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***Toxoplasma gondii* Tissue Cyst: Cyst Wall Incorporation Activity and Matrix Cytoskeleton Proteins Paving the Way to Nutrient Acquisition**

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Additional information is available at the end of the chapter

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Abstract

Toxoplasma gondii is an intracellular parasite that causes chronic infection by the development of bradyzoites housed in tissue cysts, preferably in the muscles and central nervous system. The composition and the function of the cyst wall are still not fully understood. Are *T. gondii* cysts able to incorporate nutrients through its wall? If so, how would these nutrients be traversed to cross the cyst matrix to reach the bradyzoite forms? Herein, we tested the uptake capacity of the *Toxoplasma* tissue cyst wall by employing some fluid-phase endocytosis tracers as peroxidase (HRP) and bovine serum albumin (BSA). Fluorescence images revealed these molecules on the cyst wall as well as in the cyst matrix. The subcellular localization of the tracer was confirmed by ultrastructural analysis showing numerous labeled vesicles and tubules distributed within the cyst matrix in close association with intracystic bradyzoite membrane, suggesting the cyst wall as a route of nutrient uptake. Furthermore, we confirmed the presence of cytoskeleton proteins, such as tubulin, actin, and myosin, in the tissue cyst matrix that may explain the nutrient input mechanism through the cyst wall. A better understanding of the nutrient acquisition process by the cyst might potentially contribute to the development of new therapeutic targets against chronic toxoplasmosis.

Keywords: *Toxoplasma gondii*, tissue cysts, endocytosis, macromolecules, cytoskeleton

1. Introduction

Toxoplasmosis is a worldwide human protozoan infection caused by an intracellular protozoan, *Toxoplasma gondii*. Characterized by the presence of slow replicative bradyzoites surrounded by a thick wall, the tissue cysts have a striking predilection for infecting the central nervous system and skeletal muscle cells of the host [1]. It is widely accepted that the chronic phase of the disease in an immunologically competent person is normally asymptomatic and self-resolving. However, *T. gondii* has been associated with an increase in the incidence of many relevant psychiatric disorders. This relates to the fact that the brain is an immune-privileged site for lifelong existence of *T. gondii* tissue cysts [2]. Although the current pharmacological treatments have been ineffective against the cyst form, it is possible that the tissue cyst wall acts as a biological barrier preventing the interaction of drugs with intracystic bradyzoites [3].

Despite the clinical relevance of chronic toxoplasmosis, the biology of the cyst wall as yet has not been completely elucidated [4–9]. A 116-kDa glycoprotein termed CST1 [7] and two lectins, *Dolichos biflorus* and succinylated wheat-germ agglutinin, that bind specifically to the cyst wall, were identified, and it was suggested that chitin probably represents an important component of the cyst wall [10]. There are few evidences of the composition of the cyst matrix. Parmley et al. [11] described a cyst matrix antigen named MAG1, which was also found inside the parasitophorous vacuole matrix, forming a filamentous-like protein material.

Our previous ultrastructural analysis demonstrated that the cyst wall displays endocytic activity through the engulfing of negatively charged molecules in the cystic wall [8]. These molecules are incorporated by tubules and vesicles formed from the membrane that delimits the cyst wall and localized in the granular region and posteriorly in the cyst matrix. Within the cyst, the presence of vesicles containing the tracer in close contact to the bradyzoite membrane or in its neighborhood suggests that it could be one of the incorporated molecule pathways from the host cell cytoplasm to intracystic parasites [8]. The current knowledge of mechanisms involved in the process of nutrient uptake by this parasite still presents many gaps, restricted to few reports [12, 13]. As an obligate intracellular parasite, *T. gondii* actively invades and replicates in a wide variety of nucleated vertebrate cells where it resides in a parasitophorous vacuole. The ultrastructural analysis also demonstrated that *T. gondii* is able to invade host cell nuclei, and this invasion could reflect an alternative parasite route for immune evasion or may be a nutrient source when scarce in the host cell cytoplasm [14]. The vacuole membrane is the interface between the host and parasite, playing a role in the nutrient acquisition from the host cell by the parasite. The physiological significance of the endocytosis in the *T. gondii* lifestyle with tachyzoite forms is in the incipient stages of elucidation [15, 16], as the basic mechanisms delineating endocytosis in tachyzoites, bradyzoites, and tissue cysts are still poorly understood [17].

In all eukaryotes, endocytosis and intracellular vesicle traffic are events mediated by several proteins including a complex cytoskeleton network. Cytoskeleton proteins are indispensable components of a number of vital parasite structures and functions, like cell division, membrane and cytoplasmic architectures, parasite gliding motility (glideosome), and host-cell invasion [18–20]. Besides conventional cytoskeleton elements, many components of *T. gondii* are novel proteins. Two examples are Actin-Like Protein 1 (ALP1), which plays a role during

cell division [21], and a protein called TgSIP, a structural cytoskeleton component of the parasite inner membrane complex [22].

Herein, the putative mechanisms employed by *T. gondii* cysts involved in nutrient incorporation and the cytoskeletal protein network in the tissue cysts were investigated, which could explain the endocytic activity of the tissue cyst wall and vesicle traffic in the cyst matrix. The endocytosis process and the cytoskeleton network are crucial to *T. gondii* replication, motility, invasion, and maintenance, indicative as excellent drug targets for antiparasitic therapies.

