*British Journal of Dermatology* 2001; **144:** 77–84.

# Cell-mediated immunity to the mycelial phase of *Malassezia* spp. in patients with pityriasis versicolor and controls

M.R.SAADATZADEH, H.R.ASHBEE, W.J.CUNLIFFE* AND E.INGHAM

*Division of Microbiology and *Department of Dermatology, Skin Research Centre, University of Leeds, Leeds LS2 9JT, U.K.*

Accepted for publication 9 August 2000

**Summary**

*Background* *Malassezia* is the aetiological agent of pityriasis versicolor. The mycelial phase of the organism predominates in lesions of pityriasis versicolor.

*Objectives* To evaluate the cell-mediated immune (CMI) response to the mycelial phase of *Malassezia* in patients with this disease, which has not previously been studied.

*Methods* The CMI status of 12 patients with pityriasis versicolor and 12 age- and sex-matched controls to mycelial antigen(s) of the organism was examined. The responses to the mycelial form of three strains of the organism were assessed using lymphocyte transformation and leucocyte migration inhibition assays.

*Results* The transformation responses of the lymphocytes from both patients and controls gave transformation indices $\leq 3$, although the responses of lymphocytes from patients with pityriasis versicolor to the mycelial form of *Malassezia* strains were generally higher than those of the controls. In the leucocyte migration inhibition assay, leucocytes from patients with pityriasis versicolor and controls responded to the mycelial antigens of three different *Malassezia* strains; however, there was no significant difference in leucocyte response between patients with pityriasis versicolor and controls.

*Conclusions* Patients with pityriasis versicolor do not therefore have a CMI deficiency to *Malassezia* mycelial antigens but fail to generate a protective CMI response to mycelial antigens over and above that of control individuals during active disease.

*Key words*: cell-mediated immune response, leucocyte migration inhibition, lymphocyte transformation, *Malassezia* species, mycelial antigens, pityriasis versicolor

*Malassezia*, a dimorphic and lipophilic yeast, forms part of the normal flora of human skin. The yeast phase predominates on normal skin and is found in areas rich in sebaceous lipids such as the head, trunk and upper back.[1] *Malassezia* is also the aetiological agent of pityriasis versicolor in which the mycelial phase of the organism predominates.[2] The factors which are involved in the transformation of the yeast to the mycelial phase, and in the development of pityriasis versicolor, are poorly understood.

The taxonomic classification of Guého *et al.*[3] described six lipophilic species of *Malassezia*: *M. furfur*, *M. sympodialis*, *M. globosa*, *M. obtusa*, *M. restricta* and *M. slooffiae*. The commonest residents of human skin were reported to be *M. sympodialis*, *M. globosa* and *M. restricta*, corresponding to the previously recognized serovars A, B and C.[4] Ashbee *et al.*[5] had previously studied the carriage of serovars A, B and C in patients with pityriasis versicolor and found no difference in the prevalence of the three serovars on lesional skin compared with the same skin sites of normal healthy controls. This indicated that pityriasis versicolor was not associated with an increased population density or different serovar of *Malassezia*.

The increased incidence of pityriasis versicolor in patients on corticosteroid therapy,[6] patients following renal transplantation[7] and individuals with severe malnutrition has led to the suggestion that patients with pityriasis versicolor may be immunocompromised.[8] As it is believed that the cell-mediated immune (CMI) response plays a very important and often predominant part in the host defence against fungal infection in humans,[9] several studies have been carried out on the CMI status of patients with pityriasis

Correspondence: E.Ingham. E-mail: e.ingham@leeds.ac.uk

© 2001 British Association of Dermatologists

**Table 1.** Summary of previous studies of the cell-mediated immune status of patients with pityriasis vesicolor to *Malassezia*

| Reference | Antigen used in test | Assay of cell-mediated immunity | Results |
|---|---|---|---|
| 10 | Extract of *Malassezia* yeast cells | Lymphocyte transformation | No significant difference between patients with pityriasis versicolor and controls |
| 10 | Extract of *Malassezia* yeast cells | Leucocyte migration inhibition | Patients with pityriasis versicolor less reponsive than controls |
| 11 | Extract of *Malassezia* yeast cells | Lymphocyte transformation | Patients with pityriasis versicolor less responsive than controls |
| 12 | Extract of *Malassezia* yeast cells | Lymphocyte transformation | Patients with pityriasis versicolor more responsive than controls |
| 13 | Whole yeast cells of *M. sympodialis* (serovar A), *M. globosa* (serovar B), *M. restricta* (serovar C) | Lymphocyte transformation | No significant difference between patients with pityriasis versicolor and controls. No response to *M. sympodialis* in any patient or control |
| 13 | Whole yeast cells of *M. sympodialis* (serovar A), *M. globosa* (serovar B), *M. restricta* (serovar C) | Leucocyte migration inhibition | No significant difference in response to *M. sympodialis* and *M. restricta* between patients and controls. Patients more responsive to *M. globosa* than controls |

