

Atopic Eczema/Dermatitis Syndrome and *Malassezia*

Annika Scheynius^a Catharina Johansson^a Eva Buentke^a Arezou Zargari^b
Maria Tengvall Linder^a

^aDepartment of Medicine, Unit of Clinical Allergy Research, Karolinska Institutet and Hospital and

^bLudwig Institute for Cancer Research, Stockholm Branch, Stockholm, Sweden

Key Words

Atopic eczema/dermatitis syndrome · Atopy patch test ·
Malassezia · Recombinant allergens

Abstract

Atopic dermatitis is a chronic multifactorial inflammatory skin disease, which has had a marked increase in prevalence during the last decades. Recently, a new nomenclature was recommended where the term 'atopic eczema/dermatitis syndrome' (AEDS) should be used to reflect the heterogeneity in this group of patients and where those patients without measurable IgE reactivity should be classified as either 'nonallergic AEDS' or 'non-IgE-associated allergic AEDS'. For nearly 20 years it has been discussed whether the opportunistic yeast *Malassezia*, previously designated *Pityrosporum*, is a contributing factor to AEDS. Today there are several reports that demonstrate specific serum IgE or positive skin prick test and/or atopy patch test reactions to *Malassezia* in patients with AEDS. Several IgE-binding components have been identified in extracts of *Malassezia* ranging in molecular mass between 10 and 100 kD. The genes for nine *Malassezia* allergens with molecular weights ranging from 14 to 36 kD have hitherto been identified and cloned. Six of them are now produced by recombinant techniques and used in diagnostic tests. At present the

genus *Malassezia* is subdivided into seven different species, which all have been isolated from human skin. The respective contribution of different *Malassezia* spp. to AEDS and in what proportion they share allergens remains to be clarified. We summarize here data that *Malassezia* can play a role in eliciting and maintaining eczema in patients with AEDS.

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Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease [1], which has had a marked increase in prevalence during the last decades [2]. Besides raised awareness of the disease, changes in lifestyle and an altered infection panorama have been suggested to contribute to this increase [3–5]. The pathogenesis of AD is still not fully understood. A combination of defects in the immune system and a defective skin barrier is often discussed where genetic and environmental factors are contributing to the symptoms. Since approximately 80% of the AD patients show immediate-type skin reactions to environmental allergens together with elevated serum IgE levels [6, 7], it is assumed that allergens are involved in the maintenance of the disease. It has recently been recommended that the term 'atopic eczema/dermatitis syndrome' (AEDS) should

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Correspondence to: Dr. Annika Scheynius
Department of Medicine, Unit of Clinical Allergy Research L2:04
Karolinska Hospital
SE–171 76 Stockholm (Sweden)
Tel. +46 8 51775934, Fax +46 8 335724, E-Mail annika.scheynius@ks.se

Table 1. Historical events

Year	Historical events	References
1846	Eichstedt associated the yeast cell with skin disease	28
1853	The organism was named <i>Microsporon furfur</i>	88
1889	The name <i>Malassezia furfur</i> was proposed	89
1904	<i>Pityrosporum</i> was chosen as name for the genus	90
1913	<i>Pityrosporum ovale</i> was cultured from human scales	91
1925	<i>P. pachydermatis</i> was isolated from rhinoceros	92
1951	The yeast was isolated from pityriasis versicolor and denoted <i>P. orbiculare</i>	93
1976, 1982	<i>P. ovale</i> and <i>P. orbiculare</i> were recognised as different stages in the cell cycle of the same species	94, 95
1984	The name was changed from <i>P. pachydermatis</i> to <i>M. pachydermatis</i>	96
1989	<i>P. ovale</i> and <i>P. orbiculare</i> were unified in a single species as <i>Malassezia</i>	97
1989, 1990	Diversity was found in <i>Malassezia</i>	22, 98
1990	<i>M. sympodialis</i> was included in the genus <i>Malassezia</i>	99
1995	The diversity in <i>Malassezia</i> was confirmed by rRNA sequencing	100
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	101

be used for AD to highlight that AD is not one single disease but rather an aggregation of several diseases with certain clinical characteristics in common and that those patients without measurable IgE reactivity should be classified as either 'non-allergic AEDS' or 'non-IgE-associated allergic AEDS' [8]. This nomenclature will be used here and the future will show whether it will match forthcoming knowledge about subgroups of AEDS.

