The genus *Malassezia* and human disease

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ABSTRACT

Sabouraud's *Pityrosporum* is now recognized as *Malassezia*. With taxonomic revision of the genus, newer species have been included. The role of this member of the normal human skin flora in different cutaneous and systemic disorders is becoming clearer. The immunological responses it induces in the human body are conflicting and their relevance to clinical features is yet to be explored.

Key Words: Pityrosporum, Malassezia

INTRODUCTION

The genus *Malassezia* comprises a group of superficial dimorphic fungi occurring as normal skin flora on the human body.[1] However, they can also cause infection or are associated with certain skin diseases. Rarely, they can become invasive, to cause opportunistic systemic infection in the presence of certain predisposing factors.[2] During the past two decades, this group of fungi has gained increasing importance. The nomenclature has been changed, newer species have been identified and associations of the organism with different disease entities have been described.

HISTORICAL REVIEW AND NOMENCLATURE[3]

In 1846, Eichstedt described the disease pityriasis versicolor, though he was unable to isolate the organism. Robin (1853) isolated fungal elements from these lesions and named the organism as *Microsporum furfur*, as he thought it was identical to the dermatophyte *Microsporum auduoinii*. Hence, the disease was renamed as tinea versicolor. Malassez (1874) isolated yeast cells from human dandruff scales; subsequently, spherical and oval forms of these yeasts were recognized. In 1889, Baillon included this group of yeasts under the genus *Malassezia*. Sabouraud (1904) considered this organism as a cause of dandruff and gave it a new name, *Pityrosporum malassez*. In the following years, there was controversy regarding the generic name of the fungus, *Pityrosporum* or *Malassezia*. Sternberg and Keddie (1969) detected the same antigenic components in *Pityrosporum orbiculare* and *Malassezia furfur* by the fluorescent antibody technique. In 1984 (Yarrow and Ahearn), *Malassezia* gained priority over *Pityrosporum* and was accepted as the generic name for the fungus.

There were three identifiable species in the genus *Malassezia*, namely, *M. furfur*, *M. pachydermatis* and *M. sympodialis*. In 1996, following taxonomic revision of the genus, four new species, *M. globosa*, *M. obtusa*, *M. restricta* and *M. slooffiae* were included.[4] Earlier, the first three were considered as different serovars (A, B, and C) of *M. furfur*.[5]

BIOCHEMICAL AND CULTURAL PROPERTIES

Earlier workers were unaware of the metabolic properties of *Malassezia*. Hence, the organism could

not be grown in vitro until Benham (1939) detected its lipophilic nature.[3] *Malassezia* metabolizes long chain fatty acids like oleic acid and except *M. pachydermatis*, the other six species are obligatory lipophilic.[2] A few species like *M. furfur* also produce long chain dicarboxylic acids (like azelaic acid), which inhibit pigment production in vivo and thus cause hypopigmentation.[1,2]

The organism can be grown in Sabouraud's dextrose agar (SDA) or malt agar to which chloramphenicol and cycloheximide have been added. A sterile olive oil overlay is added to meet their lipid requirement. Media with whole-fat cow's milk works better than the above. Modified Dixon agar is also ideal for the growth of *Malassezia*.[2,3]

MORPHOLOGY

The fungus is dimorphic, occurring as a saprophytic yeast form and a parasitic mycelial form. Yeast is the prime form isolated in vitro from the culture media. As detected by earlier authors, yeast cells are morphologically variable, occurring in spherical (globose), ovoid or cylindrical forms.[3] There is one way conversion of the yeasts, from spherical to oval and cylindrical forms, so that in older cultures, predominantly oval or cylindrical forms are found.[3] In vivo the yeasts multiply by monopolar budding with a collarette or scar at the base of the daughter bud, visible by electron microscopy.[3] The normal human skin flora consists predominantly of the yeast phase, the spherical form occurring on the trunk and the oval form on the scalp. The mycelial form comprises short, septate hyphae, arranged at an angle or end to end with occasional branching.[3] Pathological specimens consist predominantly of hyphae with clusters of spherical yeasts (spaghetti and meatball appearance).