versicolor to *Malassezia* (summarized in Table 1). Sohnle and Collins-Lech[10] found no difference in the lymphocyte transformation response to an extract of *Malassezia* yeast cells in patients with pityriasis versicolor compared with controls, but a lower leucocyte migration inhibition response in patients compared with controls. In another study, the same researchers[11] reported that the lymphocyte transformation response to *Malassezia* antigens in patients with pityriasis versicolor was significantly decreased when compared with controls. This was in contrast to the findings of Wu and Chen[12] who showed that the lymphocyte transformation response to a crude extract of *Malassezia* yeast cells in patients with pityriasis versicolor was increased when compared with controls. Ashbee *et al.*[13] showed that the lymphocyte transformation response to the three serovars of whole *Malassezia* yeast cells did not differ between patients with pityriasis versicolor and controls. Using the leucocyte migration inhibition assay, the leucocytes from more patients with pityriasis versicolor responded to serovar B compared with controls.

Despite the fact that it is the mycelial phase of *Malassezia* that is implicated in pityriasis versicolor, all of these studies were carried out using *Malassezia* yeast cells or their extracts. There have been no studies that have measured the immune status of patients to the mycelial antigens. The aim of this study therefore was to measure the CMI response specific to the whole mycelial form of *Malassezia* in patients with pityriasis versicolor and healthy controls, using the lymphocyte transformation and leucocyte migration assays.

## Materials and methods

### *Culture medium*

The liquid culture medium used in this study was formulated to support the transformation of *Malassezia* yeast phase into the mycelial phase in order to obtain mycelial antigens for the study. The liquid culture medium was prepared by mixing the following components in 1 L of distilled water: bacteriological peptone (10 g), yeast extract (0·1 g), ox bile (4 g) (all from Oxoid Ltd, Basingstoke, Hants., U.K.), glucose (5 g), glycerol (1 mL), glycerol monostearate (0·5 g), Tween 80 (1 mL), squalene (1 mL) (all from Sigma, Poole, Dorset, U.K.) and potassium nitrate (1 g), sodium chloride (1·3 g), ferrous sulphate (0·01 g) and magnesium sulphate (0·15 g) (all from BDH, Speke, Liverpool, U.K.). The pH of the medium was adjusted to 5·6 and the medium sterilized by autoclaving. Before use, chloramphenicol (50 mg $L^{-1}$; Sigma), cycloheximide (200 mg $L^{-1}$; Sigma) and ultrahigh temperature-treated milk (10 mL $L^{-1}$; Associated Dairies, Leeds, U.K.) were added aseptically.

### *Malassezia strains*

The strains of *Malassezia* used in this study were selected on the basis of their capacity to produce mycelia in the liquid mycelial culture medium *in vitro*. Thirty-one strains were tested in preliminary studies. Eleven strains were fresh isolates from normal human skin, eight strains were isolated from pityriasis versicolor lesions (Mycology Reference Laboratory, University of Leeds) and 12 strains

**Table 2.** Assays carried out to test the cell-mediated immune status of patients with pityriasis versicolor to three strains of mycelial-phase *Malassezia* compared with controls

| | Lymphocyte transformation assay | Leucocyte migration inhibition assay |
|---|---|---|
| Pityriasis versicolor patient PBMC ($n = 12$) | vs. Hook<br>vs. GM216 | vs. Hook<br>vs. GM216 |
| Age- and sex-matched control PBMC ($n = 12$) | vs. 2.PV.WY<br>vs. Hook | vs. 2.PV.WY<br>vs. Hook |
| | vs. GM216<br>vs. 2.PV.WY | vs. GM216<br>vs. 2.PV.WY |
| | A transformation index $\geq 3$ indicates a positive transformation response | A migration index $\leq 0{\cdot}8$ indicates a positive migration inhibition response |

PBMC, peripheral blood mononuclear cells.

were obtained from the Mycology Reference Laboratory, University of Glasgow. Nineteen of the strains were serovar A (*M. sympodialis*), eight strains were serovar B (*M. globosa*) and four strains were serovar C (*M. restricta*). Only six of the strains underwent transformation to the mycelial phase in the mycelial culture medium. These were all serovar A isolated from lesional skin. Of these, three strains consistently produced 30–40% mycelial elements in culture and these were selected for the production of the mycelial antigens. They were Hook (*M. furfur*; isolated from a psoriatic lesion), GM216 (*M. sympodialis*; pityriasis versicolor lesion) obtained from the Mycology Reference Laboratory, Glasgow and 2.PV.WY (*M. sympodialis*; pityriasis versicolor lesion) obtained from the PHLS Mycology Reference Laboratory, Leeds.

