

TITLE: COMPARATIVE ANALYSIS OF EXTRACELLULAR VESICLES FROM THE VIRULENT AND ATTENUATED VARIANT OF *PARACOCCIDIOIDES BRASILIENSIS* STRAIN Pb18

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ABSTRACT:

Extracellular vesicles (EVs) are round-shaped structures surrounded by a typical lipid membrane. They carry all kinds of molecules outside the cells, thus promoting distant cell signaling between pathogens, host cells and other microorganisms. Paracoccidioidomycosis (PCM) is a granulomatous systemic mycosis prevalent in endemic areas of Latin America and is caused by temperature-dependent dimorphic *Paracoccidioides* spp. In the present work, we compared EVs from hypervirulent *P. brasiliensis* Pb18 (vPb18) and its attenuated variant (aPb18). EVs were isolated from stationary fungal yeasts grown in Petri dishes by differential ultracentrifugation. The EVs from Pb18 of both isolates showed a size range of 30-300 nm (median of 49 nm) using nanoparticle tracking analysis (NTA), while the protein and ergosterol contents were significantly decreased in EVs produced by aPb18 (aEVs). *In vitro*, aEVs induced significantly higher production of proinflammatory mediators such as nitric oxide, TNF- α , IL-6, and MCP-1 when co-incubated with RAW 264.7 macrophages and bone marrow-derived macrophages. *In vivo*, EVs produced by the hypervirulent Pb18 (vEVs) stimulated a pro-inflammatory response that was also significantly less intense than that for aEVs; however, both EVs exacerbated murine PCM under the tested conditions, by evoking a hyperinflammatory response. The vEVs were able to stimulate recipient aPb18 cells to express active enzymes associated with an antioxidant response such as catalases; besides, some genes involved in response to oxidative stress such as the peroxiredoxin mitochondrial (*PRX1*) were up-regulated in these cells. Taken together, our results suggest that EVs from vPb18 carry immunomodulatory components that are different from those of aPb18. Therefore, EVs might play a pivotal role in the *Paracoccidioides* pathogenesis.

Keywords: Extracellular vesicles, *Paracoccidioides brasiliensis*, virulence, pathogenesis, cell signaling

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