

Original article

Immediate hypersensitivity to *Malassezia furfur* and *Candida albicans* mannans *in vivo* and *in vitro*

Background: Elevated and correlative *Malassezia furfur* (*M. furfur*) and *Candida albicans* (*C. albicans*) mannan-specific IgE have been demonstrated in atopic eczema dermatitis syndrome (AEDS) of the head, neck and shoulder (HNS) region of the skin. The significance of these antibodies *in vivo* has not been demonstrated.

Methods: Sixty-five AEDS patients with HNS distribution were included. Serum total IgE (S-IgE) and yeast antigen-specific (Cetavlon-purified mannan and whole extract antigens of *M. furfur* and *C. albicans*) IgE were measured and skin prick tests (SPT) were performed with the yeast antigens.

Results: Mannan-specific IgE and SPT were positive in 51 and 48% of patients with *M. furfur* and in 42 and 22% with *C. albicans*, respectively. Whole extract-specific IgE and SPT were positive in 85 and 95% of patients with *M. furfur* and in 91 and 57% with *C. albicans*, respectively. The highest correlation between specific IgE and SPT was seen with *M. furfur* mannan ($r = 0.60$; $P < 0.0001$). Both *M. furfur* mannan-specific IgE ($r = 0.76$; $P < 0.0001$) and SPT ($r = 0.44$; $P = 0.0005$) correlated with S-IgE.

Conclusions: Mannan-induced immediate hypersensitivity *in vivo* was demonstrated in SPT. The significant correlation between *M. furfur* mannan-specific IgE and SPT suggests that mannan is an important allergen in yeast hypersensitive AEDS *in vivo*.

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Atopic eczema dermatitis syndrome (AEDS) is a chronic inflammatory skin disease characterized by elevated specific serum IgE levels and positive skin prick tests (SPT) to a variety of allergens. Immediate hypersensitivity to saprophytic *Malassezia* yeasts is typical of AEDS involving the seborrhoeic, i.e. head, neck and shoulder (HNS), region of the skin (1–4). Specific IgE antibodies have been detected against a wide range of protein (9–96 kDa) and recombinant allergens (rMal s1, rMal s5, rMal s6) of *Malassezia* (4–6). In addition, immediate hypersensitivity reactions (specific IgE) to *Candida albicans* (*C. albicans*) protein antigens (27, 37, 43 and 46 kDa) are frequent among AEDS patients (7–9). In few existing studies with simultaneous measurements of specific IgE and SPT reactions to crude or protein yeast antigens, 45–100% of AEDS patients showed specific IgE antibodies and 40–60% were SPT positive to *Malassezia* (6, 10–13) whereas specific IgE and positive SPT reaction to *C. albicans* were found in 41–60 and 27–52% of AEDS patients, respectively (8, 9, 11).

Parallel positive SPT reactions to several saprophytic and airborne yeasts have been proposed to indicate allergenic cross-reactivity (14). *Malassezia furfur* (*M. furfur*) and *C. albicans* have been shown to cross-react

at the IgE level with mannan, a polysaccharide, being an important cross-reactive allergen in yeast hypersensitive AEDS patients (15–17).

Until now, type I immediate hypersensitivity to *M. furfur* or *C. albicans* mannan has not been studied *in vivo* with SPT. In this study, we compared an *in vitro* (specific IgE) and an *in vivo* (SPT) method in measuring immediate hypersensitivity to both carbohydrate and whole extract (protein-rich) antigens of yeasts. We examined whether specific IgE and SPT results with mannan and whole extract antigens of *M. furfur* and *C. albicans* correlated and whether these results matched with serum total IgE (S-IgE), clinical severity of eczema (SCORAD) (18) or therapeutic outcome of antifungal treatment. Our main goal was to demonstrate that mannan is capable of inducing immediate hypersensitivity reaction *in vivo*.