For nearly 20 years it has been discussed whether the opportunistic yeast *Malassezia* is a contributing factor to AEDS [9]. There was a first report on an association between AEDS and *Malassezia* in 1983 by Clemmensen and Hjorth [10]. They found that ketoconazole treatment improved eczema, especially in adult patients with a head and neck distribution of the eczema and these patients were also skin prick test (SPT)-positive to *Malassezia* extract. Several studies have supported the finding that treatment with both topical and oral ketoconazole can improve AEDS [11–13]. The severity of the eczema measured with SCORAD was decreased [11, 12] together with the levels of *Malassezia*-specific IgE and total serum IgE in AEDS patients [11, 13]. The clinical response was most significant in patients who had positive yeast cultures before the treatment [12]. Today there are several studies that demonstrate specific serum IgE or positive SPT to *Malassezia* in patients with AEDS [14–20]. Further, positive atopy patch test (APT) reactions to *Malassezia* have been reported in AEDS patients [15, 16, 21]. This review

focuses on possible pathogenic mechanisms of *Malassezia* in AEDS and on newly identified allergen components of *Malassezia* as potential diagnostic tools.

Malassezia

The yeast *Malassezia* belongs to the normal cutaneous flora of human and warm-blooded animals [22], where it colonizes stratum corneum and hair follicles and is most abundant at sebum-containing skin sites [23]. Colonization increases during puberty, when the sebum glands become active [24]. The yeast cells divide by budding, are round, oval, or cylindrical, 1–8 µm in diameter with a thick multilayered cell wall consisting mainly of carbohydrates, proteins and lipids [25, 26]. Over the years the taxonomy has been revised, and the name *Pityrosporum* has recently been altered to the former name *Malassezia* (table 1). Today the genus *Malassezia* is subdivided into seven different species (table 1), which all have been isolated from human skin. Except for *Malassezia pachydermatis* they require long-chain fatty acids for growth [27].

Although usually harmless, *Malassezia* species (spp.) can cause skin infections and even systemic infections [9]. Eichstedt [28] first reported an association between *Malassezia* and pityriasis versicolor in 1846, when he described yeast-like cells in stratum corneum from the skin scales of the patients (table 1). A number of studies have

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	101
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>	102
Mal f 3	TIMM 2782	21	Mal f 2, Mal s 5, Asp f 3 and peroxisomal membrane protein from <i>C. boidinii</i>	102
Mal f 4	TIMM 2782	35	Mitochondrial malate dehydrogenase	103
Mal s 5	ATCC 42132	18	Mal f 2, Mal f 3 and Asp f 3	104
Mal s 6	ATCC 42132	17	Cyklophilin from <i>A. fumigatus</i> and <i>Schizosaccharomyces pombe</i>	104
Mal s 7	ATCC 42132	16	No known protein	104, 105
Mal s 8	ATCC 42132	18	No known protein	104, 105
Mal s 9	ATCC 42132	14	No known protein	104, 105

shown that *Malassezia* is the causative agent of pityriasis versicolor and *Pityrosporum* folliculitis [29, 30] and plays an important role in the pathogenesis of seborrheic dermatitis and AEDS [9].

Considering the new taxonomy, it is of interest regarding diagnosis and treatment to determine which species are associated with which diseases. *Malassezia globosa* has been reported as the causative agent of pityriasis versicolor [31, 32], whereas others found *Malassezia sympodialis* to dominate on the skin of the patients with pityriasis versicolor [33]. *M. globosa*, *Malassezia furfur* and *M. sympodialis* have been most frequently isolated from seborrheic dermatitis patients [32, 33]. Concerning AEDS there are contradictory reports; in a Japanese study [32] *M. furfur* was the most common species cultured, whereas nested PCR used in another study from Japan showed that *M. globosa* and *M. restricta* could be detected on the skin in 90% of the AEDS patients, and *M. sympodialis* and *M. furfur* on 40% of the patients [34]. In studies from Canada, Russia and Sweden, *M. sympodialis* was reported as the most frequent species in both AEDS patients and healthy individuals [33, 35, 36]. Nevertheless, the isolation and detection of *Malassezia* spp. from the skin varies based on several factors: methods of detection, the efficiency of culturing *Malassezia* spp. like the use of different media, or area of sampling, the ethnic origin of the population, and the climate. The respective contribution of different *Malassezia* spp. to AEDS and in what proportion they share allergens remains to be clarified.

Malassezia Allergens

Several IgE-binding components have been identified in extracts of *Malassezia* ranging in molecular mass between 10 and 100 kD [37–40]. Different protocols for the preparation of yeast extract and the use of different species or strains of *Malassezia* contribute to varying results between studies [41]. Prolongation of culture time will, for example, lead to a loss of major allergen components, which is why we recommend that extracts should be made from log phase cultures that are 2–4 days old [41].

