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SUMMARY OF THESIS*

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INTERACTION WITH THE EXTRACELLULAR MATRIX COMPONENTS AND THE INDUCTION OF CYTOSKELETAL ALTERATIONS DURING THE PROCESS OF ADHESION AND INVASION OF *PARACOCIDIOIDES BRASILIENSIS* IN EPITHELIAL CELLS --SAMIRA ABDALLAH HANNA

Microbial virulence is a set of mechanisms that enable infectious agent to penetrate the host protection barriers and then survive against the defense mechanisms, multiply and cause disease.

Paracoccidioidomycosis presents a variety of clinical manifestations, and the fungus *Paracoccidioides brasiliensis* can reach many tissues, most importantly the lungs. Understanding of the mechanisms of dissemination is based on indirect evidence, and the polymorphic aspects of disease suggest that several virulence mechanisms are involved.

The ability of the pathogen to interact with the host superficial structures is essential to its virulence, but little is known about interactions between cells and *P. brasiliensis*. For this reason, interactions between *P. brasiliensis* and epithelial Vero and A549 cells were evaluated, with the emphasis on interactions with extracellular matrix and the induction of cytoskeletal alterations during the interaction process fungus-cells.

The kinetics of interaction showed that adhesion occurred after 30 minutes of contact between the two cell lines and the yeast, and the number of adhesion points increased on the cells after that. The fungus were inside the Vero cells after two hours and inside A549 after one hour.

The interaction between *P. brasiliensis* and epithelial cells and the components of the extracellular matrix (MEC) were evaluated through immunoperoxidase *in situ* and indirect immunofluorescence. The recognition patterns of the infected cells against anti fibronectin, anti-laminin and anti-collagen type I sera were different from those of the uninfected cells. Characteristic distributions of bound anti-fibronectin and anti-laminin on the surface of yeast cells were detected, indicating the presence of specific binding sites for each one.

Cytochalasin D, an inhibitor of actin polymerization and colchicine, which disrupts microtubules, substantially reduced invasion, indicating the participation of microfilaments and microtubules in this mechanism.

Cytokeratin filaments were not involved in fungus adhesion. However, disarrangement and disruption of the filaments were observed after longer times of fungus-cell contact.

The gp 43 activity, in concentrations ranging from 25 to 3.125 µg/mL, changed the actin cytoskeletal arrangement, and in concentrations ranging from 32.5 to 12.5 µg/mL, the integrity of cytokeratin filaments were destroyed.

The reactivity of the infected culture supernatant against anti-actin serum showed 43, 94 kDa bands and against anti-cytokeratin serum showed 40, 43, 67 and 94 kDa bands, suggesting that some components are produced during fungus-cell interaction which bind to cytoskeleton components.

Through an immunoblot assay, gp43 was also recognized by anti-actin and anti-cytokeratin sera, but not by anti-tubulin serum. Thus, it seems that this antigen could be a ligand for some cytoskeleton components and may be a candidate invasin.

Using the TUNEL with fluorescent probe technique to label cells undergoing DNA fragmentation, it was shown that *P. brasiliensis* induces apoptosis in infected cells. Nuclear fragmentation appeared after one hour and characteristic apoptotic cells were observed after two hours of contact between fungus and cells. After five hours the apoptotic bodies and rare yeast were found.

The adhesion and invasion of epithelial cells by *P. brasiliensis* may represent strategies employed to thwart the host immune response, and may help in the dissemination of the pathogen.

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