

The Effects of *Malassezia* Yeasts on Cytokine Production by Human Keratinocytes

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Yeasts of *Malassezia*, members of the microbiologic flora of the skin, cause pityriasis versicolor and have also been implicated in the pathogenesis of other superficial dermatoses; the most important ones are seborrheic dermatitis, folliculitis, and atopic dermatitis. The mechanisms by which the yeasts cause these dermatoses, however, are not yet clear, and there have been no studies on the interaction between fungi and keratinocytes, especially the effects of fungi on the production of cytokines by human keratinocytes. Recently, the genus *Malassezia* has been expanded to seven species based on molecular data. In this study, we estimated the effects of *Malassezia* yeasts on cytokine (interleukins 1 β , 6, and 8, monocyte chemotactic protein-1, and tumor necrosis factor- α) production by human keratinocytes in order to examine whether the pathogenicity of the respective *Malassezia* yeasts is different from each other and to elucidate the mechanism by which *Malassezia* yeasts cause the dermatoses with different

clinical and pathologic manifestations. Variable levels of interleukin 6 and 8, and tumor necrosis factor- α in the supernatants in response to *Malassezia* yeasts (except *M. furfur*) increased from 1 to 24 h coculture, but the monocyte chemotactic protein-1 was undetectable. Furthermore, cytokine levels in the supernatants were undetectable 1–24 h after the keratinocytes were harvested with only supernatants of *Malassezia*. These results indicate that *Malassezia* stimulates cytokine production by keratinocytes, the cytokine production needs the presence of *Malassezia*, and there are differences in ability to induce cytokine production by human keratinocytes among *Malassezia* yeasts. These differences may reflect the different inflammatory responses in *Malassezia*-associated dermatoses, resulting in different clinical and pathologic manifestations. **Key words:** *interleukin/pityriasis versicolor/seborrheic dermatitis*. *J Invest Dermatol* 116:769–773, 2001

Superficial mycoses such as cutaneous candidiasis, dermatophytosis, pityriasis versicolor, and some related infections are common diseases and are generally confined to the stratum corneum in the epidermis and cutaneous appendages (Longley, 1997). The physical and chemical structure of the skin represents a form of defense against fungal pathogens. As cutaneous fungal infections are more frequent and more severe in the patients with immunologic defects, immune responses to fungal antigens would seem to play an important part in the host defense against these infections. A number of studies suggest that the epidermis of the skin represents more than a passive barrier against entry of infecting organisms; i.e., that it also acts as an immunologic organ with some unique elements (Streilein, 1983). A variety of cell types are involved in this cutaneous immune system. This system is probably responsible for initiating immune responses that work to eliminate the infecting organisms in the immune host. In addition, such responses may also produce some

of the inflammation that results in much of the symptomatology of these infections.

The epidermis is largely composed of keratinocytes, and they not only play an important structural part in forming a physical barrier to foreign antigens and microorganisms, but also are important functionally in mediating cutaneous immune reactions. For example, keratinocytes secrete a number of soluble factors that are capable of upregulating and downregulating immune responses. Interleukin (IL) -8, a potent chemoattractant for neutrophils, was reported to be induced from keratinocytes in psoriasis and by the stimulation of other cytokines (Ansel *et al*, 1990; Anttila *et al*, 1992; Jiang *et al*, 1996). IL-1 β , IL-6, monocyte chemotactic protein 1 (MCP-1), and tumor necrosis factor (TNF)- α are also important chemical mediators in the acute inflammatory phase of the healing process on skin lesions (Barker *et al*, 1990; Anttila *et al*, 1992; Kutsch *et al*, 1993; Boxman *et al*, 1996; Jiang *et al*, 1996) and are confirmed to be released from activated keratinocytes (Jiang *et al*, 1996; Barker *et al*, 1990; Anttila *et al*, 1992; Kutsch *et al*, 1993; Boxman *et al*, 1996). To the best of our knowledge, however, there have been no studies on detailed interactions between fungi and keratinocytes, especially the effects of fungi on the production of these cytokines by human keratinocytes.

Yeasts of the genus *Malassezia* are now considered synonymous with those previously named *Pityrosporum*, which are members of the microbiologic flora of the human skin and have also been found

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Abbreviations: MCP-1, monocyte chemotactic protein 1; NHEK, normal human epidermal keratinocytes.

