Title: NULL MUTANTATIONS FOR VACUOLAR CA^{2+} TRANSPORTERS STRONGLY ATTENUATES CRYPTOCOCCAL VIRULENCE IN A SYSTEMIC MODEL OF CRYPTOCOCCOSIS

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Abstract:

Cryptococcus neoformans is an opportunistic pathogen and a leading cause of fungal infection related fatalities, especially in immunocompromised patients. The infection process starts with the inhalation of infectious particles, followed by survival and proliferation of cells within the lung, and dissemination to the central nervous system (CNS). Calcium is an intracellular messenger that controls numerous cellular processes. Two essential mediators of calcium signals in eukaryotic cells are calmodulin and the phosphatase calcineurin. The Ca^{2+}-calcineurin signaling pathway in this human fungal pathogen is essential for adaptation to the host during infection. Calcium transporters such as Pmc1 and Vcx1 regulate cytosolic calcium concentrations, providing Ca^{2+} loading into storage organelles. It has been shown that both of these vacuolar calcium transporters are required for proper virulence of *C. neoformans* assessed in an intranasal model of murine cryptococcosis. Therefore the aim of this work is to evaluate the impact of Vcx1 and Pmc1 on cryptococcal virulence and dissemination to the CNS. Murine infection was conducted by injection into the retro orbital space, which represents a systemic infection. While WT (H99 strain) and *vcx1* null mutants were able to kill mice (LT50 of 9.5 and 7 days, respectively), *C. neoformans* cells lacking the *PMC1* gene (*pmc1* and *pmc1/vcx1* null mutants) could not cause death in hosts, even after 30 days of infection. Fungal burden was accessed in lungs and brain 3 and 6 days post infection. The *pmc1* and *pmc1/vcx1* null mutants infectivity was shown to be highly impaired. Analysis of the internalization and transmigration profiles of these null mutants and WT through a monolayer of t-HUVEC cells is under analysis. This model will give us hints about possible events related to defects to cross the blood brain barrier. Moreover, we have been assessing differences in the gene expression of some calcineurin-regulated genes that could be compromised by the null mutation of *PMC1.* A specific transcriptome analysis (RNA-Seq) was conducted to evaluate gene expression alterations in the *pmc1* null mutant after incubation in tissue culture medium (DMEM) for 3 h. Collecting these results, we intend to evaluate the role of calcium transporters on *C. neoformans* virulence, and their impact on the calcineurin signaling pathway, which itself is essential for the yeast adaptation to the host environment.

Keywords: Calcium transport, *Cryptococcus neoformans*, calcineurin, virulence

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