DEMONSTRATION OF THE ORGANISM

The clinical lesions have a yellow fluorescence on Wood's lamp examination.[1] For direct demonstration of the organism, the specimen is collected from the clinical lesions by scraping or tape stripping and a KOH mount is prepared. Addition of an equal volume of Parker blue-black Quink permanent fountain pen ink to KOH (Parker's stain) enhances the visibility, as the bright blue stain is taken up by the organism.[1] From follicular lesions, a sample is collected either by scraping or by a punch biopsy. In the latter case, it is stained with Gomori's methenamine silver stain that stains the fungus black.[3]

As the organism is part of the normal skin flora, a culture from the lesions is not helpful for diagnostic purposes. For demonstration of the organism from the culture, lactophenol cotton blue stain is used.

EPIDEMIOLOGY

The fungus abounds in the skin of subjects from tropical and subtropical climates. Individual species have specific host preferences.[3] *M. pachydermatis* was first isolated from the rhinoceros.[2] It is mainly found in domestic animals, like dogs, causing otitis externa, but occasionally can infect humans.[4] *M. sympodialis* is the most frequent species occurring in humans as normal flora or sometimes in association with disease.[4] *M. furfur*, *M. globosa* and *M. restricta* also affect humans. *M. furfur* is seldom found as normal flora or in disease states. *M. globosa* and *M. restricta* are mainly pathogenic, associated with pityriasis versicolor and pityriasis capitis or seborrheic dermatitis respectively.[4] *M. slooffiae* can be demonstrated as normal flora on the trunk. This species is also pathogenic in pigs.[2]

In humans, the peak age for normal carriage or disease by *Malassezia* is the early 20s, when sebaceous gland activity is maximum.[1] Its distribution as normal flora is related to sebaceous gland density, and thus the scalp, face, central chest and back bear the highest number of fungi.[2,6] Other sites, like the hair follicles and the external ear, are also sites of colonization. The hair shaft, nail and mucosae are not affected.[2] In an epidemiological study, 97% of the normal adult population showed scalp carriage and 92% showed trunk carriage for *Malassezia*. Colonization or disease by *Malassezia* is rare at the extremes of ages.[2,3] However, in different studies, hospitalized infants have shown a positive skin culture for *M. furfur*, the incidence varying from 37% to 84%.[7,8] Many healthy infants develop a cutaneous flora comprising *Malassezia* species within the first 6 months of life.[9]

PATHOGENESIS

A variety of disease states are linked to infection or colonization by *Malassezia* (Table 1). The occurrence of clinical disease by *Malassezia* depends on the factors permitting conversion of the saprophytic yeast phase of the organism to the mycelial phase.[1] High sebum levels at puberty, excessive sweating, warmer season (May to September), application of oil, malnutrition, administration of systemic steroids or the presence of Cushing's disease, pregnant state or therapy with oral contraceptives, antibiotics or immunosuppressives are some factors that facilitate massive growth of the fungus.[1,3]

It is not clearly known whether the disease is initiated by alteration of the usual relationship between the human host and the resident yeast flora that facilitates mycelial conversion or by transmission from a source patient. Some species constituting normal flora may have a greater pathogenic potential and may be converted to the mycelial phase readily.[1] The occurrence of diseases like pityriasis versicolor in several members of a family suggests a genetic susceptibility to the infection.[1,10]

A variable quantity of *Malassezia* specific IgG antibodies are found in normal adults.[3] In disease states inflammatory and regulatory cytokines of both the Th1 and Th2 types are generated. The current view about the pathogenicity of *Malassezia* is that the organism may produce a lipid soluble, low molecular weight (< 1-2 kDa) substance under certain growth conditions, which acts as a chemotactic agent for leukocytes and thus induces inflammation.[11] In a study by Faergemann et al,[12] there was an increase in NK1+ and CD16+ cells along with complement activation in patients with seborrheic dermatitis and Malassezia folliculitis, indicating an irritant, non-immunogenic stimulation of the immune system. The cause of this irritant response is likely to be products released by *Malassezia*.

PITYRIASIS VERSICOLOR

Pityriasis versicolor (PV) is the only skin infection where *Malassezia* plays a definite causative role. It is a chronic, superficial non-inflammatory infection of the skin occurring principally on the trunk and proximal extremities of young adults. The mycelial phase of the fungus is predominant in the lesions. *M. globosa* is the main species isolated from the lesions. In one study, *M. globosa* was the only isolated species in 60% of cases of PV, and in an additional 37%, in combination with *M. sympodialis* and *M. restricta*. In other studies *M. globosa* has been isolated in more than 90% cases.[16,17] Dutta et al[18] have also reported *M. globosa* as the main species isolated from patients with PV in North India. More than one species of *Malassezia* can be isolated from a clinical specimen.[4,17,18]

The chronic relapsing course of the disease and increased incidence of PV in steroid treated and immunosuppressed patients are suggestive of failure on the patients' part to mount a protective CMI response against the fungus.[5] A recent study found that patients with PV may not actually have a deficient CMI to the mycelial antigen of *Malassezia*.[5] However, during active disease, they fail to generate a CMI response that would provide protection.

