

Pityrosporum and *Candida* specific and non-specific humoral, cellular and cytokine responses in atopic dermatitis patients

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Summary

Background Recent cytokine (RT-PCR, ELISA) analyses of inflammation in atopic dermatitis (AD) have suggested a role for IL-4, IL-5 and IFN γ . *Pityrosporum ovale* and *Candida albicans* are important allergens in some patients with AD of the seborrheic head, neck and shoulder region. In AD patients, the saprophytic yeasts induce IgE responses while they usually induce TH₁ type responses. The cytokine responses induced by yeasts in AD are sparsely investigated.

Objective To characterize the *P. ovale*- and *C. albicans*-specific and non-specific humoral, lymphoproliferative and cytokine (IL-2, 4, 5 and IFN γ) responses in AD.

Methods Fifteen AD patients and seven healthy controls (HC) were included. Ficoll-isolated PBMC were stimulated by PHA and laboratory-generated extracts of *P. ovale* and *C. albicans*. Lymphocyte proliferation was measured by ³H-thymidine incorporation and cytokine production by sandwich-ELISAs. The antigen-specific IgG and IgE antibodies were analysed by ELISA and nitrocellulose RAST.

Results *Pityrosporum ovale*- and *C. albicans*-specific IgE (both $P < 0.001$) and *P. ovale*-induced PBMC proliferation ($P < 0.02$) were elevated in AD. In general, the IL-4/IFN γ ratio induced by *P. ovale* was higher than that induced by *C. albicans* ($P < 0.01$). The PHA-induced IL-2 ($P < 0.05$) and IL-4 responses ($P < 0.005$), and the *C. albicans*-induced IL-5 response ($P < 0.02$) and IFN γ response ($P < 0.01$), were elevated in AD. A network of correlations was seen between serum total and the yeast-specific IgE, *P. ovale*-specific lymphoproliferation, PHA-induced IL-2, IL-4 and IL-5, and *C. albicans*-induced IL-5.

Conclusion The cytokine profiles found in this study support the role of TH₀ or TH₁ cells by the side of TH₂ cells in the pathogenesis of atopic dermatitis. *Pityrosporum ovale* appears to be associated more with IL-4 responses and *C. albicans* with IFN γ responses.

Keywords: *Pityrosporum ovale*, *Malassezia furfur*, *Candida albicans*, atopic dermatitis, IL-2, IL-4, IL-5, IFN- γ , IgE, IgG

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Introduction

The role of environmental allergens in AD has remained disputed, although foods and house dust mite, especially in children, have been accepted as important pathogenic factors. In AD involving the seborrheic regions of the skin,

head, neck and shoulder region, saprophytic growth and hypersensitivity to *Pityrosporum ovale* have been found, as well as some improvement of eczema by antifungal treatment [1,2,3].

Previous studies suggested that in AD, general hypersensitivity to yeasts and saprophytic growth of *Candida albicans* has clinical significance. A connection was demonstrated between saprophytic *C. albicans* growth,

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and sensitization and severity of the eczema [4]. Furthermore, it was shown that *P. ovale* and *C. albicans* cross-react at the IgE level; these findings were later confirmed by other studies [5,6,7].

The human CD4⁺ T-cells, T-helper-lymphocytes (TH), have been divided into subtypes TH₀, TH₁ and TH₂, based on their cytokine production. The production of IgE antibodies and the immediate hypersensitivity are generated via the TH₂ cells, producing IL-4 and IL-5, whereas the delayed-type hypersensitivity and cellular immune responses are generated via the TH₁ cells, producing IFN γ and IL-2. The cytokine profiles of TH clones in AD have revealed mostly TH₂-type cells and in contact dermatitis, mostly TH₁-type cells [8,9,10]. Also, it has been shown that in allergen-specific skin-derived T-cell clones, IL-10 secretion correlates positively with the release of IL-4 and IL-5 [11]. Recently, however, direct mRNA measurements from atopic skin and PBMC, ELISA analyses from peripheral blood in antigen-specific and polyclonal models have given a non-uniform perspective of a more complex network of cytokine production, suggesting a role for IFN γ in the late phase reactions [12–17].

Candida albicans is a classical recall antigen inducing TH₁ type immune responses and cell-mediated immunity which is crucial in retaining the yeast growth as saprophytic instead of invasive and infective [18]. The type and frequency of the immune responses elicited by *P. ovale* in atopic and normal subjects have been characterized in only a few studies [19–21]. In AD with head, neck and shoulder distribution, a continuous exposure to yeasts exists which induces an immune response against these saprophytes. This is likely to contribute to a different type of immune response in which both TH₁ and TH₂ responses are expected because of the controlled saprophytic fungal growth and the yeast-specific IgE.

The present study was performed in order to characterize the cellular and humoral immune responses, and the cytokine responses of PBMC, induced by *P. ovale* and *C. albicans* antigens in AD and healthy controls.

