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Pityrosporum Ovale and Atopic Dermatitis in Children and Young Adults

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Children aged 0–21 years, 60 children with atopic dermatitis (AD), 40 children with rhinoconjunctivitis and or asthma (RA) and 40 children with no atopic history (HC) were studied to evaluate the relationship between skin colonisation with *Pityrosporum ovale* and the occurrence of specific IgE antibodies to *P. ovale*. The following studies were done: culture for *P. ovale*, measurement of IgE antibodies to *P. ovale* (skin prick test, RAST), *Candida albicans*, and *Cladosporium herbarum* (RAST) and IgG antibodies to *P. ovale*. *P. ovale* could be cultured with about the same frequency in children and young adults with AD and age-matched children with or without other atopic manifestations. In spite of similar colonisation, IgE antibodies against *P. ovale* occur only in atopy and more frequently in children with AD than in those with other types of atopic disease. *Key words: Prick test; RAST; Head and neck dermatitis.*

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The etiology of atopic dermatitis (AD) is still unclear, although it is known that genetic and environmental factors influence the expression of the disease. AD has become more common globally (1) and the cumulative incidence in the Nordic countries varies between 10% and 12% (2, 3). The atopic patient has a general tendency to become sensitised due to genetic factors, allergen doses and time of exposure (4).

The lipophilic yeast, *Pityrosporum ovale,* which is a part of the normal skin microflora in adults (5–7), could play a role as an environmental allergen. This hypothesis is in accordance with studies showing that some patients with AD involving the head and neck a) have an increased incidence of positive skin prick test (SPT) with *P. ovale* extract (8), b) have specific IgE antibodies to *P. ovale* (9), c) are positive to *P. ovale* when tested by epicutaneous tests (10) and d) improve when treated with oral ketoconazole (11). Ketoconazole is an effective antifungal drug but it also has an antiinflammatory effect (12). Colonisation with *P. ovale* is less pronounced in healthy infants and children than in adults (6). Nordvall and Johansson found a strong correlation between AD and the occurrence of specific IgE antibodies to *P. ovale* in children (13).

The purpose of this investigation was a) to study the relationship between skin colonisation with *P. ovale* and the occurrence of specific IgE antibodies to this yeast and b) to evaluate the importance of *P. ovale* as an allergen in infants, children and young adults with AD, as compared to children and young adults who had rhinitis, conjunctivitis or asthma or were healthy.

MATERIAL AND METHODS

Children attending the outpatient Allergy Clinic at the Department of Pediatrics, Östra Hospital Gothenburg, or the Department of Dermatology at Sahlgrenska Hospital, Gothenburg or Rigshospital, Copenhagen were investigated. Children from the surgical ward of the Department of Pediatrics, Östra Hospital, Gothenburg served as healthy controls. The study was approved by the ethical committees in Gothenburg and Copenhagen. The investigation was performed during the period December 1988 to October 1989.

Patients with AD (Grp AD)

This group consisted of 60 children and young adults aged 7 months to 21 years (mean 10 years 6 months). The diagnosis was based on the criteria of Hanifin & Rajka (14). Six children had concomitant asthma, 8 had rhinoconjunctivitis and 6 had concomitant asthma and rhinoconjunctivitis.

Patients with rhinoconjunctivitis and/or asthma (Grp RA)

This group consisted of 40 children and young adults aged 2 to 20 years (mean 10 years 6 months) with asthma and/or rhinoconjunctivitis but no ongoing AD, although one of them had AD in early infancy. Twenty-one of the children had asthma, 8 had rhinoconjunctivitis and 11 had combined asthma and rhinoconjunctivitis.

Healthy controls with no atopic history (Grp HC)

This group consisted of 40 children and young adults aged 1.5 to 21 years (mean 11 years). Children aged 1 to 10 years were mostly inpatients from the surgical ward, scheduled for operations like hernia and strabismus.

