Chapter 1

**Giardia Intestinalis**: An Intriguing Smile-Faced Parasite

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Abstract

*Giardia intestinalis* is a human intestinal tract parasite with trophozoite and cyst stages in its life cycle. Morphological and functional alterations characterize the differentiation process from one life cycle form to another. *Giardia* is a major cause of diarrhea in humans and mammals and is a cosmopolitan parasite. The transmission is due to cyst ingestion and related to poor sanitary conditions. The cyst morphology allows the cells to survive in inhospitable environments and infect many hosts. Adults and children can acquire giardiasis, but children have more abrupt symptoms, which include problems in cognitive development. This parasite exhibits a number of unique structures and organelles, which provide an excellent model to understand cell evolution. Ventral disc, funis, median body, peripheral and encystation vesicles and mitosomes are examples of specific characteristics in this cell. Knowledge and understanding of the functions and cell mecha-
nisms could improve the efficacy of parasite transmission control, decrease the high disease burden, and contribute to the knowledge of eukaryote cell evolution.

**Introduction: A Brief History**

*Giardia intestinalis* is a protist that was described for the first time in 1681 by Antony Van Leeuwenhoek when examining his own feces by light microscopy [1]. Lambl observed the parasite in more detail and described the size, shape, and presence of a ventral disc, naming the parasite *Cercomonas intestinalis* [2]. In 1888, Blanchard changed the parasite’s nomenclature to *Giardia lamblia* as a tribute to Lambl [3]. There are several names for this parasite, such as *Giardia intestinalis, Giardia duodenalis, Giardia enterica* and *Lamblia intestinalis*. Nowadays, the preferred name is *Giardia intestinalis*. In 1926, Hegner indicated *Giardia* as a gastrointestinal parasite and an agent of giardiasis. This protest can be found in several vertebrates [4].

**Transmission and Epidemiology**

*G. intestinalis* is a common cause of diarrhea in humans and other mammals throughout the world. The parasite is transmitted by an oral-fecal route by ingestion of cysts (Figure 1). Infected individuals may or may not present symptoms but release cysts, which are found two weeks after ingestion and continue the parasite’s cycle. This period depends on the parasite’s infection of the mid gut (Figure 1).

![Figure 1: Schemeof *G. intestinalis* lifecycle. 1. Mature cyst ingestion through contaminated food/water or by direct transmission due bad hygiene conditions (oro-fecal). 2. Trophozoite exits from cyst wall. 3. Typical trophozoite after excystation presenting externalized flagella and normal ventral disc. The parasite in this phase adheres to small intestine mucosa. 4. Parasite binary division. 5. Trophozoite colonization of gut cells. 6. Trophozoite transformation into cysts. 7. Mature cyst elimination in feces. (Teixeira D and Benchimol M, unpublished). The cysts can survive in inhospitable conditions because the cyst wall. This protective structure makes the cyst resistant to cold waters and dry environments, leading successful disease transmission. Some researchers attribute the infection rates to water reservoir contamination [5], which is a concern for sanitation quality. Lower infection rates are observed when sanitary conditions are implemented [6]. As all mammals may be infected
by *G. intestinalis*, it is possible for transmission between different species to occur. The potential mechanisms for parasite transmission include human-to-human, animal-to-animal and zoonotic transmission (from animals to humans or vice-versa), as well as water or food uptake transmission in humans or animals [7].

Giardiasis is a worldwide disease, and children are more susceptible to infection by *Giardia* [8]. Several factors are linked to the prevalence rates of parasite infections, such as geographic area, group of analysis, sensitivity of the diagnostic methods, and health care accessibility. In the USA, *Giardia* infections are linked to a number of hospital interventions, with almost 5,000 cases occurring annually [9]. In 2005, more than 15,400 cases of giardiasis were reported to the governmental health department, meaning that giardiasis remains as a more prevalent enteric infective disease in the USA [10].

Giardiasis prevalence in humans (both asymptomatic and otherwise) was estimated to be 2.6, 3.3, 5.3, and 6.3% in Denmark, Finland, Norway and Sweden, respectively [11]. Prevalence rates in underdeveloped countries are different from those in developed countries. The prevalence is high in Asia, Africa and South America, with almost 200 million people presenting giardiasis symptoms and 500,000 new cases reported every year [11]. By the year 2050, it is estimated that more than 50% of the population in developed countries will be living in urban areas, with the majority in poor sanitary conditions where the parasite can be transmitted easily [11].