Materials and methods

Patients

The patients participating in the study were outpatients from the Department of Dermatology of the University Central Hospital of Turku, Finland. Fifteen patients had atopic dermatitis (AD), established by a specialist in dermatology according to the criteria of Hanifin and Rajka [22]. Seven controls with no history of allergic diseases or atopic dermatitis were included. The ages and genders of the patients are shown in Table 1. A heparinized blood sample and a serum sample were taken.

Preparation of antigens

Strain CBS 7854 of *Pityrosporum ovale* was isolated from the scalp of a patient with seborrhoeic dermatitis at the Department of Dermatology by the mycological laboratory of the University Central Hospital of Turku, and typed and deposited in CBS (Centraalbureau Voor Schimmelcultuur, Delft, Netherlands). It was cultured for one week on agar plates on the reduced fat cream supplemented medium of Leeming and Notman as previously described [23,24]. The crude cytoplasmic extract of the yeast cells was prepared after disruption in an X-press as previously described [24]. A corresponding crude cytoplasmic antigen of *C. albicans* was a *C. albicans* In House Reference preparation (IHR) of the Department of Clinical Allergology of Turku University, used in clinical studies and as an internal standard antigen of *C. albicans* [25]. The characterization of the allergenic compositions of these extracts has been described earlier [24,25]. Both extracts were dialysed and stored in lyophilized form. The extracts were found to be free of endotoxin contamination by E-Toxate kit (Sigma, St Louis, MO, USA). Phytohemagglutinin (PHA), a mitogen, was purchased from Difco Laboratories (Detroit, MI, USA).

IgE and IgG determinations

The antigen-specific IgE antibodies were analysed by nitrocellulose RAST, and antigen-specific IgG antibodies with ELISA, as previously described [26]. IgG values were presented in optical density and IgE values were presented as indexes, counted as previously described (specific cpm – blank cpm/total cpm – blank cpm) [27]. Serum total IgE was analysed by commercial RIA and expressed as kU/L (Pharmacia IgE RIA, Uppsala, Sweden).

PBMC stimulations in vitro

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized whole blood with Ficoll (Ficoll-Hypaque, Amersham Pharmacia Biotech, Uppsala, Sweden) gradient centrifugation as previously described [28]. The mononuclear cells were suspended, after washing, in RPMI (Gibco, Life Technologies, Paisley, UK) supplemented with 10% human AB serum, 2 mM L-glutamine (Gibco), 100 U/mL penicillin and 100 μ g/mL streptomycin (Nordvacc Media Skärholmen, Sweden). The cell suspension was diluted in the culture medium to 10⁶ cells/mL and applied to culture plates (Nunc, Roskilde, Denmark) together with the antigens. The 96-well plates, 100 μ L suspension/well in triplicate, were used for proliferation assays and 24-well plates, 400 μ L suspension/well in duplicate, for collection of supernatant fluids for cytokine ELISA. The cultures were incubated at 37 °C, in a humidified atmosphere with 5% CO₂, for up to 7 days. In the proliferation

assay, tritium-labelled thymidine (Amersham, UK) was used in the culture medium during the last 18 h, and the incorporated radioactivity in the cells was measured at 5 days for antigen stimulation and at 3 days for PHA stimulation. For cytokine ELISA, the culture supernatant fluids were collected after 24, 66 and 96 h of incubation for antigen stimulation, and after 24 and 66 h of incubation for PHA stimulation.

Cytokine determinations by ELISA

Cytokines released by PBMCs were measured by cytokine-specific ELISA in 96-well, flat-bottomed microtitre plates (Nunc-Immuno Plate Maxisorp, Roskilde, Denmark) as described previously [28], with slight modifications. For IL-4, the capture antibody was made of a polyclonal goat anti-IL-4 antiserum (R & D Systems Europe Ltd, Abingdon, UK), 5 mg/mL, and the tracer antibody of the anti-IL-4 rat mAb 11B4 diluted at 2 mg/mL. Calibration curves consisted of serial dilutions of purified recombinant IL-4 (Genzyme Diagnostics, Cambridge, MA, USA). A horseradish peroxidase-conjugated goat anti-rat Ig (Tago Inc., Burlingame, CA, USA) diluted 1/5000 was used for labelling. The ELISA used the mAb A35 2.0 mg/mL and the biotinylated mAb B27 1.0 mg/mL as capture and tracer antibodies, respectively. Calibration curves were made with serial dilutions of purified recombinant IFN γ (Genzyme Diagnostics). A streptavidin-alkaline phosphatase conjugate (Tago) diluted 1/5000 was used for labelling. The anti-IL-4 and anti-IFN γ antibodies were provided by Drs Jan E deVries and J Banchereau. IL-2 (Genzyme Diagnostics) and IL-5 (Pharmingen, San Diego, CA, USA) concentrations were analysed by commercially-available antibody pairs and standard kits for ELISA. The detection limits for the cytokines were 25 pg/mL for IL-2, IL-4 and IFN γ , and 12.5 pg/mL for IL-5.