Experimental design

In all patients and controls, qualitative and quantitative cultures for *P. ovale* and qualitative cultures for other fungi were performed. In Grp AD, samples were taken from the forehead, eczematous skin and uninvolved skin close to the eczema. In Grp RA and Grp HC, samples were taken from the forehead and the antecubital fossa.

Samples for qualitative cultures for *P. ovale* were taken with a curette and transferred to a glucose neopeptone yeast extract medium with lipid supplements, as previously described (15). Quantitative culture for *P. ovale* was performed with contact plates containing the same medium (PDM Pityrosporum Contact Plates, AB, Biodisk, Solna, Sweden) (16). Briefly, the contact plate was pressed against the skin for 15 s., incubated in a Bio-Bag SFJ (Marion Laboratories, Kansas, City, USA) at 37°C and read after 6 days (15, 16). Specimens for culture of other yeasts were transferred to Sabouraud's agar without supplements and incubated at 37°C for up to one week.

Immunological investigations:

i) A skin prick test (SPT) was performed on the forearm with a watersoluble extract of *P. ovale*, protein concentration 5 mg/ml (ALK Laboratories, Denmark). The results were evaluated in relation to a histamine reference equivalent to histamine hydrochloride 10 mg/ml and regarded as 3+ positive if the wheal was equal to the histamine skin reaction (17).
ii) Concentrations of total serum IgE were determined by radioimmunoassay (RIA) (Pharmacia IgE RIA 100, AB, Uppsala, Swe-

Table I. *Occurrence of P. ovale (+) on the skin in children with atopic dermatitis, rhinoconjunctivitis and/or asthma and healthy controls.*

Age (years)	AD		RA		HC	
	+	−	+	−	+	−
0– 5	2	13	1	9	0	10
6–10	2	13	2	8	1	9
11–15	10	5	6	4	9	1
16–21	11	4	7	3	9	1
Total	25 (42%)	35	16 (40%)	24	19 (48%)	21

AD: atopic dermatitis; RA: rhinoconjunctivitis and/or asthma; HC: healthy controls.

den), following the recommendations of the manufacturer. Sera were tested in duplicates and the results given in kU/l.

iii) IgE antibodies to *P. ovale*, *Candida albicans* and *Cladosporium herbarum* were measured by a radioallergosorbent test (RAST) (18,19) using commercially available reagents (Pharmacia AB) with the exception of the *P. ovale* discs, which were produced as described earlier (20). Briefly, an isolate of *P. ovale* (no. 42132) from the American Type Culture Collection (ATCC) was grown on a selective solid agar medium (15). The yeast cells were harvested, freeze-dried, sonicated in phosphate buffered saline (PBS), extracted overnight and finally coupled to cyanogen bromide-activated paper discs. The sera were tested in duplicate and the results expressed in Phadebas RAST Units (PRU)/ml or RAST classes. A positive RAST means ≥0.35 PRU/ml. In children 0 to 5 years of age, IgE antibodies to common inhalant or food allergens were determined by RIA (Phadiatop® Paediatric RIA, Pharmacia) while in the other three age-groups IgE antibodies to inhalant allergens were measured by another RIA (Phadiatop® RIA, Pharmacia). The sera were investigated in duplicate and results were expressed as positive or negative. The commercial reagents were used following the recommendations of the manufacturer.

iv) IgG antibodies to *P. ovale* were measured by means of an enzyme-linked immunosorbent assay (ELISA) measurement (21). The protein extract used as antigen was the same as the one used for SPT. The wells of Titertec polyvinyl chloride (PVC) microplates (Flow labs, Herts, England) were coated by incubation with antigen solution (10 µg/ml in PBS, pH 7.2) for 5 hours at room temperature. The wells were then washed 3 times with PBS-Tween 20 (PBS-Tw). Serum was diluted with PBS with 0.1% Bovine serum albumin (BSA) in two-fold steps, starting with a 1/400 dilution, and each dilution was tested in triplicate. After incubation over night at 4°C the plates were washed 3 times in PBS-Tw. Alkaline-phosphatase-conjugated rabbit antihuman IgG (Dakopatts, Copenhagen, Denmark) ,diluted 1/1500 with PBS-Tw, was added and allowed to incubate for 4 hours at 30°C. The plates were washed 3 times in PBS-Tw. Finally, 4-nitrophenyl phosphate substrate (Sigma, USA), 1 mg/ml in diethanolamine buffer pH 9;8 (Merck, Germany), was added and the optical density was read at 405 nm after 50 min with a Titertec photometer (Flow). As control serum we used pooled positive sera from 30 healthy adults. The absorbance readings were compared with those of the reference serum and the per cent activity calculated.