Material and methods

Patients

The patients ($n = 65$) participating the study were adult voluntary outpatients from the Department of Dermatology of the University

Central Hospital of Turku (Finland). All of them gave a written informed consent. They also attended the previously published antifungal treatment study (19). The included patients had specific IgE antibodies and positive SPT reaction to *M. furfur* mannan ($n = 24$ and $n = 31$, respectively), *M. furfur* whole extract ($n = 55$ and $n = 62$, respectively), *C. albicans* mannan ($n = 19$ and $n = 14$, respectively) or *C. albicans* whole extract antigen ($n = 59$ and $n = 37$, respectively). AEDS was established by a specialist in dermatology according to the criteria of Hanifin and Rajka (20) and EAACI position statement (21) with eczema typically located in the HNS region. The clinical severity of eczema was evaluated by calculating the SCORAD score as earlier described (18, 19).

Preparation of antigens

Malassezia furfur (CBS 7854) was grown on agar plates on a medium containing reduced-fat cream supplement as previously described (5, 22). The crude cytoplasmic extract was prepared after disruption of the yeast cells in X-Press chamber (AB Biox, Gothenburg, Sweden) as previously reported (5). *Candida albicans* whole extract was the In House Reference (IHR) preparation described earlier (23). *Malassezia furfur* and *C. albicans* mannan antigens were purified according to the Cetavlon method (24, 25).

IgE determinations

S-IgE values were analyzed by Pharmacia IgE RIA (radioimmunoassay) (Uppsala, Sweden) and specific IgE levels against *M. furfur* and *C. albicans* crude antigens by Pharmacia CAP-RAST (radio-immunosorbent test). IgE antibodies to *M. furfur* and *C. albicans* mannan were analyzed by nitrocellulose RAST as earlier described (5, 25).

Skin prick testing

The final SPT extracts were prepared by reconstituting the yeast antigens with ALK diluent (ALK, Hørsholm, Denmark) and 50% glycerol (Sigma, St Louis, MO, USA) and sterile-filtering with 0.8/0.2 μm Acrodisc® (Pall Gelman Laboratory, Ann Arbor, MI, USA). SPT was performed according to EAACI position paper (26). Antigen concentration of 1 mg/ml was used for testing. Histamine-HCl (10 mg/ml) served as a positive and ALK diluent as a negative control. Results were evaluated after 15 min and expressed as indices (mean wheal diameter/mean histamine wheal diameter) (19).

Statistical analysis

The Spearman's rank order correlation test was used for correlation analysis.

Results

Forty-two of the patients were females and 23 were males. Mean age (SD) was 31.9 (10.2) years, mean S-IgE (SD) was 2825 (2683) kU/l and mean SCORAD score (SD) was 44.1 (12.9). The percentages of *M. furfur* and *C. albicans* mannan and whole extract antigen-specific IgE and SPT positive AEDS patients are presented in Table 1. Positive results and higher levels in mannan-

Table 1. Specific IgE and SPT values of AEDS patients ($n = 65$) and percentages of positive results

Mannan-specific IgE (index)*		% Positive (>0.32)
<i>M. furfur</i>	2.3 (3.8)† ($n = 45$)	51
<i>C. albicans</i>	0.85 (1.5) ($n = 45$)	42
Whole extract-specific IgE (kU/l)		% Positive (>0.35 kU/l)
<i>M. furfur</i>	19.3 (20.1)	85
<i>C. albicans</i>	15.2 (19.5)	91
Mannan SPT (index)‡		% Positive (>0.50)
<i>M. furfur</i>	0.41 (0.40)	48
<i>C. albicans</i>	0.17 (0.30)	22
Whole extract SPT (index)		% Positive (>0.50)
<i>M. furfur</i>	0.98 (0.34)	95
<i>C. albicans</i>	0.47 (0.35)	57

* [Sample (cpm) – buffer (cpm)] $\times 10^2$ / [total activity (cpm) – buffer (cpm)].

† Mean (SD).

‡ Mean wheal diameter/mean histamine wheal diameter.

Table 2. Correlation between yeast antigen-specific IgE and SPT results

Antigen	r^*	P	n
<i>Malassezia furfur</i>			
Whole extract	0.36†	0.0042	65
Mannan	0.60	<0.0001	45
<i>Candida albicans</i>			
Whole extract	0.59	<0.0001	65
Mannan	0.27	0.075	45

* Correlation coefficient.