Statistical analysis

The Mann-Whitney *U*-test was used for unpaired comparisons, the Wilcoxon Signed Rank test was used for paired comparisons and the Spearman's Rank Order correlation test was used for correlation analyses.

Results

IgE and IgG responses (Tables 1 and 3)

Serum total IgE concentration (S-IgE) was clearly higher in AD patients compared with controls ($P < 0.002$) (Table 1). Also, *C. albicans*- and *P. ovale*-specific IgE was significantly increased in AD patients compared with healthy controls (both $P < 0.001$) (Table 1). In total, *P. ovale* was a stronger inducer of the IgE responses ($P < 0.05$) and

C. albicans more strongly induced IgG responses ($P = 0.02$) (Table 3). On the other hand, there were no significant differences in *C. albicans*- and *P. ovale*-specific IgG levels between the groups (Table 1).

Lymphoproliferative responses (Fig. 1 and Tables 2 and 3)

Lymphocyte stimulations were carried out using different concentrations of *C. albicans* and *P. ovale* antigens to find optimal concentrations for further studies (Fig. 1). Significant stimulation was initiated by both yeasts. The curve for median cpm (counts per minute) values reached the highest point at a concentration between 100 and 1000 $\mu\text{g/mL}$ with *C. albicans* and at a concentration between 10 and 100 $\mu\text{g/mL}$ with *P. ovale*. Comparison of the maximal stimulation indices in patient groups is shown in Table 2. There were no differences in unstimulated proliferations between the study groups. In AD patients, stimulation indices for *P. ovale* ($P < 0.02$) and marginally for *C. albicans* ($P = 0.05$) were significantly higher than in the group of healthy controls. *Candida albicans*-induced proliferation was higher ($P < 0.02$) than that induced by *P. ovale* in healthy controls (Table 2), while no differences were seen in AD patients (Table 2) or the total study population ($P = 0.08$, Table 3). The stimulation index values induced by PHA did not differ significantly between the groups.

Cytokine responses (Figs 2 and 3)

Figure 2 presents the kinetics of the spontaneous and PHA-induced cytokine responses in the study groups. IL-2 and IL-4 show peak responses at 24 h, and IL-5 and IFN γ at 66 h. IL-2 secretion was significantly higher ($P < 0.05$) in AD patients than in healthy controls (HC) at 24 h, and IL-4 production at 66 h ($P < 0.005$). For IL-5 and IFN γ production, there were no significant differences between the study groups. The unstimulated IFN γ responses were significantly higher in the AD patients at 66 ($P < 0.02$) and 96 ($P < 0.05$) h.

In the analysis of the cytokine production, *C. albicans* was used at a concentration 300 $\mu\text{g/mL}$ and *P. ovale* at 50 $\mu\text{g/mL}$, based on previous titrations (Fig. 3). The *C. albicans*-induced IL-5 production was significantly higher in the AD patients at 24 ($P < 0.05$) and 96 ($P < 0.02$) h compared with the healthy controls. *Candida albicans*-induced IFN γ secretions were higher in the AD patients than in the healthy controls at 24 h ($P < 0.01$).

Comparison of the peak net cytokine production induced by *C. albicans* and *P. ovale* is presented in Table 3. The *P. ovale*-induced IL-4 production was significantly higher than that induced by *C. albicans* ($P < 0.02$), whereas the *C. albicans*-induced IFN γ production was significantly higher than that induced by *P. ovale* ($P < 0.05$). The

Table 1. The study patients and controls humoral responses

Group	Sex*	Age†	IgE			IgG	
			Total‡	<i>P. ovale</i> §	<i>C. albicans</i> §	<i>P. ovale</i> ¶	<i>C. albicans</i> ¶
Atopic dermatitis (AD, <i>n</i> = 15)	F	35	6450	64.0	47.7	0.11	0.11
	F	38	2570	71.3	17.9	0.99	0.73
	F	28	2460	46.3	7.9	0.17	1.47
	M	39	5320	65.2	34.3	0.23	0.31
	F	37	920	49.0	2.7	0.38	0.34
	M	26	4440	23.5	27.7	0.14	1.16
	F	50	2080	37.3	7.7	0.11	0.16
	M	19	3560	39.4	2.2	0.23	0.21
	F	36	170	1.2	0.67	0.15	0.52
	F	36	5150	56.5	14.3	0.13	0.50
	F	29	520	2.0	5.0	0.25	0.34
	M	43	380	2.3	< 0.4	0.14	0.36
	M	27	570	5.4	62.2	0.43	0.63
	F	29	820	3.8	< 0.4	0.11	0.19
	F	32	360	< 0.4	< 0.4	0.23	0.11
Median		35	2080	37.3	7.7	0.17	0.34
Healthy controls (HC, <i>n</i> = 7)	M	35	20	< 0.4	< 0.4	0.18	0.10
	F	25	25	< 0.4	< 0.4	0.21	0.58
	F	26	95	< 0.4	< 0.4	0.15	0.40
	M	26	9	< 0.4	< 0.4	0.14	0.39
	M	25	3	< 0.4	< 0.4	0.08	0.08
	F	56	2	< 0.4	< 0.4	0.51	0.16
	F	34	8	< 0.4	< 0.4	0.36	0.78
Median		26	9	0.01	0.01	0.18	0.39
Mann–Whitney <i>U</i> -test AD vs HC			< 0.002	< 0.001	< 0.001	n.s.	n.s.