Table II. *The occurrence of P. ovale in different skin areas.*

Group	Forehead	Eczema	Uninvolved skin	Antecubital fossa
AD	20 (33%)	13 (22%)	13 (22%)	
RA	16 (40%)			10 (25%)
HC	18 (45%)			16 (40%)

Statistics

Data were analysed using Wilcoxon's test for differences between groups and Spearman's rank correlation coefficient.

RESULTS

In our study, with patients in different age-groups from 0 to 21 years , the distribution of AD varied. Twenty-three of the 60 children had eczema involving the face and or neck, but only 5 of these 23 children had a typical head and neck distribution. These 5 patients were all in the oldest age-group.

Mycology–cultures

The results of cultures for *P. ovale* are presented in Tables I and II.

Cultures did not differ significantly between the three groups. There was however a difference between the age-groups, as would be expected from earlier reports. *P. ovale* cultures were positive in 5–15% of children aged 0–10 years and in 65–90% of 11–21-year-olds. The results of the quantitative cultures from the forehead did not differ between the groups. The distribution of *P. ovale* in different areas was similar in the various groups when cultures were taken from the forehead or from healthy skin. Skin with lesions did not show an increased frequency of *P. ovale* compared to uninvolved skin.

Candida parapsilosis and *Trichosporon beigelii* were found in a total of 6 individuals and there were no differences between the groups.

Phadiatop, total serum IgE, SPT and RAST

Positive Phadiatop Paediatric or Phadiatop was seen in 66% of the children in Grp AD , in 62% in Grp RA and in 14% in Grp HC. IgE concentrations of Grp AD and of Grp RA were significantly higher than that of Grp HC ($p < 0.001$). The median concentrations of IgE were in Grp AD , 77 kU/l ($n = 47$, range 2–6100 kU/l), in Grp RA , 165 kU/l ($n = 30$,

Table III. *Results of skin prick tests with a Pityrosporum ovale extract.*

Age (years)	AD		RA		HC	
	+	−	+	−	+	−
0– 5	1	14	0	10	3	7
6–10	3	12	0	10	0	10
11–15	4	11	0	10	0	10
16–21	6	9	0	10	0	10
Total	14 (23%)	46	0	40	3 (8%)	37

Table IV. *Number of patients positive in RASTs for Pityrosporum ovale, Cladosporium herbarium and Candida albicans.*

Age (years)	RAST *P. ovale*			RAST *Cladosporium*			RAST *Candida*		
	AD	RA	HC	AD	RA	HC	AD	RA	HC
0– 5	0	0	0	0	0	0	2	0	0
6–10	1	0	0	1	1	0	2	0	0
11–15	2	0	0	2	0	0	2	0	0
16–21	5	1	0	2	1	0	3	1	0
Total	8/59 (14%)	1/38 (3%)	0/35 (0%)	5/51 (10%)	2/30 (7%)	0/33 (0%)	9/57 (16%)	1/34 (3%)	0/35 (0%)

range 2–7200 kU/l) and in Grp HC, 11 kU/l (n = 34, range 2–290 kU/l). In some cases the amount of serum taken did not allow for all analyses.

Patients with positive SPT to *P. ovale* were mainly found among 11 to 21-year-olds in Group AD (Table III). No child in Grp RA was positive and only 3 in Grp HC, all of whom had weak reactions ("one plus").

