

The Role of Fungi in Atopic Dermatitis



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KEYWORDS

• Atopic dermatitis • *Malassezia* spp • Sensitization

KEY POINTS

- *Malassezia* spp is a genus of lipophilic yeasts and the most common fungus on healthy skin.
- *Malassezia* spp. may contribute to skin inflammation and flares during the course of atopic dermatitis.
- Some *Malassezia* species release immunogenic proteins (allergens), that elicit an IgE response and react with skin immune cells.
- IgE-mediated sensitization to *Malassezia* spp. is common in atopic dermatitis patients but rare in healthy individuals.

INTRODUCTION

Atopic dermatitis (AD) is a chronic relapsing inflammatory skin disorder, characterized by intensely itchy eczema. The prevalence of AD has tripled within the last 3 decades, currently affecting up to 30% of children and 10% of adults in industrial countries.¹ The pathogenesis of AD is not fully understood. Besides some other environmental factors, the skin microbiome—the community of microorganisms colonizing the skin—has been attributed a pathogenic role in AD. The altered skin colonization with microorganisms in AD patients versus healthy individuals has been extensively investigated for bacteria, in particular *Staphylococcus aureus*. These aspects are highlighted in the article, (See Sun and Ong's article, "[Infectious Complications in Atopic Dermatitis](#)", in this issue). Recently, microbiome research extended the possible pathogenic role of fungi in AD. This research has focused on the commensal lipophilic yeast *Malassezia* spp because (i) AD patients are more frequently sensitized to *Malassezia* spp than healthy individuals, and (ii) AD patients may benefit from an antifungal therapy that is effective against *Malassezia* spp. This research led to the

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publication of a plethora of studies on the possible role of *Malassezia* spp in the development and course of AD. Several studies applied culture or molecular methods such as polymerase chain reaction (PCR) to assess possible differences in the epidemiology of *Malassezia* spp skin colonization between healthy and diseased skin such as AD. However, these studies obtained variable results presumably owing to methodical inconsistencies such as skin sampling from inconsistent body sites and the use of different cultivation methods or PCR primers. Next-generation sequencing is a molecular method that has been recently introduced into skin microbiome research, because it gives information on skin microbial communities that is complementary to cultivation or PCR. As for the bacterial skin microbiome, next-generation sequencing revealed that the skin fungal microbiome is highly specific for a particular body site.² Therefore, comparing the prevalence of *Malassezia* spp between different body sites sampled in different studies will give unreliable results. Also, the epidemiologic studies used different culture media to detect *Malassezia* spp. The authors (Glatz M, unpublished data, 2014) and others have shown that different culture media favor the growth of particular *Malassezia* spp.^{3–6} Therefore, the use of only one or a few types of culture media does not necessarily depict the whole spectrum of *Malassezia* spp present in a sample. Surprisingly, studies comparing healthy individuals and AD patients did not reveal a difference in the frequency of skin colonization with *Malassezia* spp between both groups.⁶ It therefore appears as a medical conundrum how *Malassezia* spp on the one hand seems to contribute to the pathogenesis of AD patients and on the other hand is a commensal on healthy skin. Recent research at least partially elucidated the possible pathogenetic role of *Malassezia* spp in AD.

EPIDEMIOLOGY

The skin is an ecosystem and harbors diverse and body site-specific microbial communities, which have been termed the skin microbiome. The phylogenetic profiling of the skin microbiome revealed that fungi are part of the normal skin flora at all body sites and comprise 1% to 22% of the phylogenetic composition of the skin microbiome.⁷ *Malassezia* spp almost exclusively comprises of the fungal flora of the healthy skin on most body sites. It is therefore the main eukaryotic member of the microbial flora of the skin.^{6,7} *Malassezia* spp is a genus of lipophilic yeasts (Fig. 1). Most of the *Malassezia* spp lack the genes for fatty acid synthase genes and therefore rely

