Título: Dynamin as a hostess for *Toxoplasma gondii* infection?

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The obligate intracellular protozoan parasite *Toxoplasma gondii* actively invades virtually all warm-blooded nucleated cells in a process that results in a nonfusogenic vacuole, inside which parasites continuously replicate, until signaling to egress is triggered. In this work we approached the role of the GTPase dynamin, largely known for execute the detaching of plasma membrane nascent vesicles, in order to investigate its role in the process of *T. gondii* interaction with the host cell. Although invasion of *T. gondii* is considered an active process, the parasitophorous vacuole (PV) has to detach from the host cell plasma membrane before translocation to the perinuclear sites of the infected cell. An immunofluorescence assay was performed with the aim to investigate if dynamin participates on this pinch off, and also on the translocation of the PV and parasite egress. Cells were fixed at different moments post infection, being half of the samples permeabilized with 0.1% Triton X-100 in PBS, but all of them were pre-incubated with 50 mM ammonium chloride and 3% BSA in PBS, pH 8.0, and incubated, first with the primary antibody anti-dynamin, rinsed, and then with secondary antibody goat anti-mouse IgG (H+L) conjugated to AlexaFluor 488. Actin was stained with phalloidin-red. After washing with PBS and mounting with prolong antifade, slides were examined in a Zeiss 510 LSM. The same moments of *T. gondii* cellular cycle were investigated by cryoimmuno microscopy, labeling dynamin. For this, the samples were fixed in 4% freshly prepared formaldehyde and embedded in gelatin. The samples were infiltrated overnight in 2.1 M sucrose and rapidly frozen by immersion in liquid nitrogen. Cryosections were obtained at -100°C using an Ultracut cryo-ultramicrotome, thawed in methylcellulose, blocked in PBS- 3% bovine serum albumin and then incubated in the presence of the antibody anti-dynamin for 1 hour. The cryosections were washed and incubated with 15 nm, gold-labeled with anti-mouse IgG, and observed in a FEI Tecnai electron microscope. The detection of dynamin during invasion is an indication of a role in the PV's detaching from host cell plasma membrane, in a similar way to that described for endocytosis. However, *T. gondii* egress seemed independent of dynamin participation, whereas its presence during *T. gondii* development is indicative of undescribed roles for this molecule in the tachyzoite's cell cycle.

Palavras-chaves: Toxoplasma gondii, dinamina, microscopia eletrônica

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