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Lower percentage of CD8+ T cells in peripheral blood of patients with sporotrichosis

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ABSTRACT

Purpose: To characterize the peripheral immunity and immunity response of patients with sporotrichosis, in this study we determined the lymphocyte subsets in the peripheral blood of Chinese patients with sporotrichosis.
Methods: In this retrospective study, peripheral blood was collected from 69 sporotrichosis patients (37, fixed cutaneous form; 32 lymphocutaneous) and 66 healthy controls. Lymphocyte subsets were analyzed using flow cytometry.
Results: Compared to controls, the percentage of CD8+ T cells was lower in sporotrichosis patients. The percentage of CD8+ T cells in peripheral blood tended to become lower with disease duration and disease severity, although the difference was not statistically significant for either acute, subacute and chronic patients or fixed cutaneous and lymphocutaneous patients.
Conclusion: Our data indicate that the decrease of CD8+ T cells in peripheral blood of patients with sporotrichosis is associated with disease severity, although the difference was not statistically significant for either duration or clinical forms of the disease. Combining antifungal agents and immunomodulators in patients with long disease duration and lymphocutaneous may be more beneficial than antifungal monotherapy.

1. Introduction

Sporotrichosis is one of the most frequent subcutaneous fungal infections in humans and animals caused by members of the plant-associated, dimorphic genus *Sporothrix* spp. Sporotrichosis is endemic in several areas of China, especially in the northeastern provinces of Jilin, Heilongjiang, and Liaoning [1]. The disease can be classified into 4 categories, based on clinical presentations: fixed cutaneous; lymphocutaneous; multifocal or disseminated; or extracutaneous [2]. The fixed cutaneous and lymphocutaneous forms are the most common manifestations of infection [3].

After inoculation with the conidia of *Sporothrix* spp. into the skin or subcutaneous tissue, an individual may develop either the fixed cutaneous or lymphocutaneous form of the disease depending on the depth of the inoculation, host immunological status, and strain virulence [4]. The defining feature of fixed cutaneous sporotrichosis is a single lesion confined to the site of initial inoculation. The lesion can be papular, plaque-like, nodular, or verrucous, and ulceration is very common [2]. The lymphocutaneous form of sporotrichosis is distinguished by nodular lesions that appear along the lymphatic distribution proximal to the initial lesion, with lymphangitic streaking between the nodules [3]. The average duration of sporotrichosis inoculation before the presentation is usually 5–6.41 months [1,5].

Although the host's defense mechanisms against the pathogenesis of sporotrichosis are not fully understood, histological observations indicate that there is an immune response. Specifically, the histopathological changes consist of a predominance of diffuse granulomatous and suppurative necrotizing dermatitis associated with lymphoplasmacytic infiltrate and epidermal changes [6,7]. Quintella et al. [6] reported that the numbers of lymphocytes in non-phagocytic cells correlated with the rate of lack of visible fungus in patient samples. Moreover, Miranda et al. [7] reported that lymphocytes were present in the peripheral infiltrate in 70% of sporotrichosis cases.

The present studies about pathogenic mechanisms of sporotrichosis are almost based on animal model experiments, and there

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are very few reports about humoral response in sporotrichosis patients. Animal experiments show that cell-mediated immunity is crucial for the organism to remove *Sporothrix* spp. Tachibana et al. [8] recently demonstrated that cell-mediated immunity against *Sporothrix* spp. was indicated by macrophages activated by $CD4^+$ T cells. Uenotsuchi et al. [9] showed that the cytokine profile in the peripheral blood mononuclear cells was of a dominant Th1 pattern. There are still controversies on the role of humoral response in sporotrichosis. Several studies suggest that the defect of T lymphocytes is a risk factor for sporotrichosis [10,11]. Hachisuka and Sasai [12] found that B cells were dominant in the inoculated site and the percentage of B cells was higher compared with the control. A specific humoral response induced by a 70-kDa antigen from *Sporothrix* spp. was recently observed in infected mice [13]. These data suggest that a humoral immune response also has an important role in sporotrichosis. The lymphocyte subpopulations in human subjects have not been investigated, and the role of humoral response in sporotrichosis remains to be defined.

To characterize the peripheral immunity and immunity response of patients with sporotrichosis, in this study we determined the lymphocyte subsets in the peripheral blood of Chinese patients with sporotrichosis. Flow cytometric analysis was performed to compare the profiles of lymphocyte subpopulations between sporotrichosis patients and controls.

