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Cell-mediated immune responses to *Malassezia furfur* serovars A, B and C in patients with pityriasis versicolor, seborrheic dermatitis and controls

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Abstract: It has been postulated that patients with *Malassezia furfur*-associated dermatoses have a deficient cell-mediated immune response to *M. furfur*. This study examined the cell-mediated immune responses to *M. furfur* serovars A, B and C of 10 patients with pityriasis versicolor and 10 age- and sex-matched controls; and 10 patients with seborrheic dermatitis and 10 age- and sex-matched controls. The responses to each serovar of *M. furfur* were assessed using the lymphocyte transformation assay and the leukocyte migration inhibition assay. The lymphocyte transformation responses of the patients with pityriasis versicolor to *M. furfur* serovars A, B and C (0/10, 6/10 and 5/10 respectively) were not significantly different from those of controls (0/10, 2/10 and 1/10). However, for patients with seborrheic dermatitis, significantly more patients' lymphocytes responded to serovars B and C (6/10 and 6/10 respectively) than those of controls (1/10 and 1/10). No patient or control responded to serovar A. In the leukocyte migration inhibition assay, the leukocytes from a greater proportion of patients with pityriasis versicolor (5/7) responded to serovar B than controls (2/10); and the leukocytes from a greater proportion of patients with seborrheic dermatitis (4/10) responded to serovar C than controls (0/9). Thus, this data did not indicate the presence of any cell-mediated immune deficiency to *M. furfur* in patients with pityriasis versicolor or seborrheic dermatitis, as measured by the lymphocyte transformation assay or the leukocyte migration inhibition assay. The greater responsiveness of T lymphocytes from patients may indicate that T lymphocytes might be involved in the pathogenesis of these diseases.

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Key words: *Malassezia furfur* – pityriasis versicolor – seborrheic dermatitis – lymphocyte transformation – leukocyte migration inhibition

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Introduction

Malassezia furfur has been implicated as the etiological agent in a wide range of cutaneous and systemic diseases, including pityriasis versicolor, seborrheic dermatitis, folliculitis, peritonitis and catheter-related sepsis. The studies carried out into the cell-mediated immune responses of patients with *M. furfur*-associated dermatoses have been relatively limited, in contrast to the extensive work on the humoral responses.

Sohnle & Collins-Lech (1, 2) carried out two studies into the cell-mediated immune responses of patients with pityriasis versicolor to *M. furfur*. In the first study, they found that patients with pityriasis versicolor produced less leukocyte migration inhibition factor in response to *M. furfur* than controls (1). In the second study, they examined the lymphocyte transformation response to *M. furfur* in patients with pityriasis versicolor and found that there was a statistically significant decrease in the response to *M. furfur* in patients compared to controls (2). In contrast, Wu & Chen (3) found that a group of 31 patients with pityriasis versicolor

exhibited a "higher lymphocyte responsiveness" to *M. furfur* than controls.

Two studies have examined the cell-mediated immunity of patients with seborrheic dermatitis. Bergbrant et al. (4) found that in a group of 30 patients, 13 had "subnormal mitogen stimulation responses", but they did not test the antigens of *M. furfur* in the assay. Kieffer et al. (5) determined the T- and B-lymphocyte subpopulations in 19 patients with seborrheic dermatitis and compared them to 55 subjects with atopic dermatitis and 19 controls. They found that 13 patients with seborrheic dermatitis had a low CD4:CD8 (helper:cytotoxic/suppressor) lymphocyte ratio, due to a larger number of CD8 lymphocytes.

The aims of this study were two-fold. Firstly, the T- and B-lymphocyte subpopulations were quantified for patients with pityriasis versicolor and patients with seborrheic dermatitis to determine whether they were altered in patients with the diseases when compared to age- and sex-matched controls. Secondly, the cell-mediated immune responses specific to *M. furfur* serovars A, B and C were measured in each subject to determine whether they were altered in patients with the diseases.

