Cell-mediated immunity to *Malassezia furfur* in patients with seborrhoeic dermatitis and pityriasis versicolor

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Summary

The lymphocyte transformation response to *Malassezia furfur*, *Candida albicans*, phytohaemagglutinin, concanavlin A and tuberculin purified protein derivative of 12 patients with pityriasis versicolor, 15 patients with seborrhoeic dermatitis and matched controls, was studied. Patients with pityriasis versicolor showed a significantly lower response to *M. furfur* than patients with seborrhoeic dermatitis and controls.

Introduction

Malassezia (*Pityrosporum*) species are members of the normal cutaneous flora,[1] and are also associated with the conditions pityriasis versicolor and seborrhoeic dermatitis. The genus *Malassezia* (*Pityrosporum*) can now be devided into six lipophilic and one nonlipophlic species.[2,3] The nonlipophilic species (sp.) *M. pachydermatis* is found primarily in animals and the liphophilic spp. are found primarily in humans: it is not known which of the lipophilic spp. is associated with pityriasis versicolor or seborrhoeic dermatitis. In pityriasis versicolor *Malassezia* change, according to predisposing factors, from a saphrophytic form to a pathogenic mycelial form. Predisposing factors are high temperature and humidity, profuse sweating, hereditary factors, steroid medication and malignant lymphomas. Altered cell-mediated immunity has been discussed and severe pityriasis versicolor has been described in patients with visceral leishmaniasis, a disease associated with depressed cell-mediated immunity.[4]

The connection between the lipophilic *Malassezia* spp. and seborrhoeic dermatitis has been clearly demonstrated in a number of treatment studies[5–7] but the mechanism responsible for the induction of the skin lesions is still unclear; this cannot be simply enhanced growth of the organism as a number of quantitative studies have shown no difference in the number of yeast cells between patients and healthy controls.[8,9] An abnormal immune response to *M. furfur* in patients with seborrhoeic dermatitisis is a more likely explanation. The frequent association of HIV-infection and seborrhoeic dermatitis has been suggested to be due to a suppressed cell-mediated immunity.[10,11]

Malassezia can activate complement by both the classical and alternative pathway.[12] The humoral immune response to *Malassezia* in patients with seborrhoeic dermatitis and pityriasis versicolor has been studied using different antigen preparations and different techniques: elevated titres in patients compared with controls, as well as no differences in titres, have been reported.[13–18] A cell-mediated immune response to *Malassezia* has been demonstrated by lymphocyte transformation tests in healthy individuals.[19] Lymphocyte transformation in patients with pityriasis versicolor and in patients with seborrhoeic dermatitis has been reported with divergent results.[20–24]

The aim of this investigation was to study the lymphocyte transformation response in patients with pityriasis versicolor and seborrhoeic dermatitis to a *M. furfur* extract, phytohaemagglutinin (PHA), concanavlin A (ConA), tuberculin purified protein derivative (PPD) and *Candida albicans*.

Materials and methods

Subjects

All patients with pityriasis versicolor and seborrhoeic

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dermatitis were examined by either of two experienced dermatologists. Patients who had taken systemic antifungal or immune-modulating agents during the previous month were excluded. Healthy age-matched controls were selected for all serum samples. Most of the healthy controls were hospital staff. The controls were examined by the same dermatologists as the patients and no history of or ongoing seborrhoeic dermatitis or HIV infection was reported.

Twelve patients with pityriasis versicolor, six males and six females, mean age 33 years (range, 22–45 years), and 12 controls, all female, mean age 32 years (range, 19–49 years) were included.

Fifteen patients with seborrhoeic dermatitis, 14 males and one female, mean age 39 years (range, 24–57 years), and 15 controls, three males and 12 females, mean age 39 years (range, 23–61 years) were also included in the study.

Antigens

M. furfur. M. furfur ATCC 42132 cells were harvested after 3 days growth at 37°C by washing the colonies from the surface of a solid medium containing olive oil, glycerol monostearate and Tween-80[25] and washing twice in sterile PBS, pH 7.2. The suspension of yeast cells was made as concentrated as possible (27 g/mL). The antigen was then prepared by the freeze-pressure method according to Edebo.[26] The material to be freeze-pressed is frozen such that cylindrical rods which fit into the pressure chamber are formed; a piston driven by a hydraulic pump forces the material through an orifice. At a sample temperature of 35°C and a press temperature of −20°C, ≈ 90% disruption was achieved when the yeast suspension was pressed through the orifice of the pressure chamber in a smooth flow. This antigen extract contains not only protein but all of the cell constituents.