2. Experimental design

2.1. Parasites

Mice infected with ME-49 strain *T. gondii* and maintained for 4–8 weeks in C57BL/6 were euthanized and the brain cysts isolated as described [23]. License CEUA LW 50/14 from Fundação Oswaldo Cruz Committee of Ethics for Use of Animals was authorized.

2.2. Endocytosis assays

Cysts were incubated with bovine serum albumin (BSA) labeled with fluorescein isothiocyanate (BSA-FITC), a fluid phase endocytic tracer, for 2 or 3 h at 37°C and processed for analysis in a confocal laser scanning microscopy FV300/BX51 Olympus and differential interference contrast (DIC) microscopy. Serial optical sections of each cyst incubated with BSA-FITC were converted into a volume performing 3D reconstruction. Additionally, the ultrastructural analysis was performed with two fluid phase endocytic tracers, BSA and peroxidase (HRP), both conjugated with colloidal gold (Au) particles.

2.3. Identification of cytoskeleton proteins

Brain cryosections of *T. gondii*-infected mice as well as *ex vivo*-isolated tissue cysts (pre-fixed with 4% PFA) were processed for immunolabeling. Briefly, the samples were incubated with polyclonal primary antibodies (anti-actin 1:500, anti-tubulin 1:200, anti-myosin 1:200, and microtubule-associated proteins (MAPs) 1:200), followed by incubation in CY3-conjugated secondary antibody (goat anti-rabbit IgG, 1:1000) and processed for fluorescence microscopy analysis. Control samples were processed omitting primary antibodies. Brains of uninfected animals were the controls for the experiment.

3. Results

3.1. *T. gondii* is able to incorporate macromolecules through the tissue cyst wall

T. gondii tissue cysts incubated for 2 or 3 h with BSA-FITC at 37°C were analyzed by DIC and confocal microscopy, **Figure 1A–C** and **D–F**, respectively. The BSA-FITC presented a strong

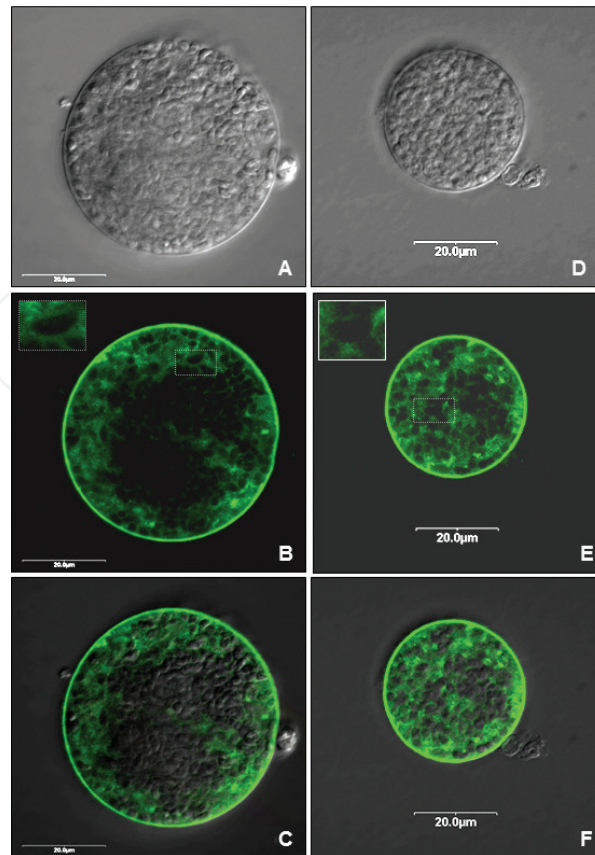


Figure 1. Confocal microscopy of *Toxoplasma* cyst incubated with BSA-FITC. (A–C) *Toxoplasma gondii* tissue cyst incubated at 37°C for 2 h and (D–F) 3 h with BSA-FITC. (A and D) Interferential microscopy (DIC) showing the cyst wall and numerous parasites inside the cyst. (B and E) Cyst fluorescence microscopy after 2 and 3 h of BSA-FITC incubation showing the tracer in the matrix, fulfilling the free spaces between the bradyzoites and throughout the cyst wall. (C and F) Overlay of fluorescence and DIC images showing the matrix and cyst wall labeled with BSA-FITC. Note that the cysts incubated for 3 h presented higher tracer incorporation. Scale bars: 20 μ m.

labeling throughout the cyst wall and also located in the cyst matrix between the intracyst bradyzoites (**Figure 1B, C, E, and F**). These features were reinforced by merge obtained from DIC and fluorescence images (**Figure 1C and F**). The incorporation of BSA-FITC by tissue cysts was time dependent (**Figure 1C and F**). During the 3D reconstruction performance of a whole cyst from a set of 351 serial sections of 0.3 μ m thickness, cyst wall transparency was strategically maintained in order to permit the visualization of the intracystic tracer (Video 1, supplementary material).

3.2. Ultrastructural analysis confirmed distinct molecule incorporation through the cyst wall

In order to examine whether the predicted protein labeling was in fact throughout the cyst wall, an ultrastructural analysis was conducted, incubating cysts with electron dense tracers, such as BSA-Au and HRP-Au. The incubation for 30 min at 4°C with BSA-Au revealed the marker at the surface as well as within invaginations of the cyst wall (**Figure 2A**). The internalization of BSA-Au occurred after 2 or 4 h of incubation at 37°C. Colloidal gold particles could

be found within small vesicles and tubules localized at the granular region independent of the time of BSA-Au incubation (**Figure 2B** and **C**).