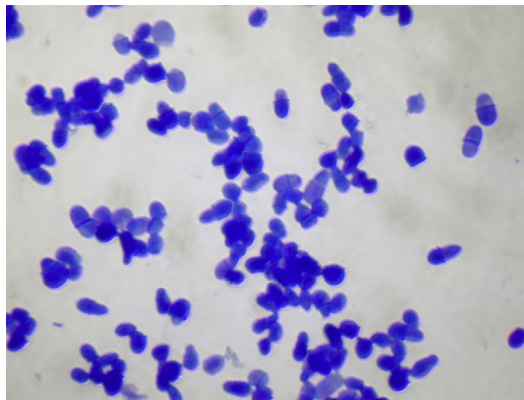


Fig. 1. *M sympodialis* culture isolated from a patient with AD, on *Malassezia* CHROM agar, grown for 72 hours at 32°C. (From Glatz M, Bosshard PP, Hoetzenecker W, et al. The role of *Malassezia* spp. in atopic dermatitis. J Clin Med 2015;4:1219; with permission.)

on exogenous fatty acid sources to satisfy their nutritive requirement.⁸ *Malassezia pachydermatis*, a species isolated from dogs and other animals,⁹ is the only known *Malassezia* spp that grows in the absence of exogenous lipids.⁸ Their need for exogenous lipids explains the predilection of *Malassezia* spp for seborrheic skin sites, such as the head and neck. The taxonomy of *Malassezia* spp has been defined in its current form in 1996, based on morphology, ultrastructure, physiology, and molecular biology.¹⁰ The genus *Malassezia* spp belongs to the phylum Basidiomycota and currently encompasses 14 species that have been isolated from human and animal skin (Table 1). Two of these species, *Malassezia globosa* and *Malassezia restricta*, are consistently found on healthy skin of individuals from the United States and Europe and are identified on almost all body sites.^{2,6} However, epidemiologic studies indicated a geographic variation in the distribution of particular *Malassezia* spp, presumably owing to climate factors. For example, *Malassezia sympodialis* has been reported in studies from Canada, Russia, and Sweden as the most frequent species, whereas in Japan *Malassezia furfur* was the most common species.⁶

RISK FACTORS FOR SENSITIZATION TO MALASSEZIA SPP

Sensitization to Malassezia spp May Correlate with the Severity of Atopic Dermatitis

As *Malassezia* spp is part of the healthy skin flora, it appears reasonable that it regularly interacts with skin immune cells such as dendritic cells or lymphocytes. Accordingly, *Malassezia* spp-specific immunoglobulin G (IgG) and IgM antibodies can be regularly detected in healthy individuals.⁸ In contrast, the rate of IgE-mediated sensitization to *Malassezia* spp is very low or even absent among individuals with healthy skin. In contrast, a high proportion of AD patients appears sensitized to this yeast,¹⁴ as demonstrated by positive skin prick tests in up to 80% of adult AD patients.^{15–18} Because skin test extracts for *Malassezia* spp are not yet commercially available and standardized, it is nearly impossible to compare the results of different skin prick test studies. Therefore, the detection of *Malassezia* spp-specific serum IgE is

Table 1
Currently identified *Malassezia* species

<i>Malassezia</i> Species	Isolated from Human Skin	Isolated from Animal Skin	Description as Species (Year)
<i>M caprae</i>	—	X	2007
<i>M cuniculi</i>	—	X	2011
<i>M dermatis</i>	X	—	2002
<i>M equina</i>	—	X	2007
<i>M furfur</i>	X	X	1889
<i>M globosa</i>	X	X	1996
<i>M japonica</i>	X	—	2003
<i>M nana</i>	—	X	2004
<i>M obtusa</i>	X	—	1996
<i>M pachydermatis</i>	—	X	1925
<i>M restricta</i>	X	—	1996
<i>M slooffiae</i>	X	X	1996
<i>M sympodialis</i>	X	X	1990
<i>M yamatoensis</i>	X	—	2004