Material and methods

Reagents

RPMI-1640 with L-glutamine, HEPES, fetal calf serum and newborn calf serum were obtained from Gibco Ltd. Lymphoprep used for the separation of monocytes and lymphocytes was obtained from Nycomed. The monoclonal antibodies and conjugate used to characterize the T-lymphocyte subpopulations were obtained from Ortho Diagnostic Systems, USA and the FITC-conjugated antibodies used to characterize the B-lymphocyte subpopulations were obtained from Behring. Latex beads were purchased from Sigma. Methanol, sodium hydroxide and ammonium chloride were obtained from British Drug Houses. Phytohemagglutinin-P was obtained from Difco and Tetanus Toxoid from the Wellcome Foundation. The ^{3}H-thymidine used in the lymphocyte transformation assay was obtained from Amersham UK and the samples counted in NE 265 from NE Technologies. The dextran and TCA were obtained from Fisons and 4.5% (v/v) human albumin solution from Blood Product Laboratories.

Patient and control subjects

Two groups of patients were studied, all of whom were attending the dermatology outpatient clinics at Leeds General Infirmary. The first group were 10 patients with pityriasis versicolor, which was defined as scaly hypo- or hyperpigmented lesions with minimal erythema. The mean age of this group was 34.0 yr (range 23–41) and all had moderate disease. All 10 patients had lesions on the chest and 8/10 had lesions on the back.

The second group were 10 patients with seborrheic dermatitis, which was defined as scaly, moderately inflamed, itchy lesions. They had a mean age of 34.9 yr (range 25–46) and also had moderate disease. The most commonly involved site in this group was the forehead (5/10), with the left and right cheeks and the chest involved in 3/10 patients. Neither group of patients was receiving medication at the time of the study or for 2 weeks previously.

Age- and sex-matched controls had no previous history of either pityriasis versicolor or seborrheic dermatitis and were not taking antimicrobial therapy at the time of sampling. The control group for the patients with pityriasis versicolor had a mean age of 33.9 yr and an age range of 23–40 yr; the control group for the patients with seborrheic dermatitis had a mean age of 34.3 yr and an age range of 26–45 yr.

Separation and enumeration of T- and B-lymphocyte subpopulations

The separation and enumeration of the lymphocyte subpopulations was carried out using standard density gradient centrifugation and indirect immunofluorescent methods. The percentage of contaminating monocytes was assessed using Latex ingestion. The T lymphocytes were labelled using monoclonal antibodies raised against the relevant CD markers and an FITC conjugate; whilst the B lymphocytes were labelled using FITC-conjugated antisera against the surface immunoglobulins.

Antigen preparation

The antigens used in both the lymphocyte transformation assay and the leukocyte migration inhibition assay were formalized, whole cell suspensions of *M. furfur* serovars A, B and C. The three serovars differ in their morphological appearance, *in vitro* growth characteristics and serological reactions. The strains used (13, 5 and 7: serovars A, B and C respectively) were all isolated from clinically normal skin of volunteers. They were differentiated into the three serovars using a panel of absorbed rabbit anti-*Malassezia* sera (6). To prepare the formalized cell suspension, each strain was grown to mid-exponential phase, harvested by centrifugation at 800 *g*, washed three times in sterile PBS and

resuspended in sterile PBS with 1% (w/v) formaldehyde. The yeasts were washed three times before use in the assays.

