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The role of *Malassezia* species in the ecology of human skin and as pathogens

E. GUÉHO,* T. BOEKHOUT,† H. R. ASHBEE,‡ J. GUILLOT,§ A. VAN BELKUM¶ & J. FAERGEMANN**
**Mycology Unit Pasteur Institute, Paris, France; †Centraalbureau voor Schimmelcultures, Yeast Division, Delft, The Netherlands; ‡Department of Microbiology, University of Leeds, Leeds, UK; §Parasitology-Mycology Unit, Alfort's National Veterinary School, Maisons-Alfort, France; ¶University Hospital Rotterdam, Rotterdam, The Netherlands; **Department of Dermatology, Sahlgrenska University Hospital, Gothenburg, Sweden*

The new taxonomic structure of the lipophilic genus *Malassezia* was presented with key characteristics for the seven described species. Among techniques used for epidemiological surveys, the pulsed field gel electrophoresis (PFGE) was found to be of little value in contrast to randomly amplified polymorphic DNA (RAPD). Immunological studies still yielded conflicting results but at least the immunomodulatory capacity of *Malassezia* yeasts appeared to be related to the cell wall lipids. A review of *Malassezia* infections together with the present consensus for their prevention and treatment was also made.

Keywords Malassezia, taxonomy, epidemiology, immunology, pathogenicity, treatment

Introduction

The taxonomy of the genus *Malassezia* (alias *Pityrosporum*) has always been a matter of controversy since its creation by Baillon in 1889 with *Malassezia furfur* as the generic type species. Taxonomic advances were finally made following genomic and ribosomal (r)RNA sequence comparisons of a large number of human and animal isolates. The genetically distinct entities matched remarkably well with morphological and serological differences documented previously within this species. The genus has, thus, been enlarged to seven species comprising the three former taxa *M. furfur*, *Malassezia pachydermatis* and *Malassezia sympodialis*, and four new taxa named *Malassezia globosa, Malassezia obtusa, Malassezia restricta* and *Malassezia slooffiae.* A rapid and inexpensive identification system has been established to separate the seven species routinely on the basis of morphological and physiological differences. These methods include detection of catalase and β-glucosidase production, tolerance to 37 °C, and ability to grow with Tween 20, 40, 80 and cremophor EL (castor oil) as unique lipid supplements. The uniqueness of the genus *Malassezia*, already demonstrated by its lipophily and cell wall ultrastructure was confirmed phylogenetically; the seven species comprised a monophyletic group when compared with a large number of basidiomycetes. Based on the new taxonomy and ease of identification, progress should be made in the near future in our understanding of the epidemiology, immunology, pathology and therapy of *Malassezia* infection.

The first epidemiological investigations taking into account all species demonstrated that systemic *Malassezia* infections were caused primarily by *M. furfur* and occasionally by *M. pachydermatis*. Pulsed-field gel electrophoresis (PFGE) confirmed the robustness of the new taxonomic structure of *Malassezia*, all species being characterized by their own karyotype. However, it was found to be of little value for epidemiological surveys, as intraspecific karyotype variability was limited. In contrast to PFGE, randomly amplified polymorphic DNA (RAPD) analysis showed genetic variation within the species which is very useful for epidemiological studies.

Studies on the specific cellular and humoral immune status of patients and healthy individuals to *Malassezia* have yielded conflicting results. Initial reports of a cellular immune deficiency to *Malassezia* in patients with pityriasis versicolor (PV) have been disputed and a greater sensitization in patients with seborrhoeic dermatitis (SD) has been reported. Controversy has also dogged work on humoral immunity with both increased titres in patients and titres in patients comparable to those of control individuals documented. The ability of *Malassezia* to act as an

Correspondence: E. Guého, Institut Pasteur, Unité de Mycologie, 25 rue du Dr. Roux, 75724 Paris Cedex 15, France.

adjuvant contrasts with its ability to down-regulate cytokine production via its lipid-rich lamellar layer, suggesting that its immunomodulatory capacity is related to cell wall lipids.

Malassezia yeasts are members of the normal human cutaneous flora and can be cultured from almost all body areas. Under the influence of predisposing factors they become pathogenic and are associated with several diseases such as PV, folliculitis, SD, some forms of atopic dermatitis, some forms of confluent and reticulate papillomatis, and even systemic infection. The most important exogenous predisposing factors are high temperatures and a high relative humidity; endogenous factors include a greasy skin, hyperhidrosis, hereditary factors, corticosteroid treatment and immunodeficiency. A high rate of recurrence characterizes cutaneous *Malassezia* infection, particularly PV. A permanent cure is therefore difficult to achieve. Consequently, prophylatic treatment is mandatory to avoid recurrence.

Taxonomy of the genus *Malassezia*

The very complex nomenclatural history of the lipophilic genus *Malassezia* was detailed by Slooff in the second edition of *The Yeasts: A Taxonomic Study* [1]. The fungal nature of PV was recognized as early as 1846, but the genus *Malassezia* was created by Baillon [2] half a century later to accommodate the filamentous fungus (and round yeast-like cells), observed in the skin lesions of PV. Unfortunately, because the lipid requirements of this fungus were not recognized at the time, neither filamentous nor yeast-like cultures could be obtained. It was the beginning of a long-lasting controversy between supporters of either the genus *Malassezia,* with the belief of many microbiologists and dermatologists that it should be reserved for the hyphal form, or the genus *Pityrosporum* created by Sabouraud [3] for organisms resembling yeasts present on the scalp. Later, when cultures were obtained successfully, most of them were only short-lived and could not benefit from any yeast identification system to distinguish entities recognized by different authors on the basis of their morphology and pathogenicity.

Consequently, two taxonomic systems have coexisted for many years. One, preferred by botanists, recognized the priority of the generic name *Malassezia* when it was shown that filaments could emerge from yeast cells [4]. In the absence of any clear differences at the time, the genus was limited to *M. furfur*, a lipid-dependent species, responsible for various cutaneous conditions of humans, and *M. pachydermatis* [5], a lipophilic species able to grow with short-chain fatty acids present in rich media, considered to be restricted to animals. *M. sympodialis,* the third *Malassezia* species described in 1990 [6] on the basis of genetic differences, was not adopted by the majority of the scientific community. The other generic name *Pityrosporum* was used preferentially by dermatologists, with *Pityrosporum ovale* [7] associated with pityriasis capitis and SD, *Pityrosporum orbiculare* [8] as the exclusive agent of PV, and *Pityrosporum pachydermatis* [9], in synonymy with *Pityrosporum canis,* adapted to animals.

