

**TITLE:** Cytokine levels in epithelial cell cultures after stimulation with secreted factors of different *Paracoccidioides* isolates

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**INTRODUCTION:** Paracoccidioidomycosis is a systemic mycosis caused by the dimorphic fungi of the genus *Paracoccidioides*. Until a few years ago, it was believed that this genus was composed only by the species *P. brasiliensis*. However, molecular and phylogenetic analyses have shown the existence of another species, *P. lutzii*. The relationship between the clinical patterns of paracoccidioidomycosis and *Paracoccidioides* species has still to be determined. However, the literature shows that *Paracoccidioides* isolates can express different molecules, leading to several responses in the host. Recently, our group demonstrated that *P. brasiliensis* is able to interact with human lung epithelial cells and to induce the secretion of the pro-inflammatory cytokines IL-6 and IL-8. The present work aimed to evaluate the IL-6 and IL-8 levels in A549 human lung epithelial cell cultures during stimulation with conditioned media obtained from cultures of different *Paracoccidioides* yeast isolates.

**MATERIAL AND METHODS:** To prepare conditioned media, yeasts of different *Paracoccidioides* isolates (Pb18, Pb03 e Pb01) were incubated for 72 hours with DMEM medium. Supernatants (conditioned media) were collected and then, incubated with A549 cells. After 16 hours, IL-6 and IL-8 levels of these cell cultures were analyzed by ELISA. Considering that the MAPKs ERK 1/2 and p38 activation are involved in the secretion of cytokines by epithelial cells, we analyzed the activation of these kinases. For this, after A549 cells incubation with *Paracoccidioides* conditioned media for different periods of time, these MAPKs were analyzed by Western blot, using anti-Phospho (P)-ERK 1/2 (Thr<sup>202</sup>/Tyr<sup>204</sup>) and anti-P-p38 MAPK (Thr<sup>180</sup>/Tyr<sup>182</sup>) antibodies.

**RESULTS AND CONCLUSIONS:** Factors secreted by different *Paracoccidioides* isolates (Pb18, Pb03 e Pb01) were capable to induce the secretion of IL-6 and IL-8 by A549 epithelial cells. Conditioned medium of isolate Pb18 (*P. brasiliensis*) induced higher levels of secretion of both cytokines than the conditioned media of Pb03 (*P. brasiliensis*) and Pb01 (*P. lutzii*). Secreted fungal factors of different *Paracoccidioides* isolates promoted the activation of the MAPKs ERK1/2 and p38, but differences in these kinases activations patterns were not pronounced among the isolates. Therefore, together, these results show that isolates of *Paracoccidioides* secrete factors that promote, in different manners, cytokine release by epithelial cells.

**Keywords:** *Paracoccidioides*, cytokines, epithelial cells, secreted factors.

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