[PS2.59]

Toxoplasma gondii interaction varies according to host cell microdomains

K.C. Dias-Cruz, W. DeSouza, M. Attias*

U.F.R.J., Brazil

Lipid rafts are dynamic nanoscale assemblies enriched in sterol-sphingolipid. The exploitation of rafts by intracellular pathogens may facilitate invasion. *Toxoplasma gondii* is a pathogen that actively invades host cells. Most host cell proteins do not pass beyond the moving junction and are excluded from the parasitophorous vacuole (PVM). However, many components of PVM derive from host cell membrane, including microdomains. We evaluated the participation of cholesterol enriched microdomains in invasion of *T. gondii* into LLC-MK2 and murine macrophages through transient depletion of host cells cholesterol with either methyl-beta-cyclodextrin (MβCD) [final concentrations of 5, 10 and 20 mM for 30 min before interaction]; or Filipin [final concentrations of 1, 3 and 6 nM for 30 min before interaction]. Reversibility for MβCD was also tested. The interaction of the parasite with the host cells after treatment with cholera toxin B subunit (CTB) that binds to GM1 ganglioside was also tested. After interaction (10 min, 50 parasites per cell), adhesion and internalization indexes were determined by light microscopy. These were significantly diminished in cells treated with MβCD compared to controls. With 20 mM of MβCD, inhibition of internalization in LLC-MK2 reached 80% and almost 100% in macrophages. Pretreatment with MβCD followed by cholesterol reposition before interaction completely reverted inhibition in macrophages, but not in LLC-MK2. Filipin did not interfere in interactions with LLC-MK2 cells, but in macrophages, inhibition reached 76%. CTB did not interfere in adhesion to LLC-MK2 cells, but internalization was inhibited in 70%. Furthermore, macrophages treated with CTB inhibited the adhesion and internalization of the parasite in almost 80%. These results indicate that host cell membrane cholesterol enriched domains participate in the process of adhesion and active invasion of Toxoplasma and the differences observed between macrophages and LLC-MK2 cells may be due to different levels of this molecule in its membranes.

Keywords: Toxoplasma, lipid rafts, adhesion, invasion