There are three major risk groups for giardiasis in developed countries. In the first group are travelers who have visited areas with high rates of *Giardia* infection. The disease takes about 9 days to present symptoms in an infected person [12]. The second and third groups are daycare children and those who practice anal sex [12-13].

**Pathogenesis, Clinical Occurrence and Treatment**

The pathogenesis of giardiasis is not well established. The major case reports are from asymptomatic infected adults, who release cysts into the environment. In symptomatic cases, the immune system is generally able to combat the infection [14]. The main symptoms of giardiasis include diarrhea, malaise, abdominal cramps, weakness, weight loss, anorexia, nausea, vomiting, fever, abdominal distension, headache and jitters. Diarrhea occurs due fixation of the parasite on the intestinal mucosal microvilli, which decreases the uptake of several nutrients, such as disaccharides, fats and vitamins [15].

The clinical effects of giardiasis seem to be more significant in children. Stunted growth and low intelligence quotients were reported in infected children, even without the classic symptoms such as diarrhea [16-17]. Psychomotor and cognitive development is also adversely affected by *Giardia* infection during childhood [18]. Giardiasis in children is also associated with low rates of hemoglobin and vitamin A [19-20].

Searching for cysts and trophozoites in fecal sam-
ples by light microscopy is a standard diagnostic method. Enzyme-linked immunosorbent assay (ELISA) is a routine detection method for *Giardia* antigens [21]. Nowadays, the basic treatment is nitroimidazole-derived drugs (metronidazole, tinidazole and ornidazole), as well as quinacrine, albendazole, furazolidone, and paromomicine. Metronidazole is used most often and is effective against protozoa and bacteria while presenting antiinflammatory properties *in vitro*. Metronidazole's mechanisms of action have been studied extensively, and it acts against the anaerobic metabolic pathway of *G. intestinalis*. It enters the trophozoite and acts upon the ferridoxin proteins, which donate electrons to metronidazole's amine group [22]. Metronidazole is activated by the reduction of this amine grouping, generating a gradient that supports intracellular drug transport [22]. The reduced metronidazole acts as an electron terminal acceptor, which links covalently to DNA [23]. This covalent linkage results in DNA damage, such as loss of helical structure, replication alterations, and strand breaking, which leads to parasite death [24].

**Biological Cycle**

The life cycle of *G. intestinalis* presents two phases. The first is the trophozoite phase, in which the parasite inhabits the small intestine (the duodenum and the initial portion of jejunum) and can occasionally reach the bile ducts and gallbladder. The other phase is the cyst, which is responsible for parasite transmission. The cyst is resistant to desiccation and is transmitted through feces (Figure 1).

Giardia infection starts with cyst ingestion through food or contaminated water [25]. Afterwards, the trophozoites excyst (emerge) from the cyst wall and colonize the small intestine. *G. intestinalis* excystation occurs in vitro when the cysts are incubated in media with low pH (mimicking the stomach pH), followed by incubation in pancreatic protease solution at a slightly alkaline pH (mimicking the small intestine) [26]. In vivo, the newly emerged trophozoites adhere to epithelial cells in the duodenum and jejunum. Several binary divisions then occur, and the adhered trophozoites form a monolayer. The establishment of the parasite monolayer provokes local inflammation and the reduction of nutrient absorption by the intestinal epithelial cells. When the trophozoites move to the final portions of the jejunum, the encystation process takes place. The cysts are released with the feces and can infect new hosts [25].

**Morphology**

**Trophozoite Morphology**

*G. intestinalis* trophozoites present a half-pear shape with bilateral symmetry and size of 12–15 μm. The parasites exhibit several unusual structures, such as the ventral disc, median bodies, funis, flange, two nuclei, and peripheral vesicles, as well ordinary structures, such as the endoplasmic reticulum, four pairs of flagella, nucleoli, ribosomes, and glycogen granules spread out in the cytoplasm (Figure 2). Typical mitochondria are not observed in this protist, but there are mitosomes, which are mitochondria-
related organelles. The presence of a Golgi complex is a controversial topic in the cell biology of *Giardia*. Some authors claim that a Golgi-like apparatus appears during the encystation process [27-28], but there is no consensus. The majority of authors claim that there is no Golgi in *Giardia*, or at least that there is no typical Golgi complex in this parasite.