### *Antigen preparations*

Whole mycelia of *Malassezia* strains Hook, GM216 and 2.PV.WY were used as antigens. The antigens were prepared by inoculation of the three strains into liquid mycelial culture medium. After 24 h the organisms were subcultured and grown for a further 24 h in the liquid culture medium. The mixtures of yeast cells and mycelia were then harvested during the exponential phase. The percentage of mycelium production was determined by counting 100 cells in five different fields of view using light microscopy and calculating the mean, which was $\approx 40\%$. The yeast cell and mycelium mixture (10 mL) was sonicated on ice for 30 s with a 150-W ultrasonic disintegrator (MSE Laboratory Products, Toddington, U.K.; 15 μm peak to peak) using a 3-mm tip diameter probe. Approximately 99% of the *Malassezia* cell population was dispersed using this procedure. A linear sucrose gradient 70% (w/v) to 110% (w/v) was used to obtain the mycelial suspension ($> 90\%$ mycelial elements). The mycelial suspension was washed six to eight times in phosphate-buffered saline (PBS; Oxoid) in order to remove all of the sucrose and culture medium components from the antigen suspension. After the last stage of the washing procedure, the mycelia were resuspended in tissue culture medium (RPMI-1640; Life Technologies, Paisley, U.K.).

### *Patients and controls*

The patients were attending the Dermatology Clinics at Leeds General Infirmary. After examination by a dermatologist they were sampled for organisms (skin lesion swab) and a sample of blood was taken. None of the patients was taking antimicrobial agents at the time, or for at least 2 weeks before sampling (to ensure that no such drugs would affect the assays of CMI response). The patient group consisted of 12 patients (seven men and five women) with pityriasis versicolor who had not been diagnosed as suffering from any other disease, e.g. immunodeficiency. This was the total number of patients attending the Dermatology Clinics who were willing to donate blood during the 24-month study period. The patients had a mean age of 36·2 years (range 24–52). The control subjects consisted of 12 age- and sex-matched volunteers (seven men and five women) with no known previous history of pityriasis versicolor. They were also not receiving any antimicrobial drugs at the time of sampling. The

healthy individuals had a mean age of 36·2 years (range 24–52).

*Lymphocyte transformation assay*

A sample of 5 mL peripheral venous blood was collected into a universal container containing 100 U of preservative-free heparin (PFH; CP Pharmaceuticals, Wrexham, U.K.). The whole blood (5 mL) was diluted with 16 mL of tissue culture medium (RPMI-1640) supplemented with penicillin (150 U $mL^{-1}$), streptomycin (200 μg $mL^{-1}$), HEPES (0·2 mol $L^{-1}$), L-glutamine (2 mmol $L^{-1}$) and 4 mL of human albumin. Antigen, negative and positive control test tubes were prepared in quadruplicate in tissue culture medium. Antigen suspension (0·5 mL), culture medium (0·5 mL) and diluted phytohaemagglutinin-P (PHA, 0·5 mL; Difco, Detroit, MI, U.S.A.; 14·5 μL $mL^{-1}$) were added to the antigen, negative and positive control tubes, respectively, and then 0·5 mL of the diluted blood sample was added to the tubes. The final concentration of mycelium in the antigen tubes was $10^7$ mycelial elements per mL. The test tubes were sealed with sterile silicone bungs and incubated at 37 °C. After the appropriate incubation time (3 days for PHA and 7 days for antigen), 1·25 μCi (50 μL) of tritiated thymidine (1 mL of TRK 61; Amersham, Amersham, U.K. in 19 mL of PBS) was added to each test tube and the tubes were incubated for 4 h at 37 °C.

