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 planter 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 × 10⁶/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% CO₂. 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 hyporesponsiveness to dermatophyte antigen was shown by measuring the release of the T cell-derived cytokines IFN-γ and GM-CSF, 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.

REFERENCES

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Zinc Status in Patients with Telogen Effluvium

Sir,

Zinc deficiency with hair loss may occur in acrodermatitis enteropathica or be acquired due to total parenteral nutrition, alcoholism, malabsorption, wound healing, hemodialysis or sickle cell anemia (1, 2). Telogen effluvium refers to the loss of hair from a disproportionately large number of follicles that have entered the telogen stage of the hair cycle in a relatively synchronous fashion (3, 4). During the earlier stages of the process, there is an increase in the percentage of telogen hair apparent on the trichogram. The increased hair fall usually follows a stressful event by about 2 to 4 months. No specific treatment is currently available.

We have here evaluated the possible role of zinc deficiency in patients with idiopathic telogen effluvium and a control group. In addition to zinc determination in blood plasma, blood cells and hair, we measured two functional indices: albumin and alkaline phosphatase (EC 3.1.3.1.) activity in serum.

MATERIAL AND METHODS

Patients

Sixteen patients (3 males and 13 females) affected by idiopathic telogen effluvium and 16 apparently healthy controls matched for age, sex, hair colour and body mass index (BMI) were recruited. The study was explained in detail by one of the study staff and all subjects gave informed consent. The diagnostic was based on trichogram, which was performed in all subjects. The average age was 30 years, with a range of 14–48 years. The average BMI was 21 kg/m², with a range of 15–28 kg/m². Subjects using dyes, bleaches or other hair treatments were excluded. Subjects with the more common presentations of telogen effluvium, i.e. pregnant or lactating women, women taking oral contraceptives, post-surgical patients, patients suffering from infectious diseases, patients on slimming diet or corticosteroid treatment were also excluded. Subjects on zinc or vitamin A treatments were also excluded.

Samples

Blood was collected between 6 and 8 a.m. from fasting subjects. Twenty ml of blood were collected into disposable plastic syringes (Becton Dickinson, Meylan, France) and transferred into three zinc-free polystyrene tubes (CML, Nemesus, France). The first tube was without anticoagulant, the second tube contained zinc-free sodium heparin (Sigma, Saint Quentin Fallavier, France) and the third tube contained 1.25 ml of zinc-free 50 g/l Na,EDTA, 2H.O (Sigma, Saint Quentin Fallavier, France). Plasma, serum, leukocytes and red blood cells were separated from each sample within 60 min of the whole blood collection. Centrifugation of the first two tubes was performed at 1,600 x g for 10 min and at 10°C. Serum alkaline phosphatase activity and albumin were determined immediately. Plasma and erythrocytes were stored frozen at -18°C until zinc analysis. Hemolyzed samples were excluded. The 11.25 ml of the third tube was mixed with 3 ml of Plasmaneg® (Roger Bellon, Neuilly sur Seine, France). Differential sedimentation of the blood was allowed to take place for 1 h, after which 7 ml of leukocyte-rich plasma was placed in a zinc-free glass tube containing 14 ml of 8.7 g/l ammonium chloride (Prolabo, Paris, France). The tube was mixed by gentle inversion and centrifuged at 400 x g for 5 min. The supernatant was discarded and the leukocyte cells were washed twice by 10 ml of ringer – lactate – glucose infusion (Aguettant, Lyon, France). Leukocytes were resuspended in 1.6 ml ringer – lactate – glucose infusion. A 100 μl aliquot of the suspension was used for leukocyte counting (Coulter counter, Coultronic, Margency, France). The remaining suspension was centrifuged at 400 x g for 15 min and the cells were stored frozen at -18°C until zinc analysis. Hair was collected 7 days after shampoo from the occipital region as close to the scalp as possible using stainless steel scissors. The hair was cut into three sections (proximal, central, distal) of approximately 20 mg. It was washed with 10 ml ace tone (Prolabo, Paris, France) to remote zinc surface contaminations and then with 10 ml of 20 g/l Triton X 100 (Prolabo, Paris, France). This procedure was then followed by four rinses with 10 ml of deionized water. The hair was then dried overnight at 50°C. The entire collection procedures were checked for zinc and were found to be free from contamination.

Analyses

To minimize analytical variations, zinc determinations of patient and matched control were performed in a batch. Zinc concentrations were