SIGNALING EVENTS IN THE INTERACTION BETWEEN *Paracoccidioides brasiliensis* AND EPITHELIAL CELLS AND MONONUCLEAR CELLS INVOLVED IN THE IMMUNE RESPONSE

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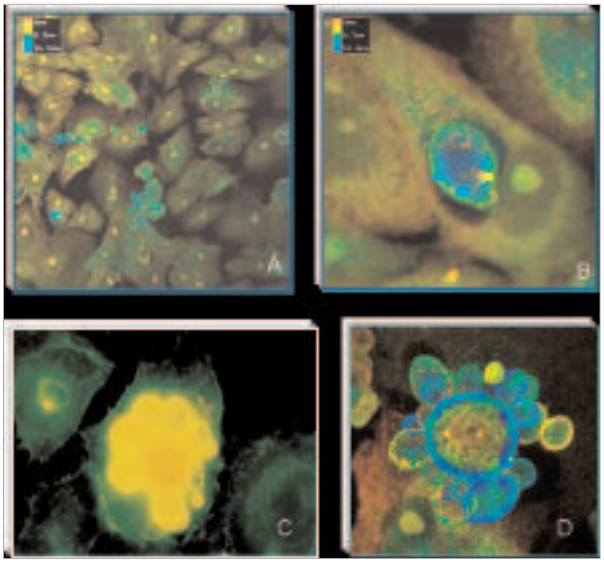

Adhesion and invasion process of P. brasiliensis *in Vero cells by confocal microscopy, after 2h (A) and 5h (B, C and D)*

Paracoccidioidomycosis (PCM) is an endemic deep mycosis caused by the dimorphic fungus *Paracoccidioides brasiliensis*. It is the leading endemic deep mycosis in Latin America, especially Brazil. The infection is acquired through the respiratory route. The conidia invade alveoli and terminal bronchi, where they transform into the yeast form. *P. brasiliensis* cause a mycosis with a wide range of clinical manifestations. Occasionally life-threatening depends on fungus virulence, its capacity to interact with the host, its invasion capacity and the immune response. With the purpose to evaluate the steps involved from the initial contact of *P. brasiliensis* with the host, the adhesion and invasion processes, our group developed interesting models of *in vitro* cell cultures. The interaction of *P. brasiliensis* with epithelial and mononuclear cells of human host seems to be determinant to the outcome of the infection and to the variability in the clinical presentation. Then, we propose to determine the genotypes of the fungus, to isolate and characterize the putative adhesins involved in this process, as well as the extra-cellular matrix components and to evaluate the fungal components that can modulate, through signaling events, the epithelial cell invading process. In parallel, we will study the participation of costimulatory molecules and the molecular aspects (Stats and caspases activation) related to PCM patients' vs. cured/ sensitized individuals lymphocyte proliferation and cytokine production after challenging with gp43, and to the apoptosis phenomena. We will also verify the role of costimulatory molecules in the induction/protection of/from apoptosis as a possible mechanism associated with the immunological imbalance of PCM. The simultaneous study fungy and host cells signaling and costimulatory pathways, will provide a better understanding of the mechanisms involved in the pathogenicity of *P. brasiliensis* and in the suppression of the Th-l response found in the patients, as compared to the healthy exposed individuals or cured patients. As a consequence, we will be able to conjunctly evaluate a higher range of cellular events associated with the pathogenesis of paracoccidioidomycosis.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Paracoccidioides brasiliensis possesses multiples factors that damage the host and contribute to its virulence phenotype. Aiming to understand the mechanisms that regulate the interaction of this fungy with host cells, we have studied the adherence, induction of cytoskeletal alterations, and differential signaling activity of the various surface molecules as well as the role of moleculy that may represent new microbial targets. Proteomic approaches allowed the characterization of fungal adhesins to extracellular matrix proteins. Adhesins expression was increased from yeasts freshly isolated from humans or after animal passage, displaying a higher adhesion capacity. Also, *P. brasiliensis* affects the cytoskeletal structure of the host cells and mechanism of invasion was shown to be microfilament and microtubule-dependent. In this study, we also show that the signaling pathways involved in the cell proliferation, growth, and cytosol signals associated with cytoskeleton were mediated by actin rearrangement, and the entry of the fungus into the epithelial cell apparently requires activation of the small family of RHO GTPases. An adaptin, a molecule that probably participates in the vesicular trafficking, and likely essential to yeast survival inside cells, was also described. In parallel, this study shows that the host-*P. brasiliensis* interactions leads to a distinct profile of costimulatory molecules expression in mononuclear cells from patients. This pattern is consistent with the hypoproliferative state ("anergic") of these cells, likely the main mechanism underlying the host failure in controlling the infection. Attempts to revert this anergy *in vitro,* by blocking the negative signaling of such molecules, were ineffective. We also detected an altered expression of STATS in patients with active disease, consistent with their biased, non-protective, TH-2 type cytokine secretion pattern. Most importantly, this pattern was modified by interfering on the local cytokine balance. The elucidation of the roles of fungal and host cells' immunoregulatory molecules may devise the development of vaccines and immunomodulatory interventions in this mycosis.

MAIN PUBLICATIONS

Monteiro-da-Silva JL, Andreotti PF, Benard G, Soares CP, Miranda ET, Mendes-Giannini MJS. 2007. Epithelial cells treated with genistein inhibit adhesion and endocytosis of *Paracoccidioides brasiliensis*. *Antonie van Leeuwenhoek*. **92**:129-135.

Andreotti PF, Monteiro-da-Silva JL, Teixeira EC, Bertolini MC, Soares CP, Benard G, Mendes-Giannini MJS. 2007. Identification of a gene encoding adaptin-like protein in the *Paracoccidioides brasiliensis* genome by randon amplified polymorphic DNA analysis. *Journal of Medical Microbiology*. **56**:884-887.

Pereira LA, Báo SN, Barbosa MS, Monteiro-da-Silva JL, Felipe MSS, Santana JM, Mendes-Giannini MJS, Soares CMA. 2007. Analysis of the *Paracoccidioides brasiliensis* triosephosphate isomerase suggests the potential for adhesin function. *FEMS Yeast Research*, **7**:1381-1388.

Barbosa MS, Báo SN, Andreotti PF, Faria FP, Felipe MSS, Feitosa LS, Mendes-Giannini MJS, Soares CMA. Glyceraldehyde-3-phosphate dehydrogenase of *Paracoccidioides brasiliensis* is a cell surface protein involved in fungal adhesion to extracellular matrix proteins and interaction with cells. 2006. *Infection and Immunity*. **74(1)**:382-389.

Mendes-Giannini MJS, Andreotti PF, Vincenzi LR, Monteiro-da-Silva JL, Lenzi HL, Benard G, Zancopé-Oliveira RM, Guedes HLM, Soares CP. 2006. Binding of extracellular matrix proteins to *Paracoccidioides brasiliensis*. *Microbes and Infection*. **8(6)**:1550-1559.

Benard G, Kavakama J, Mendes-Giannini MJS, Kono A, Duarte AJ, Shikanai-Yasuda MA. 2005. Contribution to the natural history of paracoccidioidomycosis: identification of the primary pulmonary infection in the severe acute form of the disease - a case report. *Clinical Infectious Diseases*. **40(1)**:e1-e4.

Romano CC, Mendes-Giannini MJS, Duarte AJ, Benard G. 2005. The role of interleukin-10 in the differential expression of interleukin-12p70 and its beta2 receptor on patients with active or treated paracoccidioidomycosis and healthy infected subjects. *Clinical Immunology*. **114(1)**:86-94.

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