Studies on the Lipophilic Yeast *Pityrosporum ovale* in HIV-seropositive and HIV-seronegative Homosexual Men

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Pityrosporum ovale has been implicated in the pathogenesis of seborrheic dermatitis, a dermatosis occurring with high prevalence in symptomatic HIV-infected individuals. Quantitative cultures for *P. ovale* were taken in 12 HIV-seropositive patients and in 12 HIV-seronegative controls. Sera were analysed for antibodies to *P. ovale*. In HIV-seropositive patients, lymphocyte subsets were analysed. The mean number of *P. ovale*/cm^2, the mean serum antibody titres against this yeast and the occurrence of cutaneous disorders did not differ significantly between the two studied groups. No correlation was found between the number of *P. ovale*/cm^2 and the immune status of the HIV-seropositive subjects. The absence of more serious immunological deterioration in the studied HIV-seropositive men may explain the results of this study. *Key words: Seborrheic dermatitis; Serum antibodies; Quantitative culture.* (Received March 1, 1988.)

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Dermatological symptoms occur with high frequency in patients with the acquired immunodeficiency syndrome (AIDS). The immune dysfunction induced by the human immunodeficiency virus (HIV), the infectious agent causing AIDS, renders these patients susceptible to a variety of viral, bacterial, fungal and protozoal infections. Some of these infections manifest themselves in the skin. Other cutaneous manifestations that have been reported in patients with AIDS include Kaposi's sarcoma (1), vasculitis (2), psoriasis (3), seborrheic dermatitis (4), xeroderma (2), allergic reactions (5), folliculitis (2) and nail changes (6).

Many of the skin disorders seen in AIDS patients have also been reported in HIV-infected patients earlier in the course of the disease, when the immune system has been depressed, but not to the degree of manifest AIDS.

Seborrheic dermatitis is the most common skin manifestation reported in HIV-infected individuals. Mathes & Douglass (7) diagnosed seborrheic dermatitis in 83% of AIDS patients and in 42% of patients with ARC. Farthing et al. (2) found a 20% prevalence of this skin disorder amongst HIV antibody-positive patients with PGL but without AIDS. In their study on homosexual men, Muhlemann et al. (8) found a significantly higher prevalence of seborrheic dermatitis amongst HIV-positive patients with PGL, but not in otherwise symptomless HIV-positive men when compared with HIV-negative controls.

There are now several studies indicating an association between the lipophilic dimorphic yeast *Pityrosporum ovale* and seborrheic dermatitis (9, 10). Most of these studies are treatment studies showing a good effect of antifungal agents in the treatment of dandruff and seborrheic dermatitis (10, 11).

P. ovale is the etiological agent of pityriasis versicolor (12), and this disorder has been reported in patients with AIDS as well as in HIV-positive individuals without AIDS (13). *P. ovale* also occur as part of the normal flora on the human skin (14).

One of the authours has recently described a method for quantitative culture of *P. ovale* using a special culture medium (15). In a study of regional variations on clinically normal human skin, the highest number of cultured *P. ovale* was found on the back and chest (16).

In this study, we investigated the amount of *P. ovale* on the skin in HIV-seropositive patients and in HIV-seronegative controls using the above-mentioned method for quantitative culture of this yeast. In addition, sera were analysed for antibodies to *P. ovale*. The persons studied were also clinically examined for the presence of seborrheic dermatitis, dandruff and pityriasis versicolor.

PATIENTS AND METHODS

Patients

Twelve HIV-seropositve male homosexuals attending the Department of Dermatology at Sahlgrens' Hospital were enrolled in this study. Their mean age was 34.7 years, range 24–45. None of the patients had AIDS or ARC but 6 of them fulfilled the criteria for persistent generalised lymphadenopathy (PGL) as defined by Centers for Disease Control (CDC). The other patients were asymptomatic. Twelve HIV-seronegative male homosexuals attending the same Department served as controls. Their mean age was 30.2 years, range 20–39. All were asymptomatic.

Methods

Culture material was taken from the interscapular area of the back in all subjects.

The culture technique has been described earlier (15). Briefly, a stainless ring covering a 5.5 cm^2 area of the skin was held with moderate pressure against the skin with 2 fingers. One milliliter of sterile 0.075 M phosphate buffer, pH 7.9, containing 0.1% Triton X-100 was poured into the ring and the skin was gently rubbed with a glass rod for 1 minute. The fluid was removed by pipette and the procedure repeated three times on the same area. For each sample, serial dilutions were performed in phosphate-buffered saline (PBS), pH 7.2, containing 0.1% Triton X-100. Samples (0.1 ml) from the dilutions were inoculated on a glucose-neopeptone-yeast extract agar medium containing olive oil (2%), Tween 80 (0.1%) and glycerol monostearate (2.5 g l^{-1}) (17). In addition, skin scrapings for qualitative culture were taken with a curette and transferred to the above-mentioned medium. Plates were incubated at 37°C and examined after 6 days.

Serum samples were analysed for antibodies to *P. ovale* using the indirect immunofluorescence technique. This technique has been described elsewhere (18). Briefly, antibodies were detected using fluorescein isothiocyanate (FITC)-labelled antihuman IgG (DAKO, Copenhagen, Denmark, lot 034, F202). *P. ovale* ATCC 42132 cells were used as the antigen. For detection of HIV antibodies, both a direct (Behring, Germany) and a competitive ELISA (Wellcome, England) were used. Samples positive in the ELISA were further analysed by Western blot using commercially available HIV strips (DuPont, Switzerland). In HIV-seropositive subjects, the number of lymphocytes in peripheral blood with surface antigen CD4 (helper T cells) and CD8 (suppressor-cytotoxic T cells) was determined using monoclonal antibodies (Ortho, USA) and CD4/CD8 ratios were calculated. A ratio below 0.80 was considered abnormal. The normal ranges of absolute number of CD4 and CD8 cells were set to $0.34–1.61 \times 10^9$/l and $0.21–1.06 \times 10^9$/l, respectively.