The RAST results for antibodies to *P. ovale*, *C. albicans* and *Cl. herbarium* are presented in Table IV. 10–16% of the patients with AD were RAST positive for one or more of the fungi and no significant differences were found between the 3 fungal species. In Grp RA, two patients were positive to one or more fungi and all individuals in Grp HC were negative.

IgG antibodies

A significant difference between subjects with eczema (median per cent activity 336.5 ± 188.0 (SD)) and healthy subjects (median per cent activity 158.0 ± 96.8 (SD)) was seen in the age-group 16 to 21 years ($p < 0.05$).

Table V. *Results of RASTs for Pityrosporum ovale, Candida and Cladosporium in all patients positive in skin prick tests for P. ovale. Results given as RAST class/PRU/ml.*

Total IgE KU/l	SPT	RAST *P. ovale*	RAST *Candida*	RAST *Cladosporium*
Group AD:				
28	+	0	0	0
610	+	0	2/0.7	0
n.d.	+	2/0.8	0	0
83	+	0	0	0
77	+	0	0	0
61	++	0	0	0
130	++	0	0	0
6100	+++	3/12	2/2.6	2/1.5
4100	++	3/5.5	2/1.1	1/0.5
3600	++	2/1.8	2/1.3	0
210	+++	2/0.9	0	0
210	++	0	n.d.	n.d.
140	++	2/3.3	0	0
n.d.	+++	2/0.9	0	n.d.
Group HC:				
16	+	0	0	0
n.d.	+	n.d.	n.d.	n.d.
3.8	+	0	0	0

RAST and SPT to P. ovale

The results of the RAST tests to *P. ovale* in patients with positive SPT are presented in Table V. Fourteen of these children belonged to Grp AD and 7 were positive in the RAST for *P. ovale*, compared to only one of 46 SPT-negative children in group AD.

Positive culture for *P. ovale* and positive SPT was seen in 8 patients, all from the AD group. Seven of these subjects were 11–21 years old. Data from these patients are presented in Table VI.

In all children, the correlations between SPT and IgE, SPT and RAST for *P. ovale*, and IgE and RAST for *P. ovale* were measured with Spearman's correlation coefficient. SPT and RAST for *P. ovale* and IgE and RAST for *P. ovale* showed the strongest correlation ($p < 0.001$).

The patients with head and neck distribution of AD were all 16 to 21 years old (Table VII). Of the five patients, three were positive in the SPT and RAST for *P. ovale*.

There were no significant differences between children with a head and neck distribution compared to all other children in the AD group according to the SPT and RAST for *P. ovale*. The group of children with a head and neck distribution in this investigation was, however, very small, only five.

DISCUSSION

Patients with atopic diseases are prone to produce IgE antibodies (22) but levels vary in different groups of patients (23). Although specific IgE antibodies to different antigens may possibly trigger AD flares (24) via immediate hypersensitivity reactions, other reactions may be of greater importance. According to one hypothesis, IgE bound to receptors on Langerhans cells in the epidermis may give rise to a lymphocyte-mediated reaction after reacting with the corresponding allergen (25).

Patients with atopic disease may develop specific IgE to environmental allergens. *P. ovale* has in some cases been reported to act as an allergen, most frequently giving rise to an itchy dermatitis of the face, scalp, neck and upper back (11). Recently, a strong correlation between active eczema and sensitisation to *P. ovale* was found in children aged 7–18 years (13). In a study from Japan, 54% of 46 patients with AD were prick test positive for *P. ovale*. When the patients were divided into two age-groups, those younger than 10 years showed a

Table VI. *Clinical and immunological characterisation of children with positive culture for P. ovale and positive SPT.*