The DNA was then extracted from the lymphocytes as follows. The samples were washed in 5 mL normal saline containing 2% (w/v) haemolytic saponin (Sigma) in order to lyse the red blood cells. Then the samples were centrifuged at 600 ***g*** for 5 min and resuspended and washed in 5 mL normal saline and centrifuged at 600 ***g*** for 5 min. The DNA was precipitated by adding 5 mL of cold 5% (w/v) trichloroacetic acid (BDH) and centrifuged at 1000 ***g*** for 5 min at 4 °C. This was repeated and the lymphocytes were centrifuged at 600 ***g*** for 5 min at 4 °C. To dry the DNA, 5 mL of methanol was added to each sample which was then centrifuged at 600 ***g*** for 5 min at room temperature. This stage was repeated again. The acidic DNA precipitate was dissolved and neutralized by the addition of 0·5 mL of 0·1 mol $L^{-1}$ sodium hydroxide and the tubes were incubated at 56 °C for 30 min. A sample of 0·2 mL from each tube was added to a scintillation vial and then 10 mL of Optiphase HiSafe II (EG & G Wallac) scintillation fluid was added. The samples were then placed at 4 °C for 2 h to equilibrate. The radioactivity in the samples was counted in an LKB Wallac 1217 Rackbeta Liquid Scintillation Counter. The results were expressed as counts per min (c.p.m.) per $10^5$ lymphocytes for each antigen and PHA (the number of lymphocytes added to each tube was calculated from a full blood count, which was carried out for each blood sample). The results were calculated as the transformation index (TI) for each antigen test and positive control (PHA) using the following formula:[14] TI = (c.p.m. of antigen or PHA sample)/(c.p.m. of negative control). A TI ≥ 3 was considered as a positive response.[13] The assays carried out are summarized in Table 2.

*Leucocyte migration inhibition assay*

This was carried out according to the method described by Gowland *et al.*[15] A 5-mL sample of peripheral venous blood was taken from each patient or control subject into a universal container containing 100 U of PFH. The red blood cells were sedimented by adding 2·5 mL dextran (Dextraven 150; Fisons Ltd, Coalville, U.K.). The mixture was incubated at 37 °C for 1 h. After sedimentation, the upper layer of the blood containing the leucocytes was removed to a clean test tube and 10 mL of 0·85% (w/v) $NH_4Cl$ was added. The sample was then incubated at 37 °C for 10 min in order to lyse any remaining red blood cells. The suspension was centrifuged at 400 ***g*** for 10 min and then washed using culture medium [RPMI-1640 plus penicillin (150 U $mL^{-1}$), streptomycin (200 μg $mL^{-1}$), HEPES (0·2 mol $L^{-1}$), L-glutamine (2 mmol $L^{-1}$) and 4 mL of human albumin] and this was repeated. The leucocytes were resuspended in 1 mL of culture medium supplemented with 25% (v/v) fetal calf serum. Capillary tubes (20 μL; Drummond Microcaps, Broomall, PA, U.S.A.) were filled with the leucocyte suspension and were blocked at one end with a mixture of vaseline and beeswax in order to retain the leucocytes in the capillary tubes during centrifugation. The capillary tubes were centrifuged at 400 ***g*** for 10 min and then cut at the interface between the leucocytes and the culture medium. The cut capillary tubes were then fixed into the wells of a 12-well migration plate (Sterilin, Hounslow, U.K.) containing the antigen or control suspensions. Each antigen, negative and positive control was tested in quadruplicate. The antigen suspension consisted of whole mycelial *Malassezia*, which was resuspended in culture medium (RPMI-1640 plus supplements) at a concentration of

$2 \times 10^7$ mycelial elements per mL. The negative control was culture medium alone and the positive control was tetanus toxoid (10·5 μg $mL^{-1}$; Evans Vaccines, Horsham, U.K.). The wells were overlaid with glass cover slips (no. 3, Chance Propper Ltd, Warley, U.K.) and the migration plates were incubated on a level surface at 37 °C for 18 h. The fans that the leucocytes formed as they migrated out of the capillary tubes were visualized by viewing the migration wells on an overhead projector. The area of each fan was drawn on a card and was then cut out and weighed. The migration index (MI) was calculated for each antigen test or positive control test using the following formula: MI = (weight of test)/(weight of negative control). An MI $\leq 0{\cdot}8$ was considered as a positive response.[15] The assays carried out are summarized in Table 2.

*Analysis of data*

Data (TI or MI) were proportional and therefore they were arc sin transformed as recommended by Sokal and Rohlf[16] prior to analysis. The means and 95% confidence limits were determined on the transformed data and back-transformed for presentation. The transformed data were also analysed using Student's *t*-test (patients vs. controls) and back-transformed for presentation.

## Results

*Lymphocyte transformation assay*

The results of the lymphocyte transformation assay with PHA or mycelial antigens of *Malassezia* for each patient and control were calculated as the c.p.m. per $10^5$ lymphocytes and also as the TI. There was no significant difference in the response of the patient and control lymphocytes to PHA (patients: mean TI 94·67, 95% confidence limits 80·4–117·2; controls: mean TI 111·18, 95% confidence limits 84·5–161·3).

The results of the lymphocyte transformation response for each individual patient and age- and sex-matched control to mycelial antigens of Hook strain are presented in Figure 1(A). The TIs were higher for lymphocytes from all of the patients compared with controls. Analysis of the data using Student's *t*-test showed that the mean TI of lymphocytes from patients was significantly higher than controls ($P < 0{\cdot}05$) for the Hook strain. None of the lymphocytes from patients or controls, however, responded to the mycelial antigens of Hook strain *Malassezia* with a TI $\geq 3{\cdot}0$.