DANDRUFF (PITYRIASIS CAPITIS OR PITYRIASIS SIMPLEX)

It is a subclinical, inflammatory scalp disorder, episodic,

Table 1: Disease states associated with *Malassezia*

Conditions where *Malassezia* has a definite role:		Other conditions where the role of *Malassezia* has been proposed:[13,15]	
Cutaneous	• Pityriasis versicolor	• Dandruff	• Folliculitis
Systemic	• Intravenous catheter induced fungemia with or without embolism	• Neonatal cephalic pustulosis	• Confluent and reticulate papillomatosis
	• Endocarditis	• Atopic dermatitis	• Guttate psoriasis
	• Interstitial pneumonia	• Seborrheic dermatitis and blepharitis	• Balanitis
	• Peritonitis in patients undergoing chronic ambulatory peritoneal dialysis	• Onchomycosis	• Lacrimal canaliculitis and dacryolith
		• Sinusitis	• Otitis externa
		• Nipple discharge	

recurrent or constant, that results in disruption of cohesion between corneocytes, visible as scales.[19] The disorder is most prevalent and severe among adolescents and young adults, and rare among children and the elderly.[19] Environmental factors like winter season, UV irradiation, airborne irritants and hair cosmetics are known to aggravate the condition.

Malassez (1874) had isolated the yeast forms of the species from dandruff scales.[2] It has been hypothesized that a critical quantity of the yeasts are required for the clinical manifestation of dandruff and when it exceeds this, features of seborrheic dermatitis are seen.[19] The oval yeast form of *M. restricta* has been isolated from dandruff scales.[4] Toxin production and lipase activities of the yeast inducing a pro-inflammatory state and stimulating host immune response may be operative in the pathogenesis. Environmental factors have some additive effect on the pathogenicity of the fungus.[20]

There is a definite response to topical antifungals when *Malassezia* is associated with dandruff.

SEBORRHEIC DERMATITIS

Seborrheic dermatitis (SD) is a chronic dermatitis with greasy scales in seborrheic areas in children and adults. The total count of the fungus has been found to be raised in lesions of SD.[21]

M. sympodialis and *M. restricta* are the *Malassezia* species found commonly in SD lesions.[4] In animal experiments, inoculated killed yeasts can produce histopathological features of SD. However, the levels of *Malassezia* specific antibodies are consistently raised in patients with SD.[11]

The results of studies determining the immunological response of SD patients to *Malassezia* are variable. Some authors have found slightly greater mean *Malassezia* specific IgG levels in patients with SD than in healthy controls.[22] Others were unable to detect any such difference in the levels of total humoral antibody or immunoglobulin subclasses.[23] There is also an alteration of cytokine production and their respective levels in patients with SD. Lymphocyte stimulation studies with yeast antigen have shown that lymphocytes from patients with SD produce more of IL-10 than IL-2 and IFN-γ, indicating a Th2 type of immune response in vivo.[24] Current studies indicate that the immune response to yeast antigens in SD patients remains unaltered.[11] The toxins or inflammatory mediators produced by *Malassezia* act as strong leukocyte activators and chemotactic agents, cause degranulation and thus induce inflammation.[11] The fluctuations in the disease severity are explained by the variation in the number of yeast cells and thus the amount of mediators produced under certain conditions of growth.[11]

MALASSEZIA FOLLICULITIS

In most cases, this condition is associated with SD. It is not known whether the fungus plays any causative role or whether there is merely a proliferation of *Malassezia* in the enlarged follicle. Overgrowth of the yeast causes blockage of follicular ostia. It is possible that hydrolysis of triglycerides, fatty acid synthesis and activation of the alternate complement pathway by the fungus induce inflammation, resulting in folliculitis. Histopathologically, there is a perifollicular mononuclear cell infiltrate around the infundibulum. An increased number of T helper cells and Langerhans cells have been found in skin lesions.[12] *Malassezia* yeasts are seen, but mycelial forms are usually absent. Serum IgG antibodies against *Malassezia* have been found in higher titres than in healthy controls.[12] The condition responds to 2% ketoconazole, but relapses are frequent. Hence, once weekly prophylactic treatment with such agents is recommended.