*F = female, M = male; †years; ‡kU/L; §index (see material and methods); ¶optical density.

IL-4/IFN γ ratio induced by *P. ovale* was consequently higher than that induced by *C. albicans* ($P < 0.01$).

Correlations (Table 4)

The relationships between PHA-induced net production of different cytokines were examined by comparing them in pairs in the total study population. The most significant correlation was seen between IL-4 and IL-5 production ($r = 0.52$, $P < 0.02$); in addition, IL-2 correlated significantly with IL-4 ($r = 0.50$, $P < 0.03$) and IL-5 ($r = 0.46$, $P < 0.05$). Further significant correlations are shown in Table 4. A network of correlations was found between serum total and the yeast-specific IgE, *P. ovale*-specific lymphoproliferation, PHA-induced IL-2 and IL-4, and *C. albicans*-induced IL-5.

Discussion

This comparison of yeast-specific immune responses in AD

revealed an interesting dichotomy of the studied yeasts. *Pityrosporum ovale* was a stronger inducer of responses associated with immediate hypersensitivity and TH₂-like responses, such as specific IgE and IL-4. *Candida albicans* on the other hand was a stronger inducer of TH₁-associated responses, such as specific IgG, IFN γ and lymphoproliferation in healthy individuals. Interestingly, *P. ovale* induced a strong lymphoproliferation in AD and a weak one in healthy individuals. Serum total IgE and PHA-induced IL-2 and IL-4 were elevated, confirming earlier findings in AD. In addition, *C. albicans*-induced IL-5 and IFN γ were elevated in AD patients. A network of correlations was seen between serum total and the yeast-specific IgE, *P. ovale* specific lymphoproliferation, PHA-induced IL-2 and IL-4, and *C. albicans*-induced IL-5, suggesting these to be associated with AD.

Intensively elevated total IgE concentration in serum and the appearance of IgE in skin lesions are typical features of atopic dermatitis [29]. Correlations could be found between

Table 2. The study patients and controls, lymphoproliferative responses

Group	Stimulation index*		
	PHA	<i>P. ova</i>	<i>C. alb</i>
Atopic dermatitis (AD, <i>n</i> = 15)	133	23.9	42.9
	1606	75.4	144
	794	86.0	18.3
	515	6.4	15.6
	899	140	80.2
	480	3.7	21.8
	291	3.7	8.1
	527	85.7	30.9
	2193	78.2	82.0
	335	18.8	23.3
	378	68.2	37.5
	696	35.6	70.6
	278	16.9	60.2
	820	18.0	52.5
	959	1.5	4.4
Median	696	23.9	37.5
Healthy controls (HC, <i>n</i> = 7)	770	21.4	60.1
	454	1.8	10.3
	441	14.0	54.5
	301	1.7	3.2
	182	1.8	7.5
	586	4.1	6.7
	390	1.5	5.7
Median	441	6.6	7.5
Mann-Whitney <i>U</i> -test AD vs. HC	n.s.	< 0.02	= 0.05

*Stimulation index = stimulated cpm/unstimulated cpm.

Table 3. Comparison of *Candida albicans*- and *Pityrosporum ovale*-specific IgE, IgG, lymphoproliferative and peak net cytokine responses (pg/mL) (*n* = 22)

	<i>C. albicans</i>		<i>P. ovale</i>		WSR test*
	Mean	SD	Mean	SD	
IgE (index)	10.5	17.4	21.2	26.5	<i>P</i> < 0.05
IgG (optical density)	0.44	0.35	0.25	0.20	<i>P</i> = 0.02
Stimulation index	38.2	35.1	32.0	38.6	<i>P</i> = 0.08
IL-2 (pg/mL)	72.3	112.5	40.5	77.6	<i>n.s.</i>
IL-4 (pg/mL)	38.4	27.5	60.5	52.3	<i>P</i> < 0.02
IL-5 (pg/mL)	30.0	25.2	22.2	28.2	<i>n.s.</i>
IFN- γ (pg/mL)	60.9	66.4	31.6	53.7	<i>P</i> < 0.05
IL-4/IFN- γ	1.38	1.68	3.10	3.40	<i>P</i> < 0.01