None of the lymphocytes from patients or controls responded to the mycelial antigens of GM216 strain *Malassezia* (TIs all $< 3{\cdot}0$). However, the responses of the lymphocytes obtained from 11 of 12 patients were

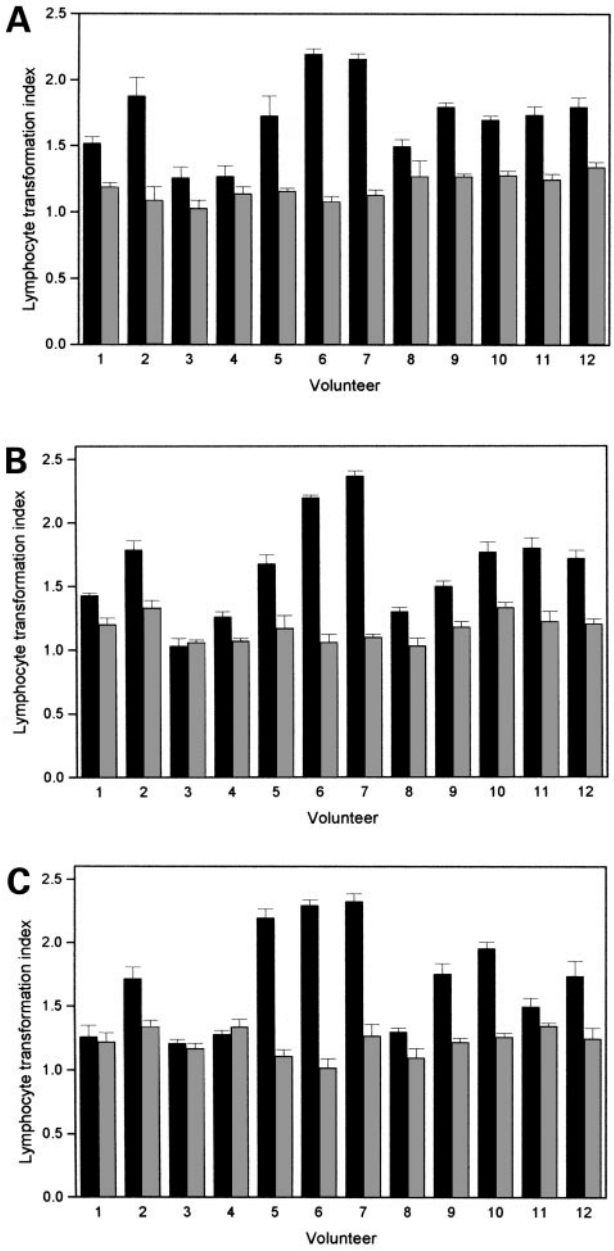


**Figure 1.** Lymphocyte transformation indices for cells from each individual pityriasis versicolor patient (black bars) and control (grey bars) to mycelial antigens of *Malassezia* strains Hook (A), GM216 (B) and 2.PV.WY (C). Results are expressed as the mean of four experiments ± 95% confidence limits.

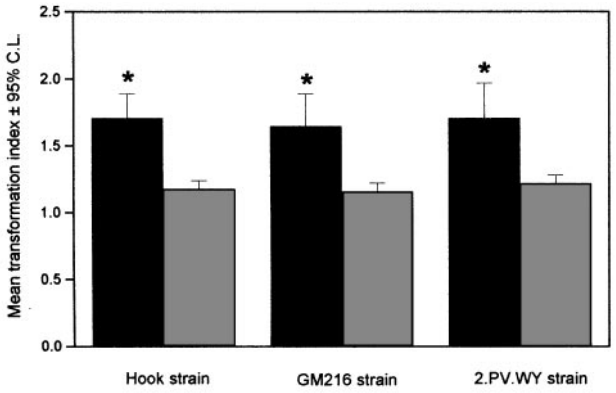


**Figure 2.** Mean lymphocyte transformation indices in response to mycelial antigens of *Malassezia* strains Hook, GM216 and 2.PV.WY for cells from patients with pityriasis versicolor (black bars) and controls (grey bars). Results are expressed as the mean lymphocyte transformation indices ($n = 12$) ± 95% confidence limits (CL). *$P < 0{\cdot}05$, Student's *t*-test.

higher than those of the controls (Fig. 1B). Analysis of the data using Student's *t*-test showed that the mean TI for patient lymphocytes was significantly higher than controls ($P < 0{\cdot}05$) with GM216.

