ARTIGO DE REVISÃO / *REVIEW ARTICLE*

Giardia lamblia: Interações parasita-hospedeiro

Giardia lamblia: *Host-parasite interactions*

/ C. P. Faria[1,2,3] / G. M. Zanini[3]
/ M. C. R. Sousa[1,2]
[1]CNC - Center for Neurosciences and Cell Biology, University of Coimbra, Portugal;
[2]Faculty of Pharmacy, University of Coimbra, Portugal;
[3]Laboratory of Parasitology, Evandro Chagas National Institute of Infectious Diseases, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.

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Correspondência:
Maria do Céu Rodrigues de Sousa
Faculdade de Farmácia da Universidade de Coimbra (FFUC), Pólo das Ciências da Saúde, Azinhaga de Santa Comba
3000-548 Coimbra, Portugal
Telefone: 351-239488400
Email: mcsousa@ci.uc.pt

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/ Resumo

Giardia lamblia (sinónimo: *G. intestinalis* e *G. duodenalis*) é considerado um dos principais agentes causadores da diarreia e é reconhecido como importante agente patogénico de surtos transmitidos pela água, que infecta animais e seres humanos em todo o mundo. Atualmente tem sido relatado um número crescente de casos de resistência a fármacos. Identificar os mecanismos de evasão, compreender a genética e as interações hospedeiro-parasita podem ajudar a esclarecer a epidemiologia deste parasita e certamente permitirá o desenvolvimento de tratamentos alternativos e a identificação de novos alvos terapêuticos.

Palavras-chave: *Giardia lamblia*; giardíase; mecanismos de evasão; patogenia

/ Abstract

Giardia lamblia (syn. G. intestinalis and G. duodenalis) *is considered one of the leading causative agents of diarrhea and is recognized as important waterborne disease pathogen that infects animals and humans worldwide. Currently, an increasing number of cases of resistance to drugs have been reported. Identify the evasion mechanisms, understand the genetics and the host-parasite interactions may help unravel the epidemiology of this parasite and certainly will allow the development of alternative treatments and the identification of new therapeutic targets.*

Key-words: *Giardia lamblia; giardiasis; evasion mechanisms; pathogenesis*

/ Introduction

Giardia lamblia (also called *G. intestinalis* and *G. duodenalis*) is the causative agent of giardiasis. This parasite has a global distribution causing an estimated 2,8x10[8] cases per annum[1], and is a leading cause of diarrhoeal disease worldwide[2]. In Asia, Africa and Latin America, about 200 million people have symptomatic giardiasis with some 500.000 new cases reported each year[3]. In developing countries, the prevalence of human giardiasis is approximately 20-30% compared with 2-5% in developed countries, where it is associated mainly with traveling and waterborne outbreaks[4,5]. Giardiasis was included as part of the WHO's Neglected Disease Initiative since 2004 because of its significant public health and socioeconomic implications[6].

In developing countries giardiasis infects in early childhood, with a prevalence of 15 to 20% in children under ten years. Children between six months and five years old and/or malnourished are more susceptible. Chronic infection can result in serious consequences on the nutritional status, physical and mental development presumably due to malabsorption of nutrients[7–9].

Clinical manifestations range from asymptomatic infections to acute or chronic diarrhoea, however the majority of cases of giardiasis have no symptoms making difficult to eradicate and control this parasite. Few virulence factors of *G. lamblia* have been identified and several mechanisms have been proposed to be important for the induction of symptoms during giardiasis[10].

Currently, an increasing number of cases of resistance to drugs have been reported. Thus, search for alternative treatment, as well as new therapeutic targets are required. In this context, the understanding of the pathogenesis and the evasion mechanisms of this parasite is of great importance.