Lymphocyte transformation assay

A sample of 5 ml of peripheral venous blood was collected from each subject into tubes containing 100 units of preservative-free heparin (CP Pharmaceuticals). The blood was then diluted with 16 ml of culture medium (RPMI containing 2% (v/v) Hepes, penicillin [150 U/ml] and streptomycin [150 μg/ml]) and 4 ml of human albumin solution to form "stock blood". The three serovars of *M. furfur* were tested in the assay; phytohemagglutinin-P (PHA), a T-lymphocyte mitogen, was used as the positive control and culture medium as the negative control. Each antigen and control were tested in quadruplicate by adding 0.5 ml of antigen or mitogen and 0.5 ml of "stock blood" into a tube. This gave a final concentration of 2×10^6 yeast cells per tube and approximately 2×10^5 lymphocytes per tube. Previous studies by Cunningham (6) showed that at this concentration the yeasts were not mitogenic to T lymphocytes when tested using cord blood. The tubes were then closed with sterile silicone bungs and incubated at 37°C. After 3 d incubation with PHA and 7 d incubation with the antigens, 1.25 μCi of tritiated thymidine was added to each tube and re-incubated for 4 h. The DNA was then extracted from the lymphocytes as follows. The cells were washed in normal saline with hemolytic saponin [2% (w/v)] to lyse the red blood cells. The lymphocytes were then centrifuged at 600 *g* for 5 min, resuspended and washed a second time. Precipitation of the DNA was carried out using 5% (w/v) TCA and the lymphocytes centrifuged at 1000 *g* for 5 min at 4°C. The lymphocytes were then resuspended and washed with a further 5 ml of 5% (w/v) TCA and centrifuged at 600 *g* for 5 min at 4°C. In order to dry the precipitated DNA, 5 ml of methanol was added to each tube and the tubes centrifuged at room temperature at 600 *g* for 5 min and this procedure was repeated. To dissolve and neutralize the acidic DNA precipitate, 0.5 ml of 0.1 M sodium hydroxide was added to each tube and the tubes incubated at 56° C for 30 min. From each tube, 0.2 ml was removed and added to 10 ml of NE 265 scintillation fluid, allowed to equilibrate at 4° C for 2 h and counted in an LKB Wallac 1217 liquid scintillation counter. Results were expressed as the transformation index for each antigen and PHA. This was calculated as:

$$\frac{\text{Mean CPM of sample} - \text{Mean CPM of control}}{\text{Mean CPM of control}}$$

A transformation index of $\geqslant 3$ was considered a positive response.

Leukocyte migration inhibition assay

A sample of 5 ml of peripheral venous blood was taken from each subject into a tube containing 100 units of preservative-free heparin (CP Pharmaceuticals). To this, 2.5 ml of dextran was added, mixed and decanted into a clean universal tube, ensuring that no bubbles were carried over. The blood mixture was then incubated at 37°C for 45–60 min to allow sedimentation of the red blood cells. The leukocyte-rich upper layer was removed to a fresh tube and made up to 10 ml with 0.85% (w/v) NH_4Cl to lyse any contaminating red blood cells. After incubation at 37°C for 10 min, the leukocytes were centrifuged at 400 *g* for 10 min and washed in culture medium. The leukocytes were centrifuged a second time and finally resuspended in 1 ml of culture medium supplemented with 25% (v/v) fetal calf serum. Capillary tubes of 20 μl volume were filled with the leukocyte suspension and plugged at one end with a vaseline and beeswax mixture. The mixture was viscous enough to prevent the leukocytes spinning out of the tubes during centrifugation at 400 *g* for 10 min. The capillary tubes were then cut at the interface between the leukocytes and the medium. The antigens used in the assay were formalized, whole cell suspensions of *M. furfur* serovars A, B and C at a concentration of 3×10^6/ml. Tetanus toxoid (10.5 μg/ml) was used as the positive control, as it is a common recall antigen, and the negative control was culture medium. The assay was carried out in 12-well migration plates with 0.5 ml of the appropriate antigen or control in each well. The cut capillary tubes were fixed in place using vaseline, with the end containing the cells opening into the antigen in the well. Four wells were used for each antigen and control with two capillary tubes in each well. The wells were overlaid with coverslips and incubated on a level surface at 37°C overnight. The fans which the leukocytes formed were visualized using an overhead projector, drawn onto card, cut out, weighed and the migration index (MI) calculated for each antigen as:

$$\frac{\text{Mean weight of tests}}{\text{Mean weight of controls}}$$

An MI of 0.8 or less was considered a positive response.

Statistical analyses

Data from the T- and B-lymphocyte numbers were recorded as percentages and were therefore arcsine-

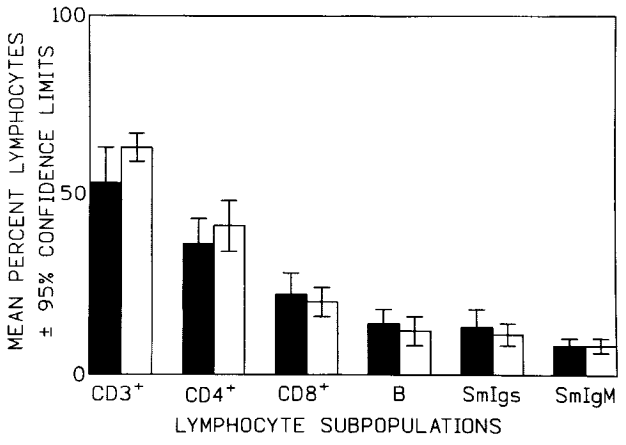

Figure 1. Lymphocyte subpopulations in peripheral venous blood from patients with pityriasis versicolor and controls.
■ Pityriasis versicolor patients.
□ Controls.
Results are expressed as the mean lymphocyte population for each group. The data was analyzed by Students t-test. Error bars represent the 95% confidence limits.