None of the subjects responded to the mycelial antigens of 2.PV.WY strain *Malassezia*. The TIs were higher for cells from 11 of 12 patients compared with the controls (Fig. 1C). Analysis of the data using Student's *t*-test showed a greater TI in the patient lymphocytes ($P < 0{\cdot}05$) when compared with the controls to 2.PV.WY.

Although the TIs for the lymphocytes obtained from patients and controls to the mycelial antigens of the three *Malassezia* strains, Hook, GM216 and 2.PV.WY, were $< 3$, the patient mean TIs were significantly higher when compared with those of controls (Fig. 2).

### *Leucocyte migration inhibition assay*

The results for the MIs of leucocytes from patients with pityriasis versicolor and controls to the mycelial antigens of Hook strain are illustrated in Figure 3(A). The MIs showed that cells from 11 of 12 and nine of 12 of the patients with pityriasis versicolor and controls, respectively, responded to the mycelial antigens of Hook strain, giving an MI of $\leq 0{\cdot}8$. All of the cells from patients and controls responded to tetanus toxoid (MI $\leq 0{\cdot}8$). Analysis of the data using the Student's *t*-test showed no significant difference between the mean MIs for the cells from patients when compared with those of controls.

Cells from 11 of 12 and nine of 12 of the patients with pityriasis versicolor and controls, respectively, responded to the mycelial antigens of GM216 strain (Fig. 3B). Analysis of the data using Student's *t*-test showed no significant difference between the MI for the cells from patients with pityriasis versicolor when compared with the controls.

Cells from 10 of 12 and nine of 12 of the patients

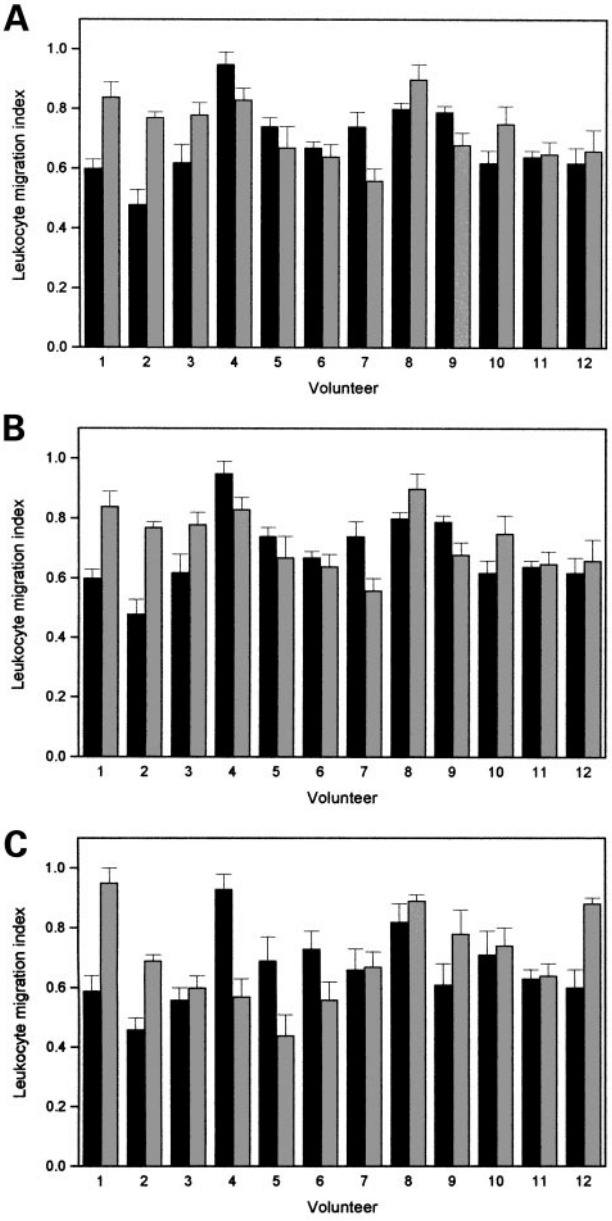


**Figure 3.** Leucocyte migration indices for cells from each individual pityriasis versicolor patient (black bars) and control (grey bars) to mycelial antigens of *Malassezia* strains Hook (A), GM216 (B) and 2.PV.WY (C). Results are expressed as the mean of four experiments ± 95% confidence limits.