/ Symptomatology

Symptomatology of *G. lamblia* infection is highly variable between individuals and can range from asymptomatic (60-80% of the cases), mild and self-limited, to severe infections with acute or chronic diarrhoea. Diarrhoea is the major symptom of giardiasis, and can occur with or without the intestinal malabsorption syndrome. Clinical signs of infection may include vomiting, dehydration, abdominal pain, flatulence usually accompanied by nausea and weight loss. There is no appearance of blood in the stool since *Giardia* is a non-invasive parasite and few virulence factors have been identified[11,12]. However, infected individuals may also develop extra-intestinal and post-infectious gastrointestinal complications. Ocular complications, arthritis, allergies, myopathy, chronic fatigue and irritable bowel syndrome (IBS) can follow an episode of giardiasis, and the mechanisms remain unknown[13,14].

The clinical manifestations are self-limiting in most of the cases, with transient intestinal complications that are usually solved completely, but because of the potential for chronic or intermittent symptoms, treatment is recommended[8,11]. Asymptomatic individuals are important reservoirs for spread of the parasite.

The giardiasis incubation period is one to three weeks after the patient ingests the cyst, and symptoms usually occur six to fifteen days after infection[11,15]. Children are more vulnerable to giardiasis than adults, and may have more serious consequences. *Giardia* infection has an adverse impact on child growth and psychomotor development, and associated with diarrhoea and malabsorption syndrome can cause iron-deficiency anaemia, micronutrient deficiencies and growth retardation[16]. In Brazil, the child population has been the group most affected by the high incidence of parasitic infections[7,17,18].

It still unclear why some individuals develop clinical manifestations while others remain asymptomatic, but there is no single and simple explanation for this broad spectrum of symptoms observed in infections caused by *G. lamblia*. Host and parasite factors may be responsible for the severity of the infection. Host factors such as age, immune and nutritional status, as well as previous infection with other *Giardia* strain and/or concurrent enteric infections with other intestinal pathogens. Parasite factors probably associated to the assemblage, virulence, pathogenicity, number of ingested cysts, replication rate, presence of variant surface proteins (VSPs) and the ability to evade the immune system[12,19].

/ Pathogenesis

Giardia infections cause intestinal barrier dysfunction via a variety of mechanisms, including increased rates of intestinal epithelial apoptosis and disruption of apical junctional complexes. The pathogenesis may be linked to factors such as: (*i*) the large amount of trophozoites attached to the intestinal epithelium being a physical barrier to absorption of nutrients[20]; (*ii*) shortening of brush border microvilli with or without coinciding villous atrophy[21,22]; (*iii*) disaccharidase impairment[21]; (*iv*) dysfunction at intestinal barrier[23,24]; (*v*) activation of the host CD8+ lymphocytes[25]; (*vi*) increased anion secretion[24] and (*vii*) increased gastrointestinal transit rates[26]. The association of all these factors triggers a series of events leading to the production of diarrhoea.

The parasite establishes a complex and dynamic interaction with host enterocytes. Cellular F actin, tight junctional Zonula–Occludens (ZO)-1, alpha-actinin and claudin protein (all of them are critical components of the sealing properties of tight junctions) disruption is modulated by a pro-apoptotic caspase 3 and a myosin-light-chain kinase (MLCK)[23,27,28]. *Giardia* products activate MLCK, which phosphorylates myosin light chain (MLC) and disrupts cytoskeletal and apical tight junctional elements in enterocytes, and subsequently increase permeability across the epithelial monolayers[28]. Therefore, the intestinal barrier disruption may indeed facilitate the translocation of luminal antigens into

underlying host tissue. In addition, increased enterocyte apoptosis in a caspase-3 and -9 dependent manner also modulate the permeability of the intestinal barrier[23,28].

The tight attachment between *G. lamblia* trophozoites and intestinal epithelial cells through its adhesive disk reduce the small intestinal absorptive surface area and induces a diffuse shortening of the epithelial microvilli, causing disaccharidase deficiencies and malabsorption of nutrients (fat-soluble vitamins, fatty acids, B12 vitamin and folic acid), water, and electrolytes[22,25,29]. The increased quantity of these nutrients in the lumen of the intestine can determine steatorrhea. Moreover, the loss of intestinal brush border surface area, disaccharidase impairment, and increased crypt/villus ratios in giardiasis are mediated by activated of CD8+ T lymphocytes of the host via parasite secretory/excretory products[25].