† Spearman's rank order correlation test.

specific IgE and mannan and whole extract SPT were seen more often with *M. furfur* than *C. albicans*. In the whole extract-specific IgE there were no differences between *M. furfur* and *C. albicans* in frequencies of positive results. This was the only assay in which positive reactions to *C. albicans* were seen frequently.

A correlation analysis was performed between specific IgE, SPT and S-IgE results. The specific IgE and SPT results of each antigen preparation had a significant or trend-like correlation (Table 2). *Malassezia furfur* mannan-specific IgE and SPT correlated better than those of *C. albicans* mannan. When whole extract antigen results were compared, *C. albicans*-specific IgE and SPT had higher correlation.

All the specific IgE results correlated significantly ($r = 0.52$ – 0.73 ; $P < 0.0005$; $n = 45$) whereas in SPT comparison significant correlations were only noticed between the mannan antigens ($r = 0.33$; $P = 0.0076$; $n = 65$) and the mannan and crude antigen of the same yeast species ($r = 0.39$ – 0.48 ; $P < 0.002$; $n = 65$).

S-IgE was found to correlate significantly with all the specific IgE results, higher correlation seen with mannans. Of the SPT results, only mannans correlated significantly with S-IgE (Table 3).

Table 3. Correlation between yeast antigen-specific IgE and SPT results and serum total IgE (S-IgE)

Antigen	Specific vs S-IgE			SPT vs S-IgE		
	r^*	P	n	r	P	n
<i>Malassezia furfur</i>						
Whole extract	0.74†	<0.0001	65	0.090	0.47	65
Mannan	0.76	<0.0001	45	0.44	0.0005	65
<i>Candida albicans</i>						
Whole extract	0.64	<0.0001	65	0.21	0.088	65
Mannan	0.73	<0.0001	45	0.25	0.048	65

* Correlation coefficient.

† Spearman's rank order correlation test.

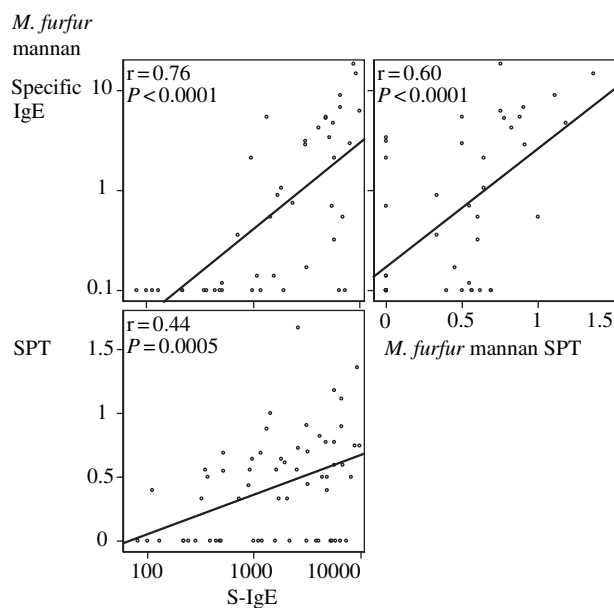


Figure 1. Correlations between *Malassezia furfur* mannan-specific IgE and skin prick test (SPT) and serum total IgE (S-IgE) results in atopic eczema dermatitis syndrome patients (Spearman's rank order correlation test). Regression lines are shown. Mannan-specific IgE and SPT indices are calculated as presented in Table 1.

Severity scoring of atopic dermatitis (SCORAD) or therapeutic outcome of antifungal treatment did not correlate with mannan-specific IgE and SPT results (data not shown).

The data of *M. furfur* mannan SPT and IgE are presented in Fig. 1.

Discussion

This is the first report demonstrating *in vivo* immediate hypersensitivity reactions in SPT with polysaccharide allergen, mannan. To our knowledge this is also the first time that mannan SPT were compared with specific IgE