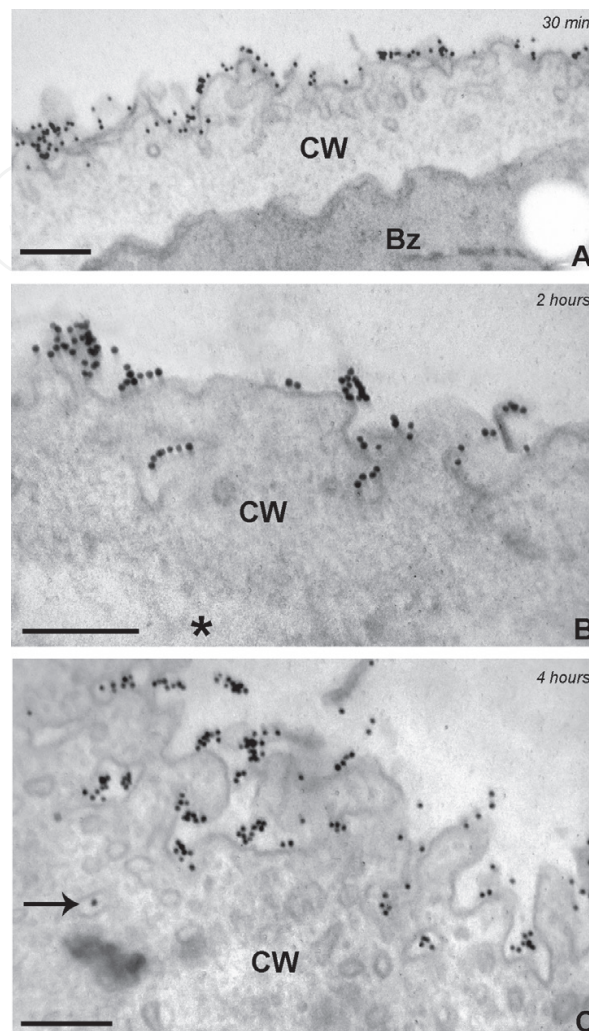


Figure 2. Transmission electron microscopy of *Toxoplasma gondii* cysts incubated with BSA-Au. (A) Tissue cyst presents many BSA-Au particles on cyst wall membrane after incubation for 30 min at 4°C. (B) Tissue cysts incubated for 2 h at 37°C displaying gold particles lining the cyst wall membrane and within the cyst membrane invaginations. (C) Tissue cysts incubated for 4 h at 37°C present BSA-Au particles in the uncoated vesicles and within tubules full of particles (arrow). Scale bars: 0.2 μm .

Similar results were obtained with HRP-Au by incubation of tissue cysts for 30 min at 4°C (**Figure 3A**). When the temperature was elevated to 37°C, the HRP-Au particles were observed within uncoated vesicles and tubules localized in the granular region of the cyst (**Figure 3B** and **C**).

3.3. Tissue cysts display a cytoskeleton network both in vivo and ex vivo

Aiming to reveal the cytoskeletal proteins in *T. gondii* tissue cysts, immunofluorescence assays were carried out with different polyclonal antibodies. Anti-myosin demonstrated the protein in

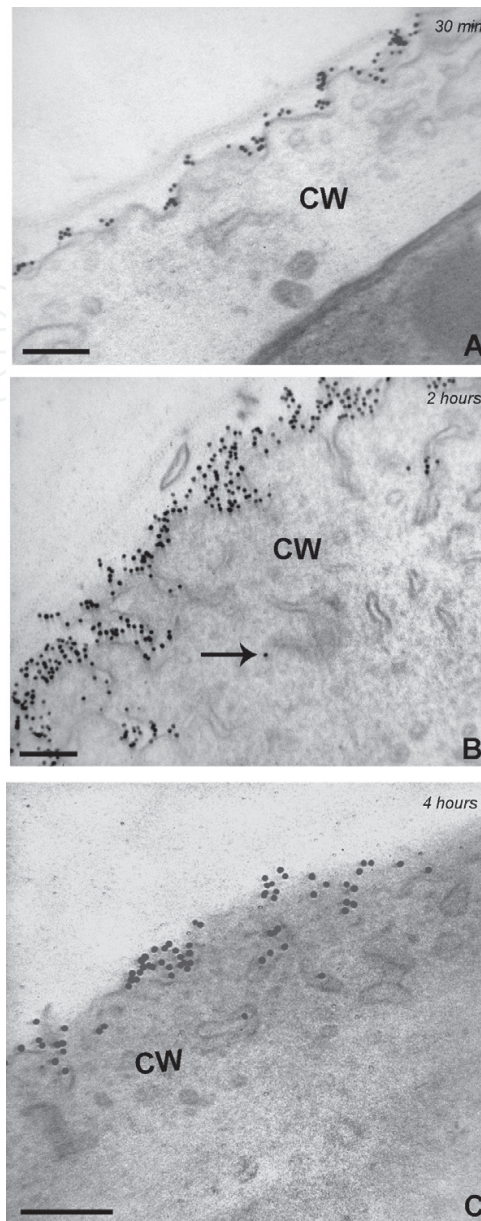


Figure 3. Transmission electron microscopy of *Toxoplasma* cyst incubated with Au-HRP. (A) Tissue cyst incubated for 18 h at 37°C with HRP-Au displaying gold particles lining the cyst membrane (arrow) and within the cyst membrane invaginations (large arrow). Scale bar: 0.2 μm . (B) Cysts present different regions of their surface with the distribution of HRP-Au (arrows) lining the cyst wall followed by spaces without any labeling. Absence of the marker in the cyst interior. Scale bar: 0.5 μm .