transformed before analysis was carried out. The data was compared using the Student t-test and then back transformed (7).

The number of subjects whose cells responded to each antigen in the lymphocyte transformation assay and the leukocyte migration inhibition assay were analyzed using the G-test with the Williams correction for small sample sizes (7).

Results

Enumeration of T- and B-lymphocyte subpopulations

The mean and 95% confidence limits for the incidence of each lymphocyte subpopulation in patients with pityriasis versicolor and controls are shown in Fig. 1. There were no significant differences in the numbers of each lymphocyte subpopulation between patients and controls ($p > 0.05$). The mean CD4:CD8 ratio for patients with pityriasis versicolor was 1.8:1 and for controls 2.1:1, indicating a normal ratio of T helper:T cytotoxic lymphocytes.

The mean and 95% confidence limits for each lymphocyte subpopulation in patients with seborrheic dermatitis and controls are shown in Fig. 2. There were no significant differences in the numbers of each lymphocyte subpopulation between patients with seborrheic dermatitis and controls ($p > 0.05$). The mean CD4:CD8 ratio for patients was 2.0:1 and for controls 2.2:1.

Lymphocyte transformation assay

The response to each antigen or PHA in the assay was expressed as the Transformation Index and an index of $\geqslant 3$ was considered a positive response. The transformation indices for patients with pityriasis versicolor and controls to the three serovars of *M. furfur* and PHA are shown in Table 1, as well as the number of subjects who responded to each antigen in each group. No patients or control responded to serovar A, 6 patients and 2 controls responded to serovar B and 5 patients and 1 control responded to serovar C. All 10 patients and 10 controls had a positive response to PHA. This pattern of responses was analyzed using the G-test with Williams correction for small samples sizes. For each serovar, the number of subjects whose cells responded was independent of whether the subject was a patient or a control.

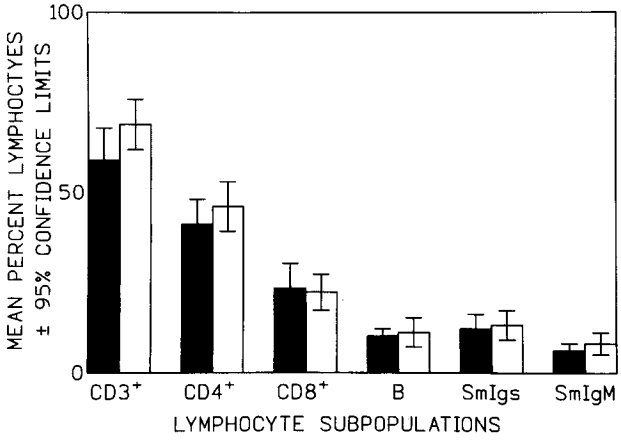

Figure 2. Lymphocyte subpopulations in peripheral venous blood from patients with seborrheic dermatitis and controls.
■ Seborrheic dermatitis patients.
□ Controls.
Results are expressed as the mean lymphocyte population for each group. The data was analyzed by Students t-test. Error bars represent the 95% confidence limits.

