



NEW HORIZONS — ALLERGY —

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Diagnostic methods for detecting sensitization to *Malassezia* in AEDS patients

Summary

Members of the yeast genus *Malassezia* (previously designated *Pityrosporum*) are believed to be a contributing factor in the atopic eczema/dermatitis syndrome (AEDS). The yeast cell involvement in the disease mechanism is not fully understood, but probably includes interaction between APCs such as Langerhans cells and the yeast or its allergenic components. Antigen presentation to other cells in the immune system will then occur, causing a T cell response and IgE production. Testing for IgE antibodies to *Malassezia*, as well as SPT and atopy patch testing with *Malassezia* can therefore be useful tools in the investigation of the disease. As there are several *Malassezia* species, the use of allergen preparations from only one species may not be sufficient to detect IgE antibodies to *Malassezia* in all sensitized patients. To obtain an optimal allergen source of all relevant *Malassezia* spp. both common allergenic components and species-specific allergens have to be considered. In the future even more representative measurements of IgE sensitization may be accomplished by the use of recombinant allergens.

This article is based on the review:

Atopic Eczema/Dermatitis Syndrome and *Malassezia*

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Introduction

Atopic eczema/dermatitis syndrome (AEDS) is the term used in the newly recommended nomenclature (1) for what was previously known simply as "atopic dermatitis". Although this chronic inflammatory skin disease has markedly increased in prevalence during the last decades, its pathogenesis is still not well understood. A combination of defects in the immune system and a defective skin barrier is often discussed, with genetic and environmental factors contributing to the symptoms. Since approximately 80% of adult AEDS patients are atopic (2, 3), it is assumed that allergens and IgE antibody-mediated mechanisms are involved in the disease.

For nearly 20 years it has been discussed whether the opportunistic yeast *Malassezia* is a contributing factor to AEDS. Today this is corroborated by reports demonstrating a strong relationship between AEDS and the occurrence of IgE antibodies to the yeast (4-10). Measurements of IgE antibodies in serum can therefore be a valuable tool in the investigation of the mechanisms of the disease.

Malassezia and AEDS

The yeast *Malassezia* belongs to the normal cutaneous flora of humans and warm-blooded animals (11), where it

colonizes stratum corneum and hair follicles and is most abundant at sebum-containing skin sites (12). Over the years the taxonomy has been revised and the previous name *Pityrosporum* has recently been altered to *Malassezia*. Today the genus *Malassezia* is subdivided into several different species, which have all been isolated from human skin (table 1) (11, 13-28).

Although usually harmless, *Malassezia* species (spp.) can cause skin infections and even systemic infections (29). A number of studies have shown that *Malassezia* is the causative agent of pityriasis versicolor and *Pityrosporum* folliculitis (30, 31) and that it plays an important role in the pathogenesis of seborrheic dermatitis and the IgE associated subgroup AEDS (29).

Entry of *Malassezia* most likely occurs through the skin, a route that is particularly accessible in AEDS patients whose skin barrier is often disrupted. Both whole yeast cells and allergenic components of the yeast are likely to pass through the skin barrier and come in contact with antigen-presenting cells (APCs), such as the Langerhans cells, in the epidermis. Upon migration to regional lymph nodes, these may then present allergens to other cells in the immune system, thereby creating an adverse memory to stimulate T cell response and IgE antibody production.