To elucidate the species concept in *Malassezia*, a taxonomic revision of the genus was undertaken using nucleic acid (partial rRNA 26S sequences and nuclear DNA) characteristics as a first step. The study was performed with a large collection of strains, including the same number from humans and animals (the latter obtained mainly from an epidemiological survey of lipophilic yeasts on animals) [10], available neotype cultures of the different species, with no holotypes being preserved, and representative cultures of morphological and serological variants described by Midgley [11] and Cunningham *et al.* [12], respectively. The molecular study distinguished seven genetic entities which correlated perfectly with the above variants [13]. However, the new taxonomy of the genus *Malassezia* was set when key characteristics were found for each taxon [14]. Consequently, the genus was enlarged to seven species when morphological and physiological characteristics were recognized [15]. Unlike the previous classification, the species no longer correlated with clinical features.

To facilitate their identification routinely, a practical identification scheme was established which, beside morphological differences, included tolerance to high temperatures, ability to express catalase activity and growth with Tween 20, 40, 60 and 80 as the sole lipid supplements in a regular medium [16]. Because some ambiguity remained between the three physiologically similar species *M. furfur, M. sympodialis* and *M. slooffiae*, this system was improved by adding in diffusion plates cremophor EL (castor oil), and characterization of β-glucosidase activity [17]. The presently recognized *Malassezia* species [15] (including the three former taxa *M. furfur, M. pachydermatis* and *M. sympodialis*, and the four new ones, *M. globosa, M. obtusa, M. restricta* and *M. slooffiae*) are presented briefly according to their increasing physiological requirements [16,17]. A few details about healthy carriage and clinical features of these yeasts may be obtained from Ashbee *et al.* [18] and Midgley *et al.* [19]; additional morphological details can be found in Midgley's papers [11,19]. The key characteristics of the species are summarized in Table 1.

Malassezia pachydermatis *(Weidmman) Dodge 1935 (Syn.* P. pachydermatis *and* P. canis*)*

The non-lipid dependent *Malassezia* strains have been recognized as a single genetic entity with priority to the

Table 1 Key characteristics of *Malassezia* species*

Species	Sab. 32 °C	Dixon 38 °C	Catalase	Esculin†	Cremophor† 1–10%	Tween 20 10%	Tween 40 0·1–10%	Tween 80 0·1%
M. pachydermatis	+	+	±	±	±	±	+	+
M. furfur	–	+	+	–	+	+	+	+
M. sympodialis	–	+	+	+	–	–	+	+
M. slooffiae	–	+	+	–	–	+	+	–
M. obtusa	–	–	+	+	–	–	–	–
M. globosa	–	–	+	–	–	–	–	–
M. restricta	–	–	–	–	–	–	–	–

*Almost all characteristics were detailed in taxonomic papers [15,16]
†For esculin and cremophor EL, see Mayser *et al.* [17]

specific epithet *pachydermatis* [20]. However, populations within this species were proved to be in the course of genetic diversification, probably due to host adaptation [21]. This species is mainly adapted to animals but can cause systemic infection in humans.

Malassezia furfur *(Robin) Baillon 1889 (syn. Microsporon furfur)*

This species, which is the generic type, groups the two neotype cultures corresponding to *P. ovale* and *P. orbiculare*. It is lipid-dependent, but the addition of a single lipid supplement to regular media is sufficient to ensure growth. It survives extreme conditions. This may explain why the species in its original sense was found to be homogeneous in former studies [22]. Strains can form filaments spontaneously. Morphology of cells is variable. This species has been shown to be involved in different pathologies, probably taking advantage of the poorer survival of the other species.

Malassezia sympodialis *Simmons & Guého 1990*

The sympodial type of budding is barely observable because the cells are very small. This species is easily identified with the above combination of tests, especially splitting of esculin within 24 h. It is the most common species on either healthy or diseased skin. However, it is too early to make statements about its pathogenity because most of the time it occurs associated with another species. *M. sympodialis* corresponds to serovar A [12].

Malassezia slooffiae *Guillot, Midgley & Guého 1996*

First recognized on animals (pigs), this species is also present on humans. It can easily be misidentified as *M. furfur* or *M. sympodialis*. In contrast to the previous species it does not grow with cremophor EL and does not split esculin. Nothing is known about its pathogenicity.

Malassezia obtusa *Midgley, Guillot & Guého 1996*

This is a very rare species which can be misidentified morphologically as *M. furfur* but differs from this species physiologically. Indeed, requirements for growth of *M. obtusa* are similar to those for *M. globosa* and *M. restricta*; the species possesses β-glucosidase.

Malassezia globosa *Midgley, Guého & Guillot 1996*

This species can be recognized by its colony and cell morphology. Colonies grow slowly and develop a deeply folded surface. Cells are spherical and have many germ tubes, especially in primary culture from patients. Because the species does not survive easily, it has been confused with other *Malassezia* species coinhabiting the same sites. It is found regularly in PV and SD. Serologically, it corresponds to serovar B [12].

Malassezia restricta *Guého, Guillot & Midgley 1996*

The specific epithet *restricta* was chosen because all characteristics are restricted (see Table 1), including catalase activity. This species is isolated regularly from the scalp and face and often occurs in association with other species. It is too early, however, to draw conclusions about its possible involvement in dandruff and SD. The species corresponds to serovar C [12].

Investigators who used Dixon's or Leeming's agars and incubation at 32–35 °C to isolate lipophilic yeasts from the skin, did not identify *M. furfur* and *M. slooffiae*, either morphologically [11] or serologically [12]. The type species *M. furfur* was shown previously to be responsible for PV [22]. One may assume that this species is masked on normal skin by more prolific species. *M. slooffiae*, which is not so frequent on humans, was simply not recognized. While most isolates from humans have been speciatied, this is not the case for animals; one isolate from an okapi (JG 583) and another from an elephant (JG 573) have not been described yet.

Molecular epidemiology of *Malassezia* infections

In modern medicine, *Malassezia* species are becoming more important among the new opportunistic fungal pathogens. *M. furfur* is a prominent species recognized during infections in the immunocompromised host [23,24] and severe infections caused by this species have been described, both in adults and juveniles [25,26]. *M. furfur* may cause a broad-spectrum of clinical phenomena, varying from PV in adults [27] to life-threatening invasive disease in neonates [28–32]. *P. orbiculare* Gordon, long-time considered to be conspecific with *M. furfur* [5], was usually reported in the older literature as the causative agent of PV.

Recent studies into the carriage rates of *M. furfur* among young adults, partly explain the increasing prevalence of this yeast among hospitalized patients. *M. furfur* is found on the skin of around 25% of children between 0 and 15 years of age [33]. A causal relationship between papulopustular eruptions of the face of neonates and infection by *M. furfur* was indicated by culturing this species from eight of 13 patients, and the positive response of these eruptions to ketoconazole therapy [34].

