Title: Modulation of zinc homeostasis in macrophages by *C. neoformans*

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Abstract

Cryptococcus neoformans is a clinically important yeast, as it is the ethiological agent of cryptococcosis. This disease is mainly characterized by meningoencephalitis with an incidence of near 1,000,000 cases/year. The infection process initiates with the deposition of infective particles in the respiratory tract. In these tissues, *C. neoformans* is exposed to host immune system and alveolar macrophages consists the first line of defense against it. Such cells internalize the yeast and form the phagolysosome structure, where *C. neoformans* can survive and replicate. During this process, zinc availability is important for both host and pathogen. To hamper pathogen growth and dissemination, host cells can reduce the zinc levels (a process named nutritional immunity). A proposed mechanism of the alteration of zinc levels inside cells is the modulation of expression of zinc transporters, as those from Zip (SLC39A) and Znt (SLC30A) family. Zip transporters are responsible for the uptake of zinc to the cytoplasm and Znt transporters for the efflux of this metal to extracellular microenvironment. Therefore, the aim of this work is to evaluate the influence of *C. neoformans* infection on zinc metabolism in macrophages. We first evaluated intracellular zinc levels in macrophages exposed to *C. neoformans* employing the zinc sensor Fluozin-3-AM. A consistent decrease of zinc levels could be observed in macrophage cells infected with *C. neoformans* compared to control macrophages and to macrophages exposed to heat-killed *C. neoformans* cells. qRT-PCR analysis of ZIP2, ZIP8, Znt1 and Znt9 genes revealed a complex pattern of expression. While live cryptococcal cells led to a reduction in ZIP2 and Znt1 transcript levels compared to control conditions, heat-killed cryptococcal cells caused an increase of ZIP2, ZIP8 and ZNT9 transcript levels. Addition of $ZnCl_2$ to macrophage:*C. neoformans* interactions led to an increased *C. neoformans* intraphagosomal proliferation rate. Collectivelly, these results confirms that macrophages employ zinc deprivation as a strategy to hamper the progression of *C. neoformans* infection process.

Keywords: zinc, zinc transporters, *Cryptococcus neoformans*.

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