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Virulence of *Sporothrix luriei* in a Murine Model of Disseminated Infection

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Abstract *Sporothrix luriei* is a rare fungus causing sporotrichosis in humans. The virulence of this fungus was evaluated in a murine model of disseminated infection. Mice were challenged intravenously with two different inocula (2×10^5 and 2×10^7 CFU/animals) but only the highest one was able to kill the animals. Infected mice died between days 12 and 16, liver and spleen being the most affected organs. In the infected tissues, a massive infiltration of fungal cells and phagocytes were observed, but not the typical "eyeglass" cells described in infected human tissue.

Keywords Animal models · *S. luriei* · Virulence · Histopathology

Introduction

Sporotrichosis is a granulomatous disease caused by species of the genera *Sporothrix* [1, 2]. These infections are mainly reported from tropical and temperate zones of South America, mostly acquired by traumatic inoculation of the fungus from soil, wood or plants. Major clinical manifestations include subacute or chronic lymphocutaneous forms. The most severe complication of the disease is a disseminated infection that especially occurs in immunosuppressed patients [3–6].

Until recently, sporotrichosis was considered to be caused by a single species, *Sporothrix schenckii*; however, some molecular studies have demonstrated that *S. schenckii sensu lato* is a complex of cryptic species [2, 7, 8]. *S. brasiliensis* and *S. schenckii sensu stricto* are the most common *species of the complex* with a different geographical distribution, degree of virulence and antifungal susceptibility. *Sporothrix luriei* is a separate species, phylogenetically related to the species of the *S. schenckii* complex [9] and reported rarely in human infection.

The first case of human infection caused by *S. luriei* was reported in a South African male by Ajello and Kaplan in 1956 [10]. So far, *S. luriei* has been reported as the causal agent in four cases of human infection, one of them being fatal [11]. Such infections were histopathologically characterized by the presence of thick-walled cells and typical large cells with an "eyeglass" configuration in tissue [10–13]. Although it is likely that these species were involved in more cases than those reported, principally because diagnosis of sporotrichosis is usually based on clinical manifestations and the fungus is not always isolated.

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In addition, the morphological similarity of the different species of *Sporothrix* complicates the diagnosis. Recently, molecular tools have been used for a more accurate identification [7, 14]. The scarcity of the infections caused by this fungus in comparison with other species of *Sporothrix* and the meaning of the presence of the "eyeglass" cells are intriguing aspects in this illness. The goal of the present study was to evaluate the virulence of *S. luriei* in a murine model of disseminated infection and to compare it with other species of *Sporothrix* from our previous findings in order to asses the potential pathogenesis of this species.

Materials and Methods

Strain and Inocula Preparation

One strain of *S. luriei* (CBS937.72), which was isolated in the first reported clinical case [10], was included. The strain was stored by lyophilization, subcultured on PDA plates and incubated at 30°C for 8–10 days. In aim to restore the virulence of the strain, which may be diminished after long period storage, a conidial suspension was intravenously administered to two mice. Seven days later, kidneys and liver were removed, homogenized and placed on PDA for strain recovery. Subcultures were used as stock to prepare the experimental inocula. The inocula were prepared by flooding the plate surface with saline solution. The resultant suspension was added into 100 ml of potato dextrose broth (PDB) and incubated in an orbital shaker (150 rpm) at 30°C for 4 days. Then, the cultures were filtered twice through sterile gauze and centrifuged at 7,000 rpm for 20 min. Conidia suspensions were adjusted to the desired concentration by haemocytometer counting. To verify the viability and size of inocula, 10-fold dilutions were placed in PDA for CFU determinations.

Animals

Six-week-old OF-1 male mice (Charles River, Criffa SA, Barcelona, Spain), weighing 28–30 g, were used. Animals were housed five per cage and provided with food and water ad libitum. After infection, animals were checked twice a day and mobility, fur aspect, orientation, skin lesions and food ingest were used for discomfort evaluation. Conditions were approved by the animal Welfare Committee of the Universitat Rovira i Virgili.

Mortality

For the survival study, two groups of 8 animals were inoculated intravenously (i.v.) via the lateral tail vein with 2×10^5 CFU/animal (low inoculum) or 2×10^7 CFU/animal (high inoculum). Animals were checked twice a day for 30 days.

Tissue Burden

Fungal load only was determined in those organs from animals infected with the inoculum that was able to cause death of animals. Two groups of animals (five per group) were infected i.v. with the high inoculum. The first group was killed on day 7 and the remaining group on day 15 post-infection; mice were killed by CO_2 anoxia, and heart, brain, kidneys, spleen, and liver were removed aseptically. Half of the organs were homogenized in 1 ml of sterile 0.9% saline, 10-fold diluted, placed on PDA plates and incubated at 30°C for 4 days for CFU determination.