Table I. Species and strains used in the study

Species	Strain ^a
<i>M. furfur</i>	VUT-98021 (CBS 1878)
<i>M. furfur</i>	VUT-98022 (CBS 7019)
<i>M. furfur</i>	VUT-98023 (CBS 6000)
<i>M. pachydermatis</i>	VUT-98028 (CBS 1879)
<i>M. pachydermatis</i>	VUT-98010 (Clinical isolate of cat)
<i>M. slooffiae</i>	VUT-98030 (CBS 7875)
<i>M. slooffiae</i>	VUT-98031 (CBS 7956)
<i>M. sympodialis</i>	VUT-98032 (CBS 7222)
<i>M. sympodialis</i>	VUT-98033 (CBS 7977)

^aVUT, School of Veterinary Medicine, University of Tokyo. CBS, Centraalbureau voor Schimmelmicrocultures.

in numerous other warm-blooded animals (Yarrow and Ahearn, 1984). An association with skin disease was first observed in 1846 when Eichstedt reported the presence of yeasts and filaments in material from the infected scales of pityriasis versicolor in which the mycelial phase predominates (Keddie, 1967). Yeast phase *Malassezia* has also been implicated in the pathogenesis of other superficial dermatoses, the most important ones being seborrheic dermatitis (severe and difficult to treat when associated with AIDS) (Groisser *et al*, 1989; Bergbrant and Faergemann, 1990), folliculitis (Back *et al*, 1985), and atopic dermatitis (Waersted and Hjorth, 1985). The mechanisms by which the commensal microorganisms cause these dermatoses, however, are not yet clear and several investigations failed to demonstrate any significant difference in humoral and cell-mediated immune responses specific to *Malassezia* in patients suffering from *Malassezia*-associated dermatoses when compared with controls (Ashbee *et al*, 1994a,b; Parry and Sharpe, 1998).

Recently, the genus *Malassezia* has been revised using morphology, ultrastructure, physical, and molecular biology. As a result, the genus has been enlarged to include seven species comprising the three former taxa: *M. furfur*, *M. pachydermatis*, and *M. sympodialis*, and four new taxa, *M. globosa*, *M. obtusa*, *M. restricta*, and *M. slooffiae* (Guilot and Guého, 1995, 1996). Epidemiologic studies regarding the frequency and distribution of each *Malassezia* yeast, however, have not been sufficiently performed yet and the pathogenicity of each is unknown at present. If the pathogenicity of the respective *Malassezia* yeasts is different from each other, different clinical and pathologic manifestations of several dermatoses caused by *Malassezia* infection could be explained by the difference of its pathogenicity. Neither its pathogenicity nor detailed interaction between *Malassezia* yeasts and human keratinocytes, however, have been studied previously, although there appear to be only two reports that connect the effects of *Malassezia* yeasts on pro-inflammatory cytokine production by human peripheral blood mononuclear cells *in vitro* (Walters *et al*, 1995; Kesavan *et al*, 1998). These data concerning the interaction between *Malassezia* yeasts and human peripheral blood mononuclear cells may be useful for elucidating the pathogenesis of bloodstream infections of *Malassezia* yeasts in low-birth-weight human neonates and immune deficiency patients (Chang *et al*, 1998; Lautenbach *et al*, 1998), but not *Malassezia*-associated dermatoses; this is because *Malassezia* yeasts are located in the stratum corneum and have usually no chance to come into contact with the peripheral blood cells (Longley, 1997). Therefore, we investigated the direct effects of *Malassezia* on the cytokine production by keratinocytes in order to examine whether the pathogenicity of the respective *Malassezia* yeasts is different from each other and to elucidate the mechanisms by which the yeasts cause several dermatoses with different clinical and pathologic manifestations, such as pityriasis versicolor, seborrheic dermatitis, etc.

MATERIALS AND METHODS

Fungal cultures The strains of *Malassezia* used in this study are listed in Table I. These species have been maintained by culturing on modified Dixon agar (Guého *et al*, 1996) at the Department of Dermatology, Teikyo University School of Medicine.