*Wilcoxon Signed Rank test.

serum IgE levels and mitogen-induced IL-2 and IL-4 production *in vitro*. This is in concordance with previous findings in atopic children whose serum IgE correlated with the ratio of *in vitro* IL-4/IFN γ production in PHA-stimulated PBMC cultures [30]. It had earlier been demonstrated that high *C. albicans*- and *P. ovale*-specific IgE levels are typical for head, neck and shoulder dermatitis (HNSD) [1,4,31,32]. In the present study, they correlated, as did serum total IgE, with *P. ovale*-induced lymphoproliferation and *C. albicans*-induced IL-5. Furthermore, the *P. ovale*-induced lymphoproliferation and *C. albicans*-induced IL-5 were also elevated in AD patients, suggesting that all these responses are involved in the pathogenesis of eczema.

No statistical differences were seen in mitogen-induced lymphocyte proliferations. This was in accordance with some previous observations and contrary to others showing reduced mitogen-induced lymphocyte proliferation and IFN γ production in cultures of PBMC from patients with and without AD [33,34]. However, the elevated *P. ovale*-specific proliferative responses in AD patients are in line with previous observations by Rokugo *et al.* [19]. On the other hand, it has earlier been reported that *C. albicans*-induced lymphoproliferative responses are suppressed in atopic dermatitis [35].

IL-4 levels were elevated in mitogen-activated PBMC cultures of the AD patients. IL-4 is clearly connected to AD as shown by the fact that IL-4-producing, allergen-specific TH₂ or TH₀ clones accumulate in lesional skin and patch-test skin in patients with atopic dermatitis [9,10,36]. The correlation of IL-2, IL-4 and IL-5 production suggests that these cytokines form a cascade of cytokines induced in the course of developing chronic inflammation. Initiation of acute skin inflammation in AD has been shown to be associated with a predominance of IL-2 and IL-4 expression [13,17,37]. In the delayed phase reaction, IFN γ production predominates and maintenance of chronic inflammation is associated with increased IL-5 expression and eosinophil infiltration [13,17,37,38]. Accordingly, *P. ovale* growth and exposure on the skin would mainly induce IL-2 and IL-4, initiating acute skin inflammation and contributing to IgE synthesis. *Candida albicans* on the other hand would induce IFN γ and IL-5, leading to late phase reaction and chronic inflammation associated with eosinophilia.

The explanation for these diverse and partly controversial findings may be that HNSD, an AD where yeasts are involved, differs from other AD because of continuous fungal exposure. In fact, this study suggests that continuous fungal exposure may induce both TH₁- and TH₂- like responses, as both elevated IgE and lymphoproliferation were seen in parallel. *Pityrosporum ovale* was a stronger inducer of IL-4 production than *C. albicans* and, accordingly,