Statistics

Wilcoxon's rank sum test was used to compare quantitative cultures and mean serum antibody titres against *P. ovale* in HIV-seropositive and HIV-seronegative men. To investigate any correlation between the number of cultured *P. ovale*/cm^2 and the absolute numbers of CD4 and CD8 cells and the CD4/CD8 ratio, Pearson's co-efficient of correlation was calculated. Level of significance 5%.

RESULTS

None of the men studied had any clinical signs of pityriasis versicolor at the time of examination. In 2 of the 12 men in the HIV-seropositive group and in 2 of the 12 HIV-seronegative subjects, dandruff was found. Two men in each group had seborrheic dermatitis in the scalp, the face and/or on the external ears. This dermatitis was pronounced in only one of these

men; he belonged to the HIV-seronegative group. Thus, no difference in clinical dermatological status was found between the two groups studied.

The mean number of cultured *P. ovale* yeast cells/cm^2 was 131±332 (mean ± SD) in the HIV-seropositive group and 22±31 (mean ± SD) in the HIV-seronegative control group, but this difference did not reach statistical significance ($p>0.05$). The qualitative culture was positive in all subjects studied, except in one of the HIV-seronegative men.

There was no statistically significant difference in the mean serum antibody titres against *P. ovale* between the two studied groups. The mean titres were 155±173 (mean ± SD) and 227±169 (mean ± SD) ($p>0.05$) in the HIV-seropositive and HIV-seronegative groups, respectively.

The analyses of lymphocyte subsets in peripheral blood of the HIV-seropositive subjects showed the mean absolute numbers of CD4 cells and CD8 cells to be $0.48\pm0.24\times10^9$/l (range $0.14–1.05\times10^9$/l) and $0.81\pm0.41\times10^9$/l (range $0.31–1.73\times10^9$/l) respectively. The mean ratio of CD4/CD8 was 0.73±0.42 (range 0.13–1.32).

No correlation was found between the number of *P. ovale*/cm^2 and the absolute number of CD4 cells ($r=-0.0894$), the absolute number of CD8 cells ($r=-0.1234$) or the CD4/CD8 ratio ($r=-0.1619$). Nor did the number of *P. ovale*/cm^2 show any correlation to the presence of PGL in the HIV-seropositive subjects.

The patients with PGL did not differ significantly from the asymptomatic HIV-seropositive men regarding the results of the lymphocyte subset determinations.

DISCUSSION

P. ovale, the etiological agent of pityriasis versicolor (12), is also implicated in the pathogenesis of seborrheic dermatitis and dandruff (9, 10). The exact role of *P. ovale* in these disorders is still unknown. However, the rationale for the belief in a pathogenic role of this yeast is the results from treatment studies showing antifungal agents to be effective in the treatment of seborrheic dermatitis and dandruff (10, 11) and the fall in the number of *P. ovale* often found in parallel with the cure of seborrheic dermatitis (11).

An increased prevalence of seborrheic dermatitis has been reported in patients with AIDS, ARC (7) and PGL (2). In this study, we did not find any difference in the occurrence of seborrheic dermatitis or dandruff between HIV-seropositive patients and HIV-seronegative controls. The HIV-seropositive patients in this study did not present any serious HIV-related symptoms. This may explain the absence of a higher prevalence of the cutaneous disorders studied in these patients. Lymphocyte subset determination revealed only minor immunological deterioration or normal immune status.

The lack of difference in the immunological parameters measured and in the occurrence of skin manifestations between asymptomatic HIV-seropositive patients and those with PGL in this study accords with a recent study (19) reporting no correlation between the presence of lymphadenopathy and clinical and immune status.

High IgG antibody titres against *P. ovale* have been found in patients with seborrheic dermatitis and dandruff (20). As shown earlier in patients with pityriasis versicolor, the production of antibodies to *P. ovale* reflects a colonization with the lipophilic yeast rather than a disease process (21). In our study, we did not find any significant difference in the mean serum IgG antibody titres against *P. ovale* between HIV-seropositive and HIV-seronegative individuals.

The cellular immune system may be of greater importance for the ability to keep *P. ovale* as a saprophyte. In lymphocyte transformation studies using *P. ovale* antigens, Sohnle & Collins-Lech (22, 23) found positive responses in patients with pityriasis versicolor as well as in normal subjects. The response was significantly lower in the patients, however. One may

speculate about the presence of a selective defect in the cellular immune response to *P. ovale* rendering some individuals prone to develop pityriasis versicolor.

Whether or not an immunological disturbance is involved in the pathogenesis of seborrheic dermatitis remains to be elucidated. But the observation that the prevalence of this skin manifestation in HIV-seropositive individuals seems to increase with the patient's immunological deterioration does not contradict this possibility.

In the light of the possible role of *P. ovale* in the pathogenesis of seborrheic dermatitis, we tentatively suggest that this disorder, when occurring in HIV-seropositive patients, may be regarded as another example of an opportunistic infection affecting these patients.

In our study, however, no significant difference was found in the mean number of cultured *P. ovale*/cm^2 between HIV-seropositive and HIV-seronegative men.

In the HIV-seropositive group studied, no correlation was found between the number of cultured *P. ovale*/cm^2 and the immunological status of the patient. This may have been because of the lack of a more pronounced immunological deterioration in the patients studied. Future follow-up of the HIV-seropositive subjects may disclose whether these results will change in connection with progressive immunological dysfunction.

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