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Mini-Review

Interaction of *Giardia lamblia* (syn. *duodenalis*, *intestinalis*) with epithelial cells

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ABSTRACT

Giardiasis is a widespread intestinal disease that is caused by the flagellate protozoan *Giardia lamblia*. For a successful pathogenicity, the parasites need to attach to the intestinal cells and evade the host's defence mechanisms. Previous studies have shown that adhesion molecules localized on the parasite surface and secretory/excretory products, play a crucial role in establishing parasitism. The intestinal junctional complex and the actin microfilaments, which sustain the junctional backbone and the brush borders, are also important during the interaction of *G. lamblia* with epithelial cells. In order to improve our knowledge in this area, it is important to carry out interaction studies using 3-dimensional epithelial cultures as well as animal models.

KEYWORDS: *Giardia*, interaction, cell junction, parasite, adhesion, ultrastructure

1. INTRODUCTION

Giardia lamblia (*syn*. *duodenalis* or *intestinalis*), the most common gastrointestinal parasite worldwide, is a widespread, non-invasive, eukaryotic microorganism that causes the waterborne disease known as giardiasis. The disease caused by *G. lamblia* is characterized by malabsorption, chronic diarrhoea, dehydration, abdominal discomfort, and weight loss [1], and affects about 300 million people every year [2]. Some infected hosts may also be merely carriers of the disease, as giardiasis may be asymptomatic and undiagnosed. Giardiasis is also widespread, and approximately 2% of adults and 6-8% of children in developed countries are infected by *G. lamblia*. It is estimated that 33% of the population in developed countries has already been infected. In USA, for example, giardiasis is the most common human intestinal parasitic disease (Centers for Disease Control and Prevention, CDC). The development of diarrhoeal symptoms in giardiasis appears to vary according to geography, and the infection might produce less severe disease in hyperendemic regions of the world via mechanisms that remain obscure [3]. Furthermore, it is known that environments such as kindergartens, schools, and other surroundings with large groups of people are ideal for the spread of infection, since the transmission of the parasite is through both oral-fecal and person-person routes. Usually in these places, inadequate hygienic conditions are favorable to propagate the disease.

Giardia lamblia presents a simple life cycle with two developmental stages: trophozoite and cyst. The animal infection starts with the ingestion of cysts present in contaminated water and vegetables. With rupture and or digestion of the cyst wall, a process known as excystation, trophozoites are

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released and the replication process starts. Under certain stimuli, some trophozoites detach from the epithelial cell surface and start a process of transformation into cysts, which are then released with the faeces.

The trophozoite is the proliferative form of the parasite which colonizes the intestine of the respective host. For this reason, trophozoites require structures for mobility. They present four pairs of flagella, which are derived from basal bodies. To attach on the intestinal epithelium, the trophozoites have a ventral and adhesive disk with a suction function. The ventral disk is supported by the cytoskeleton and is arranged in spirals.

As in all cell-to-cell interaction processes, there are at least three distinct phases. First is the attachment of the parasite to the epithelial cell surface, a process that involves cell recognition of surface-exposed components of both interacting cells. Second, cell signalling processes may trigger changes (morphological, biochemical, physiological, etc.) in both interacting cells. Third, functional alteration of the host cell occurs, which may vary from slight or reversible changes to cell death.

This short review will focus on the available information on the process of interaction of trophozoites with epithelial cells *in vitro* and *in vivo*. Some points, such as parasite structures, adhesion molecules, and models for the study of host-parasite interactions will be shortly addressed.

2. Experimental models *in vitro*

The ability to propagate pathogens *in vitro*, to study their biology and interaction with their host, is a prerequisite for understanding infectious diseases. Although there are several available experimental models to study the parasite-host relationship, an appropriate replica that reproduces strict human-host or assemblage specificities remains elusive. This limitation is not restricted to *G. lamblia* interaction studies, but is extended to those of a wide range of parasites.

Most of the present knowledge on this issue comes from experiments carried out *in vitro* using epithelial cell lines. IEC-6, a small intestine rat cultured cell line (ATCC® CRL-1592™), is an example of a common *in vitro* model. However, this cell line does not reproduce the main characteristics of an epithelium, such as polarity. In contrast, cell lines such as Caco-2 (ATCC® HTB-37™) and MDCK (ATCC® CCL-34™) reproduce the polarity of the epithelium well [4], with a clear identification of apical and basolateral regions, as well as the establishment of desmosomes and tight, adherens, and gap junctions. These cell lines have been widely used as an experimental model to study the interaction of pathogenic protozoa with intestinal epithelial cells. The disadvantages of these models are that Caco-2 is a human colorectal adenocarcinoma, and MDCK is a canine kidney lineage. The acclaimed human small intestine epithelial cell line, SCBN, which has been used in several *Giardia*-host interaction studies *in vitro* [5] was recently re-classified as a canine, or canine-like, epithelial cell line of unknown origin [6]. The human Int-407 cell line has also been used to investigate pathogenic enterobacterium and *Giardia* interaction processes [7], though these cells are derived from the jejunum and ileum of a 2 month-old human embryo. As a consequence of the varying cell lineages, there has been a wide divergence of results obtained during these studies. In addition, due to the limitations of the systems utilized, a number of factors were not considered during the experiments.