Keratinocyte cultures Normal human epidermal keratinocytes (NHEK) were obtained as cryopreserved first passage cells from Clonetics (BioWhittaker, San Diego, CA). NHEK were grown in T-75 cm² flasks using basal media (KBM-2; 0.15 mM Ca²⁺) supplemented with bovine pituitary extract (7.5 mg per ml), human recombinant epidermal growth factor (0.1 ng per ml), insulin (5 µg per ml), hydrocortisone (0.5 µg per ml), epinephrine, transferrin, gentamicin (50 µg per ml), and amphotericin-B (50 ng per ml) (KGM-2; Clonetics) at 37°C/5% CO₂. The media was replaced every 2–3 d. When near confluence (70–90%), NHEK were disaggregated using 0.025% trypsin/0.01% ethylenediamine tetraacetic acid in HEPES and either subcultured for experimental use or cryopreserved in liquid nitrogen for future purposes. All experimental procedures were performed using second to fourth passage cells.

Co-culture of keratinocytes with *Malassezia* yeasts Co-culture of NHEK with *Malassezia* yeasts was carried out according to the method by Jiang *et al* (1996). *Malassezia* yeasts were harvested after 3 d of growth in Sabouraud's liquid medium containing 0.5% of Tween 40 at 37°C. These yeast cells were washed twice in phosphate-buffered saline. The yeast cells were resuspended in serum-free keratinocyte growth medium (Clonetics), respectively.

In preliminary studies, it was determined that the number of yeast cells per NHEK needed for optimal stimulation varied widely between the tested yeast (data not shown). Based on these studies, 1:1 ratio of yeast cells to keratinocytes was chosen for subsequent experiments. Therefore, the NHEK (1 × 10⁴ cells per well) were incubated on to the 96 wells of the cell culture plate at 37°C, 5% CO₂ for 16 h. After that, 1 × 10⁴ of *Malassezia* yeasts was applied to the wells of the keratinocytes. Cell viability was then checked by the Trypan blue exclusion tests. After 1–24 h of co-culture, supernatants were obtained and kept at –20°C until use for cytokine enzyme-linked immunosorbent assay (ELISA).

Co-culture of keratinocytes with *Malassezia* culture supernatants *Malassezia* yeasts (1 × 10⁸) were cultured in 10 ml serum-free keratinocyte growth medium (Clonetics) at 37°C for 14 h. Yeast cells were centrifuged at 300 × g for 5 min to remove the cells. The supernatant was harvested and filtrated by 0.22 µm of cellulose acetate filter. The keratinocytes (1 × 10⁴ cells) were incubated with 300 µl of the supernatant at 37°C/5% CO₂. After 1–24 h of culture, the supernatants were obtained and kept at –20°C until use for cytokine ELISA.

Control samples The keratinocytes (1 × 10⁴ cells per well) were incubated in three wells and after 1–24 h of culture, supernatants were obtained and the cells were also harvested. An equivalent amount of serum-free keratinocyte growth medium (Clonetics) was added to the cells, and they were lysed by freeze/thaw two times. Lysate were centrifuged at 900 × g for 5 min to remove cellular debris. The supernatant and keratinocyte lysate were obtained as control samples for later assay.

On the other hand, 1 × 10⁴ of *Malassezia* yeasts were cultured in 300 µl serum-free keratinocyte growth medium (Clonetics) at 37°C for 24 h. Yeast cells were centrifuged at 300 × g for 5 min to remove the cells. The supernatant was harvested and filtrated by 0.22 µm of cellulose acetate filter. The supernatant was also obtained as control sample for later assay.

Lipopolysaccharide (from *Escherichia coli*; Sigma; 20 ng per well) served as a positive control.

Lactate dehydrogenase (LDH) assay This was carried out according to the manufacturer's instructions using a Cytotoxicity Detection kit (Boehringer Mannheim). LDH is a stable cytoplasmic enzyme present in all cells and is rapidly released into the cell culture supernatant upon damage of the plasma membrane. LDH activity was determined by a coupled enzymic reaction whereby the tetrazolium salt (INT) was reduced to formazan. An increase in the number of dead or damaged cells resulted in an increase in LDH activity in the culture supernatant.

ELISA for cytokines Cytoscreen Immunoassay kit (BioSource International, CA) was used. All ELISA kits were used according to the instruction manuals. The cytokines ELISA were sensitive to ≥ 15 pg per ml.

