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SUMMARY OF THESIS*

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ADHESION AND LIGATION TO FIBRONECTIN AND INVASION OF THE *Paracoccidioides brasiliensis* IN CULTURE OF VERO CELLS

The ability of a pathogen to interact with surface structures on the host plays an essential part in its virulence. An understanding of the steps of this interaction is thus crucial to the future development of treatments that inhibit the pathogen's capacity to colonize and establish an infection, ideally by blocking the surface interaction.

While the mechanisms of pathogenicity of *Paracoccidioides brasiliensis* have not yet been fully clarified, there is some evidence that the cell wall composition, and the production of soluble antigens such as a 43kDa glycoprotein (gp 43) and a serine-thiol protease, are virulence factors responsible for establishment in the host. Moreover, the capacity to adhere to and invade host tissues has been ascribed an important role in pathogenicity.

The aim of the present work was to study the process of adhesion of *P. brasiliensis* (isolate 18) to cultures of Vero cells, investigate the pattern of recognition of this fungus by specific sera and antifibronectin serum, and identify the process and probable mechanism of fungal invasion in non-phagocyte cells.

To this end a model cell culture, of epithelial cells in a monolayer over glass coverslips, and a standard embedding technique that rendered fungal invasion more evident, were developed.

Several staining techniques were used as well as immunofluorescence, immunoperoxidase "in situ" and "immunogold". Optical, transmission electron and laser confocal microscopy were all used, to manifest adhesion and invasion as clearly as possible.

Yeast-like cells of *P. brasiliensis* isolate 18 adhered to Vero cells after only 30 minutes had elapsed from the inoculation. Thus a brief contact time is enough for adhesion to occur. Observation of fungal bodies within the cytoplasm, less than 5 hours from the time of infection, demonstrated the occurrence of cellular invasion.

Fungi adhered to cells exhibited a pattern of recognition similar to fungus cultures when tested with anti-"cell-free" and anti-gp43 sera. Both the antigens are evenly distributed along the fungal wall and, in the buds, sometimes show more intense marking.

Antifibronectin serum stained strongly the Vero cells and fungus, but in distinct patterns, suggesting that this fungus has receptors for this component of the extracellular matrix and so may take part in the fungus-cell interaction.

Another element of this research was a study of the invasion of cells by *P. brasiliensis*, involving various techniques in microscopy.

The cytotoxic drug cytochalasin D was found to inhibit the invasion of Vero cells by this fungus, indicating the involvement of actin filaments in the invasion, as this drug specifically inhibits actin polymerisation in cells. This result suggests that one mechanism used by the fungus during invasion is the rearrangement of actin microfilaments.

Colonization of the surface of the mucosa is required before microorganisms are internalized by "non-professional" phagocytes. The consequence of this colonization depends largely on ligand-receptor interactions between the surfaces of the host cells and pathogens. This interaction may produce a signal in the cell membrane, provoking cytoskeleton rearrangements in the host that lead to internalization of the microorganism. Thus, it is likely that *P. brasiliensis* interacts with the fibronectin in the extracellular matrix, generating signals which stimulate cytoskeletal modification in the host cell, culminating in the invasion of the microorganism.

In this study we have been able to clarify the involvement of extracellular matrix and cytoskeleton constituents in the pathogenesis of *P. brasiliensis*, opening the way to new perspectives on the disease, such as the possibility of blocking steps in the invasion process, either as a preventative measure or in therapeutic intervention.

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