C. albicans. The protein extract of *C. albicans*, Solu-Prick (ALK, Horsholm, Denmark), was prepared by extraction of the raw material under physiological conditions, followed by dialysis and lyophilization.[27]

PPD. Tuberculin PPD RT 23 SSI was from SBL (Stockholm, Sweden).

Mitogens

Con-A was from Miles Yeada (Rehovat, Israel) and PHA was from (Difco Laboratories, MI, USA).

Lymphocyte proliferation assays

Mononuclear cells were prepared from heparinized venous blood by centrifugation on Ficoll-sodium metrizoate lymphoprep (Nyegard, Denmark). The cells were diluted to a concentration of 5×10^5 cells/mL in Iscove's modified Dulbecco's medium (Gibco, Paisley, Scotland), supplemented with 10% pooled heat-inactivated human AB serum, 2 mmol/L L-glutamine and 100 μg/mL gentamicin. Triplicate cultures containing 0.2 mL of the lymphocyte suspension and 10 μg antigen or 10 μL mitogen at a concentration of 100 μg/mL were prepared on Linbro Tissue Culture grade microtitre plates and incubated at 37°C in a 5% CO_2 atmosphere for 6 days (3 days for PHA and ConA) before 1 μCi of [H^3]thymidine (Amersham International, UK) was added to each well. After overnight incubation, the incorporation of [H^3]thymidine was determined by liquid scintillation as described by Robbins *et al.*[28] The geometric mean of the triplicate wells was determined, and the stimulation ratio was calculated by dividing the mean obtained with antigen by the mean incorporation in three wells receiving only PBS (i.e. unstimulated wells). For PHA and ConA the results were expressed as ratios between the counts of the patient sample and the mean count of three healthy blood donors analysed stimultaneously (stimulation index).

Statistical analysis

For paired data the significance was evaluated using Wilcoxon's signed ranks test. For independent means Student's *t*-test was used. $P<0.05$ was considered significant.

Results

Proliferation

In healthy volunteers and in patients with pityriasis versicolor and seborrheic dermatitis, *M. furfur* antigen induced significantly enhanced lymphocyte proliferation (mean, 32101; SD, 22421) compared to unstimulated control lymphocytes (mean, 5855; SD, 15283) ($P<0.01$). However, the figure for this stimulation was significantly lower in patients with pityriasis versicolor than for control subjects ($P<0.001$) (Fig. 1). There was no significant difference in lymphocyte proliferation after stimulation with *M. furfur* antigen between patients with seborrhoeic dermatitis and controls (Fig. 1). Lymphocyte stimulation with PHA, ConA, PPD and *C. albicans* showed no significant difference between the groups (Table 1).

Discussion

With this method, using a *M. furfur* antigen obtained by

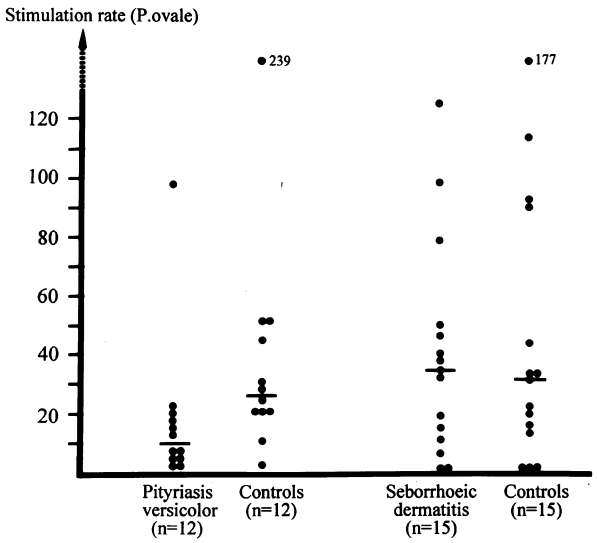

Figure 1 Lymphocyte proliferation ratio after stimulation with *M. furfur*. Results are given as the ratios between the count of the patient serum stimulated with *M. furfur* and the count of the patient unstimulated sample. Bars represent the median counts.

disintegration of cells under freeze-pressure, a significantly lower lymphocyte response was found in patients with pityriasis versicolor compared to controls but no difference was found between patients with seborrhoeic dermatitis compared to controls. In both patients with pityriasis versicolor and those with seborrhoeic dermatitis, no deficiency in cell-mediated immunity was found in lymphocyte transformation tests when two conventional mitogens, PHA and ConA, and two antigens, PPD and *C. albicans*, were used.