the cyst matrix as a diffuse staining and on the tissue cyst wall as intense heterogeneous fluorescent dots (**Figure 4A–C**). Brain cryosections from C57BL/6 mice chronically infected with *T. gondii* were incubated with the anti-myosin antibody, revealing that the distribution in tissue cysts *in vivo* followed the same pattern as *in vitro* (**Figure 4D** and **E**). For positive control, bradyzoites were incubated with the same antibody, and the myosin was localized mainly at the anterior pole (conoid region) or diffused through the parasite plasma membrane and cytosol (**Figure 4F**).

DIC microscopy (**Figure 5A**) and the immunofluorescence with the anti-tubulin antibody revealed a homogeneous labeling along the cyst wall in an area correspondent to the

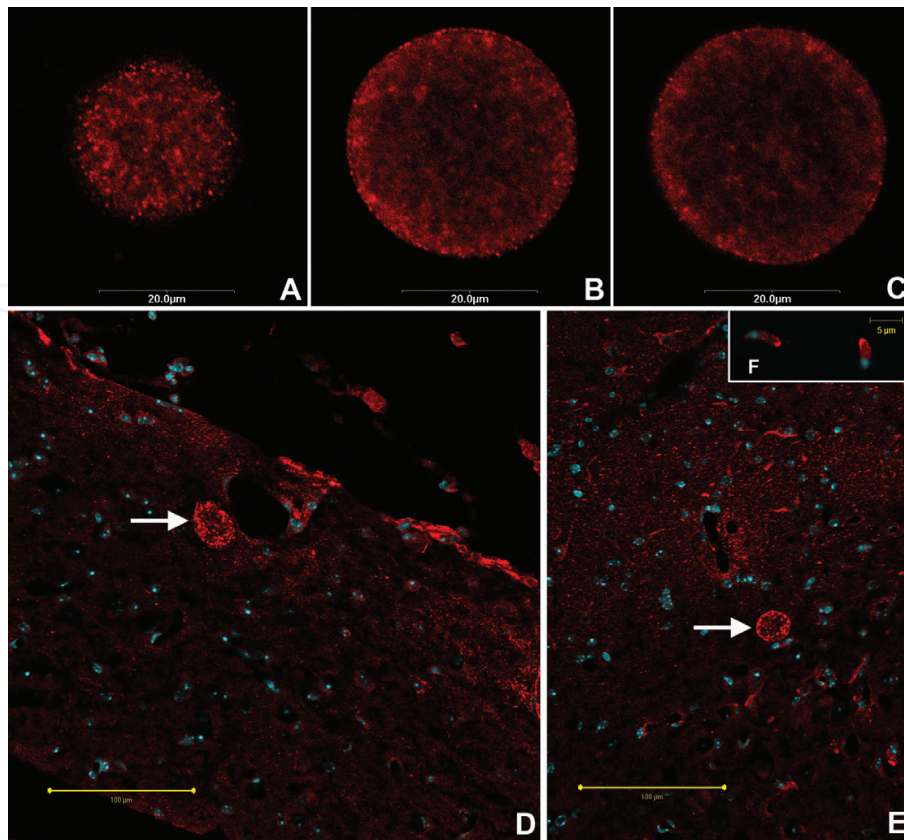


Figure 4. Immunofluorescence for detection of myosin in *T. gondii* tissue cysts. (A–C) Different confocal sections of the same isolated tissue cyst show that myosin was located along the tissue cyst wall with a dotted distribution, and the labeling was seen inside parasites as well. Scale bars: 20 µm. (D) Cryosection of a chronically infected brain showed that *in vivo* the tissue cyst wall had the same myosin distribution pattern as *in vitro*. (E) Different brain area labeled for myosin. Scale bars: 100 µm. (F) Positive control of myosin staining in *T. gondii* bradyzoites confirms the localization of the protein at the anterior pole and associated with the plasma membrane, similarly as described for tachyzoites. Scale bar: 5 µm.

granular region (**Figure 5B**). This intense wall labeling was better visualized in the merged figure (**Figure 5C**). Different virtual confocal sections of the same cyst disclosed the presence of tubulin inside bradyzoites (**Figure 5D–I**). In *T. gondii* chronically infected brains, the pattern of tubulin distribution was the same as in isolated tissue cysts (**Figure 6A**). In bradyzoites, tubulin was strongly labeled in the region corresponding to the subpellicular microtubules (**Figure 6B**), conferring the typical elongated shape of the parasite and the apical polarity of apicomplexan phylum. Microtubule-associated proteins (MAPs) form bridges linking with themselves and other structures. Tissue cysts in infected brain cryosections incubated with the anti-MAPs antibody colocalize with tubulin distribution (**Figure 6C and D**).

The most intense fluorescence labeling was for actin detection in *T. gondii* tissue cysts with homogeneous distribution along the cyst wall of isolated cysts (**Figure 7A**), presenting the same pattern of labeling as in the infected brain cryosections (**Figure 7B**). The revelation of actin inside the cyst (**Figure 7A**) suggested the detection of bradyzoites. To confirm this distribution, isolated bradyzoites were incubated with the same antibody, the labeling apparent on the conoid and extending below the apical region of the parasite body (**Figure 7C**).

