IgE-reactivity to seven Malassezia species.

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Published in:
Allergy

DOI:
10.1034/j.1398-9995.2003.00082.x

2003

Link to publication

Citation for published version (APA):

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IgE-reactivity to seven Malassezia species

**Background:** Malassezia yeasts play a role in the pathogenesis of atopic eczema/dermatitis syndrome (AEDS). The revised genus Malassezia includes several species which are natural habitants of the human skin. In this study, we evaluated the presence of immunoglobulin E (IgE) antibodies to different Malassezia spp. in AEDS patients to allow optimization of the characterization of the IgE antibody profile of IgE-associated AEDS.

**Methods:** Ninety-six adult patients, with a clinical diagnosis of AEDS, were included in the study. Seventeen of the patients had IgE antibodies to *M. sympodialis*, ATCC 42132 (m70 ImmunoCAP, Pharmacia, Diagnostic AB, Uppsala, Sweden). The IgE antibodies to seven Malassezia spp. were measured and inhibition immunoblotting was performed to investigate whether *M. sympodialis* contains all the allergen components present in the other Malassezia spp.

**Results:** Twenty per cent of 79 AEDS patients with a negative m70 ImmunoCAP test had IgE antibodies to at least one of the other six Malassezia spp. tested. Our inhibition studies indicated that Malassezia spp. to a great extent, share allergenic determinants. However, Malassezia species also contained species-specific allergens.

**Conclusion:** The use of only one species of Malassezia is not sufficient to detect all patients IgE sensitized to Malassezia. To obtain an optimal allergen preparation both common allergenic components as well as species-specific allergens have to be considered.

The genus Malassezia comprises yeasts that are natural habitants of the human skin and warm-blooded animals (1). In 1996 the genus was revised to include seven species, *M. sympodialis*, *M. globosa*, *M. restricta*, *M. slooffiae*, *M. furfur*, *M. obtusa*, and *M. pachydermatis* (2). The only non-lipid-dependent member of the genus is *M. pachydermatis*, while the remaining six species require long-chain fatty acids for in vitro growth (2, 3). Malassezia species (spp.) are considered to cause skin infections such as pityriasis versicolor and pityrosporum folliculitis (4–7) and they play an important role in the pathogenesis of seborrhoic dermatitis and ‘atopic dermatitis’ (8–12 and reviewed in 13).

The European Academy of Allergology and Clinical Immunology (EAACI) has, based on the proposal of its Nomenclature Task Force, and with representation from the EAACI Dermatology Section, decided to recommend the term atopic eczema/dermatitis syndrome (AEDS) for ‘atopic dermatitis’ (14). Atopic eczema/dermatitis syndrome is a chronic inflammatory skin disease with a wide variety of clinical manifestations. Up to 65% of patients with a clinical diagnosis of AEDS may have immunoglobulin E (IgE) antibodies specific to Malassezia yeasts (8–10, 12, 13) and treatment with ketoconazole can improve the eczema and at the same time decrease the allergen specific IgE antibody and total serum IgE levels (15, 16).

Recently, *M. furfur/Pityrosporum orbiculare* strain no. 42132, American Type Culture Collection (ATCC) was classified by biochemical characterization as *M. sympodialis* (17, J. Faergemann, personal communication). This strain has been used in several studies (13) and is the allergen source for the production of the m70 ImmunoCAP by Pharmacia, Diagnostic AB (Uppsala, Sweden) for measuring IgE antibodies specific for Malassezia in serum. Extract obtained from *M. sympodialis* induces higher T-cell responses in AEDS patients than in healthy controls (18) and positive skin atopy patch-test reactions to *M. sympodialis* have been found in AEDS patients (19). The aim of this study was to evaluate the presence of IgE antibodies to different Malassezia spp. in AEDS patients to allow optimization of the diagnosis of IgE-associated subgroup of AEDS.

**Material and methods**

Yeast strains, culture conditions and extract preparation

In addition to the *M. sympodialis* strain no. 42132 (ATCC), the following cultures from the CBS (Centraalbureau voor Schimmelcultures, Delft, the Netherlands) were used: *M. sympodialis* 7222, *M. globosa* 7966, *M. restricta* 7877, *M. slooffiae* 7956, *M. furfur* 7966.
7019, *M. obtusa* 7876, and *M. pachydermatis* 1879. All strains were cultured on modified Dixon agar (2) for 4 days at 32°C. The yeast extracts were prepared from 4-day-old cultures as previously described (20). Briefly, the cells were harvested and freeze-dried, re-suspended in phosphate-buffered saline (PBS), sonicated, centrifuged and sterile filtered (20). The protein concentration of the extracts was measured with bicinchoninic acid protein assay reagent (Pierce Chemical Company, IL) according to the manufacturer’s instructions.

