Impaired Release of Granulocyte/Macrophage Colony-stimulating Factor by Peripheral Blood Mononuclear Cells of Patients with Chronic Dermatophytosis in Response to Stimulation with Trichophytin

Sir,

The delayed-type hypersensitivity (DTH) response is recognized as one of the essential parts of the host defense against dermatophyte infections (1,2). T cell-derived cytokines are involved in the elicitation of the DTH response, and interferon-γ (IFN-γ) is regarded as a major factor in the effector phase of the DTH reaction (3). Granulocyte/macrophage colony-stimulating factor (GM-CSF), another T cell-derived cytokine, may also play a role in the elicitation of the DTH response (4). We previously demonstrated that IFN-γ and GM-CSF were released by peripheral blood mononuclear cells (PBMC) from patients with non-chronic dermatophyte infection, suggesting the involvement of these cytokines in the eradication of the dermatophytes from the stratum corneum of the skin (5,6).

The pathogenesis of chronic dermatophytosis has been partially clarified (7–10). Chronic dermatophytosis is caused mainly by *Trichophyton rubrum*. It has been proposed that *T. rubrum* induces chronic infection because it causes little inflammation, probably due to its lesser antigenicity. The site of infection may also influence the host's response. For example, patients with plantar dermatophytosis show a persistent clinical course. However, the changes of the patient's immune response may certainly favour the establishment of a chronic infection. Chronically infected patients possess a markedly reduced ability to express DTH to trichophytin, as reflected in an intradermal skin test reaction and antigen-stimulated lymphocyte transformation.

One of the key functional parameters determining the immune response to an infecting organism is the nature of cytokines produced by T cells. Recently, we have shown that the production of IFN-γ in response to stimulation with trichophytin was depressed in PBMC obtained from patients who had had a chronic dermatophyte infection, when compared with that in PBMC obtained from non-chronically infected patients (11). Our present study was focused on GM-CSF release by PBMC in patients with chronic dermatophytosis, and the pathogenesis of chronic dermatophytosis is discussed in particular in relation to the association with a possible deficiency of this immunoregulatory cytokine.

We investigated 3 patients with a dermatophyte infection (tinea pedis). All patients had *T. rubrum* infection, as demonstrated by a positive KOH examination and isolation of the causative fungi on Sabouraud's dextrose agar. Two patients, with a duration of infection of more than a year, were considered chronic cases. Peripheral venous blood was drawn from patients, and PBMC were isolated from the blood by density centrifugation. Trichophytin was prepared with *Trichophyton mentagrophytes* SM 0111 = RV 27961 (*Arthroderma vanbreuseghemii*), as reported previously (12).

PBMC (1×10^6/ml), suspended in RPMI-1640 medium (GIBCO, Grand Island, New York, USA) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% fetal calf serum, were cultured with and without trichophytin (50 μg/ml) for 72 h at 37°C in a humidified atmosphere containing 5% CO_2. Cell-free supernatants were collected and stored frozen at −70°C until needed. GM-CSF activity in the culture supernatant was determined in a solid-phase ELISA (Research and Diagnostics Systems, Minneapolis, MN, USA).

When PBMC were incubated with trichophytin, high levels of GM-CSF (118 pg/ml) were detected in the culture supernatants of PBMC from the patient with a non-chronic infection, as reported previously (6). In contrast, lower levels of GM-CSF were found in the chronically infected patients (26 pg/ml and 40 pg/ml).

Our results indicate that the production of GM-CSF by PBMC from the patients with a chronic dermatophyte infection in response to stimulation with trichophytin was impaired in contrast to that from the non-chronically infected patient. We have previously shown that the production of IFN-γ was impaired in peripheral lymphocytes obtained from the same patients with chronic dermatophyte infection (11). These findings indicate that peripheral T-lymphocytes of patients with a chronic dermatophyte infection have a reduced ability to produce IFN-γ and GM-CSF, which may play a role in the development of the DTH reaction in the skin. By measuring the release of cytokines, which are some of the key functional parameters of the immune response, this study supports the hypothesis that a partial defect in the DTH response to dermatophyte antigen may be responsible for the establishment of chronic dermatophytosis.

In our study, *in vitro* T-lymphocyte hyposensitivity to dermatophyte antigen was shown by measuring the release of the T cell-derived cytokines IFN-γ and GM-SCF, which play a role in the effector phase of the DTH reaction. Our data support the hypothesis that individuals predisposed to chronic dermatophyte infection exhibit depressed DTH to dermatophyte antigen. It is possible that a decreased release of IFN-γ and GM-CSF at the site of infection might explain the inability of chronically infected patients to eradicate dermatophytes from the skin.

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