

# ***Toxoplasma gondii* TISSUE CYST ENDOCYTOSIS: A VIEW INSIDE THE CYST**

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*Toxoplasma gondii* is a ubiquitous Apicomplexan parasite of birds and mammals, including humans. It is an important opportunistic pathogen of immunocompromised hosts [1]. After infection the *T. gondii* invades an enormous variety of cells and, eventually, forms cysts in several organs particularly brain and muscles. The factors affecting the transition of tachyzoites to the latent bradyzoite stage (in tissue cysts) remain to be defined. Tissue cysts can be 50 to 100  $\mu$ m in diameter and are separated from the host cell cytoplasm by a thick cyst wall. This wall is thought to be important in maintaining the integrity of the cyst in host cells for long periods. The cyst wall consists of a highly invaginated outer membrane underlaid with a dense osmiophilic matrix containing vesicles [2]. Up to now, few information about the endocytic capacity of *Toxoplasma* tissue cyst has been reported, which raises a number of questions regarding parasite's endocytosis processes. The first study of *Toxoplasma* cysts endocytosis were performed by Guimarães 2002 [3], showing that anionic sites present in the cyst wall are incorporated via vesicles and tubules located in the cyst matrix.

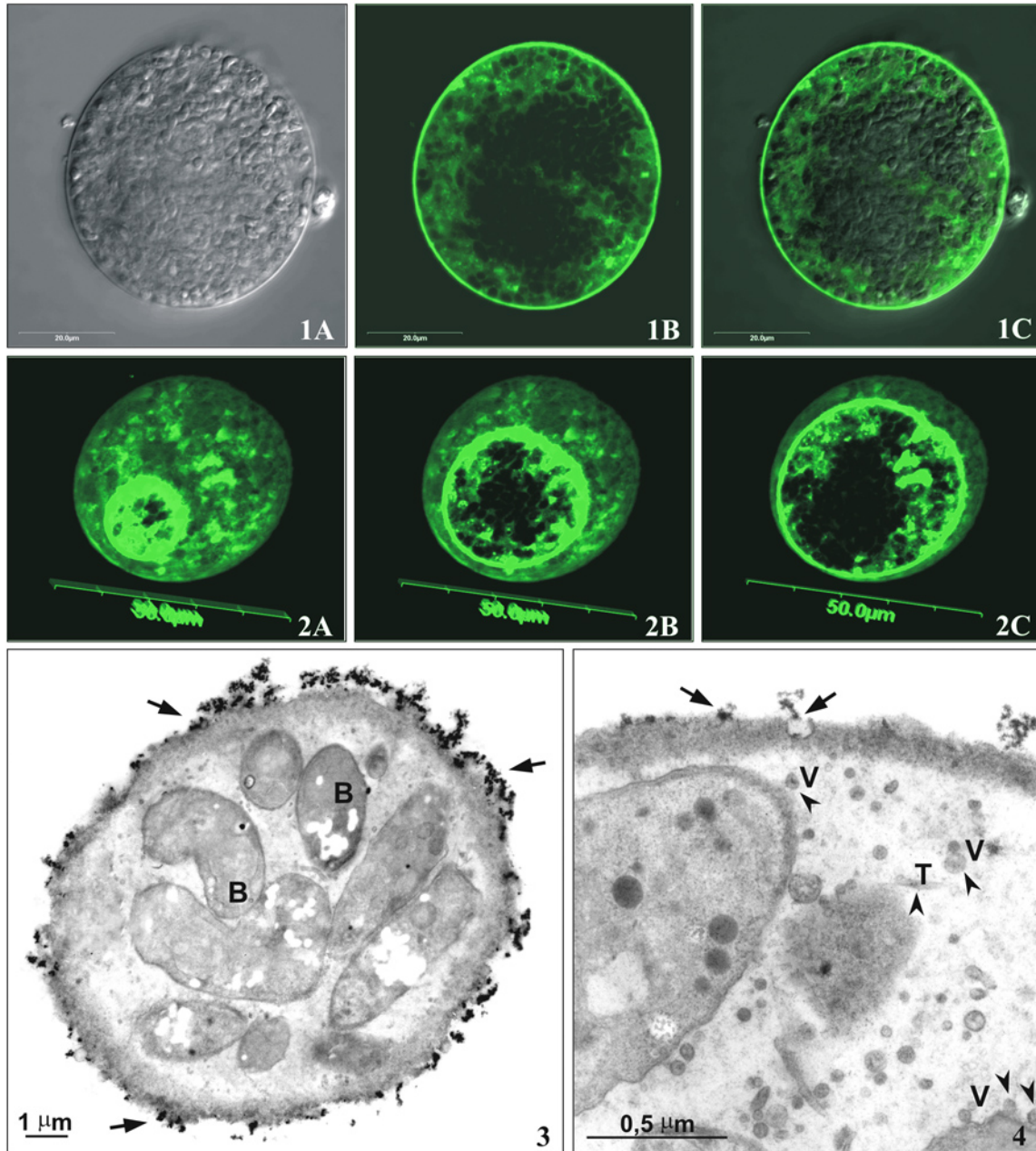
Confocal laser scanning microscope (CLSM), Optical Sections and 3D Reconstruction: Cysts isolated from mouse brain were incubated with 200  $\mu$ g/ml bovine serum albumin coupled with fluorescein (BSA-FITC) a fluid phase endocytic tracer, for 2 and 3h at 37°C, washed in PBS, fixed with 2% PFA, washed twice in PBS and applied to poly-L-lysine-coated microscopes slides and mounted in DABCO. Tissue cysts of *T. gondii* were examined with an FV300/BX51 Olympus CLSM. Differential interference contrast (DIC) and fluorescence images were obtained and performed merge images for analyse BSA-FITC distribution in cyst interior. Serial optical sections (around 200) were processed at 0.3 $\mu$ m steps, converting into a volume in which we could make virtual cuts of the reconstructed labeling and/or reconstructs the whole cyst image performed three-dimensional reconstruction (Image Pro software). Transmission electron microscopy: Cysts isolated from mouse brain were incubated with 1mg/ml native ferritin and 0.25-0.5mg/ml horseradish peroxidase (HRP-Au), fluid phase endocytic tracers, at 37°C for 5 min to 24 h. After this period, the samples were washed twice with PBS and fixed at 4°C in 2.5% glutaraldehyde buffered in 0.1 M sodium cacodylate (pH 7.2). Following fixation, the cells were rinsed in 0.1 M sodium cacodylate buffer, post fixed in 1% OsO<sub>4</sub>, dehydrated and embedded in Epoxy resin. Thin sections were stained with uranyl acetate and lead citrate, and then examined with a Zeiss EM10 transmission electron microscope.