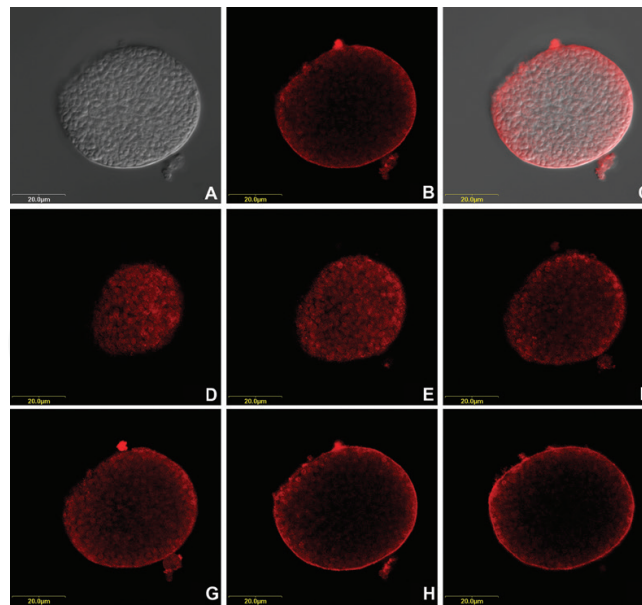


Figure 5. Immunofluorescence for detection of tubulin in *T. gondii* tissue cysts. (A) DIC microscopy, (B) fluorescence, and (C) a merge of figures A and B of an isolated tissue cyst labeling for tubulin detection showed an intense and homogeneous staining around the tissue cyst wall as well as parasite staining. (D–I) Different confocal sections of the same isolated tissue cyst confirm this distribution. Scale bars: 20 µm.

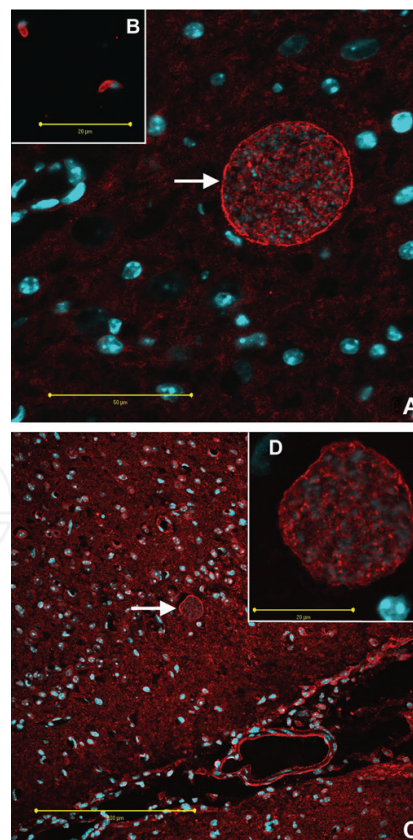


Figure 6. Immunofluorescence for detection of tubulin and microtubule-associated proteins (MAPs) in *T. gondii* tissue cysts. (A and B) Cryosections of chronically infected brains showed that, as *in vitro*, *in vivo* staining for tubulin and MAPs was intense and homogeneous around the tissue cyst wall. Scales bars 50 and 200 µm, respectively. (A') Positive control for tubulin staining in bradyzoites of *T. gondii* revealed the population of subpellicular microtubules of the parasite. Scale bars: 20 µm. (B') Detail of tissue cyst in cryosection. Scale bars: 20 µm.

