (49/72), and again only one of the ARD sera was positive. In further analyses, the mean OD(490) + 3 SD of healthy control sera was used as the detection limit.

Correlation with RAST data

The sera of 29 AD patients had been tested previously in a RAST for anti-*P. ovale* IgE.¹⁷ Concordant results were found in 79% (23/29; 10 negative, 13 positive); four sera were ELISA-/RAST+ and two sera ELISA+/RAST-. There was also a good quantitative correlation between OD(490) values in the ELISA and percentage binding of radiolabelled anti-IgE in the RAST ($r=0.74$; Spearman rank correlation).

Intracutaneous tests with *P. ovale*

Of 37 AD patients tested intracutaneously (i.c.) with *P. ovale* extract, 31 (84%) showed immediate-type reactivity. Twenty of the 31 responders, but none of the six patients with a negative i.c. test, had detectable anti-*P. ovale* IgE. Thus a good correlation was demonstrated between the ELISA and the i.c. test ($P=0.005$, Fisher's exact test), although the ELISA appeared to be less sensitive. No correlation was found between the intensity of the i.c. reactions and OD(490) values in the ELISA.

Age and anti-*P. ovale* IgE levels

Because the occurrence of *P. ovale* on the skin depends on age,^{22,23} the relation of the IgE anti-*P. ovale* levels to the age of the AD patients was studied. The highest concentrations were found among patients aged 20–30 years (Fig. 2). Although the majority (45/72) of the AD patients belonged to this age category, there was a significant predominance of moderate and high levels of anti-*P. ovale* IgE in this decade, compared to the combined other age categories ($P<0.01$; Wilcoxon's rank order sum test).

Associated ARD and severity of eczema

No significant difference was found between the ELISA results in patients being treated as out-patients (17/37 positive; median OD(490) value 0.11) and those hospitalized with an exacerbation of AD and, in most cases, involvement of a large proportion of the whole body area

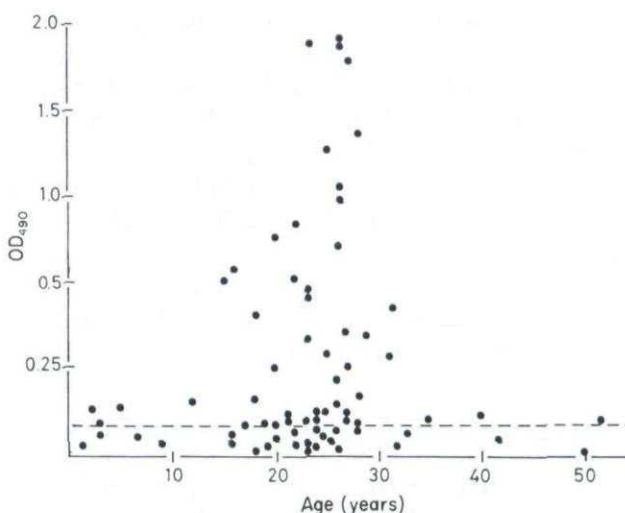


Figure 2. Anti-*P. ovale* IgE levels in AD patients as a function of age.

Table 1. Correlations between serum levels of anti-*Pityrosporum ovale* IgE (ELISA) and levels of total (PRIST) and specific (RAST) IgE in AD patients

Allergen	n*	r†	P value
Dust mite (<i>D. pteronyssinus</i>)	60	0.38	<0.001
Tree pollen	59	0.27	<0.02
<i>Artemisia</i> pollen	42	0.59	<0.0001
Grass pollen	59	0.16	<0.1
Dog dander	17	0.38	<0.02
Cat dander	17	0.45	<0.04
<i>Candida albicans</i> ‡	54	0.74	<0.0001
Total IgE	65	0.74	<0.0001

* Number of patients for whom data of both tests were available.

† Spearman's rank correlation coefficient.

‡ Determined by ELISA.

(18/35 positive; median OD(490) value 0.12). Comparison of AD patients with or without a personal history of respiratory allergy revealed a non-significant tendency ($P=0.06$; Wilcoxon) to higher levels of anti-*P. ovale* IgE in AD with associated present or previous ARD (Fig. 1).

Other parameters of atopy

Within the AD group the results (positive/negative) of i.c. tests with *P. ovale* and with various other allergens were compared. Significant correlations (Fisher's exact test) were found with i.c. tests with human dander and with grass pollen, but not with other allergens (data not shown). When the results of the anti-*P. ovale* ELISA were compared with the levels of total and specific IgE as determined by PRIST and RAST, significant correlations were found with total serum IgE, and with specific IgE against house dust, house dust mite, tree pollen, *Artemisia* pollen, and dog and cat dander (Table 1). No correlation was found with RAST results for grass pollen. Of 54 AD sera tested in an identical ELISA procedure with a coating of *Candida* instead of *Pityrosporum* extract, 14 gave an OD(490) value >0.11 . Thirteen of these 14 sera also had detectable anti-*P. ovale* IgE, and a highly significant correlation between the levels of both types of IgE was found (Table 1). As with anti-*P. ovale* IgE, anti-*Candida* IgE was only found in AD, and not in ARD patients without eczema (data not shown).