The malabsorption of nutrients and electrolytes, due parasite attachment or shortening of the brush border microvilli, creates an osmotic gradient that leads the water into the intestinal lumen resulting small intestine distension and increased peristalsis[24,26,30]. It is also possible that the diarrhoea disease in infected individuals may be a result from increased intestinal transit rate due to massive mast cell degranulation and adaptive immune responses of the host[30]. Finally, the impairment in the transport of electrolytes caused by *Giardia* leads to increased chloride hypersecretion that, with malabsorption of glucose, sodium and water may be responsible for the luminal fluid accumulation during infection[24,26].

As aforementioned, the pathophysiological mechanisms of *Giardia* infection are clearly multi-factorial, and involve host and parasite factors, as well as immunological and non-immunological mucosal processes.

Every three to five days host intestinal cells are constantly being renewed, in that way *G. lamblia* must move and re-attach frequently to avoid the transport beyond the jejunum by the peristalsis. Likewise, the mucus layer prevents the parasite from obtaining immediate access to the epithelium. Trapping of *Giardia* by mucus and their subsequent removal from the intestine by peristalsis is a good strategy by host to reduce the quantity of parasites[31]. The presence of the normal small-intestinal microbiota may be involved in the inhibition of *Giardia* growth[32]. Intestinal Paneth cell-derived, defensins, lactoferrin, proteases, lipases and bile salts constitute an efficient defence mechanism in the upper small intestine and possess anti-giardial activity *in vitro*[31,33]. In addition, immune cells produce nitric oxide (NO) that has both cytotoxic and immunomodulatory activity during intestinal infections[31,34].

The host immune response plays a key role on the clinical form or asymptomatic infection, once the most severe cases affect children and immunosuppressed. Both B cell-mediated antibody production and T cell-mediated immune responses are required to control *Giardia* infections[35]. Mast and dendritic cells play a significant role as effector cells in the immune response against *Giardia,* since IL-6 production is important for control of this infection[36,37]. This pro-inflammatory cytokine modulates B-cell maturation and switching to produce IgA, and it also mediates T-cell differentiation[33]. The main elements of the immune response include: IgA immunoglobulins that interfere with parasite adhesion to the mucosa, immunoglobulin M (IgM), and immunoglobulin G (IgG) performing cellular immune response; macrophage activity, involved in phagocytosis and antigen presentation; the activity of monocytes and neutrophils[11,38].

A higher incidence of giardiasis has been observed in patients with hypogammaglobulinemia and impaired secretion of IgA. The parasite causes a greater damage on the microvilli of these patients than in immunocompetent patients[20].

/ Evasion mechanism

Despite the wide spectrum of clinical symptoms, *Giardia* infection in human is typically characterized by little or no mucosal inflammation[39]. Microarrays analysis demonstrated that epithelial cells, when exposed to parasites *in vitro*, produce cytokines that are chemotactic for immune cells being therefore expected an increase in inflammatory status[40]. The authors suggested that *Giardia* parasites may actively subvert/limit the inflammatory response in small intestine allowing its effective colonization (Table I).

Regarding direct interaction with immune cells, *Giardia* parasites inhibit the production of pro-inflammatory IL-12 in dendritic cells, resulting in an immune response able to control the infection but devoid of strong inflammatory signals[41]. Recently, a study demonstrated that *G. lamblia* trophozoites cathepsin B (catB) cysteine proteases degraded chemoattractant interleukin-8 (CXCL8) induced by pro-inflammatory interleukin-1β, or by *Salmonella enterica* serovar *Typhimurium*, and attenuated CXCL8-induced neutrophil/polymorphonuclear leukocyte (PMN) chemotaxis[42]. In the same year, it was reported that *G. lamblia* infection decreased granulocyte tissue infiltration and cytokines and chemokines involved in PMN recruitment after intra-rectal instillation of *Clostridium difficile* toxin A and B in an isolate-dependent manner[43]. Both studies demonstrate that *Giardia* infections may attenuate PMN accumulation by decreasing the expression of the mediators responsible for their recruitment.