2. Materials and methods

2.1. Subjects

The Ethics Committee of China-Japan Union Hospital of Jilin University approved this retrospective study, and all subjects gave signed consent. Enrolled were 70 patients who received diagnoses of sporotrichosis at the Department of Dermatology and Venereology of the China-Japan Union Hospital of Jilin University from 1 July 2011 to 31 December 2013. The definitive diagnosis of sporotrichosis was established based on complaints, physical examination, histopathological examination, and fungal culture of the samples obtained through skin biopsy. To minimize the influence of immunological conditions, patients were excluded who suffered from significant infection, immune suppression, or renal, hepatic, or other medical diseases. Patients with significant infection were considered to have infectious diseases other than Sporotrichosis, with diagnoses based on clinical symptoms, signs, or laboratory examinations. Immune suppression patients were those using immune suppression medicine. Also not allowed were patients treated with systemic antifungal, corticosteroid, or other immunosuppressive therapy during the 2 weeks prior to entering the study.

The control group consisted of 66 healthy individuals selected to match the patients in age and gender (23 men, mean age 67 y, range 49–77 y; and 43 women, mean age 69 y, range 62–76 y). The participants in the control group underwent physical examination and biochemical tests that confirmed their good health condition.

To analyze the effect of disease duration on host immunity, the sporotrichosis patients were divided into a subacute-acute group (n = 42) with onset or reactivation <6 months, and a chronic group (n = 27) with sporotrichosis for ⩾6 months. Furthermore, we analyzed the sporotrichosis patients by the form of the disease [14]: fixed cutaneous (n = 37; Fig. 1), and lymphocutaneous (n = 32; Fig. 2). For both types of analyses, the groups (including the control group) were comparable in age and gender ratio.

2.2. Collection of samples

Venous peripheral blood samples (5–10 mL) of all subjects were drawn into blood collection tubes containing ethylenediaminetetraacetic acid (EDTA). All samples were examined at the Central Laboratory of China-Japan Union Hospital of Jilin University within one day of collection.

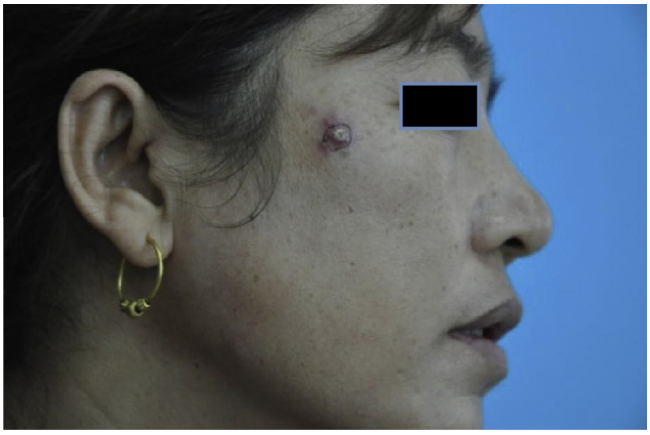

Fig. 1. Fixed sporotrichosis: a nodule on right zygomatic. Representative image.

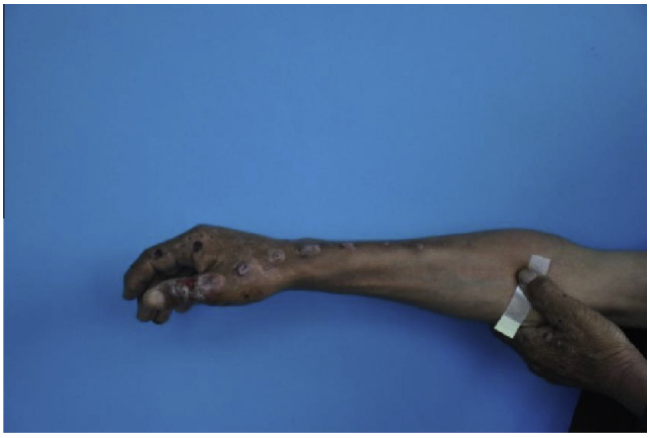

Fig. 2. Lymphocutaneous sporotrichosis: linear distribution of nodules on the left hand and arm. Representative image.

2.3. Flow cytometry

The percentages of peripheral blood subpopulations of lymphocytes were determined via flow cytometry, using a Cytomics FC500 system with CXP software (Beckman Coulter). The following antibodies were used for the determination of lymphocyte populations: anti-CD45 fluorescein isothiocyanate, anti-CD4 phycoerythrin (RD1), anti-CD8 phycoerythrin-Texas red X (ECD), anti-CD3 r-phycoerythrin-cyanine 5 (PC5), anti-CD56 RD1, and anti-CD19 ECD. Mouse IgG isotype control antibodies CD45, IgG1-PE, IgG1-ECD. IgG1-PC5 were used to estimate the amount of nonspecific binding (all the antibodies were from BD Biosciences). Cell preparations were incubated with the antibodies for 15 min in the dark at room temperature, then lysed in 500 μL FACS Lysing Solution (BD Biosciences). After washing with phosphate-buffered saline with 2% human serum albumin, the samples were immediately analyzed by flow cytometry.

2.4. Statistical analyses

The experimental data are shown as mean ± standard deviation (SD). The differences between patients with sporotrichosis and controls were analyzed by the *t*-test. Nonparametric multiple