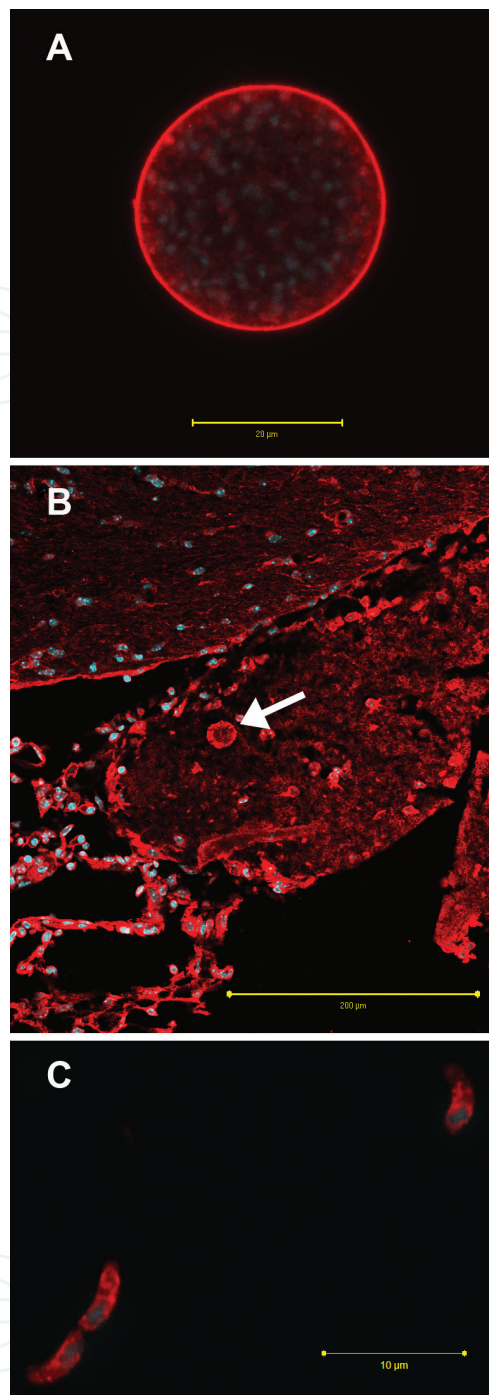


Figure 7. Immunofluorescence for detection of actin in *T. gondii* tissue cysts. (A) In isolated tissue cysts, actin detection was more intense than tubulin but still homogeneous around the tissue cyst wall. Scale bars: 20 µm. (B) In cryosections of chronically infected brains, the staining was the same as *in vitro*. Scale bars: 200 µm. (C) In positive control, anti-actin antibody labeled bradyzoites in the apical region and appeared as a diffuse staining in the parasite cytosol. DAPI stained both the cell and parasite nuclei (in blue). Scale bar: 10 µm.

3.4. Ultrastructural localization of cytoskeleton proteins through the cyst matrix

The detection by ultrastructural immunocytochemical of actin and tubulin proteins in tissue cysts revealed many gold particles distributed along the granular region and dispersed in the cyst matrix of tissue cysts (**Figure 8A**). In bradyzoites, some particles were located in the

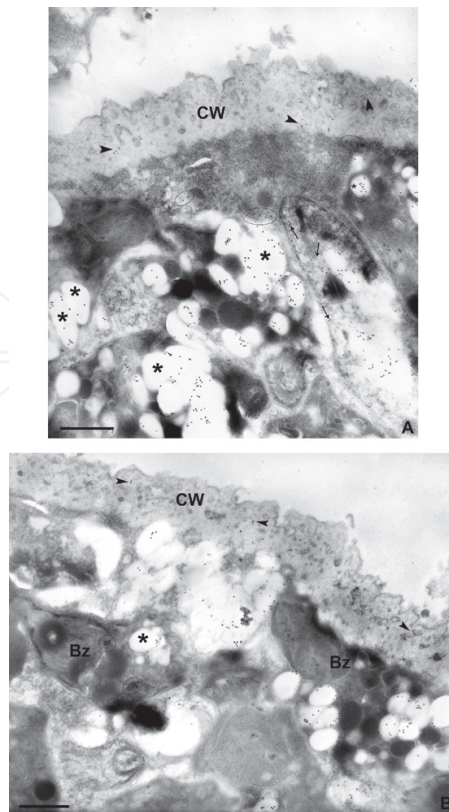


Figure 8. Transmission electron micrographs (TEM) of *T. gondii* tissue cyst labeled for actin by immunogold assays. (A) In tissue cyst, many gold particles were distributed along the granular region (arrowhead) and in the matrix (circle). In bradyzoites, some particles were located in the cytoplasm (arrows), most of them inside amylopectin granules (asterisk). (B) In bradyzoites, the gold particles were seen along the cyst granular region (arrowhead) and in clusters in the parasite cytoplasm (circle). Cyst wall (CW) and bradyzoites (Bz). Scale bars: 0.5 μm .

cytoplasm, most of them inside amylopectin granules, along the cyst granular region (arrowhead) and in clusters in the parasite cytoplasm (**Figure 8B**). The immunogold reaction for tubulin identified some gold particles on the cyst wall membrane along the granular region and in the matrix (**Figure 9A**). In bradyzoites, tubulin gold particles were detected mainly inside and around the amylopectin granules as well as in the cytoplasm and membrane (**Figure 9B**).