**Immunoblotting and inhibition assay**

Equal amounts, based on protein content, of each of the *Malassezia* extracts (24 μg/mm gel) or Dixon broth as control were separated by electrophoresis on 7.5–20% polyacrylamide gels under reduced conditions. The gels were blotted onto polyvinylidene difluoride membranes (Millipore Corp., MA). The membranes were either incubated with the serum pool (diluted 1 : 6 in PBS), or cut into strips and used for the inhibition assay. For immunoblotting inhibition *M. sympodialis* CBS 7222 extract was used to inhibit binding of IgE to allergens in the extracts of other *Malassezia* spp. The serum pool was incubated at room temperature for 2 h with different protein concentrations of *M. sympodialis* (3, 2, 1, 0.5, and 0.25 mg) or with 3 mg bovine serum albumin as a negative control. The mixtures were then incubated with the blotted membranes of various *Malassezia* extracts. Detection of IgE-binding components was performed as described previously (20). The intensity of the bands was recorded in a scanner and the percentage of the inhibition was analysed with the Image Analysis Systems Software, IASS, (Molecular Analyst Software, BIO RAD, CA).

**Table 1. Characterization of the AEDS patients**

<table>
<thead>
<tr>
<th>AEDS patients</th>
<th>No.</th>
<th>Age years median (range)</th>
<th>Gender F/M†</th>
<th>Phadiatop positive no.‡</th>
<th>Head and neck distribution no.</th>
<th>Past or present R and/or A no.§</th>
<th>Total serum IgE (kU/l) median (range)*</th>
<th>Elevated serum IgE no.§</th>
<th>m70-specific IgE (kU/l) median (range)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>m70 Negative*</td>
<td>79</td>
<td>23 (18–56)</td>
<td>57/22</td>
<td>48</td>
<td>29</td>
<td>45</td>
<td>81 (2–1314)</td>
<td>29</td>
<td>–</td>
</tr>
<tr>
<td>m70 Positive*</td>
<td>17</td>
<td>25 (18–39)</td>
<td>9/8</td>
<td>17</td>
<td>14</td>
<td>16</td>
<td>1238 (23–5700)</td>
<td>15</td>
<td>8.3 [0.6–56]</td>
</tr>
</tbody>
</table>

* m70 ImmunoCAP™, allergen source *M. sympodialis* ATCC 42132 (Pharmacia Diagnostics AB).
† Female/male.
‡ Serum IgE reactivity to 11 common aeroallergens (Pharmacia Diagnostics AB).
§ Rhinconjunctivitis and/or asthma.

Results

**Immunoblot**

Of the 79 AEDS patients negative to m70 (*M. sympodialis*, ATCC 42132), 16 (20%) were found to have IgE antibodies to one or more of the other *Malassezia* extracts. The *M. globosa* extract showed the most positive reactions (11/16, 69%) while the *M. slooffiae* extract was the least reactive (3/16, 19%) (Table 2). Among the 16 patients four were ImmunoCAP positive to only one extract (*M. globosa, M. restricta, M. obtusa*, or *M. pachydermatis*). Out of the 16 patients 12 (75%) had elevated total serum IgE, 14 (88%) had a positive Phadiatop and eight (50%) had head and neck dermatitis.

All the seventeen patients who were included because of a positive m70 ImmunoCAP had IgE antibodies to the *M. sympodialis* CBS 7222 extract (Table 2). Sixteen patients were ImmunoCAP positive to the other extracts except for *M. slooffiae* and *M. furfur* (Table 2). One patient with an IgE antibody level of 0.9 kU/l to m70 was ImmunoCAP positive only to the other *M. sympodialis* strain from CBS 7222 and not to the other species (Table 2), indicating that *M. globosa* and *M. restricta*, despite showing numerous IgE-binding components (Fig. 1), do not contain all the allergen components present in the *M. sympodialis* extract.

There was a significant correlation between total serum IgE and specific serum IgE to all seven *Malassezia* spp., using Spearman rank correlation (*r* = 0.51–0.67, *P* < 0.001). None of the spp. were more often correlated with head and neck manifestation.
Immunoblotting and inhibition assay

Immunoblotting analysis indicated that the patterns of the bands in different Malassezia spp. show similarity with each other (Fig. 1), but it seems that M. globosa (lane 6) and M. restricta (lane 7) contain more IgE-binding components than the other Malassezia spp. The control Dixon medium did not show any band with the pooled serum (data not shown).