Table 1. The transformation indices for patients with pityriasis versicolor and controls to *Malassezia furfur* serovars A, B and C and Phytohemagglutinin (PHA)

Pityriasis versicolor patients				Controls			
A	B	C	PHA	A	B	C	PHA
0	4.6	4.0	1432	0.8	31.0	19.0	1900
0.6	10.0	2.9	1176	0.4	0.2	0	773
0	2.2	0	418	0.2	0.2	0	87
0.2	0.1	0.1	1046	0.3	0.1	0	1353
0.2	11.5	3.3	577	0.2	0.2	0.3	2233
0.3	19.0	15.0	1591	0.6	1.4	0.1	510
0	2.6	10.0	637	0	0	0.1	52
0	5.0	0.6	1193	0	2.0	0	1543
0	27.0	32.0	853	0	0	0	1644
0	2.3	2.1	670	0.5	3.2	0.2	47
No of Responders							
0/10	6/10	5/10	10/10	0/10	2/10	1/10	10/10

Table 2. The transformation indices for patients with seborrheic dermatitis and controls to *Malassezia furfur* serovars A, B and C and Phytohemagglutinin (PHA)

Seborrheic dermatitis patients				Controls			
A	B	C	PHA	A	B	C	PHA
0.1	21	23	1115	0.3	0.4	0.2	459
0.2	33	3	553	0	104	53	1612
0.2	136	135	4686	0.1	0.1	0.1	143
0	5	3	1574	0.1	0	0	384
0	6	5	1037	0	0	0	216
0.2	13	5.2	880	0	0	0	106
0	0.1	0	1247	0	0	0	119
1.3	0	0	375	0	0	0	120
0.3	0.1	0	1316	0	0	0	19
0	0	0	568	0	0	0	165
No of Responders							
0/10	6/10	6/10	10/10	0/10	1/10	1/10	10/10

The transformation indices for patients with seborrheic dermatitis and controls to the three serovars of *M. furfur* and PHA are shown in Table 2, as well as the number of subjects who responded to each antigen. No patient or control responded to serovar A, 6 patients responded to serovars B and C, whilst only 1 control responded to each of B and C. All 10 patients and 10 controls responded to PHA. For patients with seborrheic dermatitis and controls, the number of subjects whose lymphocytes responded to serovar A was independent of whether the subject was a patient or a control. However, for serovars B and C there was a significant deviation from independence ($p < 0.025$), with the lymphocytes of more patients responding than did those of controls.

In each group the number of subjects whose lymphocytes responded to PHA was independent of whether the subject was a patient or a control.

Leucocyte migration inhibition assay

The migration indices to *M. furfur* serovars A, B and C and the number of subjects who responded to each antigen in each group for patients with pityriasis versicolor and controls are shown in Table 3. None of the patients responded to serovar A but 3 controls had a positive response. For serovar B, 5 patients responded and 1 control had a positive response, whilst for serovar C 2 patients and 1 control responded. The number of subjects whose leukocytes responded to each antigen were analyzed using the G-test with Williams correction for small sample sizes. The number of subjects whose leukocytes responded to serovars A and C was independent of whether the subject was a patient or a control. However, for serovar B there was a significant deviation from independence ($p < 0.05$), with the leukocytes of more patients responding than did those of controls.

The migration indices to *M. furfur* serovars A, B and C and the number of subjects who responded to each antigen in each group for patients with seborrheic dermatitis and controls are shown in Table 4. Of the patients with seborrheic dermatitis, 4 responded to serovar A, 4 to serovar B and 4 to serovar C. In comparison, 3 controls responded to serovar A, 3 to serovar B and 0 to serovar C. The number of patients whose leukocytes responded to serovars A and B was independent of whether they were patients or controls. There was a significant deviation from independence ($p < 0.025$) for serovar C, with the leukocytes of more patients responding than those of controls.

Table 3. The migration indices for patients with pityriasis versicolor and controls to *Malassezia furfur* serovars A, B and C and Tetanus toxoid (TET)

Pityriasis versicolor patients				Controls			
A	B	C	TET	A	B	C	TET
X	X	X	X	1.30	0.94	1.08	0.53
X	X	X	X	1.13	1.21	1.15	0.73
X	X	X	X	0.77	1.00	0.84	0.61
1.30	0.73	0.91	0.45	0.75	1.13	0.99	0.71
1.09	0.56	0.92	0.48	0.82	0.96	0.77	0.63
1.15	0.83	1.06	0.92	2.30	2.10	2.30	NT
1.2	0.70	1.06	1.00	1.06	0.66	1.05	0.71
0.89	0.62	0.74	0.74	1.13	1.20	1.01	0.49
1.63	0.81	1.03	0.80	1.20	1.06	1.11	0.72
1.02	0.61	0.77	0.48	0.53	0.62	0.62	0.31
No of Responders							
0/7	5/7	2/7	5/7	3/10	2/10	2/10	9/9

X=No result. NT=Not tested.