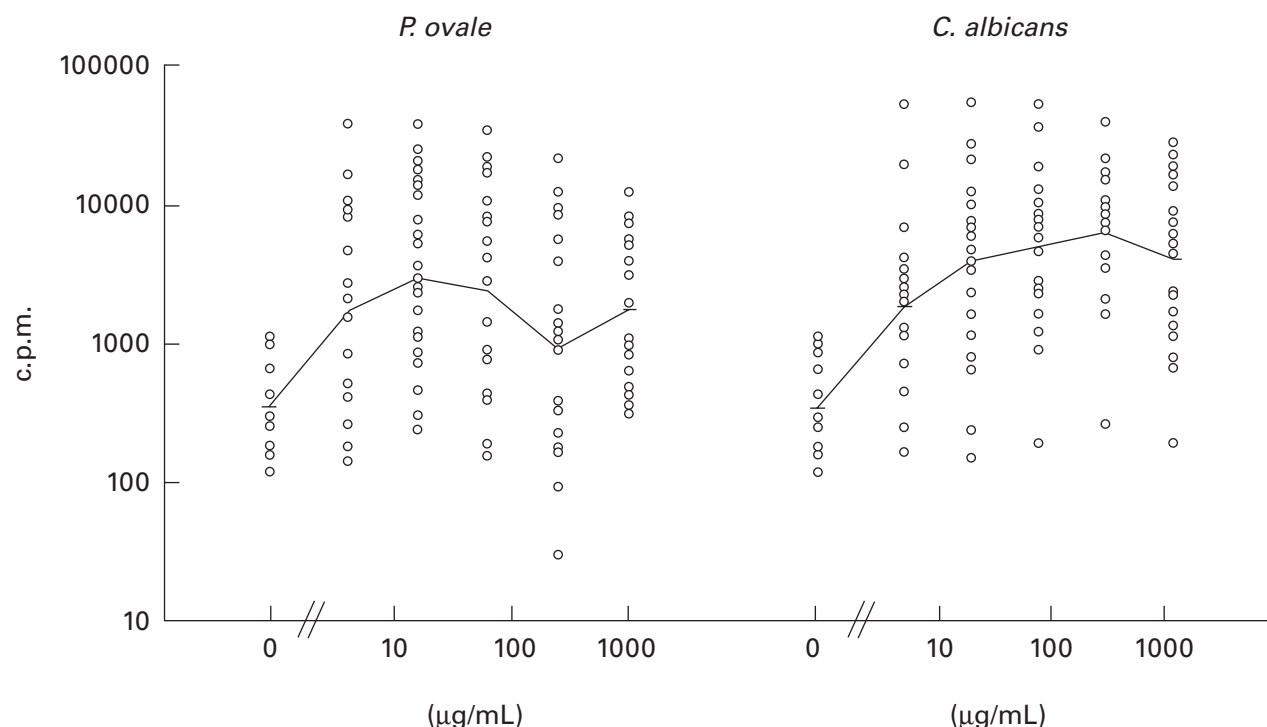


Fig. 1. The titration curves of different antigen concentrations of *Candida albicans* and *Pityosporum ovale* in lymphocyte stimulation. The median cpm (counts per minute) of each concentration are connected with a line. All study subjects ($n = 22$) are included.

with reciprocal regulation of these cytokines, *C. albicans* was a stronger inducer of IFN γ than *P. ovale*. As both TH₁- and TH₂-type immune responses seem to be involved in the immunopathogenesis, it is likely that *P. ovale* is predominantly inducing the TH₂-like response and *C. albicans* mainly the TH₁-like response.

The results of the present study on *P. ovale*-induced TH₂ responses are in line with findings of a recent study of five patients with AD and specific IgE antibodies against *P. ovale* [40]. The freshly-isolated PBMC of the patients were incubated in the presence of different antigen concentrations of *P. ovale* and the results were compared with five healthy non-atopics. Incubation of the PBMC of the atopics

led to an elevated synthesis of the TH₂-related cytokines, IL-4 and IL-10, in concordance with the present observations. In another recent study, *P. orbiculare*-reactive T-cell clones isolated from *P. orbiculare* patch tests or lesional skin were mainly TH₂-type cells, whereas T-cell clones from peripheral blood were mainly TH₁ clones [41].

The route of exposure, gastrointestinal in the case of *C. albicans*, may also be important and lead to different responses. In experimental studies, gastrointestinal challenge of mice with *C. albicans* induced protective TH₁ responses with increased numbers of IFN γ -producing T-cells in mesenteric lymph nodes and spleen [42]. After gastrointestinal T-cell priming, *C. albicans* antigens are also

	Total IgE	IgE	
		<i>P. ovale</i>	<i>C. albicans</i>
Stimulation index			
<i>P. ovale</i>	0.45 (< 0.05)	0.53 (< 0.02)	0.43 (< 0.05)
PHA-induced			
IL-2 (pg/mL)	0.66 (< 0.005)	0.65 (< 0.005)	0.70 (< 0.002)
IL-4 (pg/mL)	0.43 (< 0.05)	0.62 (< 0.005)	0.65 (< 0.005)
<i>C. albicans</i> -induced			
IL-2 (pg/mL)	n.s.	n.s.	0.43 (= 0.05)
IL-5 (pg/mL)	0.45 (< 0.05)	n.s.	n.s.

Table 4. Spearman correlations of proliferative and cytokine responses and IgE levels

