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Glatz, Martin ; Bosshard, Philipp ; Schmid-Grendelmeier, Peter

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Martin Glatz[1,2], Philipp Bosshard[3], Peter Schmid-Grendelmeier[1,2]

[1] Allergy Unit, Department of Dermatology, University Hospital of Zurich, Zurich, Switzerland

[2] Christine-Kühne Center for Allergy Research and Education CK-CARE

[3] Mycology Laboratory, Department of Dermatology, University Hospital of Zurich, Zurich, Switzerland

Correspondence to:

Martin Glatz, MD

Allergy Unit, Department of Dermatology, University Hospital of Zurich

Gloriastrasse 31, 8091 Zurich, Switzerland

Email: martin.glatz@usz.ch

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 three 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 chapter of Infectious Complications in Atopic Dermatitis by Peck Y Ong in this issue. Recently, microbiome research extended on the possible pathogenic role of fungi in AD. This research has been 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 led to the 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 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, as 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* species between different body sites sampled in different studies will give unreliable results. Also, the epidemiological studies used different culture media to detect *Malassezia* species. We [unpublished data] and others have shown that different culture media favor the growth of particular *Malassezia* species [3-6]. Therefore, the use of only one or a few types of culture media does not necessarily depict the whole spectrum of *Malassezia* species 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 1-22% of the phylogenetic composition of the skin microbiome [7]. *Malassezia* spp. almost exclusively comprises to 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 (Figure 1). Most of the *Malassezia* species lack the genes for fatty acid synthase genes and therefore rely on exogenous fatty acid sources to satisfy their nutritive requirement [8]. *M. pachydermatis*, a species isolated from dogs and other animals [9], is the only known *Malassezia* species that grows in the absence of exogenous lipids [8]. Their need for exogenous lipids explains the pedilection of *Malassezia* species 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, *M. globosa* and *M. restricta*, are consistently found on healthy skin of individuals from the U.S. and Europe, and are identified on almost all body sites [2, 6]. However, epidemiological studies indicated a geographical variation in the distribution of particular *Malassezia* species, presumably owing to climate factors. For example, *M. sympodialis* has been reported in studies from Canada, Russia and Sweden as the most frequent species, whereas in Japan *M. furfur* was the most common species [6].

Table 1. Currently identified *Malassezia* species (modified from [11-13])

Malassezia species	Isolated from human skin	Isolated from animal skin	Description as species (year)
M. caprae		X	2007
M. cuniculi		X	2011
M. dermatis	X		2002
M. equina		X	2007
M. furfur	X	X	1889
M. globosa	X	X	1996
M. japonica	X		2003
M. nana		X	2004
M. obtusa	X		1996
M. pachydermatis		X	1925
M. restricta	X		1996
M. slooffiae	X	X	1996
M. sympodialis	X	X	1990
M. yamatoensis	X		2004

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 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 desirable to assess sensitization. Fortunately, a standardized kit (ImmunoCAP® m70, Phadia) 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 which 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 to 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]. This 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 to 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 two studies on 132 children and 128 adults with AD [22 28]. The lower frequency of *Malassezia* spp. sensitization in children compared to 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 to 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 growths 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 three *Malassezia* species, namely *M. furfur, M. sympodialis* and *M. globosa*, are characterized on a molecular basis [30] (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 an 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 following.

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

Allergen	Source	Mass (kDa)	Function	Sensitization (%)	Comment	Ref
Mala f 2	*M. furfur*	21	Peroxysomal membrane protein	72		[32]
Mala f 3	*M. furfur*	20	Peroxysomal membrane protein	70		[32]
Mala f 4	*M. furfur*	35	Mitochondrial malate dehydrogenase	83		[33]
Mala s 1	*M. sympodialis*	36		unknown		[34]
Mala s 5	*M. sympodialis*	19		unknown		[35]
Mala s 6	*M. sympodialis*	18	Cyclophilin	92%		[35, 36]
Mala s 7	*M. sympodialis*	22		40-60		[35, 37]
Mala s 8	*M. sympodialis*	16		40-72		[35, 37]
Mala s 9	*M. sympodialis*	11		24-36		[35, 37]
Mala s 10	*M. sympodialis*	86	Heat shock protein70	69		[38]
Mala s 11	*M. sympodialis*	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	[39-42]

					homologe	
Mala s 12	*M. sympodialis*	67	Glucose-methanol-choline oxidoreduktase	62		[43]
Mala s 13	*M. sympodialis*	13	Thioredoxin	50	Induces auto-reactive T cells against human homologe,	[44, 45]
MGL_1304	*M. globosa*	17		62	Induces degranulation of mast cells, IL-4 release by basophils	[46, 47]

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 pro-inflammatory 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 occur, 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, *Malassezia* spp. is recognized by keratinozytes 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 TNF-alpha, IL-6, IL-8, IL-10 and IL-12p70 [40, 50]. These cytokines may contribute to skin inflammation in AD. Some other authors 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 as 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 keratinocytes [52] and human dendritic cells [48], inducing the production human beta

defensin 2 and CXLC8 [53] (Figure 2). Another possible mechanism could be the activation of the NLRP3 inflammasome in skin dendritic cells by Malassezia spp. This leads to the release of pro-inflammatory cytokines such as production of IL-1beta, and IL-4, IL-5, IL-13 which a key players in the pathogenesis of AD [54-56] (Figure 2).

We have above mentioned the IgE-mediated sensitization to various *Malassezia* spp. allergens. 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 (Figure 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 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 were substantiated by the strong correlation between AD severity and Mala s 11 sensitization [41].

Management and therapeutic approaches with antifungals in AD

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 most commonly requires topical steroids or calcineurin inhibitors. Another promising therapeutic approach is the identification and elimination of

trigger factors such as *Malassezia* spp. [27], for example by an anti-fungal therapy. The usefulness of an antifungal therapy for AD has been discussed since many years. Azole antifungals are the most common class of antifungal drugs prescribed for AD patients. In vitro, azole 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 improves eczema. However, in a placebo-controlled study the combination of topical miconazole-hydrocsortisone 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 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 two 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, as 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 steroids. Although AD severity improved in both treatment groups, this improvement was not correlated to ketaconazole but rather to the topical steroids [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-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 as 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 investigations.

Figure legends

Figure 1.

Malassezia sympodialis culture isolated from a patient with atopic dermatitis. On Malassezia CHROMagar, grown for 72 hours at 32 °C. (With permission from [65])

Figure 2.

Proposed mechanisms by which *Malassezia spp.* contributes to skin inflammation in atopic dermatitis (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 toll-like receptor 2 expressed on keratinocytes and dendritic cells what elicits the release of pro-inflammatory 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. (With permission from [65])

Figure 3.

Crystal structure of the *Malassezia sympodialis* thioredoxin Mala s 13 (Courtesy Prof. Reto Crameri, Davos, Switzerland)

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