A new receptor is involved in the uptake of *Sporothrix* spp. by human macrophages

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Introduction

Sporotrichosis is the most common human deep mycosis reported in Latin America and endemic in Brazil. The causative agents are species of the pathogenic clade of the *Sporothrix* genus: *S. schenckii*, *S. brasiliensis*, *S. globosa*, and *S. luei* [1]. Among these, *S. brasiliensis* followed by *S. schenckii* are the major species with epidemiological importance in Brazil. Interestingly, cat-transmitted sporotrichosis is related only to *S. brasiliensis* infection [2].

The innate immune system plays a key role in protection against fungal infections. In relation to *S. schenckii*, that exhibits a low virulence profile, there are several studies on the host innate immune response. For this pathogen, it has been demonstrated that TLR4 recognizes components present in lipid extracts [3]. Furthermore, TLR2 is involved in the phagocytosis of this organism by murine macrophages inducing the secretion of pro and anti-inflammatory cytokines [4]. In relation to *S. brasiliensis*, the first evidences showing the involvement of dectin-1 and the mannose receptor in the cytokine production was reported in human PBMCs [5]. Nevertheless, the mechanisms of innate immune cells in response to *S. brasiliensis* infection is still poorly understood. In the present work a model based on human monocytes-derived macrophages (hMac) was used to study the human innate immune response to the yeast parasitic phase of *S. schenckii* and *S. brasiliensis*. We recently described that the cell wall structure and organization of these two species are rather distinct, and that nutritional stress conditions can modify the cell wall architecture of both species [6]. We hereby show that thermo-labile human serum factors impact human macrophage recognition of *S. schenckii* and *S. brasiliensis*. Furthermore, cell wall modifications observed during the fungal growth interfere in the hMac response.

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Experimental design

Figure 1. Distribution of *Sporothrix* isolates in Brazil. Adapted from: Rodrigues *et al.*, *Emerg Microbes Infect.* 2014

Results

Figure 1. Phagocytosis by human monocytes-derived macrophages of *S. schenckii* and *S. brasiliensis* yeast cells, cultivated for 4 or 10 days in YPD broth, in the presence of whole human serum (wHS) or inactivated human serum (iHS). The interaction was performed for 1 hour. Results are the mean of two experiments in duplicate with SEM. Unpaired T-test: (***) $p < 0.001$.

Figure 3. TNF- α secretion by human monocytes-derived macrophages after 18 hours of interaction with *S. schenckii* and *S. brasiliensis*. Yeast cells were cultivated for 4 days (4d) or 10 days (10d) in YPD medium. The interaction assay was performed in culture medium supplemented with either whole human serum (wHS) or inactivated human serum (iHS). The results shown represent the mean of three independent experiments with SEM. ANOVA with Turkey's Multiple Comparison Test was performed for statistical analysis.

Figure 4. IL-10 secretion by human monocytes-derived macrophages after 18 hours of interaction with *S. schenckii* and *S. brasiliensis*. Yeast cells were cultivated for 4 days (4d) or 10 days (10d) in YPD medium. The interaction assay was performed in culture medium supplemented with either whole human serum (wHS) or inactivated human serum (iHS). The results shown represent the mean of three independent experiments with SEM. ANOVA with Turkey's Multiple Comparison Test was performed for statistical analysis.

Figure 5. CR3 receptor inhibition assay. Human monocytes-derived macrophages were incubated with antibodies anti-CD11b and anti-CD18, in the presence of wHS, for 1 hour at 4°C, prior to the infection. hMac were then challenged with FITC-labeled yeast cells of *S. schenckii* or *S. brasiliensis* grown for 4 days. After 4 hours of interaction the cells were analysed by FACS.

Figure 6. Cytokine secretion by human monocytes-derived macrophages challenged with LPS. hMac were incubated with 10 ng/μL of LPS in culture medium supplemented with either whole human serum (wHS) or inactivated human serum (iHS). The results shown represent the mean of three independent experiments with SD.

Figure 2. Cytotoxicity (LDH assay) of human monocytes-derived macrophages mediated by *S. schenckii* and *S. brasiliensis*. Yeast cells were cultivated for 4 days (4d) or 10 days (10d) in YPD broth, and the interaction was performed for 4 hours (4h) and 18 hours (18h), in the presence of whole human serum (wHS) or inactivated human serum (iHS). The results show the mean of two independent experiments with SEM. ANOVA with Turkey's Multiple Comparison Test was performed for statistical analysis. (*) $p < 0.05$

Discussion

Our results show that thermo-labile serum factor(s) impact hMac recognition, uptake, cytotoxicity and cytokine response to *Sporothrix* spp. Our results also demonstrate that seems to be no direct participation of the human CR3 receptor (CD11b/CD18) in the uptake of *Sporothrix* spp. by human macrophages, suggesting the participation of another receptor dependent on serum factors (ongoing experiments). To our knowledge, this is the first report describing the mechanism(s) involved in the recognition and response of human macrophages to the parasitic phase of *S. schenckii* and *S. brasiliensis*.