Distribution Pattern of Lipidic Species on Membrane Microdomains of Pathogenic Fungi

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Membrane microdomains are involved in cell signaling and adhesion. They are formed by tight packing of cholesterol, sphingosine and saturated fatty acids of (glyco)sphingolipids in association with specific proteins in cellular plasma membrane. The insoluble property in cold nonionic detergents allows separation of membrane rafts by flotation in sucrose density gradient (SDG). In order to determine the composition of membrane microdomains in *Paracoccidioides brasiliensis* and *Histoplasma capsulatum*, yeast forms were homogeneized in TNE buffer at 4°C. Cleared lysate was incubated with Brij-98 at temperatures ranging from 4°C to 45°C, or treated with methyl-ß-cyclodextrin (mßCD - sterol chelant), incubated with Brij-98 at 4°C and then submitted to SDG. Twelve fractions were collected from top to bottom and analyzed by HPTLC, SDS-PAGE and Western-blot. In microdomain fractions (4-6), it was observed enrichment of glycoinositol-phosphorylceramides, ergosterol, and proteins, such as Pma1p, a known yeast membrane raft marker. It was also observed in membrane raft fractions a decreased concentration of lipids and proteins at 40°C, but not a complete disruption as observed in mammal cells. Interestingly, fraction 5 incubated with Brij-98 at 37°C and re-centrifuged shows a complete dislodgement of lipids and proteins to soluble fractions, suggesting that other interactions, besides lipid *cis* interaction, are responsible for the packed microdomain resistance to temperature/detergent in fungi (*i.e.* cell wall and cytoskeleton interactions). It was also verified that disruption of fungal membrane rafts with mßCD promoted an inhibition by 40% of mice alveolar macrophages invasion by *H. capsulatum*, suggesting that protein and lipid organization integrity of pathogenic fungi membrane rafts represent a key membrane structure to yeast-cell interaction.

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