

Title: Different isolates of *Paracoccidioides* induces IL-8 secretion by lung epithelial cells

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Abstract: Fungi that belong to the *Paracoccidioides* genus are the etiological agents of paracoccidioidomycosis, a human systemic mycosis that occurs in Latin America. By secreting inflammatory mediators, such as cytokines and chemokines, the lung epithelial cell is one of the first cells that interact with these fungi and can respond appropriately to infection. By ELISA, we demonstrated that yeasts from different isolates of *Paracoccidioides brasiliensis* (Pb18 and Pb03) and *P. lutzii* (Pb01) distinctly promoted the secretion of the IL-8 cytokine by the lung epithelial cell line A549. By incubating yeasts with A549 cells, we observed that all isolates induce IL-8 secretion, but the direct contact between the yeast and A549 cell is only important for Pb03 isolate. We also verified that a conditioned medium containing secreted components of *Paracoccidioides* isolates can promote IL-8 secretion by A549 epithelial cells. Moreover, assays performed with yeasts added to upper compartments of Transwel® platforms, *i.e.*, inhibiting direct contact between yeasts and A549 cells, confirmed that *Paracoccidioides* secretes factors that induce IL-8 secretion by these epithelial cells. By studying the isolate Pb339 of *P. brasiliensis*, we verified that this fungus secretes proteases that interact with Protease Activated Receptors (PARs) expressed on A549. Using PAR-1, PAR-2 and PAR-4 antagonists, we found that IL-8 secretion is dependent on PAR-1 and PAR-2 activation. We also verified that the proteases secreted by Pb339 are serine and cysteine proteases. Furthermore, preliminary results show that serine proteases are also involved in IL-8 secretion promoted by other isolates (Pb18 and Pb03 isolates from *P. brasiliensis* and Pb01 from *P. lutzii*) in A549 cells. Thus, with these data, we can conclude that *Paracoccidioides* yeasts secrete proteases that can induce the secretion of pro-inflammatory cytokine IL-8 in human lung epithelial cells.

Keywords: *Paracoccidioides*; epithelial cell; cytokine; proteases; PARs

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