Table 4. The migration indices for patients with seborrheic dermatitis and controls to *Malassezia furfur* serovars A, B and C and Tetanus toxoid (TET)

Seborrheic dermatitis patients				Controls			
A	B	C	TET	A	B	C	TET
1.05	0.65	1.01	0.67	0.98	1.07	1.03	0.49
0.89	0.69	0.67	0.60	1.54	1.12	1.07	0.78
0.28	0.11	0.40	0.16	0.74	0.83	1.07	0.50
1.00	0.87	0.92	0.61	1.36	1.36	1.18	0.57
1.30	1.10	1.04	0.81	0.70	0.64	1.16	0.59
0.77	0.75	0.75	0.48	0.97	0.76	0.88	0.46
0.76	0.96	0.75	0.59	0.56	0.65	0.83	0.45
0.59	0.88	1.00	0.52	1.25	1.33	1.16	0.40
1.24	0.90	1.04	0.73	X	X	X	X
1.07	1.19	1.17	0.53	0.94	1.21	1.08	0.49
No of Responders							
4/10	4/10	4/10	9/10	3/9	3/9	0/9	9/9

X=No result.

Discussion

Immunity to fungal infections is primarily due to the hosts' cell-mediated immune response (8) and deficiencies in any part of the cell-mediated response may predispose to fungal infections. Thus, information about the cell-mediated immune responses to *Malassezia furfur* in patients with either pityriasis versicolor or seborrheic dermatitis might be important in determining the mechanisms by which the diseases arise. Few studies have been carried out into this area and the results of those studies have been conflicting. Thus, this study was carried out to assess the general and *Malassezia*-specific cell-mediated immune status of patients with pityriasis versicolor or seborrheic dermatitis and age- and sex-matched controls.

In order to compare the general cell-mediated immune status of patients and controls, the T- and B-lymphocyte populations were determined for each group. The populations of CD4 (T-helper lymphocytes) and CD8 (T-cytotoxic/suppressor lymphocytes) were used to determine the CD4:CD8 ratio to assess whether it was near the normal ratio of approximately 2:1. The population of total, and each subset, of T and B lymphocytes was found to be similar for each patient group and their respective control group, indicating that neither group of patients had any disturbance in their lymphocyte populations. The CD4:CD8 ratio was calculated for each subject and was also found to be normal. The only other study which quantified T- and B-lymphocyte populations in patients with pityriasis versicolor was that of Wu & Chen (3) who found that patients had a low CD4:CD8 ratio due to a large number of CD8 cells. Two studies have examined the lymphocyte populations in patients with seborrheic dermatitis. Kieffer et al. (5) found that in 13/19 patients there was a low CD4:CD8 ratio, due to a large number of CD8 cells. Bergbrant et al. (4) determined the T- and B-lymphocyte populations and CD4:CD8 ratio for 30 patients with seborrheic dermatitis, defining their normal ratio as 0.6–2.8. They stated that 26 patients had a ratio between 0.6 and 2.8, whilst 4 patients had a ratio greater than 2.8. However, the ratio which they defined as normal is very wide and may partially explain the discrepancies in the results between theirs and other studies.

