

**ROLE OF Src-FAMILY KINASES IN IL-8 SECRETION
BY EPITHELIAL CELLS DURING
Histoplasma capsulatum INFECTION**

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Histoplasmosis is caused by the thermally dimorphic fungus *Histoplasma capsulatum*. The absence or presence of alpha-(1,3)-glucan on its surface can alter *H. capsulatum* pathogenesis. Isolates lacking alpha-(1,3)-glucan in the yeast cell wall are designated chemotype I, these fungi multiply as non-clumping yeasts in liquid cultures and smooth colonies on solid medium. On the other hand, *H. capsulatum* isolates that have alpha-(1,3)-glucan in the cell wall are classified as chemotype II, which multiply as large clumps in liquid cultures and rough colonies on solid medium. In this study, we compared IL-8 secretion by the human lung epithelial cell line A549 induced by chemotypes I and II of *H. capsulatum*. Furthermore, we analyzed SFK (Src-Family Kinases) activation promoted by these fungal isolates and the effect of PP2, a SFK inhibitor, on A549 cell IL-8 secretion.

By flow cytometry, we analyzed two *H. capsulatum* isolates classified as chemotype II (496 and 268), and we verified that most of the yeasts (50-90%) were recognized by anti-alpha-(1,3)-glucan antibody. On the other hand, G217B, which is a chemotype I isolate, was not recognized by this antibody. Next, A549 cells were incubated with *H. capsulatum* 496, 268 and G217B, and IL-8 levels in these culture supernatants were determined by ELISA. We verified that chemotype II isolates 496 and 268 promoted an increase of IL-8 levels that were higher than the chemotype I isolate G217B. To analyze whether *H. capsulatum* yeasts were able to induce SFK activation in A549 cells, these epithelial cells were first incubated with *H. capsulatum* isolates and then, levels of SFK phosphorylated in the Tyr⁴¹⁶ residue were analyzed by Western blot. All *H. capsulatum* isolates induced SFK activation during interaction with A549 cells. In addition, PP2, an inhibitor of SFK activation, was used to determine the role of SFK activation on IL-8 secretion by A549 cells in the presence of *H. capsulatum* different isolates. We verified that PP2 decreased IL-8 levels

secreted by these cells, but this inhibitory effect was more pronounced when A549 cells were incubated with *H. capsulatum* isolates chemotype II.

Together, these results indicate that SFK activation is important for IL-8 secretion by A549 epithelial cells incubated with fungi containing alpha-(1,3)-glucan (chemotype II), suggesting that *H. capsulatum* isolates can activate different signaling pathways according to the composition of their cell wall.

Keywords: cytokine, epithelial cells, *Histoplasma capsulatum*, alpha-(1,3)-glucan, SFKs

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