Adapted from Refs. ^{11–13}

desirable to assess sensitization. Fortunately, a standardized kit (ImmunoCAP m70, Phadia AG, Steinhausen, Switzerland) for the detection of *Malassezia* spp-specific serum IgE is available, which is based on the ATCC strain 42132. Recently, a new test kit (ImmunoCAP m227) has been introduced that contains the antigens of several *Malassezia* spp and therefore is very sensitive.¹⁹ Using these commercial kits, *Malassezia* spp-specific IgE are found in up to one-third of children^{15,20–22} and two-thirds of adults with AD.^{15,19,22–24} Accordingly, a recent study on 176 adult AD patients found higher rates of IgE-mediated *Malassezia* sensitization among patients with severe compared with moderate AD.²⁵ These high rates of *Malassezia* spp-specific IgE detection in adult AD patients are consistent with the rates of positive *Malassezia* spp skin prick tests in this population (see above). Interestingly, sensitization rates against *Malassezia* spp are particularly higher in patients with head and neck type of AD.¹⁹ These higher sensitization rates may be attributed to the lipophilic properties and hence the predilection of this yeast for seborrheic skin areas, such as the head and neck region. Therefore, some authors assume that *Malassezia* spp plays a pathogenic role particularly in this clinical subtype of AD.²⁶

Pathophysiology

The pathophysiologic mechanisms underlying this high frequency of *Malassezia* spp sensitization in AD patients compared with healthy individuals remain to be elucidated. It appears that several endogenous factors such as the dysfunctional skin barrier or aberrations in the skin immune system of AD patients as well as environmental factors influence the skin colonization with *Malassezia* spp and the IgE-mediated sensitization to this yeast.²⁷ This sensitization to *Malassezia* spp may correlate to the severity of AD particularly in adults as recently shown in 2 studies on 132 children and 128 adults with AD.^{22,28} The lower frequency of *Malassezia* spp sensitization in children compared with adults and therefore the missing correlation between AD severity and *Malassezia* spp-specific IgE in children might owe to the poor growth conditions for *Malassezia* spp in children compared with adults. Children produce low amounts of sebum in their skin, and sebum production increases during puberty and is high until the age of 50.²⁹ Hence, the growth conditions for *Malassezia* spp on pediatric skin are worse than on adult skin, and this could be the reason that sensitization to *Malassezia* spp preferably occurs in adulthood.²² Several allergens of *Malassezia* spp elucidate a specific IgE response. To date, at least 14 allergens of 3 *Malassezia* spp, namely *M furfur*, *M sympodialis*, and *M globosa*, are characterized on a molecular basis³⁰ (Table 2), and 13 of these allergens are listed in the official allergen nomenclature list (<http://www.allergen.org>). These allergens may be released to a higher amount in the environment of atopic skin. For example, the allergen Mala s 12 is released in a higher amount at pH 6.0 that represents conditions of atopic skin, than in the more acidic environment of pH 5.5 of healthy skin.³¹ It remains unclear if the IgE response plays a pathogenic role in AD or rather serves as a marker for the severity of AD, but the some possible mechanisms how *Malassezia* spp allergens induce inflammation in atopic skin have been elucidated in recent years and are described in the later discussion.

***Malassezia* spp Interacts with the Skin Immune System**

Prior studies indicated that *Malassezia* spp interacts with various types of human skin and immune cells. This induces a proinflammatory immune response by these immune cells, which seems to contribute to the inflammation during AD flares. It is still unclear how the interaction between *Malassezia* spp cells and host cells occurs, but some different mechanisms have been postulated. First, *Malassezia* spp penetrated the impaired skin barrier, which is typical for AD patients. In the epidermis and dermis,