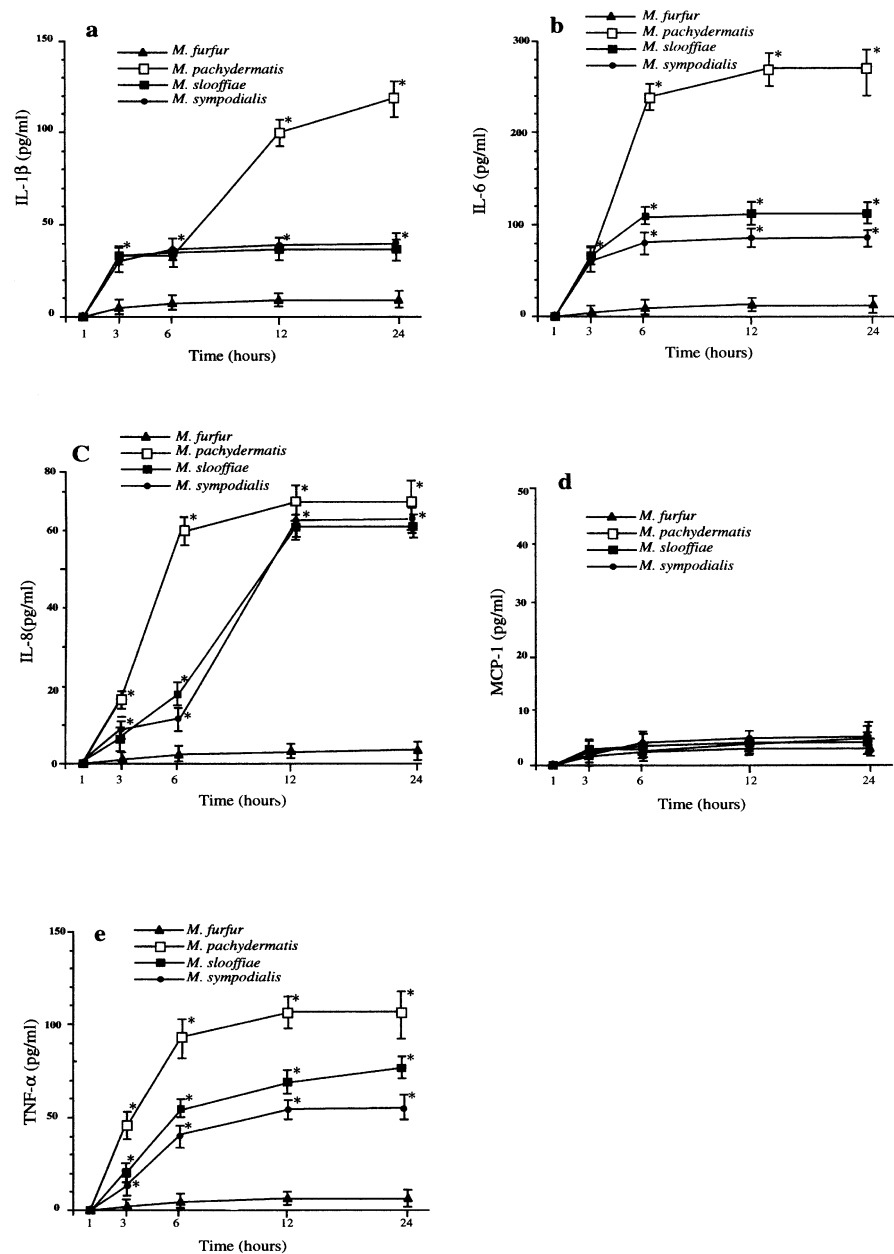


Figure 1. Effect of *Malassezia* yeasts (*M. furfur*, *M. pachydermatis*, *M. slooffiae*, *M. sympodialis*) on cytokine production by human keratinocytes. Keratinocytes were co-cultured with *Malassezia* yeasts. After 1 h, 3 h, 6 h, 12 h, and 24 h, the cytokine levels in the supernatants (a: IL-1 β , b: IL-6, c: IL-8, d: MCP-1, e: TNF- α) were measured by the ELISA method. In the supernatants co-cultured with *M. furfur*, the cytokines are low or undetectable. In the supernatants co-cultured with *Malassezia* yeasts other than *M. furfur*, however, the IL- β , IL-6, IL-8, and TNF- α are detected and increased following co-culture, whereas the MCP-1 protein is low or undetectable. Results shown are representative of five experiments. Asterisks indicate statistically significant differences ($p < 0.05$) versus untreated cells.

Statistics All experiments were carried out with five replicates; results are expressed as means $\pm$ SD. Comparisons between tests were done by the Student's *t* test, with statistical significance considered to be $p < 0.05$.