Recently, the use of induced pluripotent stem cell (iPSC)-derived culture systems, including organoids, has been proposed as a strategy to address many of these experimental bottlenecks [8]. Intestinal organoids lack cells of the immune system, but offer the possibility of studying the early events during infection as well as the direct interaction of the parasites with well-differentiated intestinal epithelial tissue [8, 9, 10].

During studies of parasite-host adhesion, quantifying the adherent trophozoites on the surface of epithelial cells is often necessary. In most cases, it is difficult to perform reproducible quantitative analyses or to identify the parasites attached to epithelial cells by means of conventional staining techniques used for light microscopy observations. We showed that environmental scanning electron microscopy (ESEM), which allows the examination of specimens at normal atmospheric pressure or in a very low vacuum without any previous treatment, can be used effectively in quantitative

studies of the parasite-host relationship (Figure 1) [11].

2.1. Major molecules involved in cell recognition and interaction

Certainly, the structural organization of the trophozoite stage of *G. lamblia* plays a pivotal role on its interaction with the epithelial cells. Indeed, it is well known that the adhesive disc, localized ventrally, enables the parasite to attach to a variety of substrates tested recently [12]. A number of parasitic surface molecules are engaged in this tight interaction, including giardins (specifically alpha, beta, delta, and gamma giardins). It has been shown that alpha giardins, a family of proteins related to annexins, are involved in the process of attachment of the protozoan to the intestinal mucosa [3, 13, 14, 15]. These proteins are also highly expressed by trophozoites. Besides, lectin-mediated attachment has been shown to be involved in the specific recognition of host cells [16, 17]. Also, there is evidence that a glucose-mannose-specific lectin and a mannose-6-phosphate-binding lectin may play a role in attachment [18, 19]. Further, a 200 kDa surface protein seems to be involved in the attachment process, since low expression of this protein significantly reduced the ability of the parasites to colonize the intestine of gerbils [20].

It is important to point out that, following attachment to the epithelium, the protozoan may, directly or indirectly, induce alterations on the epithelium via secretion of proteases [21]. This causes malabsorption and diarrhoea, as will be discussed later on. Transcriptional profiling of *Giardia*-host interactions demonstrated that a total of 200 *Giardia* transcripts were significantly changed. Some genes were up-regulated, including those identified as important during parasite adhesion to intestinal cells, such as arginine deaminase (ADI), ornithine carbamoyl transferase (OCT), enolase and cysteine proteinases [6, 22, 23]. Trophozoites use ADI and OCT to actively metabolize arginine for energy, thus depleting arginine from the growth medium. Arginine is a key metabolite required for the production of epithelial nitric oxide (NO) [22, 24], and its depletion is known to reduce proliferation of intestinal epithelial cells [25] and to induce apoptosis in human cell lines [26]. It is important to mention that human giardiasis patients show an increased rate of apoptosis of intestinal epithelial cells [22, 27].

In addition to antigenic variation and evasion from host humoral immune responses, variant surface proteins (VSPs) are also involved in *Giardia* surface attachment [3, 28]. Some VSP genes have been identified as up-regulated after interaction with intestinal cells, which may also