**Figure 2:** Ultrastructure of *G. intestinalis*. (a) Scheme of a trophozoite highlighting the main cell structures. (Teixeira D, Crepaldi P and Benchimol M, unpublished) (b) Overview of *Giardia* as seen in a thin section by transmission electron 17 microscopy (TEM). AF, Anterior flagellum; N, nucleus; A, axonemes; PV, peripheral vesicles; ER, endoplasmic reticulum; LF, Lateral flagellum. (Midlej and Benchimol, unpublished).

**Cyst Morphology**

The *Giardia* cysts are oval shaped with size of 6–10 μm and 2 to 4 nuclei. In cysts, the ventral disc is fragmented, and there has been no evidence for the funis and median bodies thus far [29] (Figures 3, Figure 5e–f). The flagella are internalized and mainly located between the parasite's plasma membrane and the cyst wall in a region called the peritrophic space (Figure 3, Figure 4).

**Figure 3:** Ultrastructure of *G. intestinalis* cysts. (a) Scheme of cyst morphology highlighting the main cell structures. (Teixeira D, Crepaldi P, and Benchimol M, unpublished) (b) General view of cyst seen with transmission electron microscopy. CW, cyst wall; N, nucleus; VD, ventral disc fragments; A, axonemes; PS, peritrophic space; F, flagella. (Midlej and Benchimol, unpublished).

The cyst wall is composed of two layers: (1) a filamentous layer with fibers of different size (7–15-nm diameter) and (2) a membranous layer composed of inner and outer cystic wall membranes [30], which are separated from trophozoite membranes by the peritrophic space (Figures 3, 5e–f). It is assumed that the transit of large molecules
could be prevented because of the cyst wall’s filamentous network, while small molecules are selected or controlled by the cystic membranes [31]. However, the selective permeability of the cyst wall is not known yet for several components, such as water, ions and small molecules, including cysticides.

**Figure 4:** G. intestinalis cyst as seen with dual beam microscopy followed by 3D reconstruction. Trophozoites were induced to encyst for 48h, and cysts were isolated. (a) A whole reconstructed cyst. (b-d) Flagella (red) inside the cyst in both peritrophic space and in the cytoplasm. (e) Ventral disc is fragmented (dark blue) and found near nuclei (brown). Cyst wall (yellow), plasma membrane (light blue) (Midlej, de Souza and Benchimol, unpublished).

**Figure 5:** Immunofluorescence of G. intestinalis using antibody against cyst wall (anti-CWP1). Figures a–d show trophozoites, while e–f show cysts. (a) Phase contrast and (c, e) highmagnification by differential interferential contrast (DIC). (b, d) Parasites in encystation process. ESVs are labeled with the antibody anti-CWP1. (f) mature cyst. Note the intense labeling in the cyst wall. ESV, excystation specific vesicle; PC, cyst wall. (Midlej and Benchimol, unpublished).
Biochemical analysis showed that the filamentous layer of the cyst wall is composed of proteins and carbohydrates, comprising 57% proteins and 43% sugar moieties. The main protein components are from the cyst wall protein (CWP) family, which include CWP1, 2 and 3 [27] (Figure 5). In addition, the presence of two new cysteine-rich proteins in the cyst wall has been demonstrated: HC-NCp [32] and cystic proteins similar to EGF and EGFCPs [33]. About 86% of the carbohydrate portion of the cyst wall is composed of a polymer of β-1, 3-N-acetyl-D-galactosamine (GalNAc), which has never been identified in other organisms [34].