Fortunately, epidemics due to *Malassezia* species are rare. The first nosocomial outbreaks of *M. pachydermatis* infection were reported in the late 1980s [35,36]. This species is encountered frequently on mammals (e.g. as a cause of otitis externa of dogs and on the skin of cats), but has also been isolated from faeces, skin and blood of neonates in intensive-care units [32,35–39]. Based on clinical observations and culture data, a cluster of *M. pachydermatis* infections was identified in a neonatal intensive-care unit [38]. The clonality of this cluster was substantiated by performing chromosome polymorphism analysis. The transmission from person to person most probably took place via the hands of medical personnel. Premature infants on parenteral nutrition are a major risk group.

The recently described lipophilic species have been recovered from a variety of clinical sources [16,19]. However, it is very likely that all these species are still considered to be *M. furfur* by clinicians and epidemiologists, which may hamper our understanding of the nature of the epidemiological and/or nosocomial spread of these organisms.

Genetic heterogeneity of clinical isolates of *Malassezia* species was demonstrated first by karyotype analysis using PFGE [32,40–42] and RAPD analysis [43]. This revealed that genotyping of *Malassezia* isolates can be performed easily and precisely. This enabled molecular tracking of strains of both *M. furfur* and *M. pachydermatis* among patients and in the clinical environment [43]. From a clinical perspective, interesting observations were made enabling effective intervention and elimination of the spread of infection. It was shown that strains of both species resisted superficial cleaning of neonatal incubators and were capable of long-term colonization of plastic surfaces. Thorough cleaning was needed to decontaminate the equipment. The observation of genetic heterogeneity of *Malassezia* isolates was later supported and extended by the analysis of partial nucleotide sequences of the large subunit rRNA [13]. Karyotypes and RAPD patterns of *M. furfur*, *M. sympodialis* and *M. pachydermatis* were found to be different. Within the *M. furfur* complex, an additional four karyotypes were observed. The seven species of *Malassezia* currently recognized, thus, have different karyotypes (Fig. 1). All have relatively small genomes, varying between 6·4 and 12·6 Mb, compared with other yeasts [32,44]. In contrast to most other fungi studied, the intraspecific karyotype variability was found to be limited in almost all species. However, within strains of *M. furfur*, two different karyotype patterns were observed which shared a number of identical bands. Typical *M. furfur* isolates possessed somewhat smaller genomes of 7·4–9·6 Mb, compared with genomes of ≈ 12·3–12·6 Mb for the atypical second type. The taxonomic significance of karyotype diversity among *M. furfur* isolates is not clear, but it may indicate ongoing diversification of populations within this species.

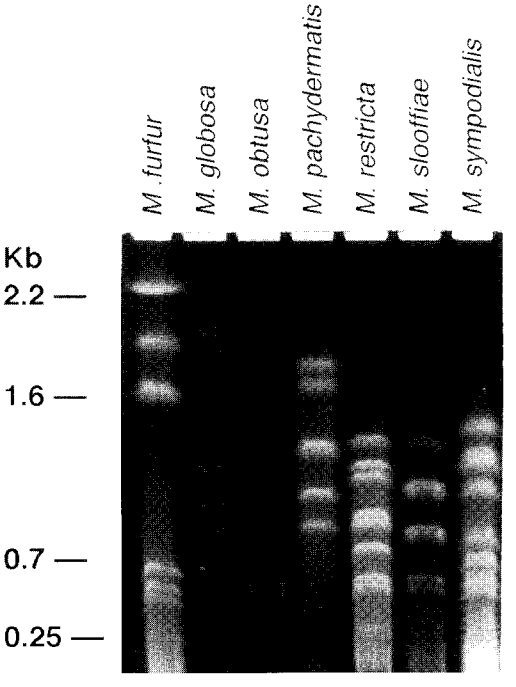

Fig. 1 Karyotypes of *M. furfur* CBS 7981, *M. globosa* CBS 7886, *M. obtusa* CBS 7876, *M. pachydermatis* CBS 7925, *M. restricta* CBS 7877, *M. slooffiae* CBS 7972 and *M. sympodialis* CBS 7859. Karyotypes of *Saccharomyces cerevisiae* and *Pichia canadensis* (as *Hansenula wingei*) (both from Bio-Rad, Veenendaal, The Netherlands) were used as size standards.

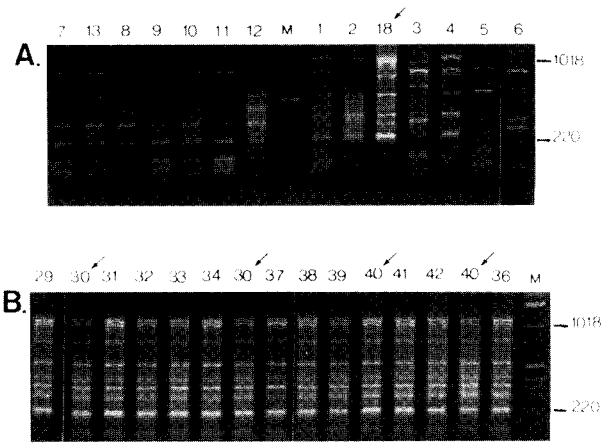

Fig. 2 Typing of *Malassezia* strains by PCR fingerprinting using primers ERIC 1R and ERIC2. A. Comparison of clinical isolates of *M. furfur* from a neonatal hospital ward (strains 7–13) and reference strains (strains 1–6). Isolate 18 (arrow) represents *M. pachydermatis*. B. PCR analysis of clinical *M. pachydermatis* strains from a neonatal hospital ward. Strains indicated with an arrow were run in duplicate.

When clinical isolates of *M. furfur* and *M. pachydermatis* from neonates and incubators on a neonatal intensive-care unit were compared with CBS collection strains of the two species, their karyotypes were found to be similar [32]. Karyotype analysis seems to be of limited value for epidemiological investigations of *Malassezia* yeasts, but remains useful for identification of species.

In contrast to PFGE, RAPD analysis of *M. furfur*, *M. pachydermatis* and *M. sympodialis* isolates maintained in the CBS collection demonstrated genetic variation within these species. However, clinical isolates of both *M. furfur* and *M. pachydermatis* obtained from neonates and incubators on a neonatal intensive-care unit gave identical banding patterns, whereas unrelated isolates displayed differences in banding patterns (Fig. 2). This suggested the presence of genuine nosocomial epidemics of both *M. furfur* and *M. pachydermatis* infection in this particular hospital ward. The regular hygienic measures taken proved to be inadequate as the *Malassezia* strains persisted over a long period of time.