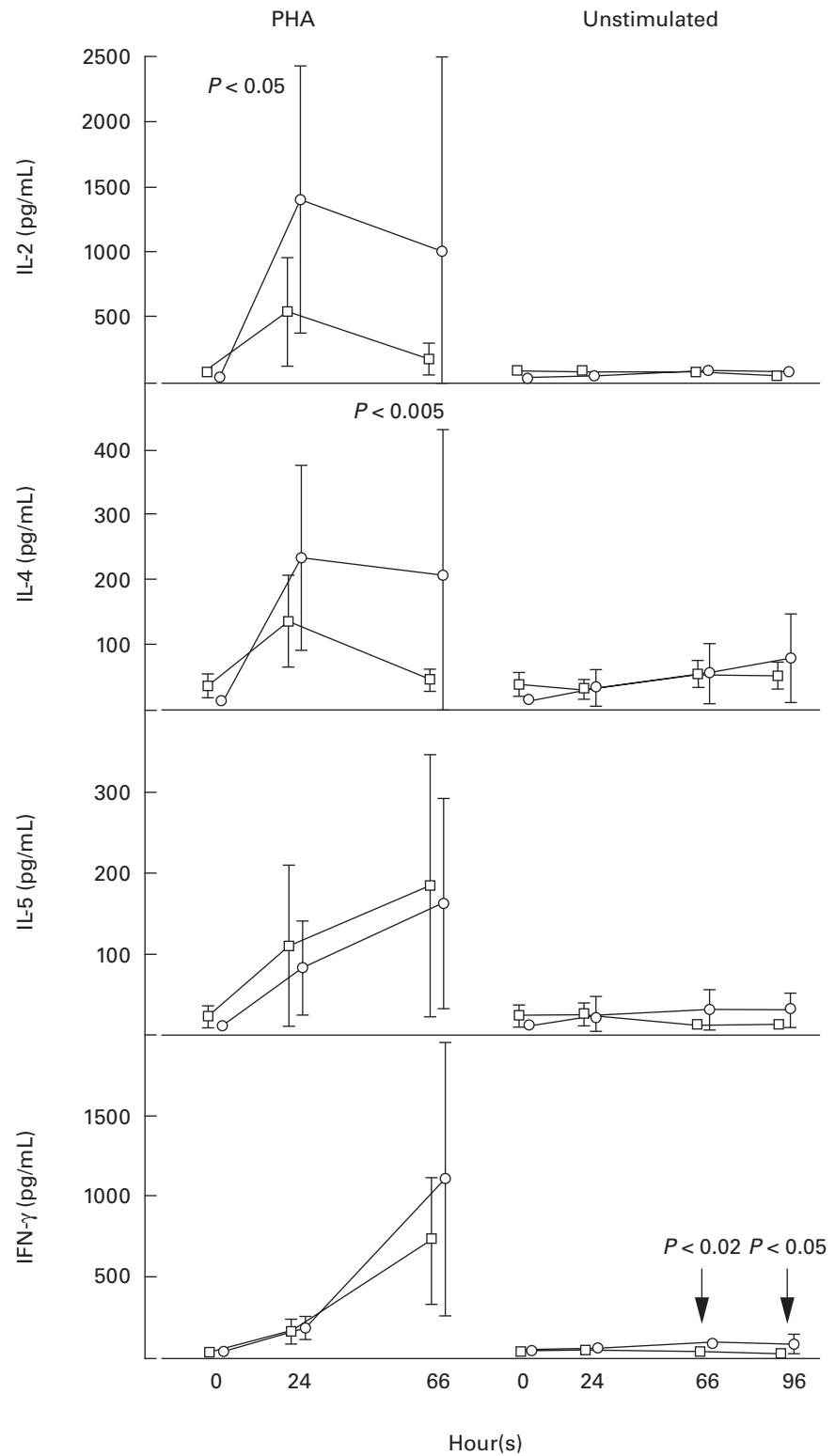


Fig. 2. The time-response curves of the PHA-induced and unstimulated cytokine responses (IL-2, 4, 5 and IFN γ) in PBMC cultures of atopic dermatitis patients (circle, $n = 15$) and healthy controls (square, $n = 7$). Cytokines were measured from the culture supernatant fluids with sandwich ELISAs. The comparison of the responses between the atopic dermatitis group and the healthy controls was done with the Mann-Whitney U -test, and significant differences are indicated in the figure.