NEONATAL CEPHALIC PUSTULOSIS

Previously considered as neonatal acne, it is a newly described benign clinical entity occurring in neonates. There is a non-follicular pustular eruption involving the face, neck and scalp.[25] Its incidence is 3% of hospitalized neonates.[26] The diagnostic criteria for differentiating this condition from others like milia, sebaceous gland hyperplasia and neonatal acne are: age of onset less than 1 month, cephalic location, smear positive for *Malassezia*, elimination of other causes of neonatal pustulosis, and response to topical

ketoconazole.[26]

M. furfur is the most commonly isolated species. *M. sympodialis* can occur in the severe form.[26] Neonatal sebum production due to maternal hormonal influences facilitates the growth of yeasts,[25] which might have been seeded from the mother or from healthcare workers.

CONFLUENT AND RETICULATE PAPILLOMATOSIS

It is a genetic defect of keratinization where there is an abnormal response to colonization by *Malassezia* and/or follicular bacteria.[27] The condition is commonly seen around puberty as asymptomatic, flat, dry papules varying in size from 1-5 mm on the mid-back, chest, sides of the neck and shoulders. The response to topical or systemic antifungals is variable.

SYSTEMIC INFECTIONS CAUSED BY MALASSEZIA

Systemic infection by *Malassezia* is becoming an increasingly recognized entity among seriously ill, low-birth-weight, hospitalized neonates or adults receiving infusion of intravenous lipid preparations as part of parenteral alimentation.[2,3] *M. furfur* is the commonest species isolated; *M. pachydermatis* has also been reported.[28] The source of the fungus in such cases is usually the patient's own skin flora or the hands of healthcare workers. The long chain fatty acids in intralipid solutions facilitate the growth of the organism along the lumen of the indwelling catheter, and depending on the host immune status there is systemic spread.[2] Examination of the removed catheter in such cases reveals adherent fungi, maximally along the distal lumen, often visible as white clumps.[3]

In the presence of fungimia, embolization occurs, the lungs being involved frequently. Yeasts can be demonstrated in the walls of small pulmonary arteries or in areas of an infarct.[3] Thrombocytopenia and leukocytosis with increased band forms are seen in children.[3] A blood culture is only infrequently positive.[3] Clinical symptoms and signs may be subtle or the patient may be asymptomatic. Morbidity associated with *Malassezia* fungimia is difficult to assess as the condition is underdiagnosed and patients usually suffer from other major illnesses.[3]

HIV INFECTION AND MALASSEZIA

The growth of *Malassezia* is known to be enhanced in immunocompromised conditions. The skin flora remains quantitatively normal in HIV infected patients. In a recent study from India, the authors found the overall incidence of *Malassezia* infection among HIV infected patients to be 13.5%; the incidence of PV, *Malassezia* folliculitis and SD was 40%, 16% and 56% respectively.[29] Among these, 9 patients had a clinical diagnosis of AIDS. A higher incidence and severity of SD has been reported in AIDS patients (30-55%[30] vs 1-3%[31] in the normal population). The incidence of SD in these patients can be correlated with CD4+ T cell counts; with > 200 cells it is 15% and with < 200 cells, it becomes 58%.[11] Thus, it appears that immunosuppression in HIV infection enhances *Malassezia* growth.[11] In the series of HIV infected patients reported by Kaviarasan et al,[29] extensive PV and SD with an aggressive course were observed in advanced stages of immunosuppression, and the authors have proposed that the presence of such features can be used as a clinical marker of AIDS in resource poor countries.

It has been proposed that aggravation of SD in HIV infected patients is not directly related to *Malassezia* growth.[11] In disease states, the level of toxic products of *Malassezia* rises, increasing the prevalence and severity of SD.[11] Moreover, the HIV infected state increases level of interferons and tumor necrosis factor-α, which are known to alter the lipid metabolism, increasing serum triglyceride and cholesterol levels. This increases the patient's sensitivity to inflammatory mediators released by *Malassezia*.[11]

Though the genus *Malassezia* has been associated with many cutaneous and systemic disorders, its exact role in the causation of most of these conditions is yet to be elucidated. Several studies have been conducted on the immunopathogenesis of the disorders associated with these fungi but the results of many of these are contradictory. Further research on the pathogenesis of

Malassezia related illnesses should overcome this problem.

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