Histopathology Study

In order to evaluate the histopathological changes in different tissues, two groups of 15 animals were infected i.v. with high or low inoculum. Macroscopically changes as size, weight, color, presence or absence of hemorrhage or abscesses were assessed. On days 7, 15 and 30 after challenge, five animals per group were killed as previously described. Half of each of the above-mentioned organs was fixed with 10% buffered formalin. Samples were dehydrated, paraffin-embedded, sliced into 2 μm sections and stained with hematoxylin and eosin (H-E), Periodic acid-Schiff (PAS) and Grocott methenamine silver. Sections were examined by light microscopy for histopathological changes.

Statistical Analysis

Organ burden data were $\log_{10}$-transformed and compared by the two-tailed Mann–Whitney *U*-test, using Graph Pad Prism 5 for Windows. Survival was compared using a two-tailed log-rank test. *P* values < 0.05 were considered significant.

Results

Viability of Inocula

For the low inoculum, the viability was 1.3×10^6 CFU/ml and for high inoculum 11×10^8 CFU/ml.

Mortality

The mortality rate of mice infected with *S. luriei* correlated with inocula size (Fig. 1). All the animals challenged with the low inoculum (2×10^5 CFU/animal) survived to the end of experiment (30 days), while the high inoculum (2×10^7 CFU/animal) caused 100% mortality in 12–16 days.

Tissue Burden

Mice infected with the high inoculum showed fungal loads in all studied organs on day 7 post-infection. The mean CFU/g was high in all the studied organs, ranging from 5.56 ± 0.35 to 6.34 ± 0.27 CFU/g. The most affected organs were liver and heart followed by brain, kidneys, lungs and spleen (Fig. 2). Fungal load increased significantly in all organs at day 15 post-infection in comparison with those obtained on day 7 post-infection ($P < 0.007$). Furthermore, in these cases, there was a significant increase in burden, with liver being the most infected organ (8.06 ± 0.9 CFU/g), followed by brain, lungs, kidneys, heart and spleen.

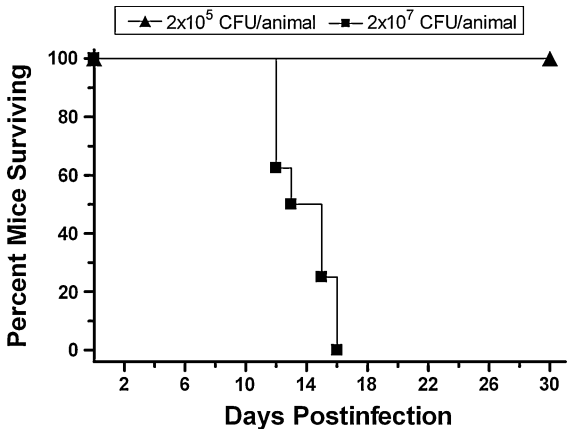

Fig. 1 Cumulative mortality of mice infected with *S. luriei* (FMR 9290). Mice were infected with 2×10^5 CFU/animal or 2×10^7 CFU/animal

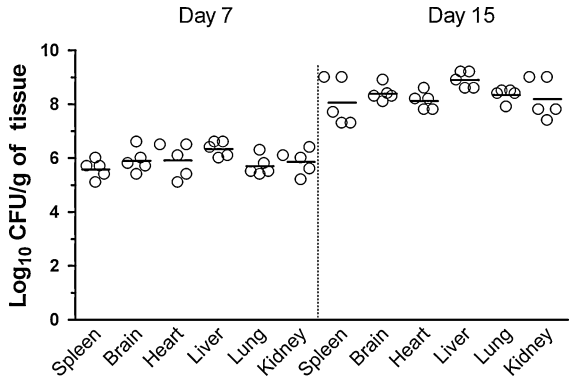

Fig. 2 Quantitative fungal recovery from different organs of mice infected with *S. luriei* (FMR 9290) 2×10^7 CFU/g of tissue

Histopathology

All animals, regardless the inoculum size, developed cutaneous lesions on the tail in 9–12 days post-infection, which progressed to ulcerative lesions with a visible swelling.

At 7 days post-infection, animals infected with the low inoculum showed a marked splenomegaly and hepatomegaly with a few nodules approximately 1 mm in diameter in both organs. Microscopically, microgranulomatous lesions with a necrotic center and few free fungal cells were noticed in the interstitial spaces of tissue. However, abundant yeast-like elements were widely observed in the cytoplasm of the Kupffer cells probably as a result of intense phagocytosis. Neither lesions nor histological changes were found in other organs. After 15 days of infection, reduction in the size and number of nodules in liver and spleen was observed corresponding to a decreasing number of fungal elements in tissue. Remission of lesions and scarce fungal cells was observed 30 days after infection in the surviving animals.