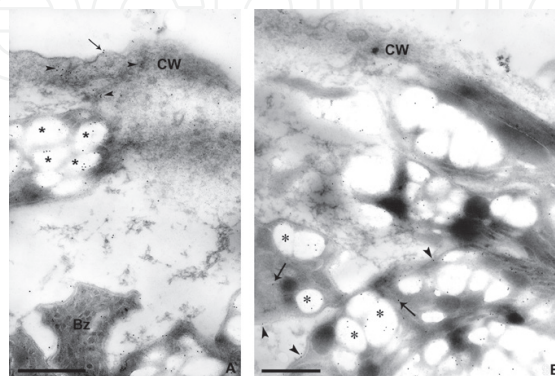


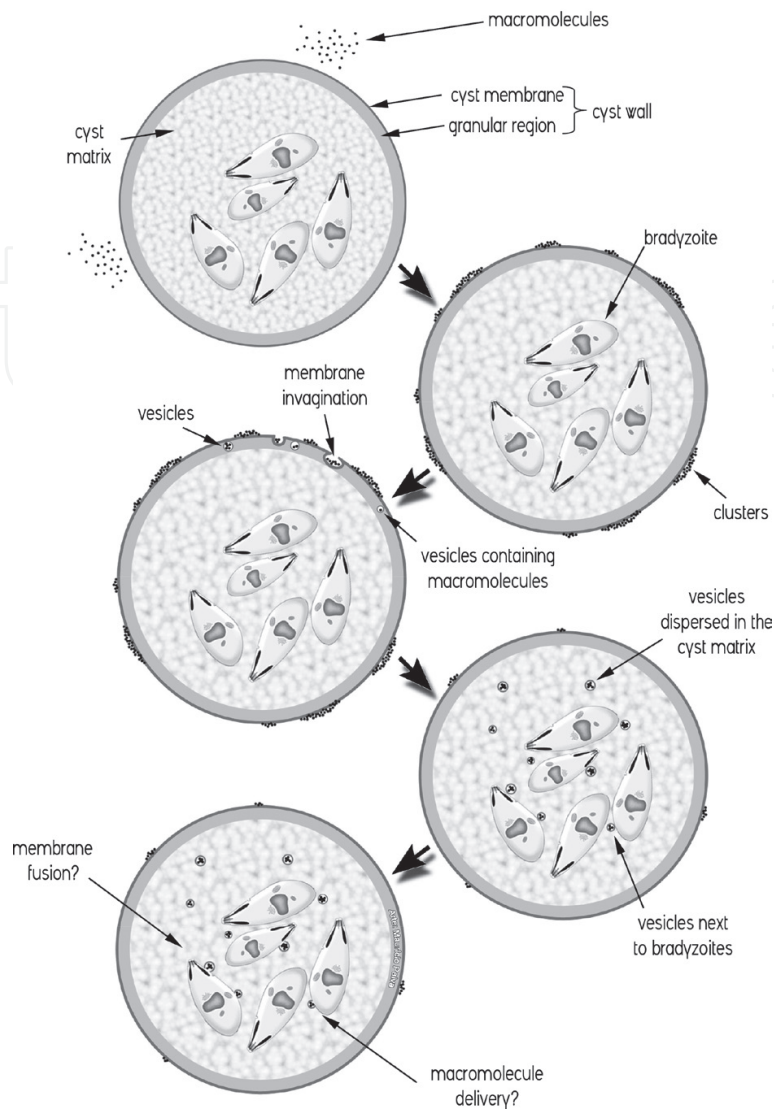
Figure 9. Transmission electron micrographs (TEM) of *T. gondii* tissue cyst labeled for tubulin by immunogold staining. (A) In the tissue cyst, gold particles are seen on the cyst wall membrane (arrows) along the granular region (arrowheads). In the bradyzoites, gold particles were present mainly inside and around the amylopectin granules (asterisk). (B) Bradyzoites cytoplasm (arrow) and membrane (arrowhead) showed some gold particles. Cyst wall (CW) and bradyzoites (Bz). Scale bars: 0.5 μm .

4. Discussion

This study reveals the ability of the *T. gondii* cyst wall to incorporate macromolecules *in vitro* as well as the presence of cytoskeleton elements in tissue cysts. A critical question in the lifelong persistence of host *Toxoplasma* cysts is how these intracellular parasites are able to survive for long periods within the cyst. The nutrients required by bradyzoites surrounded by the cyst wall may be necessary for its maintenance, multiplication, and amylopectin granule synthesis [23, 24]. The cyst wall, due to its limiting, highly invaginated membrane, provides an enhancement of the cyst surface area, affording the traffic of material between the parasite intracyst and the host cell cytosol [25–27]. Our results show that the *T. gondii* cyst wall is able to incorporate fluids and macromolecules from the external environment via the formation of vesicles and tubules, corroborating findings [8], when there is endocytic activity of the cystic wall, a dynamic process that occurs in *ex-vivo* cysts. These properties might be an alternative method for parasite survival, remaining enclosed within cysts for a long period of time or, as proposed, for the entire life of the infected host [28, 29]. These data point to the endocytosis, described for mammalian cells [30, 31] as well as the endocytic pathway of other pathogenic protozoa [32], as a mechanism of tissue cysts during intracellular development for nutrient acquisition from the host cell.

The exposure of *Toxoplasma* cysts to BSA-FITC for 2 or 3 h exhibited for the first time, the incorporation dynamics of this endocytic marker located at and attached initially to the cyst wall and afterward, the transit within vesicles toward the cyst matrix. Ultrastructural images from cysts incubated with the two different fluid-phase endocytic tracers (BSA-Au and HRP-Au) demonstrated that they were capable to associate with the cystic wall and be internalized via vesicle and tubules. The incubation of tracers and cysts at 37°C for periods 1–4 h displayed a heterogeneous labeling with formation of clusters on the cyst wall. Guimarães et al. [8] obtained similar images after cyst incubation with cationized ferritin. This labeling type may be due to the motility of cyst wall components or even the presence of different surface micro domains. Some images suggested fusion between the vesicles originating from invagination of the cyst wall with the parasite membrane. However, we have been unable to visualize vesicles discharging the marker or the marker inside the parasite. The present results corroborate previous data of our group with cationized ferritin showing vesicles and tubules containing particles in close contact with the membrane bradyzoite intracystics [8]. The previous data added together with those presented in this work suggest an active process of membrane fusion involved in the bradyzoite macromolecule uptake. It could be one of the pathways for parasite nutrient acquisition through the molecules available in the host cell cytoplasm (see **Scheme 1**, supplementary material). Moreover, we believe that the tubules and vesicles between the filaments of the cyst wall play a key role in delivering internalized molecules from the cyst wall to the intracellular bradyzoites and vice versa.