**Giardia**’s Unique Structures and Organelles

**Ventral Disc**

Two-thirds of the parasite’s ventral face is occupied by the ventral disc (Figure 2a, Figure 6, Figure 7a, Figure 8a, Figure 9a). This structure is 8–10 μm, concave shaped, and located in the anterior part of the cell (Figures 2a, 6a). The disc is composed of both microtubules and micro-ribbons arranged in a spiral. There are no microtubules in its central area, which is called the bare zone (Figure 6). The micro-ribbons are protein bridges that link the disc microtubules [35]. The micro-ribbons are made of giardins, which are specific proteins found only in the ventral disc and are 29 to 38 kDa [36]. The ventral disc has an important role in *G. intestinalis* karyokinesis [37].
Figure 7: Cytoskeleton of *G. intestinalis* by high-resolution scanning electron microscopy after partial removal of the plasma membrane by detergent treatment. (a) The funis microtubules (Fn) are anchored to the posterior-lateral flagella (P). (Adapted from Benchimol et al., 2004) (b) Median body (MB). (Adapted from Piva and Benchimol, 2004) (c) Cytoplasmic filaments contacting the median body (MB) as seen with Helium Ion Microscope. A ring array is noted (arrowhead). (d) The lateral microtubules of funis are interconnected by small bridges (arrowheads). VD, ventral disc; V, ventral flagella; P, postero-lateral flagella; Fn, funis; N, nucleus; C, caudal flagella. (Figures c-d Adapted from from Gadelha et al., 2015).

Figure 8: Transmission electron microscopy of (a-b) vegetative *G. intestinalis* and (c) encysted cell with ESVs identified by its size and electrondensity. Peripheral vesicles (arrows) present positive labeling for acid phosphatase cytochemistry (Figure b). N; nucleus, ER, Endoplasmic reticulum; ESV, encystation specific vesicles; F, flagella. (Middlej and Benchimol, unpublished).
Figure 9: Mitosomes (M) in trophozoites of *G. intestinalis* seen by TEM. They present two membranes and located (b) centrally and (a, c) peripherally. Arrows, peripheral vesicles; N, nucleus; ER, endoplasmic reticulum; F, axonemes of flagella; VD, ventral disc. (Benchimol, M. unpublished).

**Funis**

The funis structure was described in 1973 by Holberton and is composed of two sets of microtubules located in the ventral region of the parasite next to the caudal flagella (Figures 6b–7). Some authors claim that it has a potential structural function [38] and supports the caudal flagella movement because of its localization and linkage with the lateral flagella [39]. However, there is still no clear evidence of its function.

**Median Body**

The median body is a structure that is composed of a set of irregular disposed microtubules located in the central region of the parasite. The function of this structure has not been clarified yet, but there are some proposals. For example, the median body could act as a microtubule reservoir for the generation of a new ventral disc during parasite mitosis [40] and/or function as a microtubule organizer center (MTOC) [41]. The size of the median bodies varies from 0.2 to 1.8 μm in thickness and 0.8 μm to 8.0 μm in length, and it can occupy about 60% of the cell width and and 8.4% of its length [42] (Figure 7a).

**Peripheral Vesicles**

An endocytic system is uncommon in *G. intestinalis*. *Giardia* does not present specific sites for particle entry like a cystostome. Instead, the parasite exhibits an intriguing and unique organelle system composed of numerous vacuoles right below the plasma membrane called peripheral vesicles [43]. These vesicles are elongate and present an oval shape with size of 100–200 nm [44] (Figures 8, 9a). Peripheral vesicles participate in the endocytosis process, digestion, and retrograde transport in *Giardia* [45].
Cytochemical studies revealed the presence of several enzyme types found in the peripheral vesicles, such as acid phosphatases, disulfide bound proteins, and glucose-6-phosphatase. Moreover, it was shown that these vesicles present similar functions to early and late endosomes [45]. The peripheral vesicles have an essential role during differentiation of cysts into trophozoites since there is a dephosphorylation of CWPs by acid phosphatases of these vesicles, which allows cell exit during the encystation process [46].

**Mitosomes**

Tovar et al. (2003) described the mitosomes in *Giardia*, which is a relatively new finding in the cell biology of the parasite [47]. Little is known about specific functions of this organelle. The name mitosome (synonym: “crypton”) was proposed to indicate that this organelle is a highly reduced mitochondrion [48]. Nowadays, there is agreement that mitosomes are mitochondria-related organelles, as are the hydrogenosomes in *Trichomonas* [49]. The identification of mitosomes in *Giardia* was supported by the characterization of protein machinery compounds responsible for gathering the iron-sulfur complex (IscS and IscU proteins) [50]. In addition to those proteins, chaperones such as Cpn60 and HSP70 were also described in this organelle [51].

