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Humoral immune response against soluble and fractionate antigens in experimental sporotrichosis

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Abstract

Sporotrichosis is a chronic granulomatous mycosis caused by the dimorphic fungus *Sporothrix schenckii*, which is widely distributed in nature, and presents a saprophytic mycelial form on plant debris and soil. The immunological mechanisms involved in the prevention and control of sporotrichosis are not yet fully understood. In this study, mice were studied after infection with *Sporothrix schenckii*. In the first week after infection, fungal loading increased and thence decreased drastically 14 days after infection. Analysis by immunoblotting showed that the sera of all mice tested had antibodies reacting only with a 70 kDa antigen, with predominance of IgG1 and IgG3. Taken together, our results show that antigens from *S. schenckii* induced a specific humoral response in infected mice.

Keywords: Sporotrichosis; Humoral immune response; Antibody

1. Introduction

Sporotrichosis a chronic granulomatous mycosis caused by the dimorphic fungus *Sporothrix schenckii* which is widely distributed in nature and presents a saprophytic mycelial form on plant debris and soil. The traumatic inoculation of conidia and hyphae of *S. schenckii* results in the development of this subcutaneous mycosis and within the infected tissue the fungus differentiates into its yeast form and may spread to other tissues. The systemic form of sporotrichosis may evolve from an initial cutaneous lesion or be associated with the inhalation of conidia [1–3]. Recently, more severe clinical forms of this disease have been associated with immunocompromised patients, such as human immunodeficiency virus (HIV)-infected patients, suggesting that *S. schenckii* is an emerging opportunistic pathogen [4].

Cultivation and immunological techniques are of great importance for the diagnosis of *S. schenckii* infection. The latter techniques require an understanding of the antigenic structure of the etiological agent [5]. Apparently, mono-rhamnosyl rhamnomannan is the principal component causing cross reactivity. Predominant in the yeast phase of the fungus [6], this component has not had, as of yet, its molecular weight determined [7]. It has, however, been precipitated and purified from the yeast wall of *S. schenckii* using Concanavalin A. Three principal subunits with masses 84, 70 and 58 kDa have been identified [8]. Other investigations suggest that the surface polysaccharides vary during the morphological differentiation of the fungus [6]. Additional studies of the yeast antigens describe a range of proteins with masses between 22 and 70 kDa [9].

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The immunological mechanisms involved in the prevention and control of sporotrichosis are not fully understood. Previous studies have suggested that cell-mediated immunity plays an important role in the protection of the host against this fungus. Tachibana et al. [10] have recently demonstrated that acquired immunity against *S. schenckii* is expressed by T-cell-active macrophages.

Even though many studies have investigated antibody (Ab) responses in fungal infections, critical features such as Ab fine specificity and/or Ab isotype were seldom observed. Moreover, because many different clinical and experimental settings have been addressed, no general conclusion has yet been reached [11]. Some Abs (for instance, the anticryptococcal ones) may be disease enhancing. Nonetheless, recent data strongly support the existence of protective Abs against two of the major opportunistic agents of fungal disease. In addition to strengthening the few significant findings published previously, this new evidence is important because it highlights new protective antigens as well as possible novel sources and mechanisms of Ab-mediated protection [12–14].

The role that the humoral immune response plays in sporotrichosis is until now uncertain. Characterisation of *Sporotrix* antigens which are active in disease would permit the understanding of the importance of antibody response, which might be of value in forecasting the prognosis and for the selection of an optimal therapeutic regimen. In this study, we evaluated antibody production and determined the antigenic components involved in the humoral immune response in experimental infection.

2. Materials and methods

2.1. Organism

Sporothrix schenckii strain M-64, previously isolated from a human case of sporotrichosis, was originally obtained from the Department of Dermatology of the Faculty of Medicine at the University of São Paulo. Yeast forms of *S. schenckii* were grown for 3 days in brain-heart infusion agar (BHI) at 37 °C.

2.2. Animals

Twelve BALB/c female mice with 8–12 weeks of age were used. Mice were obtained from the University of São Paulo animal facilities.

2.3. Soluble antigen preparation

For the preparation of yeast-phase soluble antigen, yeast cells were grown in yeast nitrogen-Casamino Acids-glucose (YCG) medium consisting of (grams per litre of distilled water); yeast nitrogen base, 6.7; Casamino acids, 2.5; glucose, 50; and 1 mL of a vitamin mixture (containing [per 100 mL] thiamine hydrochloride, 50 mg; riboflavin, 50 mg; calcium pantothenate, 50 mg; nicotinic acid, 50 mg; pyridoxine hydrochloride, 10 mg; *p*-aminobenzoix acid, 10 mg; inositol, 10 mg; folic acid, 1 mg; biotin, 0.4 mg) [9]. Cells from 48-h-old cultures on BHI agar were suspended in BHI broth and inoculated into tubes containing 20 mL of BHI broth followed by incubation with agitation at 35 °C for 48 h. Yeast grown in BHI broth was inoculated into 200 mL of YCG in 1-L Erlenmeyer flasks. These starter cultures were incubated for 3 days with shaking (100 rpm, orbital shaker, New Brunswick Scientific, Edison, NJ, USA). Yeast cells were centrifuged and transferred to 3 L of YCG medium (in 4-L Erlenmeyer flasks) and incubated at 35 °C with shaking (as before) for 7 days. The yeast cells were removed by filtration and antigen preparation was stocked at −20 °C until use.

2.4. Animal infection

Groups of six BALB/c mice were infected intraperitoneally with 5×10^6 yeast forms of *S. schenckii* suspended in PBS (2.65 g Na2HPO4, 0.35 g NaH2PO4.H2O and 8.18 g NaCl in 1 L of distilled water). The control group received PBS only. Blood samples were collected individually at 7, 14, 21 and 28 days after infection and their serum was stocked at −20 °C until use.

2.5. Fungal loads in the organs of infected animals

On the 7th, 14th, 21st and 28th day after infection, animals were sacrificed and the fungal load in organs (liver and spleen) was determined by measuring colony forming units (CFU). Briefly, the organs were collected, weighed and disrupted using a tissue grinder. The cell suspensions were plated on BHI agar. Colonies were counted from the 4th day until no fungal growth was detected. The results were expressed in CFU/g of tissue.

3. Elisa

An indirect solid-phase enzyme-linked immunosorbent assay (ELISA) was performed on serum samples by standard methods as previously described [15]. An optimal concentration of the soluble *Sporothrix* antigen (0.5 μg/mL) (prepared as above) was added to a microplate well (in 0.2 mL) (Polystyrene microtiter plates, Costar). This concentration of *Sporothrix* protein was determined by checkerboard titration of twofold dilutions of antigen and high-titered infected mouse serum. The plates were incubated overnight at 4 °C and washed in phosphate-buffered saline (PBS) containing 0.05%