We have used the American Type Culture Collection (ATCC) yeast strain No. 42132 originally isolated by Dr. Jan Faergemann, Department of Dermatology, Gothenburg, Sweden, from a patient with pityriasis versicolor. An extract from this strain is used in the ImmunoCAP 'm70' test for measuring allergen-specific serum IgE (Pharmacia Diagnostics AB, Uppsala, Sweden). The strain was first designated *Pityrosporum orbiculare*, later *M. furfur*, but recent retyping indicates that the proper name should be *M. sympodialis* [20; Dr. Jan Faergemann, pers. commun.]. The genes for nine *Malassezia* allergens with molecular weights ranging from 14 to 36 kD have hitherto been identified and cloned (table 2). Mal f 2–4 are cloned from a *M. furfur* strain No. 2782 from the Teikyo Institute for Medical Mycology (TIMM), Tokyo, Japan, whereas the others are cloned from the ATCC strain 42132 now named *M. sympodialis*. Interestingly, 4 of the so far cloned allergens show no significant sequence similarity to known proteins, whereas others share simi-

larities with each other or other yeast proteins (table 2). The uniqueness of some of the allergens makes them highly interesting in terms of diagnostic tools. It is also a challenge to elucidate the function of the newly discovered yeast proteins, whether they are, for example, enzymes of importance for survival of the yeast cell and whether the interaction with the host stimulates their production. The *Malassezia* allergens cloned by us from the ATCC strain 42132 are now produced by recombinant techniques and used in diagnostic tests [42, 43].

Possible Pathogenic Mechanisms

The route of entry of the yeast *Malassezia* most likely occurs through the skin, especially bearing in mind that the skin barrier of AEDS patients is often disrupted [1]. Little is, however, known about the mechanism by which *Malassezia* overcomes the skin barrier. A recent study tried to assess this question by studying *M. furfur* invasiveness in keratinocytes, and found support for the hypothesis that *Malassezia* can cause an initial break in the barrier function of the epidermis [44]. Both whole yeast cells and allergenic components of the yeast are likely to pass the skin barrier and come in contact with professional antigen-presenting cells (APCs), such as Langerhans cells in the epidermis. We have found that the first cloned *Malassezia* allergen, Mal s 1 (36 kD), a major allergen that is localized to the cell wall [45], can be released into the culture medium (own unpubl. results) and thus most probably also in the skin.

It is of great importance to gain knowledge about the events involved in turning a nonsensitized individual into a sensitized one in order to prevent or improve conditions like AEDS. The interaction between foreign substances and dendritic APCs is the starting point for the immune system to decide whether and what type of immune response will be mounted. We have, therefore, studied the initial steps in the interaction between dendritic APCs and *Malassezia* upon the first encounter. Human monocyte-derived dendritic cells (MDDCs) [46] have been used as a model to study this interaction. We found that the majority of immature MDDCs, reflecting Langerhans cells in the skin, internalized whole *Malassezia* yeast cells within 1 h, whereas mature MDDCs, mimicking dendritic cells in the regional lymph nodes, have essentially lost the capacity to take up *Malassezia* and its components [47, 48]. The binding of *Malassezia* extract and mannan, an IgE-binding cell wall component that is highly immunogenic [49], is inhibited in a dose-dependent manner by

methyl- α -D-mannopyranoside, suggesting uptake via the mannose receptor. However, the interaction was never completely inhibited indicating the involvement of additional receptors. Uptake of the nonglycosylated rMal s 5 (18 kD), on the other hand, is mainly mediated via non-specific fluid phase pinocytosis [47]. The process of internalizing *Malassezia* is associated with MDDC maturation, production of proinflammatory and immunoregulatory cytokines like TNF- α , IL-1 β and IL-18, but not IL-12p70, presumably favoring induction of a Th2-type immune response [48]. IL-18 has generally been considered as a Th1-inducing cytokine and in mice has been shown to synergize with IL-12 in inhibiting IgE production [50]. In the absence of IL-12, however, it was reported that IL-18 stimulates IL-4 production and histamine release by mast cells as well as IgE production [51, 52]. MDDCs incubated with *Malassezia* were also shown to induce proliferation in autologous lymphocytes [48]. This chain of events most likely contributes to the inflammatory reaction in AEDS. Furthermore, as these experiments were performed in the absence of IgE, they imply that sensitization of AEDS patients to *Malassezia* can be mediated by immature dendritic cells in the skin. The recent finding that dendritic cells can produce IL-2 after microbial stimuli and that this production is required for proper T cell activation highlights the importance of dendritic cells as key players in the induction of immune responses [53] as well as data implicating dendritic cells in playing a major role in the induction of peripheral tolerance [54].