The two methods used in this study to assess the cell-mediated immune response of subjects to *M. furfur*, the lymphocyte transformation and leukocyte migration inhibition assays, have been used widely to measure cell-mediated immune response to specific antigens and mitogens (1, 2, 9, 10, 11). This present study defined a transformation index of $\geqslant 3$ as a positive response to the antigen. Using this criterion, no patient with pityriasis versicolor or control had a positive transformation response to *M. furfur* serovar A. Although it was not statistically significant, more of the patients responded to serovars B and C than controls. For patients with seborrheic dermatitis, the lymphocytes of significantly more patients responded to serovars B and C than those of controls, but did not differ in their responses to serovar A. Cunningham (6) previously used this system to determine the cell-mediated immune response to *M. furfur* A, B and C in normals and found that the peak response occurred on d 7 of incubation. Thus, the transformation responses were determined on d 7 to record the peak responses. Sohnle & Collins-Lech (1) were unable to find any differences in the lymphocyte transformation responses to *Malassezia* between 12 patients with pityriasis versicolor and 15 controls. However, in a subsequent study including 18 patients with pityriasis versicolor and 42 controls, they found a significantly lower response in the patients when compared to controls (2). This difference occurred on d 6 of incubation, but the peak response did not occur until d 9. As the transformations were not significantly different at the peak response on d 9, the difference observed may have been solely due to the rate with which the lymphocytes transformed rather than the presence of a cell-mediated immune deficiency specific to *M. furfur* in patients. Sohnle & Collins-Lech (2) suggested that the difference in response at d 6 was due to a lower number of lymphocytes sensitized to *M. furfur* in patients compared to controls. However, because this difference was not maintained at the peak of the response, their explanation seems untenable. Another possible explanation is that the organism used by Sohnle & Collins-Lech was serovar A, which was used in this study and did not stimulate the lymphocytes of any patient or control. Thus, the apparent deficiency in the lymphocyte transformation responses to *M. furfur* (2) may simply have reflected the lack of reactivity of serovar A in the assay rather than a generalized cell-mediated immune deficiency. The study of Wu & Chen (3) found that patients with pityriasis versicolor had a higher response to *M. furfur* than controls in the lymphocyte transformation assay, which is in agreement with the findings of this study.

In this study, the patients and controls all had similar levels of responses to PHA. However, Hashimoto et al. (12) reported that 1 patient with pityriasis versicolor had a lower response to PHA than a control, but conclusions based on a single example are highly dubious. In contrast, Wu & Chen (3) found that the transformation responses to PHA in 31 patients with pityriasis versicolor were not depressed

when compared to 30 controls. Bergbrant et al. (4) performed a study on 30 patients with seborrheic dermatitis and found that 13 of them had a lower mitogen stimulation response than normals. However, they noted that, of the 13 with a low response 6 had a mean age of 70, compared to a mean age of 46 for the whole group. Thus, the lower stimulation might be due to age rather than the presence of seborrheic dermatitis *per se*.

The second method used to measure cell-mediated immune responses to *M. furfur* was the leukocyte migration inhibition assay. Using this assay, it was found that a significantly greater proportion of patients with pityriasis versicolor responded to serovar B than controls; but their responses to serovars A and C were not different from controls. For patients with seborrheic dermatitis, a greater proportion of them responded to serovar C than did controls; but they did not differ in their responses to A or B from controls. The finding of this study for patients with pityriasis versicolor conflict with that of Sohnle & Collins-Lech (1) who found that 12 patients with pityriasis versicolor had a significantly lower migration inhibition response to *M. furfur* than did controls. Sohnle & Collins-Lech stated that most of their patients were receiving topical therapy at the time of the study, but did not state what the treatments were. Many antifungal drugs have been shown to have effects other than those on the fungus, such as inhibition of mitogen-induced (13) and antigen-induced lymphocyte proliferation (9) and stimulation of neutrophil migration *in vivo* (14). If the patients in the study of Sohnle & Collins-Lech (1) were using antifungals with these properties their observations may have been affected by the drugs.

In conclusion, this study did not support the findings of the previous studies that patients suffering from either pityriasis versicolor or seborrheic dermatitis had a cell-mediated immune deficiency to *M. furfur*, as measured by the lymphocyte transformation assay or leukocyte migration inhibition assay. Generally, both patients and controls responded more to serovars B and C than A. This was not due to a greater prevalence of serovars B and C on the subjects' skin, as a previous study demonstrated that the population densities of serovars B and C were significantly lower on the chest and back than serovar A; and all three serovars reached similar population densities on the head sites (15). Thus, these results suggest that serovars B and C may be more immunogenic than serovar A. The results seen in this study demonstrating that the lymphocytes of a greater number of patients than controls responded to *M. furfur* may suggest that, if these responses were mirrored in the lesions of these diseases, then T lymphocytes might be involved in the pathogenesis of the diseases. If examination of the T lymphocytes in the lesions of seborrheic dermatitis revealed that they were specific to *M. furfur*, this would also be the first direct evidence implicating *M. furfur* in the disease.

Acknowledgments

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