RESULTS

Cytokine production by NHEK co-cultured with *Malassezia* yeasts After 1–24 h of co-culture of NHEK with each *Malassezia* yeasts, the IL-1 β , IL-6, IL-8, MCP-1, and TNF- α protein levels in the supernatant were determined by ELISA. **Figure 1** depicts the results of this experiment. In the supernatants co-cultured with *M. furfur*, the levels of cytokines examined in this study (IL-1 β , IL-6, IL-8, MCP-1, and TNF- α) were low or undetectable (**Fig 1**). In the supernatants co-cultured with *Malassezia* yeasts other than *M. furfur*, however, the IL-1 β , IL-6, IL-8, and TNF- α were detected and increased following co-culture, whereas the MCP-1 protein was low or undetectable in the supernatants co-cultured with any *Malassezia* yeasts (**Fig 1**). The cytokine levels were higher in the supernatant co-cultured with *M. pachydermatis* than in that with *M. slooffiae* and *M. sympodialis* (**Fig 1**).

Cytokine production by NHEK co-cultured with *Malassezia* culture supernatant After 1–24 h, keratinocytes were incubated with culture supernatant of each *Malassezia*, the IL-1 β , IL-6, IL-8, MCP-1, and TNF- α were low or undetectable (**Fig 2**).

Controls In the controls where only keratinocyte culture supernatant, *Malassezia* culture supernatant, and cell lysate, respectively, present, no IL-1 β , IL-6, IL-8, MCP-1, or TNF- α proteins were detected at any time after culturing. Lipopolysaccharide (1 μ g per ml) induced significant amounts of IL-1 β , IL-6, IL-8, and TNF- α but not MCP-1.

Cell viability The release of cytokines was not due to cell lysis; LDH, as a cytoplasmic marker, was found to be present in the supernatant of stimulated cells at levels similar to those detected in the supernatant of unstimulated cells. Overall, growth and viability of NHEK, as assessed by the LDH assay, did not vary significantly and NHEK remained viable throughout the 24 h co-culture period.