Table 1. Historical events

| Year | Historical events | References |
|------------|--|------------|
| 1846 | Eichstedt associated the yeast cell with skin disease | 13 |
| 1853 | The organism was named <i>Microsporon furfur</i> | 14 |
| 1889 | The name <i>Malassezia furfur</i> was proposed | 15 |
| 1904 | <i>Pityrosporum</i> was chosen as name for the genus | 16 |
| 1913 | <i>Pityrosporum ovale</i> was cultured from human scales | 17 |
| 1925 | <i>P. pachydermatis</i> was isolated from rhinoceros | 18 |
| 1951 | The yeast was isolated from pityriasis versicolor and denoted <i>P. orbiculare</i> | 19 |
| 1976, 1982 | <i>P. ovale</i> and <i>P. orbiculare</i> were recognised as different stages in the cell cycle of the same species | 20, 21 |
| 1984 | The name was changed from <i>P. pachydermatis</i> to <i>M. pachydermatis</i> | 22 |
| 1989 | <i>P. ovale</i> and <i>P. orbiculare</i> were unified in a single species as <i>Malassezia</i> | 23 |
| 1989, 1990 | Diversity was found in <i>Malassezia</i> | 11, 24 |
| 1990 | <i>M. sympodialis</i> was included in the genus <i>Malassezia</i> | 25 |
| 1995 | The diversity in <i>Malassezia</i> was confirmed by rRNA sequencing | 26 |
| 1996 | Four new species <i>M. globosa</i> , <i>M. restricta</i> , <i>M. obtusa</i> and <i>M. slooffiae</i> were described | 27 |
| 1997 | The first recombinant <i>Malassezia</i> allergen Mal s 1 was produced | 28 |

Considering the new taxonomy, it is of interest with regard to diagnosis and treatment to determine which species are associated with which diseases. *Malassezia globosa* has been reported as the causative agent of pityriasis versicolor (32, 33), but also *M. sympodialis* have been found to colonize the skin of patients with this disease (34). *M. globosa*, *M. furfur* and *M. sympodialis* have been most frequently isolated from patients with seborrheic dermatitis (33, 34).

There are, however, contradictory reports concerning AEDS. In a Japanese study *M. furfur* was the most common species identified by culturing from skin samples (33), whereas another study from Japan showed that *M. globosa*- and *M. restricta*-specific DNA segments could be detected by PCR on samples from the skin of 90% of the AEDS patients examined, while *M. furfur* was only found in 40% of the cases (35). In studies from Canada, Russia and Sweden, *M. sympodialis* was reported as the most frequent species in both AEDS patients and healthy individuals (34, 36, 37).

The cause for these divergent findings is likely to be the fact that procedures for isolation and detection of *Malassezia spp.* from the skin show great variations with respect to the method of identification, the efficiency of culturing the yeast, the use of different media, the area of sampling, the ethnic origin of the population, and the climate. The relative contribution of different *Malassezia* species to AEDS and to what degree they share allergens remains to be clarified.

Allergens

A wide range of IgE-binding components have been found in extracts of *Malassezia* (38-41), resulting in considerable variations in allergenic content and difficulties in standardization of the extract. Besides, the use of only one species is probably not sufficient to detect most patients with IgE sensitization to *Malassezia*. To obtain an optimal

allergen source from *Malassezia*, both common allergenic components and species-specific allergens need to be considered. In the future these problems may be solved with the use of recombinant allergens.

Several IgE-binding components have been identified in extracts of *Malassezia*, ranging between 10 and 100 kD in molecular mass (22-25). The genes for nine *Malassezia* allergens with molecular weights ranging from 14 to 36 kD have so far been identified and cloned (table 2) (42-46). Interestingly, four of the so far cloned allergens show no significant sequence similarity to known proteins, whereas others share similarities with each other or other yeast proteins. The uniqueness of some of the allergens makes them highly interesting as potential diagnostic tools and further studies are in progress.

IgE antibody testing

Allergen-specific IgE antibodies to *Malassezia* in serum from patients with AEDS was first described in 1990 (4). *Malassezia*-specific IgE antibodies can be detected in serum in 32-68% of patients with AEDS (4, 7-10) and positive skin prick test (SPT) to *Malassezia* can be found in approximately 30-80% (29, 9). IgE antibodies to *Malassezia* have not been reported in healthy controls or seborrheic patients (5-7, 46).

Patients with IgE antibodies to *Malassezia* are often found among AEDS patients with head and neck manifestation and with high total serum IgE levels, and only occasionally among atopic patients with respiratory diseases without AEDS (4, 7, 8). Among children with AEDS, *Malassezia*-specific IgE antibodies are more common after puberty (47, 48).

