LETTERS TO THE EDITOR

Local Expression of Migration Inhibitory Factor and Th1 Type Cytokine mRNA in Sporotrichosis Lesions

Sir,

Sporotrichosis is a deep mycosis caused by Sporothrix schenckii, in which granulomatous lesions develop exclusively in the skin; it rarely affects systemic organs except in patients treated chronically with steroids for a long period. Many patients with sporotrichosis show a delayed skin reaction to sporothricin. The delayed type hypersensitivity (DTH) response to S. schenckii is one of the host defense mechanisms in sporotrichosis (1-3). T-cell-derived cytokines (such as IFN-γ and IL-2) are involved in the elicitation of the DTH response (4). It has been reported that peripheral blood mononuclear cells (PBMC) from patients with sporotrichosis produce high levels of IFN-γ and IL-2 in response to stimulation with sporothricin (1). The host response to infection appears to be regulated by specific patterns of local cytokine production (5, 6). However, local cytokine production by the sporotrichosis lesion is not known.

Skin biopsy specimens were obtained after informed consent from a 57-year-old male patient with lymphocutaneous sporotrichosis. The patient had an S. schenckii infection, revealed by isolation on Sabouraud dextrose agar. He was treated with oral terbinafine, 125 mg/day. During the treatment period, a biopsy specimen of the lesion was obtained every month and cultured for isolation of S. schenckii. After being embedded in OCT medium (Miles, Elkhart, IN), specimens were snap frozen in liquid nitrogen and stored at −70°C. S. schenckii was not detected 3 months after the initiation of treatment. Total RNA was extracted from each biopsy specimen by the method of Chomczynski & Sacchi (7), with some modification. To facilitate the rapid lysis of the cells isolated from tissue, 50 pieces of 4-μm cryostat sections were placed in 4 M guanidinium isothiocyanate buffer. The samples were treated with DNase (Promega Co, Madison, WI) for 60 min at 37°C. RNase inhibitor (Boehringer Mannheim Co, Indianapolis, IN) was present during all enzymatic manipulations of RNA. cDNA was synthesized from oligo-DT (Pharmacia)–primed RNA by incubation at 43°C with M-MLV reverse transcriptase (GIBCO BRL, Life Technologies Inc., Gaithersburg, MD) and 0.5 mM of dNTPs (Pharmacia AB, Uppsal, Sweden) for 1 h. The PCR method was carried out as described by Yamamura et al. (8). The PCR reaction mixture contained 10 mM of Tris-HCl, pH 9.0, 50 mM of KCl, 2.5 mM of MgCl₂, 0.1% (W/V) gelatin, 0.2 mM of dNTPs, 25 pM of 5′ and 3′ oligonucleotide primers, and 2.5 U of Taq polymerase (Promega). cDNA was amplified by PCR, using oligonucleotide primers specific for β-actin, CD38, IL-2, IFN-γ, IL-4, IL-5, and macrophage migration inhibitory factor (MIF). A DNA thermocycler 480 (Perkin-Elmer Cetus, Norwalk, CT) was used for 35 cycles of denaturation at 94°C for 1 min and annealing/extension at 55°C (in the case of IL-2 and IFN-γ) or 65°C for 2 min (in the case of β-actin, CD38, IL-4, IL-5 and MIF).

The sequences of cytokine-specific primer pairs 5′ and 3′ are as follows: β-actin: GGGGACATGTGAGGACAGTCATGCAGCT, and ATCCTCATGTCGCTGACCTCAG, and IFN-γ: GGTGGGTCGCTACTGAGGCCTCATC; IL-2: ACTCAGAGGATGTCGCTCATT; and AGGT- AATCCATGTCGCTCAGA, IFN-γ: GTATATCTGTCGCT- TTCCA, and ACCGGAAATTAGTGCAGGCTT; and CTGCCTCTGTCGCTGACCTCAG; IL-5: ATGAGGATCTGTCGCTG; and TCA- CTGTCGCTGACCTCAG; and ATCCATGTCGCTGACCTCAG; and GGCAGAAGGT- GAGGATCTGTCGCTGACCTCAG; and GGCAGAAGGT- GAGGATCTGTCGCTGACCTCAG. The PCR product was subjected to electrophoresis in 1.5% agarose gel and visualized by staining with ethidium bromide.

In the sporotrichosis lesion, IL-4 and IL-5 mRNA expression was not detected, and expression of MIF and Th1 type cytokines (IL-2, IFN-γ) predominated. In the lesion that improved after treatment with terbinafine, MIF mRNA expression was not detected and IL-2, IFN-γ mRNA expression became weak. These results suggest antigen-specific local expression of MIF, IL-2 and IFN-γ.