Extracts of M. sympodialis ATCC 42132 of different protein concentrations were used to inhibit the binding of IgE to Malassezia spp. allergens (Fig. 2A–H). When the inhibitor was used at the concentration of 0.5 mg, 66% of the IgE-binding to M. sympodialis CBS 7222 was inhibited (Fig. 2A, lane 4), and with 3 mg of inhibitor 79% inhibition was obtained (Fig. 2A, lane 7). The binding of serum IgE to M. globosa, M. restricta, M. slooffiae, M. furfur, M. obtusa, and M. pachydermatis was reduced by 22, 27, 37, 64, 82, and 53%, respectively, when the serum pool was preincubated with 0.5 mg of the inhibitor (Fig. 2B–G, lane 4). Increasing the concentration of the inhibitor to 3 mg resulted in inhibition of M. globosa, M. restricta, M. slooffiae, M. furfur, M. obtusa, and M. pachydermatis by 54, 60, 93, 87, 96, and 77%, respectively (Fig. 2B–G, lane 7). In the homologous inhibition, 82% extinction of the bands was obtained when 0.5 mg of M. sympodialis ATCC 42132 was used as inhibitor (Fig. 2H, lane 4), and this increased to 95% using 3 mg (Fig. 2H, lane 7). The results show that M. sympodialis ATCC 42132 at concentrations lower than 3 mg is a rather poor inhibitor of IgE binding to M. globosa and M. restricta, as the patterns were reduced by 60% or less even at the highest inhibitor concentration (Fig. 2B and C, lane 7). In contrast, the binding of IgE to M. furfur, M. obtusa, and M. pachydermatis was significantly reduced even with the lowest concentration of the M. sympodialis inhibitor (0.25 mg) (Fig. 2E–G, lane 3).

Discussion

As the genus Malassezia has been revised and expanded to include several species, all present on human skin, a need has arisen to consider the usage of correct species for the identification of IgE antibodies to Malassezia in patients with AEDS. In this study, we demonstrated that the use of only one species is not sufficient to detect all the patients with IgE antibodies to Malassezia. Twenty percent of 79 AEDS patients with a negative m70 ImmunoCAP test (M. sympodialis ATCC 42132) had IgE...
antibodies to other Malassezia spp. A number of studies have been published on the occurrence of Malassezia species on the skin of AEDS patients and normal controls. Among these, Sugita et al. used nested PCR and showed that M. furfur, M. globosa, M. restricta, and M. sympodialis are common inhabitants of the skin of both AEDS patients and healthy subjects (24). In that study, M. globosa and M. restricta were detected on the skin of 90% of the patients, M. sympodialis and M. furfur were detected on 40% of the patients, whereas M. slooffiae DNA was found in less than 7% of the patients and not in the healthy controls (24). Aruzumanian showed M. sympodialis to be the most frequent species in both AEDS patients and healthy individuals and M. globosa was infrequently isolated from normal skin (25). However, isolation and detection of Malassezia spp. from the skin varies, based on several factors such as methods of detection, the efficiency of culturing of Malassezia, the use of different media, area of sampling, and geographical differences. Previous serological work by Koyama et al. on five different Malassezia spp. demonstrated that in patients with AEDS, 83% of sera had IgE antibodies to M. globosa, 74% to M. sympodialis, 65% to M. furfur, 56% to M. restricta, and 50% to

Figure 2. Inhibition of IgE binding to Malassezia spp. extracts by M. sympodialis ATCC 42132. A pool of five sera all with total serum IgE value ≥2300 kU/l and positive m70 ImmunoCAP = 9 kU/l were preincubated with 0.25, 0.5, 1, 2, and 3 mg protein (lane 3–7 respectively) of M. sympodialis ATCC 42132 extract or with 3 mg BSA (lane 2) as control. The preincubated sera were used to inhibit binding of IgE to the blotted membrane of different Malassezia spp. (A–H). The first lane in each set is the binding of the pool serum without inhibitor to different Malassezia spp. The molecular mass markers are indicated.


to Malassezia spp. to Malassezia in patients with AEDS (23). It remains to be established to what extent the so far identified and cloned allergens from Malassezia ATCC 42132 are also present in the other Malassezia spp. Recently, Sugita et al. isolated a new member of the genus Malassezia from the skin of patients with AEDS and proposed the name M. dermatis for this novel species (36). Further investigations are required to clarify the presence of IgE reactivity to this species.

In conclusion our study shows that the use of only one species of Malassezia is not enough to detect all patients IgE sensitized to Malassezia. To obtain an optimal allergen preparation both common allergenic components as well as species-specific allergens have to be considered.

Acknowledgments

We thank Hojjat Eshaghi for excellent technical assistance, and Maria Lundberg at MIAB, Uppsala, Sweden, for performance of the ImmunoCAP assay. This study was supported by grants from the Swedish Medical Research Council (grant no. 7924), the Swedish Asthma and Allergy Association, the Swedish Foundation for Health Care Sciences and Allergy Research, the Swedish Council for Work Life Research, and the Karolinska Institutet.

References