IgE antibodies to the allergen are formed in sensitized individuals. These antibodies can bind to Langerhans cells and monocytes, where the membrane-bound IgE improves the allergen-presenting capacity of the APCs upon a second encounter with the allergen [55–59]. Langerhans cells express all three known types of IgE receptors: the low-affinity receptor (Fc ϵ R2 or CD23), the high-affinity receptor (Fc ϵ R1), and the epsilon-binding protein (ϵ BP) [60]. In already sensitized individuals, an association between allergen-specific serum IgE and positive APT reactions has been reported [21, 61–63]. It has been suggested that a positive APT reaction requires the presence of epidermal IgE+ Langerhans cells in clinically non-involved skin, but that other yet unknown factors are also involved [63]. Such a factor could be the presence of allergen-specific Th2 cells, which can release cytokines that promote an inflammatory reaction. However, in their study Langeveld-Wildschut et al. [63] could establish house dust mite allergen-specific T cell lines from uninvolved skin from only half of the patients with positive APT reaction. If this reflects the true situation in the skin,

there may also be an early recruitment of allergen-specific T cells from the circulation. Recently, we also found a clear association between the presence of specific IgE and positive APT reaction to *Malassezia*. Nevertheless, the Th2-like peripheral blood mononuclear cell (PBMC) response to *Malassezia* showed a stronger correlation with the APT reaction than did the specific IgE levels or SPT reaction [43]. This supports the hypothesis that the Th2-like T cell response is one of the crucial factors for allergen-specific eczema reactions in AEDS. Those T cells may either provide a pool for rapid recruitment to the skin, or reflect a spillover of cells harbored in the AEDS skin, or both, and might together with epidermal IgE+ Langerhans cells be essential for the APT reaction.

Elevated proliferation of PBMC in response to in vitro stimulation with *Malassezia* extract has been found in AEDS patients with specific serum IgE to *Malassezia* [16, 64–66] in combination with a Th2-like cytokine profile [65–67]. The Th2-like cytokines IL-4 and IL-13 support IgE production, while IL-5 promotes growth and differentiation of eosinophils [68], one of the early skin-infiltrating cells in the APT reaction [21, 69]. AEDS patients have elevated levels of eosinophils in the blood, but few eosinophils are found in chronic lesional skin [70]. However, dermal deposition of major basic protein and eosinophil cationic protein in chronic lesional skin suggests the involvement of eosinophils in AEDS [70, 71]. In addition, high numbers of eosinophils have been found in skin from AEDS patients after positive APT reactions to inhalant allergens [69] and *Malassezia* [21], indicating recruitment of eosinophils in the acute phase of the disease.

T helper cells are thought to play an important role in the pathogenesis of AEDS. Allergen specific T cells cloned from acute AEDS lesional skin show a Th2/Th0-like cytokine profile [64, 72], while the in situ expression of IL-4 in chronic AEDS lesions is accompanied by the expression of interferon- γ [73, 74], also suggesting an involvement of Th1 cytokines in the chronic phase. A possible scenario would be an influence by Th2 cytokines from allergen-specific T cells in the acute phase of AEDS. The infiltration of eosinophils contributes to an increased expression of IL-12 that may promote the Th2/Th1 shift in the chronic AEDS lesion [68, 75].

Diagnostic Methods for Detection of *Malassezia* Allergy

Positive SPT reactions to *Malassezia* were first demonstrated in adult AEDS patients with head and neck dermatitis [10, 76]. Allergen-specific IgE to *Malassezia* in serum from patients with AEDS was first described in 1990 [14]. *Malassezia*-specific serum IgE in a frequency of 32–68% can be detected in patients with AEDS [14, 17–20] and positive SPT to *Malassezia* can be found in approximately 30–80% of AEDS patients [9, 19]. IgE-reactivity to *Malassezia* is not reported in healthy controls [15–17, 21] or seborrheic dermatitis patients [15, 21]. Patients with specific IgE to *Malassezia* are often found in AEDS patients with head and neck manifestation [15, 19, 20] and with high total serum IgE levels [14, 17, 18], and only occasionally in patients with atopic respiratory diseases without AEDS [14, 17, 18]. Among children with AEDS *Malassezia*-specific IgE is more common after puberty [77, 78]. The variations seen in the different studies are mainly due to the selection of patients, reflecting the heterogeneity of the disease. But variations between the studies can also be due to different techniques for the preparation of extracts and not least the use of different species or 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 *Malassezia slooffiae* in 50% [79].