Pat. no.	Age	Age at onset	*P. ovale*			SPT	Involved areas	IgE KU/l	RAST[b] *P. ovale*	RAST *Candida*	RAST *Cladosporium*
				Qual. culture	Quant. culture						
1	3 yrs	2 month	1[a]	0	0	+	arms legs	28	0	0	0
			2	<5 col.	0						
			3	0	0						
2	12 yrs	2 month	1	<5 col.	3	++	arms legs	130	0	0	0
			2	0	0						
			3	0	0						
3	14 yrs	1½ yrs	1	<5 col.	8	+++	arms legs trunk	6100	3/12	2/2.6	2/1.5
			2	0	0						
			3	0	0						
4	20 yrs	1 year	1	–	5	++	head-neck infra-gluteal	3600	2/1.8	2/1.3	0
			2	<5 col.	10						
			3	<5 col.	1						
5	20 yrs	5 yrs	1	0	0	+++	head-neck	210	2/0.9	0	0
			2	<5 col.	6						
			3	<5 col.	2						
6	19 yrs	2 yrs	1	<5 col.	0	++	arms face	210	0	n.d.	n.d.
			2	<5 col.	4						
			3	5–10 col.	9						
7	17 yrs	1 month	1	<5 col.	8	++	hands legs	140	2/3.3	0	0
			2	<5 col.	5						
			3	<5 col.	0						
8	19 yrs	4 month	1	0	0	+++	neck trunk	n.d.	2/0.9	0	n.d.
			2	0	0						
			3	<5 col.	1						

[a] 1: Forehead
2: Eczem
3: Uninvolved skin

[b] Data given as RAST class and PRU/ml

significantly lower incidence of positive SPT than those in the older age-groups, 39% compared to 64% (26).

The lipophilic yeast *P. ovale* is a saprophyte found on the skin of nearly all adults (5–7) and in children after puberty. In infants, *P. ovale* is less common. In a report by Faergemann and Fredriksson (6), *P. ovale* could not be cultured in children younger than 5 years. It could, however, be cultured from 10% of 5-years-old children and from 23% of 10-year-old children,and in children 15 years or older it could be cultured in nearly all cases. In another study, 53% of healthy infants aged 1–24 months were found to harbour *P. ovale* (27). In the present investigation, we found the skin to be colonised with *P. ovale* in 5–15% of children aged 0–10 years and in 65–90% of 11–21-years-old patients, irrespective of the occurrence of AD or other atopic disease. This finding clearly shows that AD per se does not predispose to colonisation with *P. ovale*. However, since fatty acids used in emollients may promote growth of *P. ovale* (28), it is possible that increased colonisation with *P. ovale* may occasionally be due to treatment rather than to the disease itself. Although there was no significant difference between total IgE in patients with AD and in those with respiratory allergy, the children with AD were more prone to have specific IgE antibodies against *P. ovale*, as measured by prick tests and RAST. In our study, 23% of AD-patients were SPT positive. However, this figure is probably slightly too high since weak, "one plus," reactions were also found in 8% of the healthy controls. If these weak reactions are excluded 15% of our AD-patients are still SPT positive for *P. ovale*. These patients are all in the age-group 11–21years .(Table III). This finding agrees with the results of Nordvall and Johansson (13) who found that atopic children

Table VII. *Immunological data of five patients with head and neck distribution of atopic dermatitis.*

Total IgE KU/l	RAST[a] *P. ovale*	RAST *Candida*	RAST *Cladosporium*	SPT
4100	3/5.5	2/1.1	0	++
120	0	0	0	neg
3600	2/1.8	2/1.3	0	++
210	2/0.9	0	0	+++
260	0	0	0	neg

[a] Data given as RAST class and PRU/ml.

with IgE-antibodies to *P. ovale* had an unusually high incidence of AD. In our study, not all patients with positive SPT were culture positive for *P. ovale*. However, these patients have probably earlier been exposed to *P. ovale*.