results in AEDS patients, correlative specific IgE and SPT results have earlier been presented only with the crude antigens of *M. furfur* ($r = 0.47$) and *C. albicans* ($r = 0.38$) (9). It was shown that the specific IgE and SPT results of each yeast antigen preparation correlated, *M. furfur* mannan results better than those of whole extract antigen, suggesting specific IgE-binding. The *in vivo* allergenicity of mannan was further supported by the facts that *M. furfur* mannan SPT was positive in 48% of AEDS patients and that *M. furfur* and *C. albicans* mannan SPTs had a significant correlation with S-IgE and with each other. Previously, it was suggested that IgE antibodies directed against allergens containing cross-reactive carbohydrate determinants (CCD) had poor biological activity as these glycoallergens neither released histamine nor yielded positive SPT reactions thus resulting in false-positive specific IgE values (27, 28). However, since then, the biological activity and clinical relevance of CCD-specific IgE has been re-evaluated and anti-carbohydrate IgE containing sera have been shown to induce histamine release and lose that ability if the allergen was exposed to periodate oxidation prior to IgE binding and histamine release assay (29–31).

It was previously shown that *M. furfur* induces a higher IgE production than *C. albicans* which is a more potent IgG-inducer (32). This is in line with the present finding that specific IgE and SPT results of *M. furfur* mannan correlated better with themselves and with S-IgE than those of *C. albicans*. In addition, previously, the specific IgE level against *C. albicans* (protein-rich) whole extract antigen was found to be higher than against purified mannan polysaccharide in line with the good correlation between specific IgE and SPT results of whole extract antigen noticed in this study (33).

Malassezia furfur ($r = 0.42 - 0.74$) and *C. albicans* ($r = 0.66 - 0.84$) specific IgE have been shown to correlate with S-IgE (5, 34–37). In this study all the specific IgE results associated significantly with S-IgE and, more importantly, *M. furfur* and *C. albicans* mannan SPTs also correlated with S-IgE, which is a novel finding. To assess the possible interfering role of high S-IgE in specific IgE and SPT correlations (38), patients with S-IgE $> 10\,000$ kU/l ($n = 2$) were not included in the study and the rest of the patients were divided into three groups on the basis of S-IgE [S-IgE < 500 ($n = 17$), 500–4000 ($n = 27$) and > 4000 ($n = 21$) kU/l]. No significant differences in correlations between groups were detected (data not shown).

The merely moderate correlations between specific IgE and SPT results found in the present study may be a consequence of methodological, antigen- and/or patient-dependent factors. Assays for specific IgE, including RAST, are *in vitro* tests where the antigen is coupled on a paper or nitrocellulose disc or sponge and recognized by specific IgE in the serum, whereas SPT is an *in vivo* test in the skin where the mast-cells with FcεRI-bound IgE are the main effector cells in immediate hypersensitivity

reaction. Yeast antigens possess several IgE binding epitopes and it might be possible that the epitopes recognized *in vitro* and *in vivo* could differ. In addition, with similar levels of specific IgE the amount of allergen required for a positive SPT result may differ 100-fold between patients (39). Therefore, the lack of threshold end-point dilution in skin prick testing may have affected especially the *C. albicans* mannan results of this study. On the contrary, gastrointestinal tract colonizing *C. albicans* could be better recognized in the mucosal barrier than in the skin where it is rarely found (8). The lack of correlation between SCORAD score or antifungal treatment outcome and mannan-specific IgE and SPT results might indicate that also non-IgE-mediated responses appear during the change of clinical course of mannan-sensitive AEDS.

Based on statistically significant correlations between yeast antigen-specific IgE and SPT results, it can be concluded that in our patients *M. furfur*, and especially its mannan, could be a clinically important yeast antigen aggravating AEDS and that *C. albicans* antigens could be for a large part cross-reactive. Previous ELISA- and RAST-inhibition studies where *M. furfur* allergens were the most potent fluid-phase inhibitors support this presumption (16, 17). Furthermore, there were no corre-

lations between whole extract antigen SPT – unlike specific IgE – results or between mannan and whole extract SPTs of different yeast species suggesting SPT to be more specific than specific IgE in assessing immediate hypersensitivity reactions toward allergens used in this study. Therefore, both tests are recommended for optimal estimation of sensitization.

Malassezia furfur mannan SPT correlated with *M. furfur* mannan-specific IgE and S-IgE showing the first evidence of the histamine-releasing capacity of yeast mannan polysaccharide-specific IgE. These findings suggest that *M. furfur* mannan is an important allergen in yeast hypersensitive AEDS involving the HNS region of the skin.

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