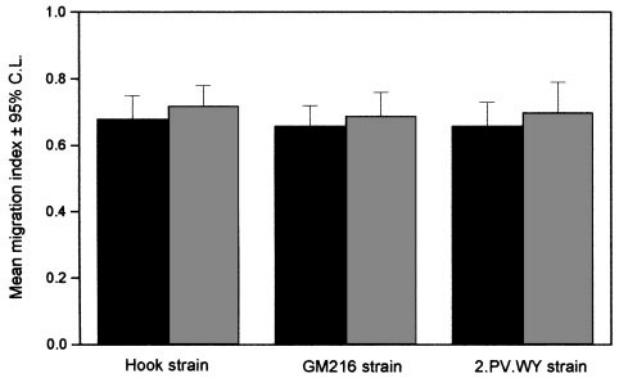


**Figure 4.** Mean leucocyte migration indices in response to mycelial antigens of *Malassezia* strains Hook, GM216 and 2.PV.WY for cells from patients with pityriasis versicolor (black bars) and controls (grey bars). Results are expressed as the mean leucocyte migration indices ($n = 12$) ± 95% confidence limits (C.L.).

with pityriasis versicolor and controls, respectively, responded to the mycelial antigens of 2.PV.WY strain (Fig. 3C). Analysis of the data using Student's *t*-test showed no significant difference between the MI for the cells from patients with pityriasis versicolor when compared with the controls.

The mean MIs for the leucocytes obtained from patients and controls to the mycelial antigens of the three *Malassezia* strains, Hook, GM216 and 2.PV.WY, were $\leq 0{\cdot}8$. However, no significant difference was seen between the mean MIs of patients and controls (Fig. 4).

## Discussion

Pityriasis versicolor is a non-inflammatory chronic superficial infection of the skin.[17] The disease may be treated topically but the relapse rate is high.[18] Systemic treatment with ketoconazole lowers the relapse rate but its long-term use is not recommended.[18] The chronicity and relapse of the disease suggests that patients fail to mount a protective CMI response against the organism. Previous studies of the specific CMI response to *Malassezia* in patients with pityriasis versicolor have given conflicting results.[10–12] The antigen that was used in previous studies was, however, either whole yeast cells or yeast cell extracts. The major difference in the study described here is that a great deal of effort was directed towards obtaining the mycelium of *Malassezia* for use as the antigen in assays of the CMI status of patients and controls.

Two methods were utilized, lymphocyte transformation and leucocyte migration inhibition. These methods have been widely used to determine CMI response to microbial antigens *in vitro*. In this study, the whole mycelia of three strains of *Malassezia* were used. All three strains were serovar A because this was the only species that transformed from the yeast to the mycelial phase in the mycelial culture medium. All of the patients' leucocytes responded normally to PHA (lymphocyte transformation) and tetanus toxoid (leucocyte migration inhibition). However, the lymphocytes from patients with pityriasis versicolor and controls failed to give a TI $\geq 3{\cdot}0$ to all three strains of *Malassezia* serovar A mycelium. There was, however, a significantly greater mean TI for the patient group compared with the controls. Ashbee *et al.*[5] reported that there were no positive responses to the yeast cells of *Malassezia* serovar A in pityriasis versicolor patients; the results of the present study using mycelial antigens of serovar A are in agreement.

Most of the patients had leucocytes that responded to *Malassezia* serovar A mycelium in the leucocyte migration inhibition assay. However, there was no significant difference between the mean of MIs for the cells from patients with pityriasis versicolor compared with the controls (for all three *Malassezia* mycelial antigens tested). These data conflict with the results of Sohnle and Collins-Lech[10] who reported that cells from 12 patients with pityriasis versicolor had significantly reduced migration inhibition responses to *Malassezia* yeast cell extracts compared with controls. Ashbee *et al.*[5] reported a significantly greater leucocyte migration inhibition response to the yeast cells of *Malassezia* serovar B with leucocytes from patients with pityriasis versicolor compared with controls, but the responses to serovars A and C were no different between patients and controls.

The difference in the results using the two assays of CMI response indicated that leucocyte migration inhibition was a more sensitive assay than lymphocyte transformation for the detection of CMI response to *Malassezia* serovar A antigens. This has been reported previously for CMI response to *Malassezia* serovar A yeast cells. The leucocyte migration inhibition assay is believed to be an *in vitro* correlate of delayed-type hypersensitivity (type IV hypersensitivity)[19] and measures antigen-specific 'effector' CD4+ T cells that are able to release migration inhibition factor directly following appropriate antigen recognition (the assay is performed over a 24-h period). Lymphocyte transformation, however, measures the incorporation of radiolabelled thymidine into the DNA of proliferating antigen-specific lymphocytes. Why the lymphocytes of patients and controls should respond in the leucocyte

migration inhibition assay but not in the lymphocyte transformation assay is not immediately obvious. *Malassezia* cells (hyphal elements or yeast cells), however, possess a lipid-rich capsule, which has been shown to suppress proinflammatory cytokine release by mononuclear phagocytes.[20,21] It is possible that this is also a mechanism for the apparent lack of lymphocyte proliferation, by inhibition of lymphocyte-activating cytokine (e.g. interleukin-1) release from antigen-presenting cells. This phenomenon would not necessarily have a direct effect in the leucocyte migration inhibition assay.