Our results are in contrast with the results of Wu and Chen, who found that 30 patients with pityriasis versicolor had a higher lymphocyte responsiveness to *Malassezia* antigen than the controls.[21] Ashbee, who found no significant difference in immune response in 10 patients with pityriasis versicolor, used intact whole cells suspensions of *M. furfur* serovar A, B and C.[22] Sohnle and Collins-Lech reported similar results, with no difference in lympocyte transformation responses to a *Malassezia* antigen between 12 patients with pityriasis versicolor and 15 healthy contols.[20] However, in a subsequent study they found significantly lower responsees to a *Malassezia* antigen in 18 patient with pityriasis versicolor compared to 42 healthy controls after 6 days of incubation, but not after 9 days.[29]

In patients with seborrhoeic dermatitis, Wikler *et al.* demonstrated a reduced lymphocyte transformation response to a *Malassezia* antigen in eight out of 12 patients, compared to eight healthy controls.[24] Similar results have been reported by Neuber *et al.* who observed an increased lymphocyte response to a *Malassezia* antigen in a control group, but not in patients with seborrhoeic dermatitis.[23] Ashbee found that lymphocytes from significantly more patients with seborrhoeic dermatitis responded to Malassezia serovar B and C compared to healthy controls.[22]

Our finding of a significantly lower lymphocyte responsiveness to a *M. furfur* extract in patients with pityriasis versicolor compared to healthy controls could be due to the antigen preparation. We used a *M. furfur* antigen obtained by disintegration of cells under freeze-pressure. The advantage of freeze-pressing is that it is more effective than sonic disintergration and it preserves enzymes and other intracellular components better. The extract is very crude but it contains all parts of the cell and is therefore used as a reference extract. In earlier studies, mainly intact whole cell extracts have been used.[20–24] When intact whole cell extracts are used the components in the surface of the cell will be dominant. It has recently been shown that *M. furfur* is capable of supressing release of pro-inflammatory cytokines by peripheral blood mononuclear cells and that removal of lipids from the cell wall negates this ability.[30]

The diminshed response found in this study in patients with pityriasis versicolor could be one of the reasons for the minor inflammatory response in this disease and the enhanced growth of *M. furfur*.

The mechanism by which *M. furfur* induces the lesions in seborrhoeic dermatitis is still unclear. In this

Table 1 Stimulation ratio in patients with pityriasis versicolor, seborrhoeic dermatitis and controls (mean ± SD).

	C. albicans	PPD	PHA	ConA
Patients with pityriasis versicolor ($n = 12$)	20.9 ± 22.5	16.9 ± 23.6	1.0 ± 0.4	1.3 ± 0.5
Matched controls to patients with pityriasis versicolor ($n = 12$)	21.0 ± 31.3	33.7 ± 43.0	1.2 ± 0.4	1.2 ± 0.3
Patients with seborrhoeic dermatitis ($n = 15$)	16.1 ± 9.9	18.8 ± 11.6	0.7 ± 0.4	0.7 ± 0.4
Matched controls to patients with seborrhoeic dermatitis ($n = 15$)	22.0 ± 13.6	28.9 ± 14.6	0.8 ± 0.4	0.7 ± 0.4

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study, we could not show an altered immune response to a *M. furfur* extract. *M. furfur* is known to have lipase activity and this activity, together with the ability of *M. furfur* to activate complement, could contribute to nonspecific skin inflammation. The role of cytokines in the inflammatory process in seborrhoeic dermatitis is interesting: interleukin 1, interleukin 6 and tumour necrosis factor-α have been shown to be supressed in studies in which *M. furfur* was cocultured with peripheral blood mononuclear cells from healthy individuals.[30]

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