Our group observed that *G. lamblia* does not trigger macrophages activation via canonical pro-inflammatory signaling cascades such as mitogen-activated protein kinases (MAPKs) and transcription nuclear factor-κB (NF-κB), being this reflected by minimal induction of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) and of cytokines/chemokines such as IL-1β, IL-6, tumor necrosis factor alpha (TNF-α) and chemokine (C-C motif) ligand 4 (CCL4)[44].

TABLE I - *GIARDIA* SPP. EVASION MECHANISMS

FUNCTIONS	MECHANISMS
Inhibition of host immune system	• Secrete cysteine proteases that degrades CXCL8* thereby attenuating the accumulation of PMN* • Cleavage of $p65^{RelA}$ NF-κB subunit that attenuates gene expression such as iNOS* and COX-2*
Protection from nitroxidative stress through inhibiting NO production	• Consume arginine as energy source • Secrete ADI* and OCT* enzymes that metabolize arginine to produce ATP*
Protection from oxidative stress	• Produce flavoHb that metabolizes NO to nitrate • Secrete flavodiiron and NADH oxidase that are able to convert O_2 to H_2O • Secrete SOR*, which reduces $O_2^{-\cdot}$ to H_2O_2 • Produce Prxs* that degrade H_2O_2, $ONOO^-$ and alkyl-hydroperoxide compounds
Protection against IgA, proteases, oxygen and free radicals	• Antigenic variation (switching of the expression of genes encoding VSPs*)
Parasitic transmission and survival to internal (digestive system) and external environment	• Cyst formation
Subversion/limitation of inflammatory response	• Unknown trophozoites products

*CXCL8, chemoattractant interleukin-8; PMN, neutrophil/polymorphonuclear leukocyte; iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase-2; ADI, arginine deiminase; OCT, ornithine carbamoyltransferase; ATP, adenosine triphosphate; SOR, superoxide reductase; Prxs, peroxiredoxins; VSP, variant surface proteins

For instance, *Giardia* trophozoites were shown to evade host immune responses by inhibiting NO production in human intestinal epithelium cells (IEC)[45]. The L-arginine is the primary substrate for the production of NO by the enzyme nitric oxide synthase (NOS), however *G. lamblia* trophozoites are able to degrade the amino acid as an energy source. The parasite rapidly reduces the amount of arginine in the growth medium decreasing the proliferation of IECs[46]. NO is an immunoregulatory substance of the immune system, inhibiting both growth and encystation of *G. lamblia* trophozoites as well as excystation of cysts *in vitro*, and is responsible for the cytotoxicity of the macrophages[45].

It is well documented that *G. lamblia* secretes enzymes of the arginine metabolic pathway (arginine deiminase [ADI] and ornithine carbamoyltransferase [OCT]), upon interaction with intestinal epithelial cells[47]. These two enzymes are able to metabolize arginine to produce adenosine triphosphate (ATP) thereafter decreasing the amount of arginine. Moreover, it was proposed that *G. lamblia* trophozoites produce a flavohemoglobin protein (flavoHb) capable of degrading NO[48] and attenuating T-cell proliferation[49]. This protein has a well-known NO reductase activity and degrades NO to nitrate, protecting the parasite from nitrosative stress[48].