These results suggest that Pityrosporum yeasts may play a pathogenic role in older children and adults with AD. *P. ovale* is uncommon in young children and sensitisation to *P. ovale* may therefore be uncommon, in contrast to the age-group 11–21, where *P. ovale* colonisation is seen in the majority of individuals. In our study and in the study by Rokugo et al (26), the older patients were more often SPT positive. The reason for the pronounced difference between AD patients and other atopic individuals as regards sensitisation to *P. ovale* is unknown. However, patients with AD have an altered skin barrier, which may facilitate sensitisation through the skin. This explanation gains support from our finding of an increased rate of sensitisation in AD, but a similar rate of colonisation with *P. ovale* in AD and in other atopic diseases.

Since the finding of an increased incidence of sensitisation in AD was not restricted to *P. ovale* but was also observed for *Cl. herbarium* and *C. albicans*, it seems likely that sensitisation occurs against a variety of microorganisms colonising the skin, with ensuing possible pathogenic consequences (29). The positive tests for the different fungi could also be a result of cross-reactivity (30).

Faergemann found a statistically significant difference in IgG antibody titers to *P. ovale* between adult controls and children, interpreted as due to colonisation with *P. ovale* in adults (31). In view of this finding we compared IgG antibody titers between the corresponding age-groups. IgG antibodies were significantly elevated in Grp AD age 16–21 years as compared to healthy children. This probably reflects increased exposure through eczematous skin and a tendency for IgG to follow IgE production.

AD passes through different phases as the patient grows older (32). The head and neck distribution is most often seen in adolescents and young adults. In our study, we only found five patients with a typical head and neck distribution, and we could not find any significant difference between these children and the other children in the eczema group,as regards incidence of IgE antibodies to *P. ovale*. Adult patients with the typical head and neck distribution of their eczema have, however, in other studies been found to be significantly more often prick test positive for *P. ovale* than patients with AD of other distributions(10).

In conclusion, we found that *P. ovale* could be cultured from the skin with about the same frequency in children and young adults with AD and in age-matched subjects with or without other atopic manifestations. In spite of the similar rate of colonisation, however, IgE antibodies against *P. ovale* occur only in atopy and more frequently in children with AD than in those with other types of atopic disease.

These data support earlier suggestions that *P. ovale* may play a pathogenic role in AD. Colonisation as well as sensitisation seem to be rare in small children, but since the incidence of both events increases with age, allergy to *P. ovale* should be considered a possible pathogenic factor, particularly among older children and adults with the head and neck distribution of AD.

