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LS-7-P-3336 Ultrastructural aspects of Toxoplasma gondii egress

Caldas L. A.[1,3], Seabra S. H.[2], Attias M.[3], De Souza W.[1,3]

[1]Instituto Nacional de Metrologia, Qualidade e Tecnologia, Inmetro, Rio de Janeiro, Brazil, [2]Centro Universitário da Zona Oeste, Rio de Janeiro, RJ, Brazil, [3]Instituto de Biofísica Carlos Chagas Filho; Universidade Federal do Rio de Janeiro, Brazil

Email of the presenting author: mattias@biof.ufrj.br

The obligate intracellular protozoan parasite *Toxoplasma gondii* is capable of infecting almost every nucleated warm blooded cells and has raised medical and veterinary importance. Nevertheless, its egress from host cell is still poorly understood. Herein, we investigated some general aspects of the dynamics of egress by confocal and elecron microscopy. For Field Emission Scanning electron microscopy (FESEM), between 30 and 48 hours post-infection, LLCMK2 monolayers were washed and fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer pH 7.2, post-fixed for 1 h with 1% OsO_4 in 0.1 M cacodylate buffer pH 7.2 and 0.8% potassium ferrocyanide, dehydrated in ethanol, critical point dried in CO_2, sputtered with carbon and observed in a Jeol 6340 field emission scanning electron microscope. Some samples had the surface detached by adhesive tape, which allowed the observation of the host cell interior. For confocal microscopy, the monolayers were pre-incubated with 50 mM ammonium chloride and 3% BSA in PBS pH 8.0 for 45 min. The samples were then incubated with primary antibodies at a 1:100 dilution for 1 h, rinsed, and incubated with 1:400 secondary antibodies at room temperature for 1 h. Observation was carried out in a Zeiss 510 LSM 510 NLO. By FE-SEM, in natural egress, we observed the detachment of one of the parasites from the residual body with the parasitophorous vacuole membrane and the tubular network still present (Figure 1). Comparing natural egress with calcium ionophore induced egress in the second one tubular disassembly precedes parasite detachment. The participation of the host cell cytoskeleton was also checked, indicating a dual role in *T. gondii* egress: it can be a substrate for intracellular gliding of the parasite, contributing to its escape, and also a barrier for *T. gondii* during egress since escape from host cells occurred preferentially at actin free zones (Figure 2).

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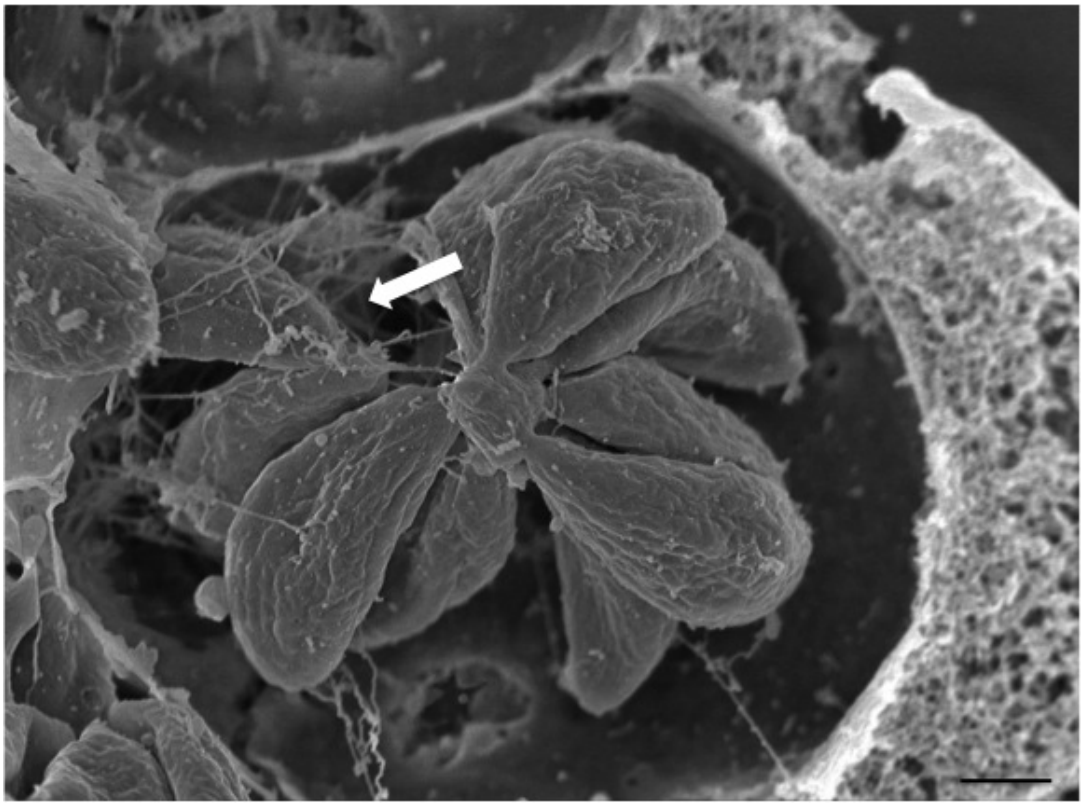

Fig. 1: A rosette of T. gondii inside a parasitophorous vacuole, from which parasites, after 30hpi, begin to detach (arrow). Bar. 1µm

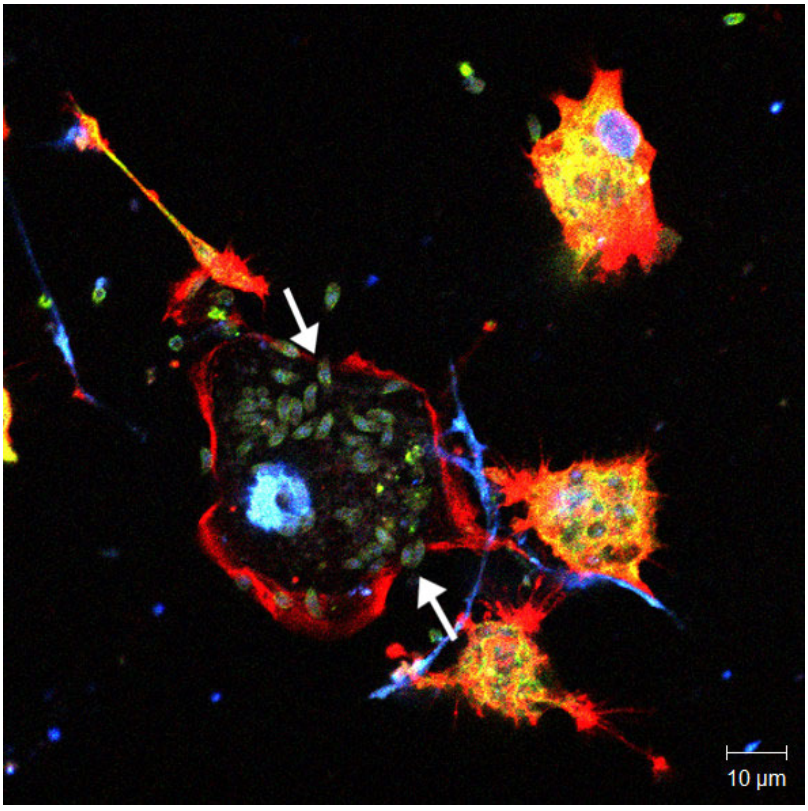

Fig. 2: T. gondii induced egress after cytochalasin D treatment. Parasites (blue) escape through actin (red) free zones (arrows).