In order to survive oxidative stress *Giardia* possesses an arsenal of enzymes such as flavodiiron[48] and NADH oxidase[50] that are able to convert O_2 to H_2O[51]. Superoxide reductase (SOR) reduces $O_2^{-\cdot}$ to H_2O_2[52], and subsequently peroxiredoxins (Prxs) degrade H_2O_2, $ONOO^-$ and alkyl-hydroperoxide compounds[51].

Another important mechanism for evasion of the host immune response is the antigenic variation, a clonal phenotypic variation which the parasite expressed successively alternative forms of their surface antigens without genotype changes. Antigenic variation in *G. lamblia* involves variant surface proteins (VSPs) that are cysteine-rich integral membrane proteins. These proteins cover the entire surface of the parasite and conferred protection against IgA and proteases produced by the host[10]. Furthermore, the cyst is the infective stage of *Giardia*, being responsible for transmitting the parasite. The cyst formation, also called encystment, is also an essential mechanism for *Giardia* adapts to both environment, external and internal (digestive system)[53]. Thus undergoing a significant change during its life cycle in order to survive.

/ *Giardia lamblia* genetic diversity and symptoms

G. lamblia is considered a species complex, whose members show little variation in their morphology, but have a remarkable genetic

TABLE II - RESULTS OF SEVERAL WORLDWIDE STUDIES IN AN ATTEMPT TO ASSOCIATE ASSEMBLAGES WITH SYMPTOMATOLOGY

COUNTRY	STUDY POPULATION	RESULTS	REFERENCES
India	12 young adults with and without symptoms	Positive association between assemblage A and diarrhoea	Paintlia et al. 1998
Netherlands	18 participants ranging in age from 8 to 60 years with symptoms	Assemblage A isolates were detected in patients with intermittent diarrhoeal complaints, while assemblage B isolates were present in patients with persistent diarrhoeal complaints	Homan and Mank 2001
Australia	353 children under 5 years, with and without symptoms	Assemblage A isolates were more likely to have diarrhoea	Read et al. 2002
Turkey	56 symptomatic and asymptomatic individuals	Individuals with assemblage A was associated with diarrheal symptoms, whereas assemblage B was seen in asymptomatic infections	Aydin et al. 2004
Bangladesh	2534 symptomatic patients of all ages	Patients with assemblage A were more likely to have diarrhoea, and assemblage B patients was associated with asymptomatic *Giardia* infection	Haque et al. 2005
Ethiopia	80 participants of all ages, with and without symptoms	Symptomatic infection was more associated with assemblage	Gelanew et al. 2007
Cuba	95 primary-school children with and without symptoms	Patients with assemblage B were more prevalent among symptomatic children	Pelayo et al. 2008
Peru	845 children between 1 month and 9 years old, with and without symptoms	Association between assemblage A and diarrhoea	Pérez-Cordón et al. 2008
Argentina	60 participants with ages ranging from 1 to 33 years	Assemblage B were associated with symptomatic people	Minvielle et al. 2008
Spain	108 patients with ages ranging from 2 to 72 years, with and without symptoms	Assemblage A were associated with symptomatic infections, and assemblage B with asymptomatic	Sahagún et al. 2008
India	452 children up to 3 years old, with and without symptoms	Individuals with assemblage A had diarrhoea more frequently	Ajjampur et al. 2009
Saudi Arabia	1500 school children with and without symptoms	Assemblage B had a strong correlation with the symptoms	Al-Mohammed 2009
Malaysia	321 symptomatic and asymptomatic individuals with ages ranging from 2 to 72 years	Assemblage B was more present in symptomatic giardiasis	Mohammed Mahdy et al. 2009
Nepal	1096 patients greater than 12 years of age with diarrhea or other gastrointestinal symptoms	Most infections were classified as assemblage B	Singh et al. 2009
UK	819 children of all ages, most of the cases had symptoms (<2% asymptomatic)	Assemblage A was more frequently associated with fever than assemblage B	Breathnach et al. 2010
Argentina	244 school children aged 3 to 11 years old	Assemblage B was associated with diarrhea, vomiting and weakness	Molina et al. 2011
Sweden	214 patients of all ages with and without symptoms	Flatulence was more common in children aged 0–5 years infected with assemblage B	Lebbad et al. 2011
Cuba	452 symptomatic and asymptomatic children	Association between infection by assemblage B and the presence of diarrhoea or flatulence or abdominal pain	Puebla et al. 2014
Ethiopia	92 patients with gastrointestinal symptoms with ages ranging from 0.5 to 80 years	Assemblage B was more associated with symptomatic infection	Flecha et al. 2015
UK	406 patients of all ages with symptoms	Vomiting was reported more frequently by the cases infected with assemblage B, who also reported a longer duration of illness	Minetti et al. 2015