In short, our results indicated that PFGE may be a useful tool for identifying species of *Malassezia*, whereas PCR-mediated techniques seem appropiate for epidemiological investigations.

The interaction of *Malassezia* with the immune system

The genus *Malassezia* exists as both a skin commensal and as the aetiological agent of cutaneous and systemic disease. Whilst immunity to *Malassezia* has been studied extensively in healthy individuals and patients with cutaneous disease, nothing is known about whether an immune response occurs during systemic disease, or about the type and extent of this response.

Interaction with the cellular immune system

One of the first studies to examine cellular immunity to *Malassezia* was that of Sohnle & Collins-Lech [45]. These authors used lymphocyte transformation (LT) and leucocyte migration inhibition (LMI) assays to dialysed antigenic extracts from *Malassezia*. In the LT assay, control individuals had a measurable cell-mediated immune response to the antigens, which was not significantly different to that of patients with PV. With the LMI assay, however, patients produced less leucocyte migration inhibitory factor than the controls. In a subsequent study, Sohnle & Collins-Lech [46] found that patients had a significantly lower LT response on day 6 compared with the controls, but the peak responses on day 9 were not significantly different. They suggested that patients with PV had fewer lymphocytes sensitized to *Malassezia* than controls, which led to the 'conventional wisdom' that these patients had a cell-mediated immune deficiency to *Malassezia*. This explanation seems untenable in view of the lack of a difference between the peak responses and may simply reflect differences in the rate of transformation of the lymphocytes, rather than overall sensitization or number. In contrast, Wu & Chen [47] used the LT assay and found that 31 patients with PV had a 'higher lymphocyte responsiveness' to *Malassezia* than 30 healthy controls. Ashbee *et al.* [48] used both the LT and LMI assays to measure cellular immunity to *M. furfur* serovars A, B and C and found that patients with PV did not have a deficient response to *Malassezia*, and in contrast, seemed to be more responsive to certain serovars than controls.

Only one study has examined the cell-mediated immunity specific to *Malassezia* in patients with SD. Ashbee *et al.* [48] used the LT and LMI to determine the responses of 10 patients with SD to the three serovars of *Malassezia* and compared the responses to those of 10 healthy controls. They found that lymphocytes from significantly more patients responded to serovars B and C in the LT than from controls, and lymphocytes from more patients responded to serovar C in the LMI than from controls. Thus, patients with SD appear to be more reactive to *Malassezia* than healthy controls. The lesions of SD tend to be more inflamed than those of PV, with more infiltrates and, thus, present more opportunities for *Malassezia* to sensitize the immune system.

Bergbrant *et al.* [49] studied the T-cell mitogenic responses in 30 patients with SD and found that 13 had lower responses than the control subjects. However, six of those with lower responses were considerably older than the rest and this may explain their lower mitogenic stimulation.

Interaction with the humoral immune system

Malassezia-specific antibodies are present in serum from both normal individuals and patients with *Malassezia*-associated diseases. Children 2–3 years old have demonstrable titres of IgG and IgM, the levels of which do not change in adult life. IgM is the only *Malassezia*-specific antibody where the levels vary with age, with lower titres in individuals over 60 years. Titres of IgA appear to be extremely low at all ages [50].

The literature on antibody titres in patients with *Malassezia*-associated disease is very confused, due largely to widely differing methods, antigenic preparations and classification of the organisms used by different groups. For patients with PV, two types of result have been obtained: those demonstrating elevated antibody titres in patients [51,52] and those showing no differences between patients and controls [53,54]. The same dichotomy exists in the literature on SD, with some groups finding raised antibody titres in patients [49,55] and some groups observing no differences [54,56]. Thus, no consensus emerges as to how *Malassezia* interacts with the humoral immune system during either PV or SD. The presence of *Malassezia* on normal skin and its ability to elicit antibodies in the absence of disease, suggests that humoral immunity to it is simply a consequence of its presence as a member of the skin commensal flora and is not related to the pathogenesis of either PV or SD.

Interaction with the non-specific immune system

Contrary to the extensive investigations of cellular and humoral immune responses to *Malassezia*, very few studies have examined the non-specific immune response. It has been shown that *Malassezia* is able to activate the alternative complement pathway, as well as the classical pathway [57], although the extent to which this occurs varies from strain to strain (R. Ashbee, unpublished results).

Malassezia has been shown to act as an adjuvant in experimental animals, protecting them from subsequent bacterial infections [58] and tumours [59]. This property, generally associated with persistent microorganisms, suggests that *Malassezia* is relatively resistant to killing by phagocytes. Evidence supporting this contention comes from a study by Richardson & Shankland [60] who showed that only 5% of phagocytosed *Malassezia* cells were killed by neutrophils after internalization for 2 h. Two mechanisms might be involved in this resistance to phagocytosis. Azelaic acid, produced by *Malassezia*, decreases the production of oxygen radicals by neutrophils *in vitro* [61], an important oxygen-dependant killing pathway. Secondly, certain lipids depress reticuloendothelial activity [62] or cause reticuloendothelial blockade [63]. The cell wall of *Malassezia* has an unusually high content of lipid (15–20%) which may affect the ability of neutrophils to kill this yeast.

Cytokine production in response to *Malassezia* has only been examined recently. Studies coculturing *Malassezia* with peripheral blood mononuclear cells (PBMNC) from healthy individuals have yielded interesting results. Supernatants from the cocultures were assayed for levels of interleukin 1β (IL-1β), IL6 and tumour necrosis factor-α (TNF-α) and all were shown to be suppressed [64,65]. This ability to suppress the release of pro-inflammatory cytokines may well contribute to the relatively non-inflammatory nature of PV and SD. Removal of the lipid from the cell wall by solvent extraction has been shown to reverse the suppressive effect on IL1 and IL6, leading to significantly increased production of these cytokines by PBMNC [66]. The ability of *Malassezia* to suppress cytokine release by PBMNC, whilst also possessing adjuvant properties seems paradoxical. It may be a reflection of the different conditions under which the organism was grown in the respective studies. The presence of large quantities of lipid in the growth medium may result in the 'suppressive' characteristics, whilst limited lipid might result in 'adjuvant' characteristics. It is interesting to speculate whether corresponding conditions on the skin might have similar effects. This finding of the involvement of cell wall lipid in immunomodulation by *Malassezia* seems likely to open up new areas of research into the interaction of *Malassezia* with the immune system.