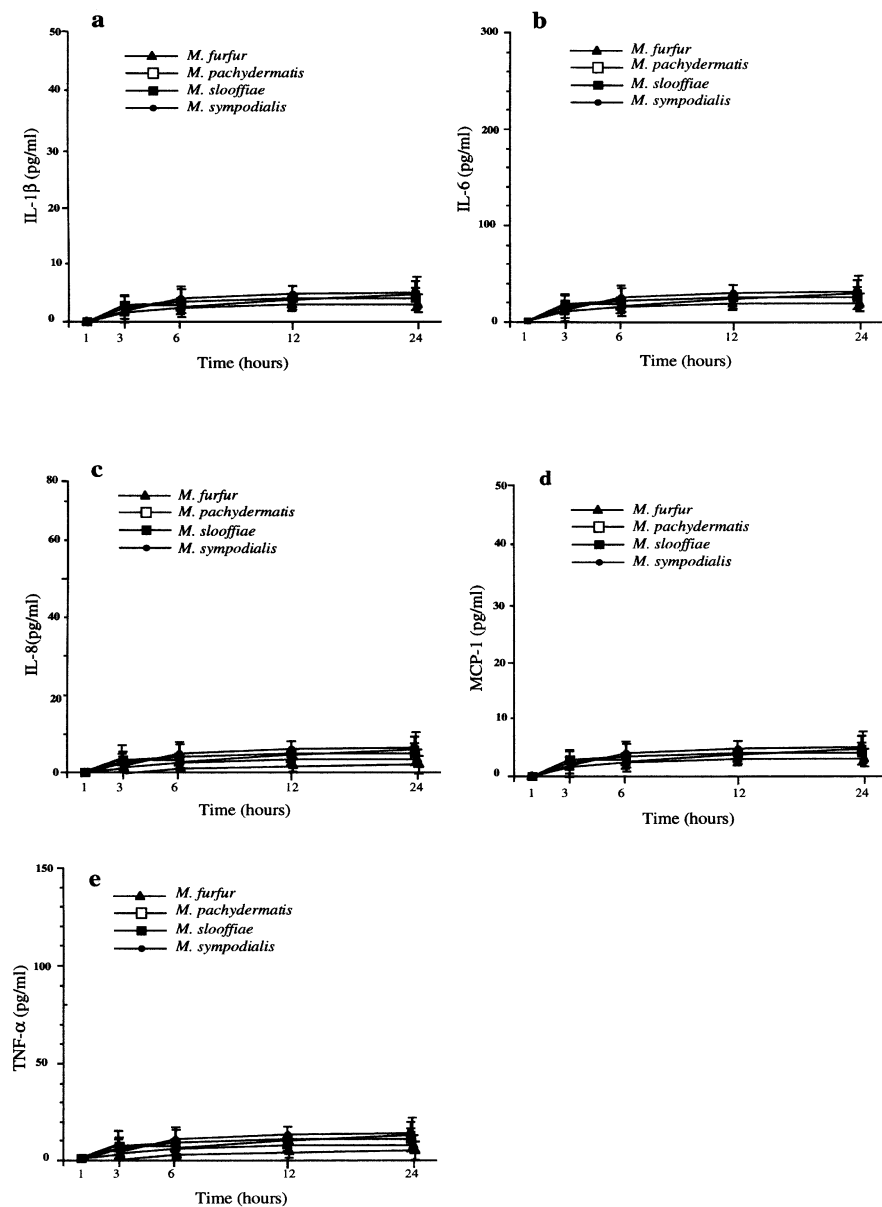


Figure 2. Effect of culture supernatants of *Malassezia* yeasts (*M. furfur*, *M. pachydermatis*, *M. slooffiae*, *M. sympodialis*) on cytokine production by human keratinocytes. Keratinocytes were cultured with the supernatant of the *Malassezia* yeasts. The cytokine levels in the supernatants (a: IL-1 β , b: IL-6, c: IL-8, d: MCP-1, e: TNF- α) were measured by the ELISA method, 1 h, 3 h, 6 h, 12 h, and 24 h after keratinocytes were harvested with only culture supernatants of *Malassezia* yeasts. All cytokines examined in this study are low or undetectable at any time after culturing. Results shown are representative of five experiments.

DISCUSSION

The etiology of seborrheic dermatitis is poorly understood. Although there are arguments that *Malassezia* yeasts are not the causative organisms but are merely present in large numbers, there are data that support the concept that *Malassezia* also contributes to the pathogenesis of seborrheic dermatitis. First, several compounds with anti-fungal activity are effective treatments (Ford *et al*, 1984; Marks *et al*, 1985; Skinner *et al*, 1985; Faergemann, 1986). Secondly, the reduction in the number of *Malassezia* on the skin during treatment correlates with clinical improvement (Marks *et al*, 1985; Skinner *et al*, 1985; Faergemann, 1986). Thirdly, if seborrheic dermatitis recurs, it does so when numbers of the organism increase again (Faergemann, 1986). These data suggest strongly that anti-fungal treatment for seborrheic dermatitis act by an anti-fungal mechanism. It is presently unknown, however, how *Malassezia* yeasts, which are not only a member of the normal human cutaneous flora, but also a causative organism of pityriasis versicolor, cause the dermatitis.

In this study, we estimated the effect of *Malassezia* on cytokine production by human keratinocytes and demonstrated that causative fungi of superficial mycoses could induce cytokine production by human keratinocytes and that there were differences in their ability to induce cytokine production by human keratinocytes among the *Malassezia* yeasts. These differences in cytokine production may reflect different inflammatory responses, i.e., inflammatory infiltrate in the skin among several dermatoses related to *Malassezia* infection. For example, the IL-1 β , IL-6, IL-8, and TNF- α were detected in the supernatant, and increased following co-culture with *M. pachydermatis*, *M. slooffiae*, and *M. sympodialis*, whereas the cytokines in the supernatants co-cultured with *M. furfur* were low or undetectable. These results indicate that *M. furfur* causes no inflammatory response in the skin and may be a member of the microbiologic flora of the skin. The other *Malassezia* yeasts, however, can cause clinically and histologically different dermatoses, according to their ability to induce cytokine production by human keratinocytes. For example, some *Malassezia* yeasts stimulate IL-8 production by human keratinocytes, resulting in a

neutrophilic infiltration in the *Malassezia*-associated dermatoses, such as seborrheic dermatitis and *Malassezia* folliculitis. Interestingly, MCP-1, a potent chemoattractant for monocytes, could not be detected in the supernatants co-cultured with any *Malassezia* yeasts examined in this study. These results correspond to the histopathologic features of seborrheic dermatitis: superficial perivascular and interstitial infiltrate of lymphocytes sometimes accompanied by a few neutrophils in the dermis and focal exocytosis of lymphocytes and occasional pyknotic neutrophils within parakeratotic foci in the epidermis, but no monocyte infiltrate is observed (Ackerman *et al*, 1997; Cohen *et al*, 1997). Therefore, the effects of *Malassezia* on cytokine production by human keratinocytes could explain the pathogenic mechanisms by which *Malassezia* yeasts cause seborrheic dermatitis.

