

## Cytokine Production of Peripheral Blood Mononuclear Cells in a Patient with Sporotrichosis in Response to Stimulation with Sporotrichin

Sir,

Sporotrichosis is a granulomatous mycotic infection, mainly of the skin, caused by *Sporothrix schenckii*. The immunological mechanisms involved in the prevention and control of sporotrichosis are not fully understood. However, a delayed-type hypersensitivity (DTH) response is recognized as an important factor in the host defence against *S. schenckii* (1, 2). T cell-derived cytokines are involved in the elicitation of the DTH response, and interferon- $\gamma$  (IFN- $\gamma$ ) is regarded as a major factor in the effector phase of the DTH reaction (3). Other cytokines, such as interleukin-2 (IL-2) and granulocyte/macrophage colony-stimulating factor (GM-CSF), also contribute to the DTH response (4). In this study, the release of these cytokines in response to stimulation with sporotrichin was measured in peripheral blood mononuclear cells (PBMC) from a patient with lymphocutaneous sporotrichosis, and the involvement of cytokine in the eradication of *S. schenckii* from the skin is discussed.

A 48-year-old female patient with lymphocutaneous sporotrichosis was referred to the Dermatology Clinic of Kyushu University Hospital. The patient had an *S. schenckii* infection, revealed by isolation on Sabouraud dextrose agar. Sporotrichin was prepared with *S. schenckii* ATCC 10268, as reported previously (5). PBMC ( $1 \times 10^6$ /ml), suspended in RPMI-1640 medium (GIBCO, Grand Island, NY) supplemented with 100 U/ml penicillin, 100  $\mu$ g/ml streptomycin, and 10% fetal calf serum, were cultured with and without sporotrichin (50  $\mu$ g/ml) for 72 h at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>. Cell-free supernatants were collected and stored frozen at -70°C until use. IFN- $\gamma$  activity in the culture supernatant was measured with a radioimmunoassay (RIA) test kit (Centocor, Melverne, PA). IL-2 activity was measured with a RIA test kit (IRE-Medgenix, Brussels, Belgium), and GM-CSF activity was determined in a solid-phase ELISA obtained from Research and Diagnostics Systems (Minneapolis, MN).

When PBMC were incubated with sporotrichin, high levels of IFN- $\gamma$ , IL-2, and GM-CSF were detected in the culture supernatant of PBMC from this patient (Table I). When PBMC were incubated without sporotrichin, there were no significant levels of these cytokines.

Our data indicated that IFN- $\gamma$ , IL-2, and GM-CSF were released by PBMC from the patient with sporotrichosis in response to stimulation with sporotrichin.

IFN- $\gamma$ , produced by Th1 cells, plays an important role in the

effector phase of the DTH reaction (3). Other T cell-derived cytokines may also play a role in the elicitation of the DTH response (4). Such factors may include the Th1 cell-derived cytokine IL-2 or the Th1/Th2 cell-derived cytokine GM-CSF (4). In this study, we showed that our patient had circulating sporotrichin-specific T lymphocytes capable of producing IFN- $\gamma$ , IL-2, and GM-CSF, which are known to play a role in the development of the DTH reaction in the skin. With this secretion pattern, sporotrichin-specific T lymphocytes from the patient resembled the Th1 cell subset.

The DTH response appears to play a critical role in host resistance to *S. schenckii* infection. DTH-mediating Th1-like cells may serve as the initiating event through the release of IL-2 into the local environment, with subsequent activation of other Th1-like cells. IL-2 stimulates the production of GM-CSF and IFN- $\gamma$  by DTH-mediating Th1-like cells. GM-CSF and IFN- $\gamma$  may attract and/or activate macrophages and neutrophils (6, 7). Both neutrophils and macrophages can phagocytize and kill *S. schenckii* (8, 9). In this way, the DTH response mediated by cytokine production acts as an effector mechanism.

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Table I. Cytokine release by peripheral blood mononuclear cells

Cultured with	IFN- $\gamma$ (U/ml)	IL-2 (U/ml)	GM-CSF (pg/ml)
(-)	0.3	<0.8	11
Sporotrichin	8.9	1.7	197