Malassezia as pathogens

The lipophilic yeast genus *Malassezia* is now divided into seven different species, six being strictly lipid-dependent with a requirement for long-chain fatty acid supplementation in the medium to ensure the growth [67], and one (*M. pachydermatis*) for which the lipids present in rich media such as Sabouraud glucose agar are sufficient. This latter species is isolated only rarely from humans. In contrast, all six of the lipid-dependent species have been isolated from humans. These yeasts are members of the normal human cutaneous flora and can be cultured from almost all body areas [68,69]. Under the influence of predisposing factors they become pathogenic and are associated with several diseases such as PV, *Malassezia* folliculitis, SD, some forms of atopic dermatitis, some forms of confluent and reticulate papillomatosis, and even systemic infection. The non-lipid-dependent species *M. pachydermatis* has occasionally been implicated in cases of systemic infection. However, the new taxonomy must be applied to epidemiological surveys before we can conclude whether all seven *Malassezia* species have clinical relevance.

Pityriasis versicolor

PV is a chronic superficial fungal disease usually located on the upper trunk, neck or upper arms [70,71]. The disease has a worldwide distribution. In tropical areas it has been reported in 30–40% of the population whereas its incidence in temperate climates is much lower (1–4%). PV is generally a disease of postpubertal and mature individuals when the sebaceous glands are most active and is seen generally in otherwise healthy people. It is found on skin where sebaceous glands are present, preferentially in areas occluded by clothing. However, in the tropics it is more often localized on the face. Lesions are slightly scaling, papular and nummular. They may coalesce to involve large areas of the body and vary in colour from red to brown or white. The spores and short hyphae of *Malassezia* can be detected by microscopy. Due to predisposing factors the fungus changes in PV from round blastoconidia to the mycelial form. The most important exogenous factors are high temperatures and a high relative humidity which probably explain why PV is more common in the tropics. The most important endogenous factors are greasy skin, hyperhidrosis, hereditary factors, corticosteroid treatment and immunodeficiency.

Without treatment, PV is a chronic disease and after treatment recurrence is a problem, reaching 60% 1 year after treatment and 80% after 2 years. The diagnosis is based primarily on the typical clinical picture in combination with bright yellow fluorescence under Wood's light examination and direct microscopy. Direct microscopy is of major importance. Potassium hydroxide (20%) wet smears can be used, but the tape method is usually preferred. Thin layers of the stratum corneum containing the fungus can be obtained by stripping with tape. The section of the tape containing fungal elements can be stained with methylene blue (1%, 1 min); the round budding cells and hyphae ('spaghetti and meatballs') can be identified easily.

There are numerous ways of treating PV topically, with many different formulations of antifungal agents [70,71]. However, depigmentation will remain for several months after treatment. The patients should treat the whole trunk, neck, arms and legs down to the knees, even when small areas are involved. Systemic therapy is indicated primarily for extensive lesions, for lesions resistant to topical treatment, and for frequent relapse. However, with short-term treatment the risk of side-effects with systemic therapy may be minimized and oral antifungals may therefore be used even for other indications [72–74]. Recurrence is due to the presence of predisposing factors which may be difficult to eradicate. A permanent cure is therefore difficult to achieve, explaining the chronicity of this infection. Consequently, a prophylactic treatment regimen is necessary to avoid recurrence [75].

Malassezia *folliculitis*

Malassezia folliculitis is characterized by follicular papules and pustules localized on the back, chest, upper arms, sometimes the neck, and more seldom the face. It is often associated with a troublesome itching [70,76]. Under the influence of predisposing factors, *Malassezia* folliculitis may be explained by extensive growth following dominance of the yeast in the hair follicle. Local occlusion may play an important role. The inflammation may be due to metabolites produced by the fungus and to free fatty acids produced as a result of its lipase activity. The disease is more common in tropical countries and one study from the Philippines has shown a prevalence of 16·5% in patients visiting a dermatology clinic. In temperate climates, it is more common during the summer months. Acne aestivalis, with a clinical picture corresponding to *Malassezia* folliculitis, was described originally as a condition following heavy sun exposure. The disease is also seen as a complication in patients receiving oral corticosteroid or immunosuppressive treatment. It is also a complication seen quite often in patients with acquired immunodeficiency syndrome (AIDS).

The typical lesions are small follicular papules and pustules. Pruritus is often the main complaint and there is generally a discrepancy between the small lesions and the often troublesome itching. Distribution of lesions is predominant on the trunk, and itching and lack of comedones differentiate the condition from acne. However, the two diseases may often coexist with non-itching acne lesions on the face and itching lesions of *Malassezia* folliculitis on the trunk and upper arms. The diagnosis is based on a typical clinical picture with itching papules and pustules as the dominant symptoms, direct microscopy and culture in combination with histopathology, and the effect of antimycotic treatment. If a biopsy is cut serially and stained with methenamine-silver, black, round, budding yeast cells and sometimes even hyphae will be found in dilated follicles.

The effect of antimycotic treatment is often dramatic [70,76]. Most cases respond well to topical treatment. In patients with extensive lesions or in those who do not respond to topical treatment, oral ketoconazole or the triazoles fluconazole or itraconazole may be effective alternatives [70]. Lesions and itching will recur in most patients if treatment is not given intermittently. Therefore, a prophylactic treatment schedule, such as topical treatment once or twice a week, is mandatory.

Seborrhoeic dermatitis

There are now many studies indicating that *Malassezia* plays an important role in SD [70,77–79]. Many of these are treatment studies which describe the effectiveness of antimycotics, paralleled with a reduction in number of

yeasts, and recolonization leading to a recurrence of SD [70,78]. Other studies indicate that disorders of the immune function are important. The increased incidence of SD in patients with immunosuppressive disorders suggests that the relationship between *Malassezia* and the immune system is important [49,79]. Human immunodeficiency virus-positive patients, especially patients with AIDS, have an increased incidence of SD. Patients with neurological disorders such as Parkinson's disease, multiple sclerosis, stroke and even patients with mood depression also have an increased incidence of SD.

An increase in number of natural killer T-cells and decreased phytohaemagglutinin and concanavalin-A stimulation was found in peripheral blood from a large number of patients with SD in one study [79]. Low *Malassezia* serum IgG antibody titres were also detected in patients compared with controls. Other studies have found a reduced lymphocyte stimulation reaction when lymphocytes from patients with SD were stimulated with a *Malassezia* yeast extract [80]. Additionally, IL-2 and interferon-γ production by lymphocytes from patients was markedly depressed and IL-10 synthesis was increased after stimulation with *Malassezia* extract. The amount of lipid on the skin of patients with SD was significantly higher than on the skin of controls. In conclusion, impaired cell-mediated immunity may facilitate survival of these fungi on the skin and also contribute to a low T-cell dependent antibody response. The inflammatory response to *Malassezia* products would not be down-regulated and therefore an increased inflammatory response would occur and the dermatitis triggered.