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REFERENCES

1. Hanifin J. Epidemiology of atopic dermatitis. Monogr Allergy 1987; 21: 116–131.
2. Schultz Larsen F, Holm N, Henningsen K. Atopic dermatitis. A genetic-epidemiologic study in a population-based twin sample. J Am Acad Dermatol 1986; 15: 487–494.
3. Åberg N, Engström I, Lindberg U. Allergic disease in Swedish school children. Acta Paediatr Scand 1989; 78: 1446–1452.
4. Kjellman M. Prediction and prevention of atopic allergy. Allergy 1982; 37: 463–473.
5. Roberts SOB. *Pityrosporum orbiculare*: Incidence and distribution on clinically normal skin. Br J Dermatol 1969; 81: 264–269.
6. Faergemann J, Fredriksson T. Age incidence of *Pityrosporum orbiculare* on human skin. Acta Derm Venereol (Stockh) 1980; 60: 531–533.
7. Leeming JP, Notman FH, Holland KT. The distribution and ecology of *Malassezia furfur* and cutaneous bacteria on human skin. J Appl Bacteriol 1989; 67: 47–52.
8. Waersted A, Hjorth N. *Pityrosporum orbiculare* – a pathogenic factor in atopic dermatitis of the face, scalp and neck? Acta Derm Venereol (Stockh) 1985; Suppl 114: 146–148.
9. Schrenker T, Kalveram KJ, Hornstein OP, et al. Spezifische IgE-Antikörper gegen *Pityrosporon orbiculare* bei Patienten mit atopischem Ekzem. Z Hautkr 1989; 64: 478–479.
10. Kieffer M, Bergbrant I-M, Faergemann J, et al. Immune reactions to *Pityrosporum ovale* in adult patients with atopic and seborrheic dermatitis. J Am Acad Dermatol 1990; 22: 739–42.
11. Clemmensen O, Hjorth N. Treatment of dermatitis of the head and neck with ketoconazole in patients with type I sensitivity to *Pityrosporon orbiculare*: Semin Dermatol 1983; 2: 26–29.
12. Cutsem Van J, Gerven Van F, Cauwenbergh G, et al. The anti-inflammatory effects of ketoconazole. J Am Acad Dermatol 1991; 25: 257–261.
13. Nordvall L, Johansson S. IgE antibodies to *Pityrosporum orbiculare* in children with atopic diseases. Acta Paediatr Scand 1990; 79: 343–348.
14. Hanifin J, Rajka G. Diagnostic features of atopic dermatitis. Acta Derm Venereol (Stockh) 1980; Suppl 92: 42–47.
15. Faergemann J. Lipophilic yeasts in skin disease. Semin Dermatol 1985; 4: 173–84.
16. Faergemann J. The use of contact plates for quantitative culture of *Pityrosporum ovale*. Mykosen 1987; 30: 298–304.
17. Aas K, Belin L. Standardization of diagnostic work in allergy. Acta Allergol 1972; 27: 439–468.
18. Johansson SGO, Bennich H, Berg T. In vitro diagnosis of atopic allergy. III Quantitative estimation of circulating IgE antibodies by the radioallergosorbent test. Int Arch Allergy Appl Immunol 1971; 41: 443–451.
19. Wide L, Bennich H, Johansson SGO. Diagnosis of allergy by in vitro test for allergen antibodies. Lancet 1967; II: 1105–1107.
20. Johansson S, Karlström K. IgE-binding components in *Pityrosporum orbiculare* identified by an immunoblotting technique. Acta Derm Venereol (Stockh) 1991; 71: 11–16.
21. Johansson S, Faergemann J. Enzyme-linked immunosorbent assay (ELISA). For detection of antibodies against pityrosporum orbiculare. J Med Vet Myc 1990; 28: 257–260.
22. Juhlin L, Johansson SGO, Bennich H, et al. Immunoglobulin E in dermatoses. Arch Dermatol 1969; 100: 12–16.
23. Uehara M. Heterogeneity of serum IgE levels in atopic dermatitis. Acta Derm Venereol (Stockh) 1986; 66: 404–408.

24. Hanifin J. Atopic dermatitis. Allergy 1988; 43, suppl. 8: 36–38.
25. Bruynzeel-Koomen CAFM. Bruynzeel PLB. A role for IgE in patch test reactions to inhalant allergens in patients with atopic dermatitis. Allergy 1988; 43, suppl 5: 15–21.
26. Rokugo M, Tagami H, Usuba Y, et al. Contact sensitivity to *Pityrosporum ovale* in patients with atopic dermatitis. Arch Dermatol 1990; 126: 627–632.
27. Ruiz-Maldonado R, Lopez-Matinez R, Chavarria P, et al. *Pityrosporum ovale* in infantile seborrhoeic dermatitis. Pediatric Dermatol 1989; 6: 16–20.
28. Roed-Petersen J. Tinea versicolor and body lotions. Acta Derm Venereol (Stockh) 1980; 60: 439–440.
29. Savolainen J, Lammintausta K, Kalimo K, et al. Immunoblotting analysis of IgE response to *Candida albicans* in atopic dermatitis. submitted.
30. Koivikko A,Kalimo K, Nieminen E, et al. Allergenic cross-reactivity of yeasts. Allergy 1988; 43: 192–200.
31. Faergemann J. Antibodies to *Pityrosporum orbiculare* in patients with Tinea Versicolor and controls of various ages. J Invest Dermatol 1983; 80: 133–135.
32. Rajka G. Natural history and clinical manifestations of atopic dermatitis. Clin Rev Allergy 1986; 4: 3–26.