The role of the IgG response to *Malassezia* in the pathogenesis of *Malassezia*-associated diseases is unclear. *Malassezia*-specific IgG in serum is found in healthy individuals, patients with AEDS and patients with other *Malassezia*-induced diseases [21, 80]. The *Malassezia*-specific IgG levels do not differ between patients with AEDS and healthy controls or patients with seborrheic dermatitis and the specific IgG levels in the AEDS patients do not correlate with the patch test responses to *Malassezia* [21]. Thus, the determination of *P. orbiculare*-specific IgG seems to have no value in the diagnosis of sensitization to *Malassezia* in AEDS.

The APT reaction, an eczematous skin reaction induced by the application of aeroallergens on nonlesional skin of AD patients, was first described in 1982 by Mitchell et al. [81] and has thereafter been evaluated in many studies [82, 83]. Positive APT reaction to *Malassezia* has been reported in AEDS patients: 13% of the investigated patients when tested on intact skin with whole yeast cells [15], 64% after chamber scarification [16] and 53% [21] on tape-stripped skin using yeast extract. Both the ImmunoCAP™ method for detecting serum IgE and the SPT are

designed to demonstrate allergen-specific IgE. The APT reaction is thought to be dependent on antigen presentation mediated by specific IgE bound to epidermal Langerhans cells [55, 63], but in addition the presence of allergen-specific T cells seems to be important for the reaction [43, 62]. Recently, we have found positive APT reactions to *Malassezia* in AEDS patients without head and neck dermatitis and/or with low total serum IgE levels. We also detected positive APT reactions to *Malassezia* in AEDS patients without detectable *Malassezia*-specific serum IgE and in some cases also without positive SPT reactions to *Malassezia* [84]. This reaction pattern is in accordance with that described for other inhalant allergens [83, 85, 86]. Concerning the AEDS patients with positive APT reactions to *Malassezia* despite negative SPT reaction and undetectable specific serum IgE to *Malassezia* one can speculate about the mechanisms. The reactions might depend on minute amounts of allergen-specific IgE, not detected by SPT but sufficient for antigen presentation, a Th2-dominated type IV reaction initiated in the absence of specific IgE, or even a classical Th1-dominated type IV reaction. The possibility of *Malassezia* hypersensitivity should not be forgotten 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* hypersensitivity in certain subgroups of AEDS patients.

Diagnostic Advantages with Recombinant Allergens

There is a high need for standardization of reagents and methods in diagnosing hypersensitivity to *Malassezia* among patients with AEDS. The *Malassezia* extracts contain a wide range of IgE-binding components [37–40] with variations in allergenic contents and difficulties in standardization of the extract [41]. Besides, the use of only one species is probably not enough to detect patients with IgE reactivity to *Malassezia*. To obtain an optimal allergen source from *Malassezia* spp. both common allergenic components and species-specific allergens have to be considered. These problems may be solved in the future with the use of recombinant allergens [87].

In a recent study where the specific serum IgE reactivity to *Malassezia* extract was compared with that of rMal s 1 and rMal s 5–9 in 156 adult patients with AEDS, of whom 74 (47%) patients had IgE antibodies to *Malassezia* extract, it was found that 86 (55%) had detectable IgE antibodies to any of the rMal s allergens [42]. The highest

sensitivity was found for rMal s 9, suggesting that rMal s 9 is the most important allergen of those so far studied and therefore a good candidate to be used in further diagnosis.

In parallel with serological studies, we have tested the three recombinant allergens rMal s 1, rMal s 5 and rMal s 6 in SPT and APT studies. We have found that all three recombinant allergens tested can induce positive SPT and APT reactions in AEDS patients. Together they were sufficient to detect approximately 70% of the AEDS patients with positive SPT reaction to *M. sympodialis* extract and, interestingly, an additional 12% of the patients who were SPT negative to the extract [84]. A mixture of proper Mal allergens might be a valuable tool for detection of allergen-specific serum IgE and SPT reactions in the future.

Concluding Remarks

Evidence has been accumulated that *Malassezia* is a contributing factor in AEDS. It is still an enigma how, where and when an individual becomes sensitized to allergens of this opportunistic fungi. Most probably the sensitization is through the skin barrier where APC like Langerhans cells interact with *Malassezia*, migrate to regional lymph nodes and present allergens to other cells in the immune system creating an adverse memory to react with IgE production and T cell response. Interestingly, we have found eczema patients with no detectable specific IgE reactivity to *Malassezia* in serum and negative SPT reaction but with a positive APT reaction to *Malassezia* [84]. We believe that *Malassezia* and its allergenic components can function as tools to dissect pathogenic mechanisms in AEDS and provide help to identify different subgroups of patients.

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