SD is characterized by inflammation and desquamation in areas with a rich supply of sebaceous glands, namely the scalp, face and upper trunk. Dandruff is the mildest manifestation of the disease. It is a common disease, with a prevalence of 2–5% in different studies [79]. The skin lesions are distributed on the scalp, eyebrows, nasolabial folds, cheeks, ears, presternal and intrascapular regions, axilla and groin. The lesions are red and covered with greasy scales. Itching is common on the scalp. Complications include lichenification, secondary bacterial infections and otitis externa. The course of SD tends to be chronic with regular flare-ups. In temperate climates it improves during the summer months. The disease will flare-up during periods of mental and physical stress.

Antifungal drugs active against *Malassezia* yeasts are effective in treating most cases of SD and prophylactic antifungal treatment reduces the recurrence rate of this disease much more than corticosteroids [70,77,78].

Atopic dermatitis

There are now several studies indicating that *Malassezia* yeasts may be an important allergen in some patients with atopic dermatitis, especially in adult patients with atopic dermatitis localized to the scalp, face and neck [81]. We have found that 78% of patients with this distribution of atopic dermatitis were prick-test positive to a *Malassezia* protein extract [81]. In a double-blind study many of these patients were cured with oral ketoconazole [82]. Recently Kroger *et al.* showed that *P. ovale* extracts increased IL-4, IL-10 and IgE synthesis in patients with atopic dermatitis [83].

In conclusion, our knowledge about the pathogenesis and treatment of PV, *Pityrosporum* folliculitis, SD and atopic dermatitis has increased tremendously during the last 10 years. Today we have more effective treatments for the more severe forms of these diseases. However, due to the presence of various predisposing factors, recurrence is a major problem and to avoid this a prophylactic treatment schedule is mandatory.

Contributors

The contributors to this symposium were: **E. Guého,** *Taxonomy and phylogeny of the genus* Malassezia; **T. Boekhout,** *Molecular epidemiology of* Malassezia *infections*; **H.R. Ashbee,** *The interactions of* Malassezia *with the immune system*; **J. Faergemann,** Malassezia *as pathogens*. The co-convenors were **E. Guého** and **J. Faergemann**.

References

1 Slooff WC. Genus 6. *Pityrosporum* Sabouraud. In: Lodder J, ed. *The Yeasts, A Taxonomic Study*, 2nd edn. Amsterdam: North-Holland Publ 1970 1167–1186.

2 Baillon H (ed.). *Traité de botanique médicale cryptogamique*. Paris: Octave Doin 1889 234–235.

3 Sabouraud R. *Les maladies desquamatives. Maladies du cuir chevelu*. Paris: Masson et Cie 1904.

4 Keddie FM. Electron microscopy of *Malassezia furfur* in tinea versicolor. *Sabouraudia* 1966; **5:** 134–45.

5 Yarrow D, Ahearn DG. *Malassezia* Baillon. In: Kreger-van RIJ NJW, ed. *The Yeasts, A Taxonomic Study*, 3rd edn. Amsterdam: Elsevier Sc Publ BV 1984 882–885.

6 Simmons RB, Guého E. A new species of *Malassezia*. *Mycol Res* 1990; **94:** 1146–9.

7 Castellani A, Chalmers AJ. (eds) *Manual of Tropical Medicine*, 2nd edn. London: Baillère, Tindall and Cox 1913 1747.

8 Gordon MA. The lipophilic mycoflora of the skin. 1. *In vitro* culture of *Pityrosporum orbiculare* n. sp. *Mycologia* 1951; **43:** 524–35.

9 Weidman FD. Exfoliative dermatitis in the Indian rhinoceros (*Rhinoceros unicornis*), with description of a new species: *Pityrosporum pachydermatis*. In: Fox H, ed. *Rep Lab Museum Comp Zoo Soc*. Philadelphia 1925; 36–45.

10 Guillot J, Chermette R, Guého E. Prévalence du genre *Malassezia* chez les mammifères. *J Mycol Méd* 1994; **4:** 72–9.

11 Midgley G. The diversity of *Pityrosporum (Malassezia)* yeasts *in vivo* and *in vitro*. *Mycopathologia* 1989; **106:** 143–53.

12 Cunningham AC, Leeming JP, Ingham E, Gowland G. Differentiation of three serovars of *Malassezia furfur*. *J Appl Bacteriol* 1990; **68:** 439–46.