These findings are taken as evidence of antigen-specific (Th1 type) local expression. It is noteworthy that virtually identical results have been obtained also from lesions of skin biopsies of patients with active atopic dermatitis (8), which presumably is a Th2 type of inflammation. Mosmann et al. (9) reported that murine CD4+ T cells can be divided into two functionally different subsets (Th1 and Th2). Th1 cells secrete IL-2, IFN-γ and are the principal effectors of cell-mediated immunity against intracellular microorganisms and of DTH reactions. MIF combines effector cells, macrophages, to lesions and facilitates TNF-α secretion, acts together with IFN-γ to promote nitric oxide release, and augments macrophage killing of intracellular pathogens (10, 11). It has been reported that nitric oxide exerts bactericidal action of fungi such as Candida albicans by inhibiting the mitochondrial respiratory chain of eukaryotic cells in the effector phase of DTH (12, 13). These mechanisms may contribute to the elimination of S. schenckii in a sporotrichosis lesion.

Fig. 1. PCR analysis of cytokine mRNAs from sporotrichosis lesion. Skin biopsy specimen of the lesion was obtained every month. Total RNA extracted from each biopsy specimen was reverse transcribed to cDNA, cDNA for β-actin (marker for all cells), CD38 (a marker of T cells), IL-2, IFN-γ, MIF, IL-4 and IL-5 was amplified by PCR. The predicted size of the PCR products were 546, 316, 266, 356, 347, 317 and 465 bp, respectively. Molecular weight markers (M) are shown in the last lane on the right in panel. Lanes 1, 2, 3, 4, 5, 7, 8, 10, 12 and 14: Skin biopsy specimen was obtained from non-treated sporotrichosis lesion. Lanes 2, 4, 6, 8, 10, 12 and 14: Skin biopsy specimen was obtained from the lesion that was three months after the initiation of treatment.

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lesion. It is suggested that in sporotrichosis patients with no underlying diseases in particular, S. schenckii-specific DTH induced in lesions participates in preventing organisms from spreading to other systemic organs.

REFERENCES


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Congenital Ectopic Nails of the Fingers Associated with Bone Deformities

Sir,

Ectopic nail, known as congenital onychoheterotopia, was referred to as polyonychic congenita sine polycystyly in cases reported by Ohyza in 1931 (1). A congenital ectopic nail is an additional nail independent of the normal nail, a relatively rare condition. Several authors (1–4,10) have described this condition, mainly in Japan, as an asymmetrical, non-hereditary, non-familial change involving one finger or toe, unlike most congenital disorders. We describe a patient with ectopic nails on the palmar tips of the right fifth and left fourth finger.

CASE REPORT

A 26-year-old Japanese woman presented with hard keratotic papules on the tip of the right fifth finger and the left fourth finger. Physical examination revealed a small hard oval, keratotic lesion, measuring 4 x 3 mm, with a rough surface at the center of the palmar aspect of the right fifth finger, which showed a clubbed appearance at the tip (Fig. 1A), and a thin small flattened keratotic lesion, just beneath the nail at the center of the lateral aspect of the left fourth finger (Fig. 2A) without motor disturbance in the affected fingers. These hyperkeratotic lesions on the fingers had existed since birth and grew with the same speed as the other normal nails, so that she had to trim them occasionally. On closer examination and with information from the patient, they were determined to be nails. Radiography disclosed a Y-shaped bifurcation of the distal phalanges of the right fifth finger (Fig. 1B) and an M-shaped depressed deformity in the phalanges of the left fourth finger (Fig. 2B), corresponding to the location of the ectopic nail.

Fusing of the distal interphalangeal joint of the bilateral fourth and fifth toes and latent bursitis of the sacral bone were also found on roentgenogram. Her family history was not contributory and no previous injuries to the affected fingers were noted. She was not seeking surgical removal of the ectopic nails at this time.

DISCUSSION

In 1931, Ohyza (1) reported an ectopic nail, referring to it as polyonychic congenita sine polycystyly. Subsequently, Kikuchi (2) described two similar cases, for which he proposed the term "ectopic nail". Affected patients show no association of motor disturbance or other anomalies involving the fingers or toes. The present case can be differentiated from congenital onychodysplasia of the index finger (COIF or Iso-Kikuchi syndrome (9)), because the localization of the ectopic nails in COIF is restricted to the dorsal aspect of the finger. Similar conditions, such as polyonychia associated with syndactyly and polydactyly, can be differentiated by their clinical and radiographic appearance. In addition, acquired digital fibrokeratosis and supernumerary digits are differentiated, since the lesions described had the clinical appearance of nail-plate with similar hardness and continuous growth.

Previously, some authors (1–8, 10) have described cases of Ohyza’s type congenital ectopic nail. A patient with ectopic