Table 2
Allergens from *Malassezia* spp and their relevance in atopic dermatitis

Allergen	Source	Mass (kDa)	Function	IgE-Mediated Sensitization (%)	Comment	References
Mala f 2	<i>M furfur</i>	21	Peroxisomal membrane protein	72		32
Mala f 3	<i>M furfur</i>	20	Peroxisomal membrane protein	70		32
Mala f 4	<i>M furfur</i>	35	Mitochondrial malate dehydrogenase	83		33
Mala s 1	<i>M Sympodialis</i>	36		Unknown		34
Mala s 5	<i>M sympodialis</i>	19		Unknown		35
Mala s 6	<i>M sympodialis</i>	18	Cyclophilin	92		35,36
Mala s 7	<i>M sympodialis</i>	22		40–60		35,37
Mala s 8	<i>M sympodialis</i>	16		40–72		35,37
Mala s 9	<i>M sympodialis</i>	11		24–36		35,37
Mala s 10	<i>M sympodialis</i>	86	Heat shock protein70	69		38
Mala s 11	<i>M sympodialis</i>	23	Manganese superoxide dismutase	43–75	Induces dendritic cell maturation, release of IL-6, IL-8, IL-12p70, TNF- α by dendritic cells, auto-reactive T cells against human homologue	39–42
Mala s 12	<i>M sympodialis</i>	67	Glucose-methanol-choline oxidoreductase	62		43
Mala s 13	<i>M sympodialis</i>	13	Thioredoxin	50	Induces autoreactive T cells against human homologue	44,45
MGL_1304	<i>M globosa</i>	17		62	Induces degranulation of mast cells, IL-4 release by basophils	46,47

Malassezia spp is recognized by keratinocytes and immune cells such as Langerhans cells, dermal dendritic cells, natural killer cells, and fibroblasts.⁴⁸ A second possible mechanism of *Malassezia* spp–human cell interaction might be mediated by proteins of *Malassezia* spp that are packed and released in nanovesicles.⁴⁹ It was demonstrated that these nanovesicles stimulate dendritic cells and mast cells to release of tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), IL-8, IL-10, and IL-12p70.^{40,50} These cytokines may contribute to skin inflammation in AD. Some other investigators suggest that Toll-like receptors (TLRs) such as TLR2 recognize *Malassezia* spp.⁵¹ TLRs are members of the large family of pattern recognition receptors, which play a key role in the innate immune system because they recognize molecules that are commonly shared by pathogens. Some recent findings substantiated the relevance of TLRs for the immune response of human cells against *Malassezia* spp. For example, *Malassezia* spp induces the expression of TLR2 and TLR4 on human keratinocytes⁵² and human dendritic cells,⁴⁸ inducing the production human beta defensin 2 and CXCL8⁵³ (Fig. 2). Another possible mechanism could be the activation of the NLRP3 inflammasome in skin dendritic cells by *Malassezia* spp. This inflammasome activation leads to the release of