13 Guillot J, Guého E. The diversity of *Malassezia* yeasts confirmed by rRNA sequence and nuclear DNA comparisons. *Antonie van Leeuwenhoek* 1995; **67:** 297–314.
14 Guého E, Faergemann J, Lyman C, Anaissie EJ. *Malassezia* and *Trichosporon*, two emerging pathogenic basidiomycetous yeast-like fungi. *J Med Vet Mycol* 1994; **32** (Suppl. 1)**:** 367–78.
15 Guého E, Midgley G, Guillot J. The genus *Malassezia* with description of four new species. *Antonie van Leeuwenhoek* 1996; **69:** 337–5.
16 Guillot J, Guého E, Lesourd M, Midgley G, Chévrier G, Dupont B. Identification of *Malassezia* species: a practical approach. *J Mycol Méd* 1996; **6:** 103–10.
17 Mayser P, Haze P, Papavassilis C, Pickel M, Gründer K, Guého E. Differentiation of *Malassezia* spp. – selectivity of Cremophor EL, castor oil and ricinoleic acid for *M. furfur*. *Br J Dermatol* 1997; **137:** 208–13.
18 Ashbee HR, Ingham E, Holland KT, Cunliffe WJ. The carriage of *Malassezia furfur* serovars A, B and C in patients with pityriasis versicolor, seborrhoeic dermatitis and controls. *Br J Dermatol* 1993; **129:** 533–40.
19 Midgley G, Guého E, Guillot J. *Malassezia* species. In: Hay RJ and Ajello L, eds. *Topley & Wilson's Microbiology and Microbial Infections*. Superficial keratinophilic fungi, London: Arnold Publ 1997 Vol. 4, Part IV, Chap 12 : 201–11.
20 Guillot J, Guého E, Chermette R. Confirmation of the nomenclatural status of *Malassezia pachydermatis*. *Antonie van Leeuwenhoek* 1995; **67:** 173–6.
21 Guillot J, Guého E, Chévrier G, Chermette R. Epidemiological analysis of *Malassezia pachydermatis* isolates by partial sequencing of the large subunit ribosomal RNA. *Res Vet Sci* 1997; **62:** 22–5.
22 Guého E, Meyer SA. A reevaluation of the genus *Malassezia* by means of genome comparison. *Antonie van Leeuwenhoek* 1989; **55:** 245–51.
23 Perfect JR, Schell WA. The new fungal opportunists are coming. *Clin Infect Dis* 1996; **22:** s112–s118.
24 Hazen KC. New and emerging yeast pathogens. *Clin Microbiol Rev* 1995; **8:** 462–78.
25 Schoepfer C, Carla H, Bezou MJ, *et al. Malassezia furfur* septicemia after bone marrow graft. *Arch Pediatr* 1995; **2:** 245–8.
26 Shaparago NI, Bruno PP, Bennett J. Systemic *Malassezia furfur* infection in an adult receiving total parenteral nutrition. *J Am Osteopath Assoc* 1995; **95:** 375–7.
27 Akpata LE, Gugnoin HC, Srivastava R, Utsalo SJ. Dermatomycoses among industrial workers in cross River State, Nigeria. *Mycoses* 1992; **35:** 371–4.
28 Dankner WM, Spector SA, Fierer J, Davis CE. *Malassezia* fungaemia in neonates and adults: complication of hyperalimentation. *Rev Infect Dis* 1987; **9:** 743–53.
29 Surmont I, Gavilenes A, Vandepitte J, Eggermont E. *Malassezia furfur* fungaemia in infants receiving intravenous lipid emulsions. A rarity or just underestimated? *Eur J Pediatr* 1989; **148:** 435–8.
30 Stuart SM, Lane AT. *Candida* and *Malassezia* as nursery pathogens. *Semin Dermatol* 1992; **11:** 19–23.
31 Nichols JM, Yuen KY, Saing H. *Malassezia furfur* infection in a neonate. *Br J Hosp Med* 1993; **49:** 425–7.
32 Boekhout T, Bosboom RW. Karyotyping of *Malassezia* yeasts: taxonomic and epidemiological implications. *Syst Appl Microbiol* 1994; **17:** 146–53.
33 Silva V, Di Tilia C, Fischman O. Skin colonisation by *Malassezia furfur* in healthy children up to 15 years old. *Mycopathologia* 1996; **132:** 143–5.
34 Rapelanoro R, Mortureux P, Couprie B, Malleville J, Taieb A. Neonatal *Malassezia furfur* pustulosis. *Arch Dermatol* 1996; **132:** 190–3.
35 Larocco M, Dorenbaum A, Robinson A, Pickering LK. Recovery of *Malassezia pachydermatis* from eight infants in a neonatal intensive care nursery: clinical and laboratory features. *Pediatr Infect Dis J* 1988; **7:** 398–401.
36 Michelsen PA, Viano-Paulson MC, Stevens DA, Diaz PS. Clinical and microbiological features of infection with *Malassezia pachydermatis* in high-risk infants. *J Inf Dis* 1988; **157:** 1163–8.
37 Guého E, Simmons RB, Pruitt WR, Meyer SA, Ahearn DG. Association of *Malassezia pachydermatis* with systemic infections of humans. *J Clin Microbiol* 1987; **25:** 1789–90.
38 Welbel SF, McNeil MM, Pramanik A, *et al.* Nosocomial *Malassezia pachydermatis* blood stream infections in a neonatal intensive care unit. *Pediatr Infect Dis J* 1994; **13:** 104–8.
39 Marcon MJ, Powell DA. Human infections due to *Malassezia* spp. *Clin Microbiol Rev* 1992; **5:** 101–19.
40 Kiuchi A, Taharaguchi S, Hanazawa R, *et al.* Chromosome-sized DNA of *Malassezia pachydermatis* by pulsed-field gel electrophoresis. *J Vet Med Sci* 1992; **54:** 1219–20.
41 Boekhout T, Renting M, Scheffer WA, Bosboom R. The use of karyotyping in the systematics of yeasts. *Antonie van Leeuwenhoek* 1993; **63:** 157–63.
42 Howell SA, Qiun C, Midgley G. Karyotypes of oval cell forms of *Malassezia furfur*. *Mycoses* 1993; **36:** 263–6.
43 Van Belkum A, Boekhout T, Bosboom R. Monitoring spread of *Malassezia* infections in a neonatal intensive care unit by PCR-mediated genetic typing. *J Clin Microbiol* 1994; **32:** 2528–32.
44 Yoganathan T, Lind H, Buck GA. An electrophoretic karyotype and assignment of ribosomal genes to resolved chromosomes of *Pneumocystis carinii*. *Mol Microbiol* 1989; **3:** 1473–80.
45 Sohnle PG, Collins-Lech C. Cell-mediated immunity to *Pityrosporum orbiculare* in tinea versicolor. *J Clin Invest* 1978; **62:** 45–53.
46 Sohnle PG, Collins-Lech C. Analysis of the lymphocyte transformation response to *Pityrosporum orbiculare* in patients with tinea versicolor. *Clin Exp Immunol* 1982; **49:** 559–64.
47 Wu C, Chen Kt. Lymphocyte proliferation to crude extract of *Pityrosporum* species and natural killer activity in tinea versicolor. *J Med Assoc Thailand* 1985 70 (Suppl. **3**)**:** 45.
48 Ashbee HR, Ingham E, Holland KT, Cunliffe WJ. Cell-mediated immune responses to *Malassezia furfur* serovars A, B and C in patients with pityriasis versicolor, seborrhoeic dermatitis and controls. *Exp Dermatol* 1994; **3:** 106–12.
49 Bergbrant I-M, Johansson S, Robbins D, *et al.* An immunological study in patients with seborrhoeic dermatitis. *Clin Exp Dermatol* 1991; **16:** 331–8.
50 Cunningham AC, Ingham E, Gowland G. Humoral responses to *Malassezia furfur* serovars A, B and C in normal individuals of various ages. *Br J Dermatol* 1992; **127:** 476–81.
51 Da Mert GJ, Kirkpatrick CH, Sohnle PG. Comparison of antibody responses in chronic mucocutaneous candidiasis and tinea versicolor. *Int Arch Allergy Appl Immunol* 1980; **63:** 69–76.
52 wu yc chen Kt. Humoral immunity in patients with tinea versicolor. *J Dermatol* 1987; **12:** 161–6.
53 Furukawa F, Danno K, Imamura S, Soh Y. Histological and serological studies of *P. orbiculare* in cases of pityriasis versicolor. *J Dermatol* 1981; **8:** 27–30.
54 Ashbee HR, Fruin A, Holland KT, Cunliffe WJ, Ingham E. Humoral immunity to *Malassezia furfur* serovars A, B and C in