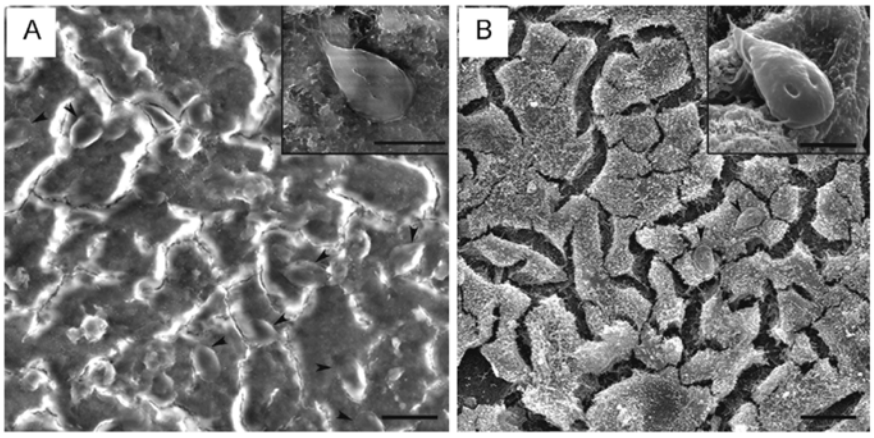

Figure 1. Samples observed using an environmental scanning electron microscopy (ESEM) (A), where a large number of adherent trophozoites are observed (arrowheads). Conventional SEM (B) showed fewer parasites (2 cells), which are indicated by arrowheads. The preparation of samples for SEM resulted in an increased retraction of cellular contacts during dehydration, in addition to the detachment of parasites. Main bars: 20 µm; inset bars: 5 µm. Reprinted from Maia-Brigagão, C. and de Souza, W. 2012, Micron, 43, 494, with permission from Elsevier.

account for the variation in symptoms during giardiasis [23]. *Giardia* ADI also appears to contribute to antigenic variability via its role in modifying VSP proteins [29].

2.2. *Giardia* and junctional complex disturbance

The junctional complex of the intestinal epithelium is responsible for the maintenance of the epithelium organization and the integrity of the cell-cell contacts. This complex consists of three main structures: tight junctions, adherens junctions, and desmosomes. The tight junction is the most apical structure, and is responsible for sealing and regulating the transiting of molecules among the cells through the cell-cell contacts. Adherens junctions, also at the apical complex, are associated with actin microfilaments, while desmosomes are linked to intermediary filaments, which confer greater resistance to the epithelium [30, 31, 32].

Disturbances at the cell-cell contacts may be evaluated through the integrity of tight junctions, since this effect is one of the first evidences of epithelium injury. Some pathogenic organisms, including some viruses, bacteria, and protozoa, have the ability to damage the intestinal barrier function by disrupting the tight junctions. They have developed an extensive and complex array of strategies to alter this region and succeed in establishing parasitism. One of the main consequences resulting from the interaction between parasites and host cells is the reduction of the transepithelial electric resistance (TER), leading to an increase in the paracellular flux.

Studies involving *Giardia* and intestinal cells demonstrated that this parasite is also able to raise the permeability of the epithelium [27, 33, 34, 35, 36, 37, 38], though the results differ when a kidney cell line (MDCK) was used [39]. It is important to point out that some contradictory results found in the literature may result from the use of different experimental systems. The various research groups perform the experiments using different *Giardia* strains (including some that do not infect humans), diverse times of interaction, varied parasite-host cell proportions during the assays, and different mammalian cell lines. Because of the dissemblance of parameters, it is sometimes difficult to achieve a conclusive answer for some questions about *Giardia* interaction. But it is generally a consensus that this parasite increases the intestinal permeability.

Our group proposed that *Giardia* attachment reduces the transepithelial resistance of intestinal monolayers *in vitro* after interaction, but the disturbance is not only restricted to the apical junctional complex. We observed that the parasite also disrupts the monolayers at the adherens junction and at desmosomal levels [34]. We reported a kind of ramification of the ZO-1 protein in Caco-2 cells after interaction of *G. duodenalis*, tending to migrate into the cytoplasm, as shown by 3-dimensional (3D) reconstruction of confocal images (Figure 2). Different patterns of distribution of junctional proteins were still observed in other works, where immunostaining for ZO-1 in epithelial monolayers exposed to *Giardia* (trophozoites or sonicates) exhibited focal disruptions of the protein, in addition to punctuated distribution and cytoplasmic accumulation [33, 35, 37, 40, 41].

In contrast to other groups, we did not observe alterations in expression of any protein analyzed during the experiments (claudin-1, ZO-1, E-cadherin, β-catenin and desmocollin-2/3), although their intracellular distributions were modified. This argument is supported by a study reporting changes in expression of a large range of epithelial genes during *Giardia* infection [42]. In this work, the authors did not find any relevant differences in the levels of expression of junctional proteins in intestinal cells after interaction with the parasite. Yet, reduction of claudin-1 expression during chronic giardiasis has been reported [27].

The perijunctional actin belt is also a critical factor for apical cellular junction formation [43, 44, 45] for the proper regulation of paracellular permeability [46, 47]. Some studies showed that *Giardia* adherence on the surface of the epithelium may induce the disarrangement of the actin microfilaments, which may be related to disorganization of the microvilli [27, 34, 35, 37, 38, 40]. The tight and/or adherens junctional proteins affected during parasite interaction, as well as the *Giardia*-mediated increment of intestinal permeability, may result from the disorder of actin filaments.