In addition, the cytokine levels were higher in the supernatant co-cultured with *M. pachydermatis* than in that with *M. slooffiae* and *M. sympodialis*. *M. pachydermatis* was first described in 1925 in a rhinoceros with exfoliative dermatitis (Weidman, 1925) and was subsequently found to be associated with otitis media and otitis externa in dogs (Abou-Gabal *et al*, 1979). In general, these animal dermatoses are clinically and histologically more severe than *Malassezia*-associated human dermatoses. Therefore, the difference in ability to induce cytokine production between *M. pachydermatis* and the other *Malassezia* yeasts might reflect the difference in severity between animal and human dermatoses.

On the other hand, after 1–24 h, keratinocytes were incubated with only culture supernatants of *Malassezia* yeasts, the IL-1 β , IL-6, IL-8, MCP-1, and TNF- α were low or undetectable. These results suggest that *Malassezia* cannot release a factor to stimulate cytokine production by human keratinocytes and that cell to cell contact between *Malassezia* yeasts and human keratinocytes play an important part in cytokine production by human keratinocytes.

Our findings provide a clue to the solution in the heated debate as to whether the commensal microorganisms, *Malassezia* yeasts, directly cause not only pityriasis versicolor, but also other dermatoses such as seborrheic dermatitis, folliculitis, atopic dermatitis, etc.