patients with pityriasis versicolor, seborrhoeic dermatitis and controls. *Exp Dermatol* 1994; **3:** 227–33.
55 Midgley G, Hay RJ. Serological responses to *Pityrosporum (Malassezia)* in seborrhoeic dermatitis demonstrated by ELISA and Western blotting. *Bull Soc Fr Mycol Méd* 1988; **17:** 267–76.
56 Bergbrant I-M, Faergemann J. Seborrhoeic dermatitis and *Pityrosporum ovale*: a cultural and immunological study. *Acta Dermatol-Venereol* 1989; **69:** 332–5.
57 Sohnle PG, Collins-Lech C. Activation of complement by *Pityrosporum orbiculare. J Invest Dermatol* 1983; **80:** 93–7.
58 Takahashi M, Ushijima T, Ozaki Y. Biological activity of *Pityrosporum*. I. Enhancement of resistance in mice stimulated by *Pityrosporum* against *Salmonella typhimurium. Immunology* 1984; **51:** 697–702.
59 Takahashi M, Ushijima T, Ozaki Y. Biological activity of *Pityrosporum*. II. Antitumour and immune stimulating effect of *Pityrosporum* in mice. *J Nat Cancer Inst* 1986; **77:** 1093–7.
60 Richardson MD, Shankland GS. Enhanced phagocytosis and intracellular killing of *Pityrosporum ovale* by human neutrophils after exposure to ketoconazole is correlated to changes of the yeast cell surface. *Mycoses* 1991; **34:** 29–33.
61 Akamatsu H, Komura J, Asada Y, Miyachi Y, Niwa Y. Inhibitory effect of azelaic acid on neutrophil functions: a possible cause for its efficacy in treating pathogenetically unrelated diseases. *Arch Dermatol Res* 1991; **283:** 162–6.
62 Stuart AE, Biozzi G, Stiffel C, Halpern BN, Mouton D. The stimulation and depression of reticulo-endothelial phagocytic function by simple lipids. *Br J Exp Pathol* 1960; **41:** 599–604.
63 Spratt MG, Kratzing CC. Oleic acid as a depressant of reticuloendothelial activity in rats and mice. *J Reticuloendothelial Soc* 1975; **17:** 135–40.
64 Walters Ce, AshbeeHr Eady Ea. *et al. Malassezia furfur* suppresses *in vitro* interleukin-6 release by peripheral blood mononuclear cells. *J Invest Dermatol* 1995; **105:** 492A.
65 Kesavan S, Walters CE, Holland KT, Cunliffe WJ, Ingham E. *Malassezia furfur* suppresses the release of interleukin-6 (IL-6), interleukin-1β (IL-1β) and tumour necrosis factor-alpha (TNF-α) from human peripheral blood mononuclear phagocytes *in vitro. J Invest Dermatol* 1996; **107:** 447A.
66 Kesavan S, Holland KT, Cunliffe WJ, Ingham E. The effects of de-lipidisation on the immunomodulatory activity of *Malassezia* species. *J Invest Dermatol* 1997; **108:** 389A.
67 Porro MN, Passi S, Caprill F, *et al.* Growth requirements and lipid metabolism of *Pityrosporum orbiculare. J Invest Dermatol* 1976; **66:** 178–82.
68 Gordon MA. The lipophilic microflora of the skin. *Mycologia* 1951; **43:** 524–34.
69 Faergemann J, Aly R, Maibach HI. Quantitative variations in distribution of *Pityrosporum orbiculare* on clinically normal skin. *Acta Dermatol-Venereol* 1983; **63:** 346–8.
70 Faergemann J. *Pityrosporum* infections. In: Elewski BE, ed. *Cutaneous Fungal Infections.* New York: Igaku-Shoin 1992 69–83.
71 Faergemann J. Tinea versicolor (pityriasis versicolor). In: Demis DJ, ed. *Clinical Dermatology.* Philadelphia: Lippincott-Raven 1995 1–9.
72 Jones HE. *Ketoconazole Today. A Review of Clinical Experience.* Manchester: ADIS Press 1987.
73 Delescluse J. Itraconazole in tinea versicolor. A review. *J Am Acad Dermatol* 1990; **23:** 551–4.
74 Faergemann J. Treatment of pityriasis versicolor with a single dose of fluconazole. *Acta Dermatol-Venereol* 1992; **72:** 74–5.
75 Faergemann J, Djärv L. Tinea versicolor: treatment and prophylaxis with ketoconazole. *Cutis* 1982; **30:** 542–5.
76 Back O, Faergemann J, Hörnqvist R. *Pityrosporum folliculitis*: A common disease of the young and middle-aged. *J Am Acad Dermatol* 1985; **12:** 56–61.
77 Shuster S. The aetiology of dandruff and the mode of action of therapeutic agents. *Br J Dermatol* 1984; **111:** 235–42.
78 Faergemann J. Seborrhoeic dermatitis and *Pityrosporum orbiculare*: Treatment of seborrhoeic dermatitis of the scalp with miconazole-hydrocortisone (Daktacort), miconazole and hydrocortisone. *Br J Dermatol* 1986; **114:** 695–700.
79 Bergbrant I-M. Seborrhoeic dermatitis and *Pityrosporum ovale*: cultural, immunological and clinical studies. *Acta Dermatol-Venereol* 1991; **71** (Suppl.)**:** 1–167.
80 Neuber K, Kröger S, Gruseck E, Abeck D, Ring J. Effects of *Pityrosporum ovale* on proliferation, immunoglobulin (IgA, G, M) synthesis and cytokine (IL-2, IL-10, IFNγ) production of peripheral blood mononuclear cells from patients with seborrhoeic dermatitis. *Arch Dermatol Res* 1996; **288:** 532–6.
81 Kieffer M, Bergbrant I-M, Faergemann J, *et al.* Immune reactions to *Pityrosporum ovale* in adult patients with atopic and seborrhoeic dermatitis. *J Am Acad Dermatol* 1990; **22:** 739–42.
82 Hjorth N, Clemensen OH. Treatment of dermatitis of the head and neck with ketoconazole in patients with type I hypersensitivity for *Pityrosporum orbiculare. Semin Dermatol* 1983; **2:** 26–9.
83 Kroger S, Neuber K, Gruseck E, Ring J, Abeck D. *Pityrosporum ovale* extracts increase interleukin-4, interleukin-10, and IgE synthesis in patients with atopic eczema. *Acta Dermatol-Venereol* 1995; **75:** 357–60.