Discussion

Several recent reports have described a high frequency of immediate and/or delayed type anti-*Pityrosporum* reactivity in patients with AD. Rokugo *et al.*¹⁸ reported

delayed-type reactions to scratch-patch tests in 64% of AD patients, and positive skin prick tests (SPT) in 54%¹⁸ but no data was given as regards anti-*P. ovale* IgE. Kieffer *et al.*¹⁹ found a similar high frequency of positive SPT (65%), and *P. ovale*-specific IgE was demonstrated in 64%, using a basophil histamine release assay, which correlated well with the SPT results.¹⁹ Nordvall and Johansson²⁰ assessed anti-*P. ovale* IgE in 105 sera from atopic children with or without eczema. Their results agreed with our previous and present findings in that a positive RAST was found mainly in patients with AD or AD + ARD (38%), and only incidentally (6%) in patients with ARD alone. In our previous study,¹⁷ the majority of adult AD patients reacted positively to intracutaneous tests (87%) or SPT (59%) with *P. ovale* extracts. None of the atopic controls with ARD but no eczema responded to SPT and only 12% reacted to i.c. tests.¹⁷ RAST testing was performed with a culture filtrate of *P. ovale*, and binding of IgE was found in 65% of AD sera but was virtually absent from the sera of atopic controls without skin disease.

Because we preferred a non-radioactive immunoassay, a sensitive ELISA was developed with microtitre plates coated with *P. ovale* extract. Only small amounts of serum (less than 0.05 ml) were required for duplicate measurements in coated and in non-coated control wells. The assay can be easily performed with the same allergen solution as used for *in vivo* diagnostic tests. Although we used partial denaturation in alkaline coating buffer in the present study the ELISA can also be performed with *P. ovale* extract coated in PBS (unpublished data). Despite the technical differences, the ELISA results correlated well with the previously used RAST method, with only 20% (6/31) of AD sera giving discordant results; five of these six sera had OD(490) values and binding percentages near the lower detection limits of both assays.

Our results may have been influenced by the relatively large proportion of severe cases of AD, as nearly 50% of the sera were obtained from hospitalized patients with exacerbating AD. No difference was found, however, between the levels of anti-*P. ovale* IgE in the out-patients and in-patients with atopic dermatitis suggesting no relationship with the severity of the disease.

Our data confirm the previously reported specificity of anti-*P. ovale* IgE for AD. The marked correlations with total and specific serum IgE suggest that atopy with a predisposition to produce IgE antibodies is necessary for the production of anti-*P. ovale* IgE, but its association with AD indicates that other factors are also involved. The presence of IgE against skin microflora might be one

of the aetiological factors that distinguish AD patients from atopics without skin disease. Alternatively, the occurrence of anti-*P. ovale* IgE might be a secondary phenomenon, due to an increased exposure to the yeast allergens. Its relation to age suggests that the skin colonization rate is of importance, because the highest anti-*P. ovale* IgE levels were found in patients aged 20–30 years, and *P. ovale* colonization in healthy controls reaches its maximum in the third decade of life.^{22,23} Preliminary studies have shown an increased colonization with *P. ovale* in lesional, compared to the healthy skin in these patients (J.E.M.H. van Bronswijk and E. Young, unpublished data). It is not known whether lesions induced by scratching may provide a suitable environment for the enhanced growth of *P. ovale*. However, even if increased exposure to *P. ovale*-derived allergens is a secondary phenomenon, the subsequent IgE-mediated skin reactions to *P. ovale*-derived allergens may contribute significantly to the severity of exacerbations. The beneficial effect of oral anti-mycotic treatment^{16,24} is suggestive for a causative role for *P. ovale* in some forms of AD. Trials are currently being carried out with topical ketoconazole to determine if this is an effective treatment.

The correlation between levels of anti-*P. ovale* IgE and levels of IgE binding to *Candida* allergens agrees with the data reported by Nordvall and Johansson.²⁰ Anti-*Candida* immediate-type reactivity has been suggested to be associated with bronchial asthma.^{25–27} However, so far we have found anti-*Candida* IgE, like anti-*P. ovale* IgE, only in AD, and not in respiratory allergy without eczema. A more extensive study with a larger series of asthma patients should reveal whether anti-*Candida* IgE only occurs in asthmatics who also have eczema. Preliminary inhibition ELISA experiments have indicated that anti-*Candida* IgE in a number of AD patients is completely cross-reactive with extracts of *P. ovale* (G. Doekes, unpublished data). This would suggest that type I immunoreactivity to *P. ovale* might result from sensitization to *Candida* species. On the other hand, anti-*P. ovale* sensitization might strongly influence the immunoreactivity of AD patients towards other commensal or infective micro-organisms like *C. albicans*, e.g. in secondarily infected skin lesions. Whatever the origin of the IgE-mediated reactions with common microbial antigens, their role in AD may be significant.

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