Animals infected with the high inoculum showed intense splenomegaly and hepatomegaly and numerous nodules in all studied organs. Microscopically, large necrotic areas were found in liver, spleen, kidneys and heart, 7 days post-infection. In addition, tissue areas were substituted by fungal elements consisting of mature, round and immature, oval or cigar-shaped cells, which were especially abundant in liver. Although tissue damage was evident in liver, inflammatory cells were not observed. In brain and lungs, only a few yeast cells were observed (data no

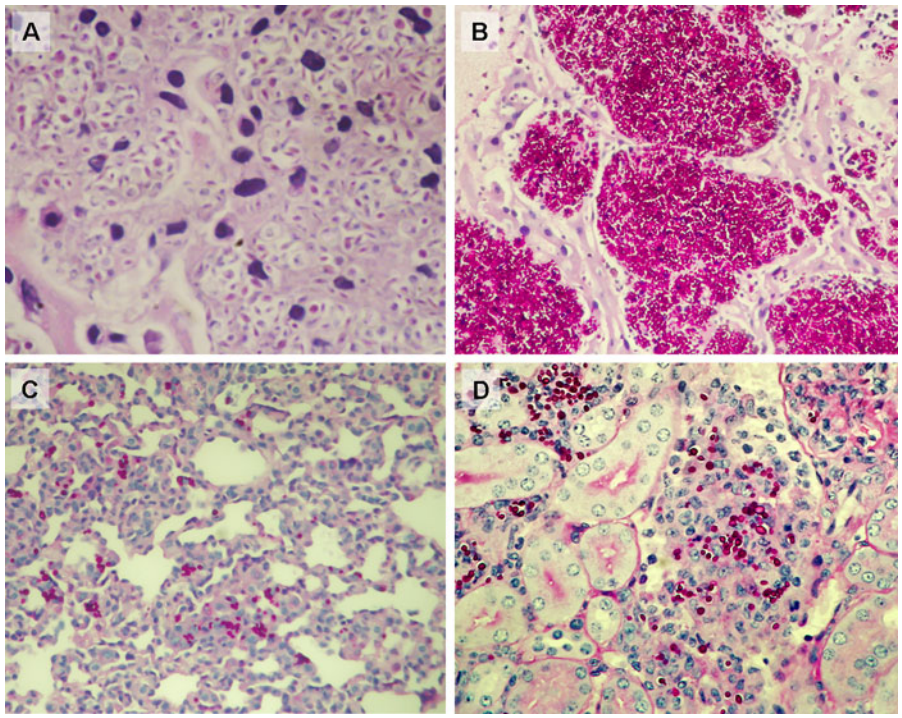

Fig. 3 Histological findings for *S. luriei* in mice infected with 2×10^7 CFU/animals at 15th day. **a** Fungal infiltration by fungal cells in liver but with no inflammatory response. **b** Massive infiltration with yeast-like cells replacing hepatocytes **c** Decrease in the alveolar spaces due to increased interstitial tissue due to the presence of fungal cells. **d** Scattered infiltration of fungal cells in kidney tubules. The stains used are hematoxylin eosin (**a**) and periodic acid–Schiff (**b–d**)

showed). At day 15, the number of nodules increased in all organs, especially in liver and spleen where a complete invasion of granulomas was observed. In all studied organs, a massive infiltration of fungal cells was observed particularly in liver where phagocytes and fungal elements invaded the hepatic tissue reducing it to scattered islets of hepatocytes. These fungal elements were mostly located in the Kupffer cells (Fig. 3a, b). In the other organs, there was no alteration in tissue structure but there was a large presence of fungal cells (Fig. 3c, d). The presence of cells with typical "eyeglass" shape, which had been observed in the human cases, was not observed in this experimental infection [10, 11]. It was not possible to carry out the histopathological study 30 days after infection, because all the animals had succumbed to the infection.

Discussion

We have developed a murine model of disseminated infection by *S. luriei* that was used to demonstrate the virulence and the high degree of tissue invasion of that fungus. Similar to a previous study that tested other species of the genus, i.e. *S. brasiliensis*, *S. mexicana* and *S. schenckii* [15], low inocula of *S. luriei* (2×10^5 CFU/animal) was not able to cause mice mortality and the lesions produced disappeared a few days after challenge. In contrast, infection with high doses (2×10^7 CFU/animal) allowed *S. luriei* to invade and proliferate in internal organs causing tissue destruction. Infected mice died between 12 and 16 days after infection, which was a similar, or even shorter, period of time to that obtained in infections carried out with *S. schenckii* or *S. brasiliensis* with the same inoculum size [15]. These histopathological findings were similar to those previously obtained during the experimental infection with *S. brasiliensis* but differed from those seen in the *S. schenckii sensu stricto* infection, which mainly evolved showing granulomas with a necrotic center [15]. Although "eyeglass cells" have been described as the most typical histopathological feature at identifies *S. luriei* in the four clinical cases

reported to date, we did not observe such cells in the tissues of our infected mice.

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