In conclusion, the findings of the present study showed that cells from most patients with pityriasis versicolor and controls were able to respond to the mycelial antigens of *Malassezia* strains as determined by leucocyte migration inhibition. However, there was no significant difference in the responsiveness of patients compared with controls. This indicated that patients with pityriasis versicolor did not have a CMI deficiency to *Malassezia* mycelial elements. However, the lack of an increased responsiveness in patients with active disease may indicate that a protective CMI response to mycelial-phase *Malassezia* may not be generated in patients with pityriasis versicolor.

## Acknowledgments

The authors acknowledge the Ministry of Health and Medical Education of the Islamic Republic of Iran for its financial support through the present study, and also Dr M.Richardson and Dr T.Bhattacharyya for their generous donation of *Malassezia* strains.

## References

1 Leeming JP, Notman FH, Holland KT. The distribution and ecology of *Malassezia furfur* and cutaneous bacteria on human skin. *J Appl Bacteriol* 1989; **67**: 47–52.

2 Faergemann J. Tinea versicolor and *Pityrosporum orbiculare*—mycological investigations, experimental infections and epidemiological surveys. *Acta Derm Venereol (Stockh)* 1979; **86** (Suppl.): 1–23.

3 Guého E, Midgley G, Guillot J. The genus *Malassezia* with description of four new species. *Antonie Van Leeuwenhoek* 1996; **69**: 337–55.

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

5 Ashbee HR, Ingham E, Holland KT, Cunliffe WJ. The carriage of *Malassezia furfur* A, B and C in patients with pityriasis versicolor, seborrhoeic dermatitis and controls. *Br J Dermatol* 1993; **129**: 533–40.

6 Boardman CR, Malkinson FD. Tinea versicolor in steroid treated patients. *Arch Dermatol* 1962; **85**: 84–92.

7 Koranda FC, Dehmel EM, Kahn G, Penn I. Cutaneous complications in immunosuppressed renal homograft recipients. *JAMA* 1974; **229**: 419–24.

8 Faergemann J. *Pityrosporum* infections. *J Am Acad Dermatol* 1994; **31**: 18–20.

9 Cassone A. Cell-mediated immunity mechanisms in fungal infections. In: *Fungal Disease: Biology, Immunology, and Diagnosis* (Jacobs PH, Nall L, eds). New York: Marcel Dekker, 1997: 113–36.

10 Sohnle PG, Collins-Lech C. Cell mediated immunity to *Pityrosporum orbiculare* in tinea versicolor. *J Clin Invest* 1978; **62**: 45–53.

11 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.

12 Wu YC, Chen KT. Lymphocyte proliferation to crude extract of *Pityrosporum* species and natural killer activity in tinea versicolor. *J Med Assoc Thailand* 1987; **70** (Suppl. 3): 47.

13 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.

14 Hudson L, Hay E, Hay FC. Lymphocytes and effector cells. In: *Practical Immunology* (Hay E, Hay FC, eds). Oxford: Blackwell Science, 1989: 127–81.

15 Gowland G, Ward RM, Holland KT, Cunliffe WJ. Cellular immunity to *P. acnes* in the normal population and patients with acne vulgaris. *Br J Dermatol* 1978; **99**: 43–7.

16 Sokal RR, Rohlf FJ, Wilson J Cotter S In.: *Biometry The Principles and Practice of Statistics in Biological Research* (Wilson J, Cotter S, eds), 2nd edn. New York: W.H.Freeman, 1981: 128–78.

17 McGinley KJ, Lantis LR, Marples RR. Microbiology of tinea versicolor. *Arch Dermatol* 1970; **102**: 168–71.

18 Faergemann J. Current treatment of cutaneous *Pityrosporum* and *Candida* infections. *Acta Derm Venereol (Stockh)* 1986; **121**: 109–16.

19 Hallab N, Jacobs JJ, Black J. Hypersensitivity to metallic biomaterials: a review of leukocyte migration inhibition assays. *Biomaterials* 2000; **21**: 1301–14.

20 Kesavan S, Walters CE, Holland KT, Ingham E. The effects of *Malassezia* species on pro-inflammatory cytokine production by human peripheral blood mononuclear cells *in vitro*. *J Med Vet Mycol* 1998; **36**: 978–1006.

21 Kesavan S, Holland KT, Ingham E. The effects of lipid extraction on the immunomodulatory activity of *Malassezia* species *in vitro*. *J Med Vet Mycol* 2000; **38**: 239–47.