Mitosomes are small, 200-nm organelles that are located between the axonemes of *Giardia* and distributed throughout the cytoplasm (Figure 9). This organelle is delimited by a double membrane [47] and presents several proteins involved in the transport machinery, such as TOM and TIM family proteins [52]. Several mitochondrial characteristics and functions have not been found in mitosomes, such as ATP synthesis, the citric acid cycle, oxidative phosphorylation, heme biosynthesis, DNA, lipid metabolism and the amino acid and urea cycles [49]. The biosynthesis of Fe-S clusters is a unique mitochondrial function that appears to be retained in mitosomes [53].

**Encystation Specific Vesicles**

When encystation starts, large vesicles known as encystation specific vesicles (ESVs) appear before the cyst wall forms [54] (Figure 5, Figure 8c, Figure 10, Figure 11). The CWPs are synthetized in the endoplasmic reticulum. The ESVs bud off from their locations in the initial period of encystation and carry the CWPs [55]. The mechanism of ESV budding is not well known yet, but there are two hypotheses: (1) a lateral segregation could occur, followed by the concentration of cyst wall material in specialized subcompartments in the endoplasmic reticulum [56]; and/or (2) the transport of CWPs to ESVs could occur by COPII vesicles in a homotypic fusion [57].

The process of ESV maturation is less controversial. About 15–24 h after encystation, membrane peripheral proteins are recruited to ESVs, such as Bip- HSP70, Sec61 and COPII [58-59]. Thus, the ESVs and their contents start to mature and the CWPs are post-translationally modified. Evidence of the post-translational modifications in ESVs include the presence of disulfide isomerases (PDI2) in the
ESVs [60], the cleavage of C terminal CWP2 by a specific encystment protease [61] and the phosphorylation of the newly synthetized CWPs [46].

![Figure 10: Super Resolution – Structured illumination microscopy of encysted G. intestinalis. ESVs (red) and ECVs (green) are observed.
(a) The ECVs are preferentially located near ESVs and nuclei. (b,c) High-magnification of Figure a. Note the discontinuous labeling of ESVs (Figure b). A close proximity of ESVs and ECVs is seen in Figure c (Midléj V and Benchimol M, unpublished).](image)

There are different points of views concerning the ESVs. One is that they are Golgi-like [58]. This hypothesis is supported because (1) ESVs are associated with COPI and COPII, which are part of a protein family that participates in Golgi traffic [57]; (2) the ESVs are affected by brefeldin A, a drug known to inhibit anterograde movement of Golgi cisterns [57]; and (3) ESV biogenesis and maturation depend of GTPases Sar1 and Arf1, respectively [62]. However, the ESVs present some characteristics that are not related to a classic Golgi complex. The ESVs appear only during the *Giardia* encystation process, there are no glycosyltransferases in *G. intestinalis* and the ESVs do not present morphological features that define it as a Golgi complex according parameters used for other eukaryotes. Thus, it is difficult to confirm that the ESVs are Golgi-like organelles.

**Encystation Carbohydrate-Containing Vesi-
cles**

For a long time, there has been discussion about how the carbohydrates moieties in the cyst wall are synthetized and delivered to the *Giardia* surface, which is a missing piece of the *Giardia* encystation puzzle. The lack of a specific *Giardia* GalNAc marker has hindered understanding of the cyst-wall sugar-formation mechanism [58]. However, our group used a lectin, *Dolichos biflorus* agglutinin (DBA), which specifically recognizes GalNAc glycopolymers and made it possible to detect a new set of vesicles called encystation carbohydrate-containing vesicles (ECVs) [63]. Although ECVs are distinct from ESVs, they originate from the endoplasmic reticulum by budding off during early stages of the encystation process [63]. The ECVs are small membrane-bound organelles that are 0.2 to 2 μm in size. When observed by transmission electron microscopy, they are electron lucent and do not present CWPs, just sugar moieties [63] (Figure 10 and Figure 11).
Final Remarks

There are still several open questions concerning *Giardia* biology, such as (1) how the ESV and ECV contents are secreted and how the cyst wall is formed; (2) what the real functions of the median bodies and funis are and what happens with these structures during the differentiation process; (3) what the role of mitosomes is in *Giardia* cell biology; and (4) whether or not there is a Golgi complex in *G. intestinalis*. These are a few examples of how far researchers are in solving enigmatic questions in this notable protozoan parasite.

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