REFERENCES

- Abou-Gabal M, Chastain CB, Hogle RM: *Pityrosporum (pachydermatis) "canis"* as a major cause of otitis externa in dogs. *Mykosen* 22:192–199, 1979
- Ackerman AB, Chongchitnant N, Sanchez J, Guo Y, Bennin B, Reichel M, Randall MB: *Histologic Diagnosis of Inflammatory Skin Diseases*, 2nd edn. Baltimore: Williams & Wilkins, 1997, pp 722–726
- Ansel J, Perry P, Brown J, *et al*: Cytokine modulation of keratinocyte cytokines. *J Invest Dermatol* 94:101S–107S, 1990
- Anttila HSI, Reitamo S, Erkkö P, Ceska M, Moser M, Baggiolini M: Interleukin-8 immunoreactivity in the skin healthy subjects and patients with palmoplantar pustulosis and psoriasis. *J Invest Dermatol* 98:96–101, 1992
- Ashbee HR, Fruin A, Ingham E, Holland KT, Cunliffe WJ: Humoral immunity to *Malassezia furfur* serovars A, B and C in patients with pityriasis versicolor, seborrheic dermatitis and controls. *Exp Dermatol* 3:1–7, 1994a
- 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, seborrheic dermatitis and controls. *Exp Dermatol* 3:106–112, 1994b
- Back OJ, Faergemann J, Hornqvist R: *Pityrosporum* folliculitis: a common disease of the young and middle aged. *J Am Acad Dermatol* 12:56–61, 1985
- Barker JNW, Sarma V, Mitra RS, Dixit VM, Nickoloff BJ: Marked synergism between tumor necrosis factor- α and interferon- γ in regulation of keratinocyte-derived adhesion molecules and chemotactic factors. *J Clin Invest* 85:605–608, 1990
- Bergbrant IM, Faergemann J: The role of *Pityrosporum ovale* in seborrheic dermatitis (review). *Semin Dermatol* 9:262–268, 1990
- Boxman ILA, Ruwof C, Boerman OC, Löwik CWGM, Ponc M: Role of fibroblasts in the regulation of proinflammatory interleukin IL-1, IL-6 and IL-8 levels induced by keratinocyte-derived IL-1. *Arch Dermatol Res* 288:391–398, 1996
- Chang HJ, Miller HL, Watkins N, *et al*: An epidemic of *Malassezia pachydermatis* in an intensive care nursery associated with colonization of health care worker's pet dog. *N Engl J Med* 338:706–711, 1998
- Cohen LM, Skopicki DK, Harist TJ, Clark WH Jr: Noninfectious vesiculobullous and vesiculopustular diseases. In: Elder D (ed.) *Lever's Histopathology of the Skin*, 8th edn. Philadelphia: Lippincott-Raven Publishers, 1997, pp 215–216
- Faergemann J: Seborrheic dermatitis and *Pityrosporum orbiculare*: treatment of seborrheic dermatitis of the scalp with miconazole-hydrocortisone (Daktacort), miconazole and hydrocortisone. *Br J Dermatol* 114:695–700, 1986
- Ford GP, Farr PM, Ive FA, Shuster S: The response of seborrheic dermatitis to ketoconazole. *Br J Dermatol* 111:603–607, 1984
- Groisser D, Bottone EJ, Lebowitz M: Association of *Pityrosporum orbiculare* (*Malassezia furfur*) with seborrheic dermatitis in patients with acquired immune deficiency syndrome (AIDS). *J Am Acad Dermatol* 20:770–773, 1989
- Guého E, Midgley G, Guilot J: The *Malassezia* with description of four new species. *Antonie Van Leeuwenhoek* 69:337–355, 1996
- Guilot J, Guého E: The diversity of *Malassezia* yeast confirmed by rRNA sequences and nuclear DNA comparisons. *Antonie Van Leeuwenhoek* 67:297–314, 1995
- Jiang Y, Russell TR, Graves DT, Cheng H, Hong S, Levitz AM: Monocyte chemoattractant protein 1 and interleukin-8 production in mononuclear cells stimulated by oral microorganisms. *Infect Immun* 64:4450–4455, 1996
- Keddie FM: Electron microscopy of *Malassezia furfur* in tinea versicolor. *Sabouraudia* 5:134–137, 1967
- Kesavan S, Walters CE, Holland T, Ingham E: The effect of *Malassezia* on pro-inflammatory cytokine production by human peripheral blood mononuclear cells in vitro. *Med Mycol* 36:97–106, 1998
- Kutsch CL, Norris DA, Arend WP: Tumor necrosis factor- α induces interleukin-1 α and interleukin-1 receptor antagonist production by cultured human keratinocytes. *J Invest Dermatol* 101:79–85, 1993
- Lautenbach E, Nachamkin I, Schuster MG: *Malassezia pachydermatis* infection. *N Engl J Med* 339:270, 1998
- Longley BJ: Fungal disease. In: Elder D (ed.). *Lever's Histopathology of the Skin*, 8th edn. Philadelphia: Lippincott-Raven Publishers, 1997, pp 5517–5525
- Marks R, Pearce AD, Walker AP: The effects of a shampoo containing zinc pyrithione on the control of dandruff. *Br J Dermatol* 112:415–422, 1985
- Parry ME, Sharpe GR: Seborrheic dermatitis is not caused by an altered immune response to *Malassezia* yeast. *Br J Dermatol* 139:254–263, 1998
- Skinner RB Jr, Noah PW, Taylor RM, Zanolli MD, West S, Guin JD, Rosenberg EW: Double-blind treatment of seborrheic dermatitis with 2% ketoconazole cream. *J Am Acad Dermatol* 12:852–856, 1985
- Streilein JW: Skin-associated lymphoid tissues (SALT): origins and functions. *J Invest Dermatol* 80:12S–16S, 1983
- Waersted A, Hjorth N: *Pityrosporum orbiculare*—a pathogenic factor in atopic dermatitis of the face, scalp and neck? *Acta Dermatol Venereol* 114:146–148, 1985
- Walters CE, Ingham E, Eady A, Cove J, Kearney JN, Cunliffe WJ: In vitro modulation of keratinocyte-derived interleukin 1 α (IL-1 α) and peripheral blood mononuclear cell-derived IL-1 β release in response to cutaneous commensal microorganisms. *Infect Immun* 63:1223–1228, 1995
- Weidman FD: Exfoliative dermatitis in the Indian rhinoceros (*Rhinoceros unicornis*) with description of a new yeast species, *Pityrosporum pachydermatis*. *Rep Lab Mus Comp Pathol Zool Soc* 53:36–44, 1925
- Yarrow D, Ahearn DG: Genus 7: *Malassezia* baillon. In: Kreger-Van Rijn NJW (ed.). *The Yeasts, a Taxonomic Study*, 3rd edn. Amsterdam: Elsevier Science Publishers, 1984, pp 882–885