variability[12,19]. This species is divided into eight distinct genetic assemblages (A-H), but only assemblages A and B are known to infect humans. The remaining assemblages are likely to be host specific, as assemblages C and D predominantly occur in dogs and other canids, assemblage E in hoofed livestock, assemblage F in cats, assemblage G in rats and assemblage H in marine mammals[54,55].

G. lamblia presents a heterogeneous clinical manifestations and one hypothesis is that the parasite assemblages could play a part in the development of symptoms. Assemblages A and B have been considered genetic variants of the same species. However, the latest studies suggest that the genomic differences between assemblages A and B are sufficient to classify them into two different species[56,57]. Some authors believe that the genomic differences between strains WB (assemblage A) and GS (assemblage B) may explain some of the phenotypic differences[58,59].

Studies trying to associate *G. lamblia* assemblages with symptoms had been done all over the world[60–62]. Several studies have shown a strong correlation between assemblage A and symptomatic infection, and between assemblage B and asymptomatic infection[60,63–66] (Table II). In contrast, other studies have reported an association between assemblage B and symptomatic infections[62,67–69]. No correlation was observed between assemblages and symptoms in the studies conducted in Brazil[70], Cuba[71] and Nicaragua[72]. But despite the effort, there is still a lack of concordance on this issue and many questions remain to be answered.

Recently, it was demonstrated that *G. lamblia* assemblage A trophozoites attenuate secretion of the CXCL8 through a secreted catB cysteine protease, directly attenuating CXCL8-induced PMNs chemotaxis[42]. In contrast, these effects were not observed with *G. lamblia* assemblage B GS/M infections. Moreover, *Giardia* assemblage A decreased granulocyte infiltration and cytokines and chemokines involved in PMN recruitment[43]. Likewise, Ma'ayeh and colleagues[73] observed phenotypic and transcriptional differences between *G. lamblia* assemblage A (WB strain) and assemblage B (GS strain), with the latter being more tolerant to H_2O_2 and exhibiting higher basic transcript levels of antioxidant genes.

It is well documented that HIV infected patients have a weakened immune system due to the depletion of CD4 T cells and this makes them more susceptible to a range of infections. Of the non-opportunistic intestinal parasites, *G. lamblia* is one of those most commonly found in HIV infected patients[84–87]. Some studies trying to associate *G. lamblia* assemblages with clinical manifestation in patients with the HIV virus were done[88–90]. A cross-sectional study of *G. lamblia* infection positively correlated assemblage B with HIV infection[90], and probably the lower CD4 T count is advantageous for assemblage B replication[91].

/ Conclusions

Modulation of the host immune response will benefit the parasite by extending the length of infection and allowing greater time for transmission to a new host. Unlike most intestinal pathogens, *Giardia* induces diarrhoea without necessarily causing significant infiltration of neutrophils or macrophages. As aforementioned, the recent findings indicate that *Giardia* actively modulates host inflammatory responses. Certainly further studies are needed to show whether the different assemblages induce different immune responses and whether these differences are related to differences in symptoms.

Identify the evasion mechanisms, understand the genetics of this parasite and the host-parasite interactions may help unravel the epidemiology of this parasite and certainly will allow the development of alternative treatments and new therapeutic targets.

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