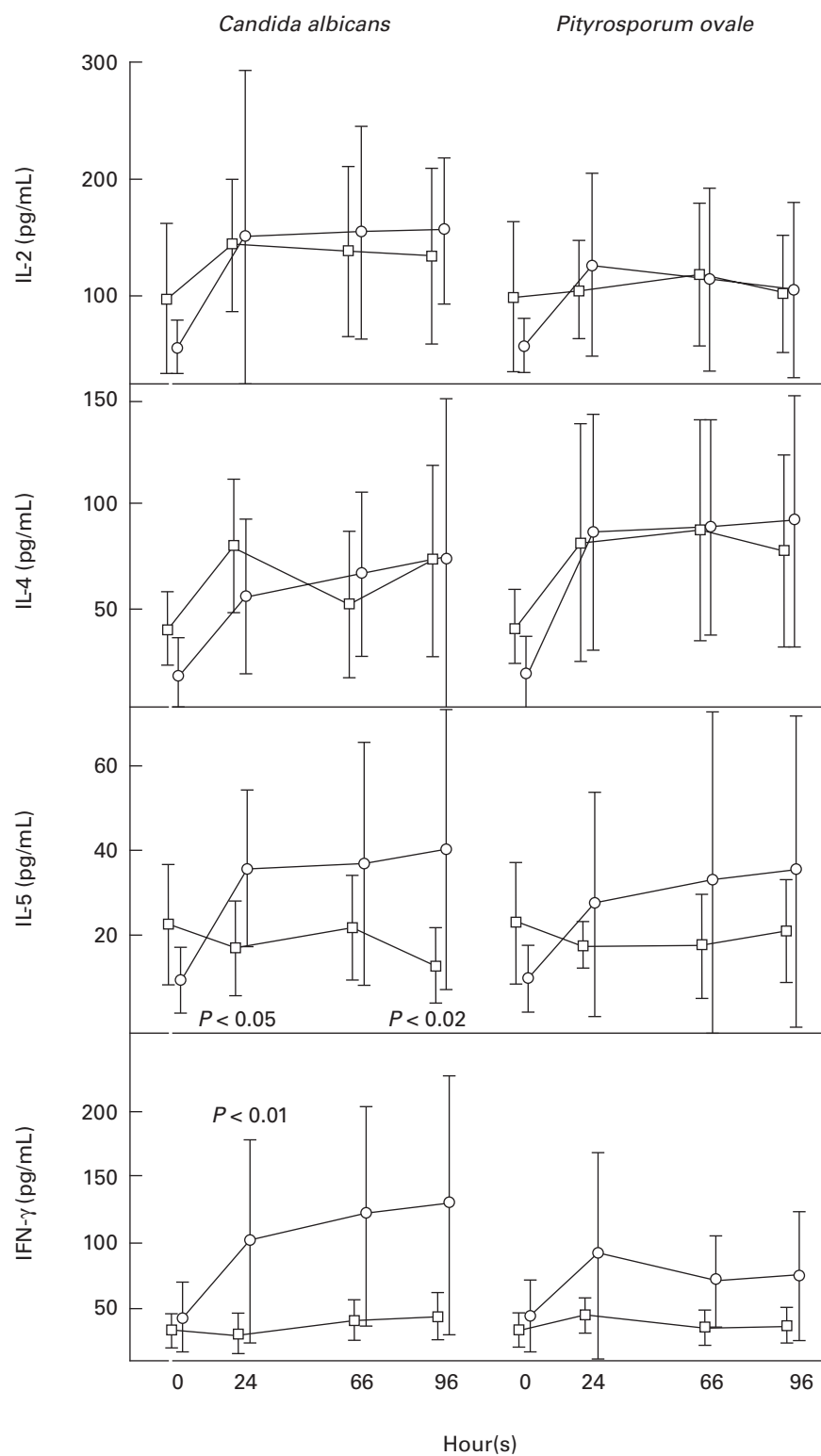


Fig. 3. The kinetics of the *Candida albicans*- and *Pityrosporum ovale*-induced cytokine responses (IL-2, 4, 5 and IFN γ) in PBMC cultures of atopic dermatitis patients (circle, $n = 15$) and healthy controls (square, $n = 7$). *Candida albicans* was used at a concentration of 300 $\mu\text{g/mL}$ and *P. ovale* at 50 $\mu\text{g/mL}$, and cytokines were measured from the culture supernatant fluids with sandwich ELISAs. The comparison of the responses between the atopic dermatitis group and the healthy controls was done with the Mann–Whitney U -test, and significant differences are indicated in the figure.

likely to induce strong proliferative responses in the skin, as Langerhans cells are more effective than PBMC in inducing proliferative T-cell responses to *C. albicans* [43]. *Candida albicans*, also by inducing an intact TH₁ response, would control complete overgrowth and tissue invasion of the saprophytic yeasts. Cure in murine candidiasis is associated with efficient clearance of the yeast from infected organs, and detection of strong TH₁ responses. The production of IL-4 by TH₂ cells may limit TH₁-associated protective immunity [18]. This is also apparent in patients with chronic mucocutaneous candidiasis, in which *Candida* antigens trigger a predominantly also TH₂-instead of a TH₁-type cytokine response [44]. In line with these findings, elevated IgE levels have previously been detected in human invasive candidiasis patients, suggesting TH₂ activation as well [26]. It seems that a controlled mucous growth of saprophytes requires an intact TH₁ response, while a TH₂ response leads to totally uncontrolled superficial or invasive growth, as seen in chronic mucocutaneous or invasive candidiasis. Supporting this hypothesis, a TH₁ immune response is seen in superficial vaginal infections in which yeast growth remains limited [45].

It appears that in some cases of AD, hypersensitivity reactions to yeasts are important pathogenic factors. The straightforward division of TH cells to TH₁ and TH₂ cells may not be directly applicable to these patients, as both *C. albicans* and *P. ovale* antigens induce lymphoproliferation, IgE, IL-2, IL-4, IL-5 and IFN γ . However, there seems to be a difference between these antigens so that *P. ovale* is a stronger inducer of TH₂-type responses and *C. albicans* is a stronger inducer of TH₁-type responses. *Pityrosporum ovale* would thus mainly induce acute skin inflammation and contribute to IgE synthesis. *Candida albicans* on the other hand would be associated with the late and delayed phase reactions and chronic inflammation. The *C. albicans*-induced intact TH₁ response would control the growth of the saprophytic yeasts.

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