This cyst wall property opens new perspectives to the investigation of cytoskeletal element involvement in the process of nutrient incorporation by *T. gondii* tissue cysts. Thus, research was initiated concerning the molecular motors that move vesicles and tubules along actin filaments or microtubules, which might mediate the movement from the wall to the matrix cyst. Immunofluorescence of infected brain cryosections and isolated tissue cysts revealed that the tissue cyst wall and matrix are surrounded by a network of cytoskeleton proteins containing at least actin, tubulin, and myosin. Previously, our group demonstrated that the cyst wall is



Scheme 1. *Toxoplasma* cyst endocytosis. Schematic representation illustrating macromolecules endocytosis by *Toxoplasma* cyst showing each step of this process: (A) *Toxoplasma* cyst exposed to macromolecules; (B) binding of macromolecules on the cyst wall and cluster formation; (C–E) macromolecule incorporation through the cyst membrane invagination followed by the formation of vesicles which migrate from the granular region toward the cyst matrix where they were visualized next to bradyzoites.

formed by negatively charged molecules, and that these molecules are incorporated in the matrix through an endocytic process, involving tubules and vesicles formed from the membrane delimiting the cyst wall [8]. Additionally, we verified that these vesicles and tubules were at different locals of the cyst wall, intimating that they are certainly moving from the outer membrane of the cyst toward the bottom of the cyst wall. Moreover, some vesicles were seen in the matrix very close to the bradyzoite plasma membrane.

Until now, there has been no evidence of the fusion of these vesicles with the parasite membrane. However, we suggest that it might be an important pathway for host cell nutrient acquisition. Nutrient required for bradyzoites is extremely important, as the parasite is confined inside the tissue for long periods and can persist throughout the life of the

host [1]. Di Cristina et al. [33] observed that fluorescence molecules, such as D-luciferin, have access to bradyzoites within intact cysts both *in vitro* and *in vivo*. Lemgruber et al. [9] described that the tissue cyst wall incorporation is dependent upon the molecular weight of the tracers. Our data are therefore consistent with both of these studies.

In this way, our interest was to explain how these molecules are incorporated by the cyst wall and also how they can move within the cyst matrix considering that the cyst is not one cell but a structure that maintains various parasites in its interior. The endocytic activity of the cyst wall requires a stimulus induced by molecular signaling which involves cytoskeleton proteins as described for eukaryotic cells. Both actin and tubulin contribute to intracellular vesicle trafficking and endocytic activity in a series of dynamic events in the endomembrane system. The strong positive staining of tubulin and actin, revealed with polyclonal mammalian antibodies throughout the cyst matrix, indicated the presence of a cytoskeleton network within the cyst. Further investigation would be needed to evaluate the origin of these proteins. Eukaryotic cells exhibit tubulin dimers that can be easily altered by posttranslational modifications that differentially mark distinct microtubule subpopulations [34, 35]. Each microtubule subpopulation is organized for specific functions, such as the mitotic spindle, tracks for vesicular transport plus the basal body, and associated flagellar axoneme. In *T. gondii*, there are at least five discrete tubulin-based structures including the conoid, cortical microtubules, intraconoid microtubules, and centrioles, as well as a spindle in replicating parasites [19]. Studies may be required to confirm whether or not tissue cyst matrix cytoskeleton proteins originate from the parasite or from mammalian host cells during the parasitophorous vacuole formation.

Numerous protozoan parasites have the ability to form the cyst stage that normally allows them to survive under adverse environmental conditions. Chávez-Munguía et al. [36] showed that during the encystation process of *Entamoeba invadens*, the cyst wall components are concentrated and transported in specific vesicle, which contain a fibrillar material. This material is then deposited on the parasite plasma membrane through the aperture of the vesicles. Vesicles with a similar appearance may also be present in the cytoplasm of *E. invadens* mature cysts and in the parasite *Acanthamoeba castellanii*. Benchimol [37] also described this structural mechanism involved in the deposition and transport of material to the *Giardia lamblia* pseudocyst wall. After releasing their contents, the membranes may reseal to form empty vesicles, which may be endocytosed by the parasite and/or remain as empty vesicles. Hausen et al. [38] suggested that *G. lamblia* cysts treated with the benzimidazole derivative drug Albendazole, which binds to α - β -tubulin heterodimers of assembling microtubules, link to cyst tubulin, strongly interfering in the excystation process of the parasite. The demonstration of actin, tubulin, and myosin in the composition of the *T. gondii* cyst wall and their distribution in the cyst matrix, as shown here, by immunofluorescence in the *ex-vivo* and *in vivo* systems, is a potential key to the understanding of the incorporation and traffic of molecules by the *T. gondii* cyst wall.

Actin is a highly conserved microfilament protein that plays an important role for gliding motility and in *T. gondii* tachyzoites host cell invasion [39]. By indirect immunofluorescence, actin has been reported to be primarily concentrated in the anterior third of the tachyzoite and in a diffuse staining pattern throughout the cytoplasm [40, 41], our immunofluorescence for

actin in the bradyzoite stage revealing the same staining pattern. We also considered that the strong fluorescence staining along the cyst wall could be a false positive and may be caused by adsorptions of the antibody. This hypothesis was discarded when an infected brain was sectioned, and the cyst stain pattern was the same as for intact isolated tissue cysts.

In conclusion, *T. gondii* cyst wall endocytic activity has been demonstrated to provide insight in the parasite cell biology. The relationship of the cyst wall dynamics and cytoskeleton elements maintained in *ex-vivo* cysts corroborate the hypothesis that the cyst wall incorporates and circulates molecules, which could guarantee the parasite survival and persistence during *T. gondii* host infection. In addition, this data potentially provides prospects for new strategies and drug design for toxoplasmosis treatment.

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