

TITLE: Role of TLRs and integrins in IL-8 secretion by A549 epithelial cells infected with *Paracoccidioides brasiliensis*

AUTHORS: Barros BCSC; Almeida BR; Suzuki E.

INSTITUTION: Universidade Federal de São Paulo, Escola Paulista de Medicina, Departamento de Microbiologia, Imunologia e Parasitologia, Disciplina de Parasitologia

Paracoccidioides brasiliensis is one of the etiological agents of paracoccidioidomycosis, the most prevalent systemic mycosis in Latin America. Human infection occurs when the mycelial form of this fungus is inhaled by the host and, once in the lungs, develops into yeast. In this scenario, host epithelial cells form not only a physical barrier against pathogens, but also contribute for the immune system by producing inflammatory mediators, such as cytokines and chemokines. The interaction among pathogens and host cells occurs, initially, with receptors such as integrins and Toll-like receptors (TLRs), which activate their respective cell signalling pathways. Recently, our group demonstrated that yeasts of *P. brasiliensis* induced an increase of $\alpha 3$ integrin expression in A549 cells after 5 hours of infection. Curiously, after 24 hours of infection, we verified that this integrin expression was reduced in A549 cells. By using Transwell[®] inserts, we observed that $\alpha 3$ integrin expression reduction seems to be dependent of the contact between the fungus and the epithelial cell. As *P. brasiliensis* promoted an IL-8 secretion in $\alpha 3$ and $\alpha 5$ integrins-dependent manners, in the present work, we verified the role of TLRs in this event. First, by Western blot, we observed that *P. brasiliensis* induced an increase of TLR2 and TLR4 expression in A549 cells and, when TLR2 was silenced, we verified, by ELISA, a reduction of IL-8 levels, indicating that this TLR also participates in this cytokine secretion by epithelial cells. Later, by immunoprecipitation, we observed an interaction between $\alpha 3$ integrin and TLR2 after 5 hours of infection. Thus, *P. brasiliensis* can modulate host inflammatory response by promoting interaction between receptors and, consequently, cytokine secretion in epithelial cells.

Keywords: *Paracoccidioides brasiliensis*; Integrins; Toll-like Receptors; Epithelial cells; Interleukin-8.

Development Agencies: FAPESP, CAPES and CNPq