The results from different studies vary considerably, mainly due to the selection of patients, which reflects the heterogeneity of the disease. But the variation can also be due to different techniques used for the preparation of

Table 2. Cloned allergens from *Malassezia*

| Allergen | Derived from yeast strain | Size (kD) | Sequence similarity | References |
|----------|---------------------------|-----------|--|------------|
| Mal s 1 | ATCC 42132 | 36 | No known protein | 28 |
| Mal f 2 | TIMM 2782 | 20 | Mal f 3, Mal s 5 Asp f 3 from <i>Aspergillus fumigatus</i> and peroxisomal membrane protein from <i>Candida boidinii</i> | 42 |
| Mal f 3 | TIMM 2782 | 21 | Mal f 2, Mal s 5, Asp f 3 and peroxisomal membrane protein from <i>C. boidinii</i> | 42 |
| Mal f 4 | TIMM 2782 | 35 | Mitochondrial malate dehydroxygenase | 43 |
| Mal s 5 | ATCC 42132 | 18 | Mal f 2, Mal f 3 and Asp f 3 | 44 |
| Mal s 6 | ATCC 42132 | 17 | Cyklophilin from <i>A. fumigatus</i> and <i>Schizosaccharomyces pombe</i> | 44 |
| Mal s 7 | ATCC 42132 | 16 | No known protein | 44, 45 |
| Mal s 8 | ATCC 42132 | 18 | No known protein | 44, 45 |
| Mal s 9 | ATCC 42132 | 14 | No known protein | 44, 45 |

extracts and not least the use of different species and strains of *Malassezia*. In patients with AEDS, serum IgE antibodies to *M. globosa* have been detected in 83%, to *M. sympodialis* in 74%, to *M. furfur* in 65%, to *M. restricta* in 56% and to *M. slooffiae* in 50% (49).

Unclear role of IgG antibody response

While there appears to be a close relationship between IgE sensitization to *Malassezia* and manifestations of AEDS, the role of the IgG response in the pathogenesis of *Malassezia*-associated diseases remains unclear.

Malassezia-specific IgG antibodies are found in serum of patients with AEDS and other *Malassezia*-induced diseases, but also in healthy individuals (46, 50). The levels of IgG antibodies to *Malassezia* do not differ between patients with AEDS and healthy controls or patients with seborrheic dermatitis. Further, IgG antibody levels in AEDS patients show no correlation with atopy patch test responses to *Malassezia* (46). Thus, the determination of IgG antibodies seems to have no value in the diagnosis of sensitization to *Malassezia* in AEDS.

Atopy patch test can find additional patients

Both the ImmunoCAP® method for detection of serum IgE antibodies and SPT are designed to demonstrate allergen-specific sensitization. The atopy patch test (APT) reaction, an eczematous skin reaction induced by the application of allergens on nonlesional skin, is considered to be dependent on antigen presentation mediated by IgE antibodies bound to epidermal Langerhans cells. In addition the presence of allergen-specific T-cells seems to be important for the reaction.

Positive APT reaction to *Malassezia* has been reported in 13 % of AEDS patient when tested on intact skin with whole yeast cells, in 64% after chamber scarification and in 53% on tape stripped skin using yeast extract (5, 6, 46).

Recently positive APT reactions to *Malassezia* in AEDS patients without head and neck dermatitis and/or with low total serum IgE levels were found. Also positive APT reactions to *Malassezia* in AEDS patients without detectable *Malassezia*-specific IgE antibody in serum were detected and in some cases without positive SPT reactions to *Malassezia* (51). The possibility of allergy to *Malassezia* should therefore not be excluded in patients without head and neck dermatitis and/or with low total serum IgE levels. The addition of APT to the test battery makes it possible to reveal a previously overlooked impact of *Malassezia* allergy in these subgroups of AEDS patients.

Concluding remarks

In recent years much evidence has been presented which highlights the importance of *Malassezia* as a contributing factor in AEDS and many reports demonstrate a strong relationship between the IgE associated subgroup of AEDS and the occurrence of IgE antibodies to the yeast. Measurement of IgE antibodies to *Malassezia* in serum can therefore be a valuable tool in investigations of the disease. However, as there are several *Malassezia* species, the use of extracts from only one species is probably insufficient to detect IgE antibody reactivity in all patients sensitized to *Malassezia*.

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