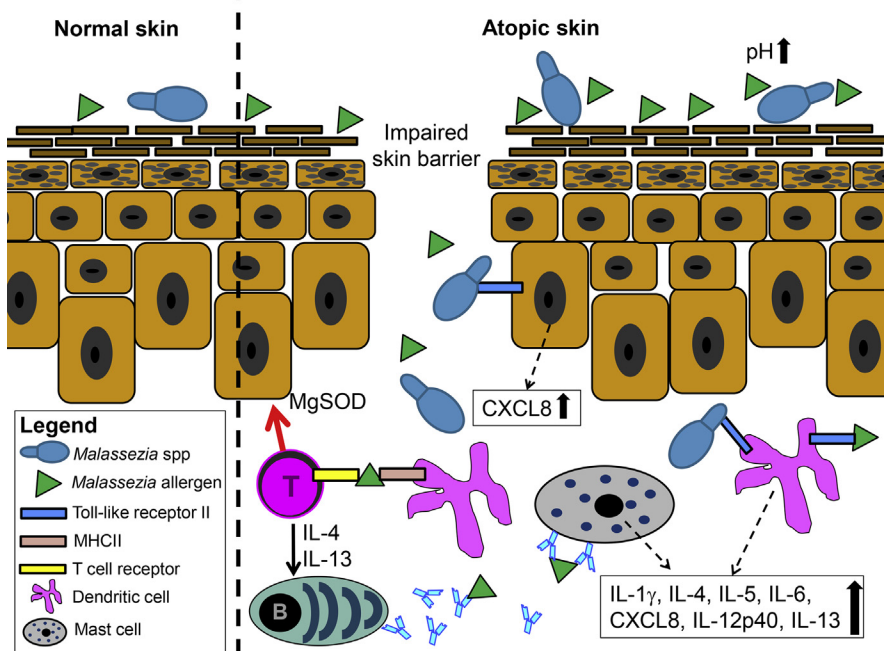


Fig. 2. Proposed mechanisms by which *Malassezia* spp contributes to skin inflammation in AD patients. The increased pH in atopic skin contributes to increased allergen release by *Malassezia* spp. These allergens, supposedly together with whole *Malassezia* spp cells, penetrate the epidermis through the disturbed skin barrier in AD patients. *Malassezia* spp cells and their allergens may be recognized by TLR2 expressed on keratinocytes and dendritic cells that elicit the release of proinflammatory cytokines. *Malassezia* spp components elicit the production of *Malassezia* spp-specific IgE antibodies through the dendritic cell and T-cell-mediated activation of B cells. These IgE antibodies may also contribute, possibly through mast cells, to the inflammation in atopic skin. Finally, autoreactive T cells can cross-react between fungal and human manganese-dependent superoxide dismutase (MgSOD) and hence sustain skin inflammation. (From Glatz M, Bosshard PP, Hoetzenecker W, et al. The role of *Malassezia* spp. in atopic dermatitis. J Clin Med 2015;4:1221; with permission.)

proinflammatory cytokines such as production of IL-1 β , and IL-4, IL-5, and IL-13, which are key players in the pathogenesis of AD^{54–56} (see Fig. 2).

The authors have mentioned the IgE-mediated sensitization to various *Malassezia* spp allergens above. These allergens may also directly stimulate IgE-independent immune mechanisms. For example, Mala s 13 is a fungal thioredoxin that is very similar to its human counterpart (Fig. 3). When human CD4⁺ T cells recognize the fungal thioredoxin, they may cross-react to the human enzyme, which is expressed by human keratinocytes. This cross-reaction will induce a T-cell-mediated skin inflammation, which is commonly seen in AD.⁴⁵ A similar induction of autoreactive T cells and T-cell-mediated inflammation was observed for another *Malassezia* spp allergen, Mala s 11, which is a manganese-dependent superoxide dismutase. The significance of these allergens for skin inflammation in AD was substantiated by the strong correlation between AD severity and Mala s 11 sensitization.⁴¹

Management and Therapeutic Approaches with Antifungals in Atopic Dermatitis

The basis of every effective AD therapy is the use of skin emollients. They rehydrate the skin and repair the impaired skin barrier. In case of clinically manifest skin inflammation during AD flares, an anti-inflammatory treatment is necessary. This treatment most commonly requires topical corticosteroids or calcineurin inhibitors. Another promising therapeutic approach is the identification and elimination of trigger factors such as *Malassezia* spp,²⁷ for example, by an antifungal therapy. The usefulness of an antifungal therapy for AD has been discussed for many years. Azole antifungals are the most common class of antifungal drugs prescribed for AD patients. In vitro, azole