Images from confocal microscopy along with the 3D reconstruction of tissue cysts incubated with BSA-FITC showed the tracer localized at the cyst wall, as well as within the cyst matrix, which after 3h of incubation appeared to be more diffusely than 2h, suggesting a time dependent incorporation (Fig. 1 and 2). On the other hand, ultrastructural analysis showed a heterogeneous labelling with HRP-Au, displaying areas with the marker in the membrane of the cyst wall and others areas displaying extends regions without any labelling. No HRP-Au complex was seen inside the cyst. Native ferritin was detected distributed all over the cyst wall, predominantly in patches of different sizes and localized inside vesicles and tubules in the cyst matrix, sometimes very close to the bradyzoites suggesting a possible fusion between their membranes (Fig. 3 and 4). Our present study brings important data related to endocytosis in *T. gondii* contributing in the future design of therapeutic approach during the treatment of toxoplasmosis.

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## **References:**

- [1] Luft, B.J., R.G. Brooks, F. K. Conley, R. E. McCabe and J. S. Remington. (1984). JAMA 252: 913-917.
- [2] Dubey, J.P., D.S. Lindsay, and C. A. Speer (1998). Clin. Microbiol. Rev. 11: 267-299.
- [3] Guimarães EV (2002). Master Thesis. Fiocruz



**Figures 1A, B and C:** *Toxoplasma* tissue cyst incubated with BSA-FITC for 3h at 37°C. Differential interference contrast (DIC) (1A) and fluorescence images (1B) were obtained and performed merge images (1C) for analyze BSA-FITC distribution in cyst interior. Cyst wall membrane and matrix labeled. **Figures 2A, 2B and 2C:** Optical sections of a whole *Toxoplasma* tissue cyst by 3D reconstruction. The transparency of cyst wall allows the view of the interior of the cyst displaying the matrix labeled. **Figure 3:** *Toxoplasma* tissue cysts incubated with native ferritin. Cyst wall displaying labeling of its whole surface predominantly in patches (arrows). B=bradizoite. **Figure 4:** Cyst wall displaying labeling of its surface with native ferritin, predominantly in patches (arrows) and in an invagination of the cyst membrane (arrow). Native ferritin localized inside vesicles (V) and tubules (T) in the cyst matrix, sometimes very close to bradyzoites intracyst (head arrows), suggesting a possible fusion between their membranes.