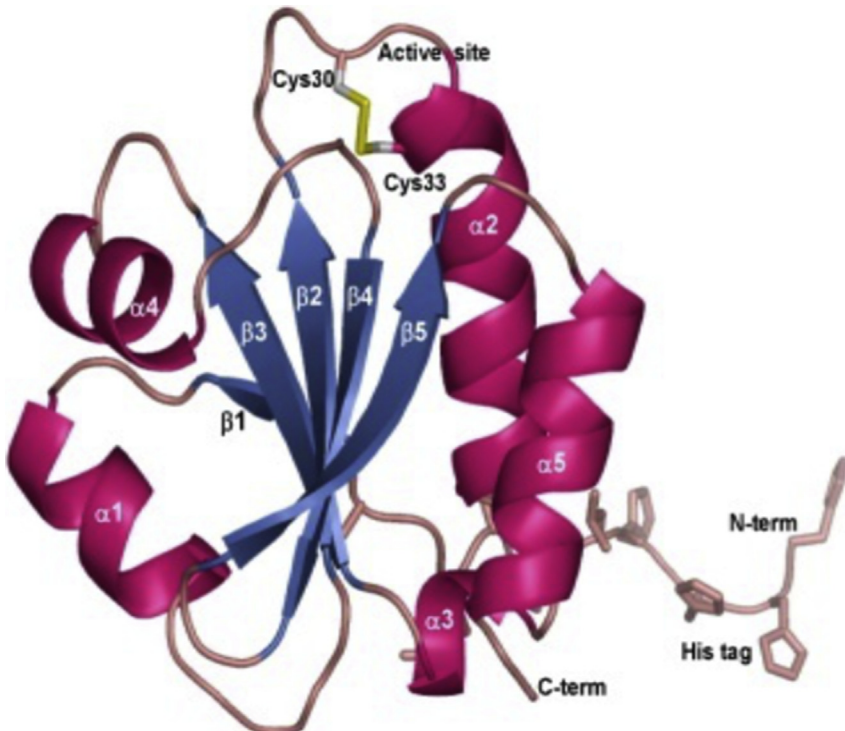


Fig. 3. Crystal structure of the *M. sympodialis* thioredoxin Mala s 13. (Courtesy of Prof Reto Cramer, PhD, Davos, Switzerland.)

antifungals are effective against *Malassezia* spp,^{29,57} but susceptibility testing of *M pachydermatis*, a species most commonly isolated from dogs, showed that strains isolated from dogs with AD were less susceptible to azole antifungals than strains isolated from healthy dogs.⁵⁸ In humans, the resistance of *Malassezia* spp to antifungals has not been investigated to date.

Several trials investigated the effects of topical or systemic azole antifungals on AD patients and compared it to placebo. However, these trials gave somehow ambiguous results. As a clinical experience, the topical application of ketoconazole on the face of patients with AD of the head and neck type improved eczema. However, in a placebo-controlled study, the combination of topical miconazole-hydrocortisone cream with ketoconazole shampoo was not superior to hydrocortisone alone in patients with head and neck-type AD.⁵⁹ The benefit of a systemic antifungal treatment for the AD patient has been investigated in a randomized, placebo-controlled trial on 36 AD patients treated with ketoconazole versus 39 AD patients treated with placebo. AD severity improved significantly in the ketoconazole group but not in the placebo group.⁶⁰ For another trial, a total of 53 AD patients were treated with either 2 different dosages of itraconazole or placebo. The improvement of AD severity was significantly higher in itraconazole-treated patients than in the placebo group.⁶¹ The positive effect of azole antifungals on AD could also owe to the anti-inflammatory properties of ketoconazole or itraconazole, because these drugs inhibit the production of IL-4 and IL-5 by T cells.⁶²

The positive effects of antifungals were not confirmed by another study on 15 AD patients treated with ketoconazole versus 14 AD patients treated with placebo. Both treatment groups received topical corticosteroids. Although AD severity improved in both treatment groups, this improvement was not correlated to ketoconazole but rather to the topical corticosteroids.⁶³ The ambiguous results of these clinical trials might be attributed to a selection bias and low patient numbers. It can be speculated that antifungal therapies are more effective in a particular subgroup of AD, for example in patients with a head and neck type of eczema. More recently published studies were of less quality, for example, they comprised retrospective observations and lacked a standardized scoring system to assess the severity of AD.⁶⁴ More randomized, placebo-controlled studies on large patient populations are needed to reliably assess the benefit of an antifungal therapy in AD.

In summary, there is little doubt that *Malassezia* spp plays a role in AD because it may interact with the local skin immune responses and barrier function, and sensitization against this skin-colonizing yeast can correlate with disease activity. Also, antifungal therapy shows beneficial effects in some patients. However, the pathogenetic mechanism and mutual interaction between *Malassezia* spp and AD still remain partly unclear and need further investigation.

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