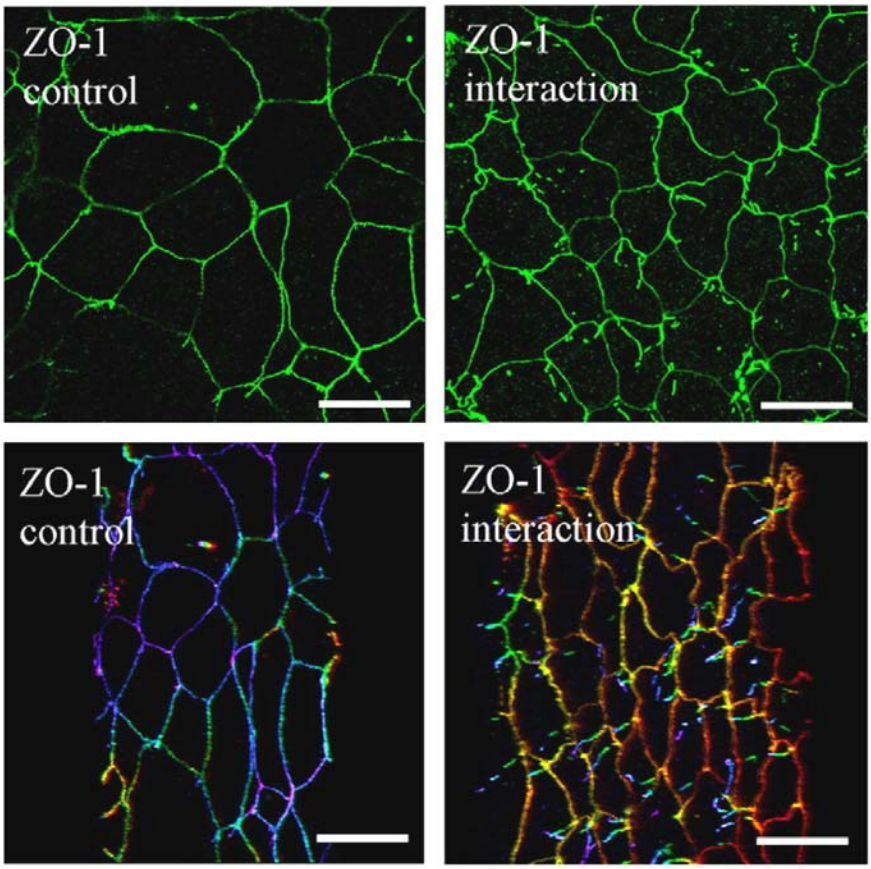

Figure 2. The distribution of tight junction protein ZO-1 was affected after 24 h of interaction with the parasites, changing to a branch-like pattern. This may be seen more clearly in the colour coded reconstructions of total slices of ZO-1 labeling, and with a rotation angle of 7°, which corresponds to control and interaction images, respectively. Bars: 10 μm. Reprinted from Maia-Brigagão, C., Morgado-Díaz, J. A. and de Souza, W. 2012, Parasitol. Int., 61, 280, with permission from Elsevier.

2.3. Ultrastructure and other aspects of the *Giardia* and the host cells during interaction

The protozoan touches the epithelial cell surface through any portion of its cell body, but there is a preference to use the region where the adhesive disk is located to establish the permanent contact with the host cells. Both scanning and transmission electron microscopies observations have shown that microvilli retract and even disappear from the parasite adhesion zone (Figure 3). As long as *Giardia* disturbs the cellular junctions, it is also possible to observe the permeation of the ruthenium red dye into the host cell-cell contacts (Figure 2).

In the gastrointestinal tract, the induction of enterocyte apoptosis is a highly regulated process that contributes to the homeostatic turnover of epithelial cells [48]. Genes associated with apoptosis are up-regulated in cells exposed to *Giardia* products [42], and it is known that dysregulated apoptosis may also modulate intestinal epithelial barrier permeability. This event increases rates of epithelial apoptosis just after exposure to trophozoites, both *in vitro* and in patients with chronic giardiasis [3, 27, 36, 49, 50]. Adhesion of *G. lamblia* to the brush border of enterocyte-like cells involves the lipid raft membrane microdomains of the trophozoite, leading to disorganization of the apical F-actin cytoskeleton that, in turn, results in a dramatic loss in the distribution of functional brush border-associated proteins [40].

Research aiming to understand the events of phosphorylation and dephosphorylation during the interaction between *G. lamblia* and epithelial cells

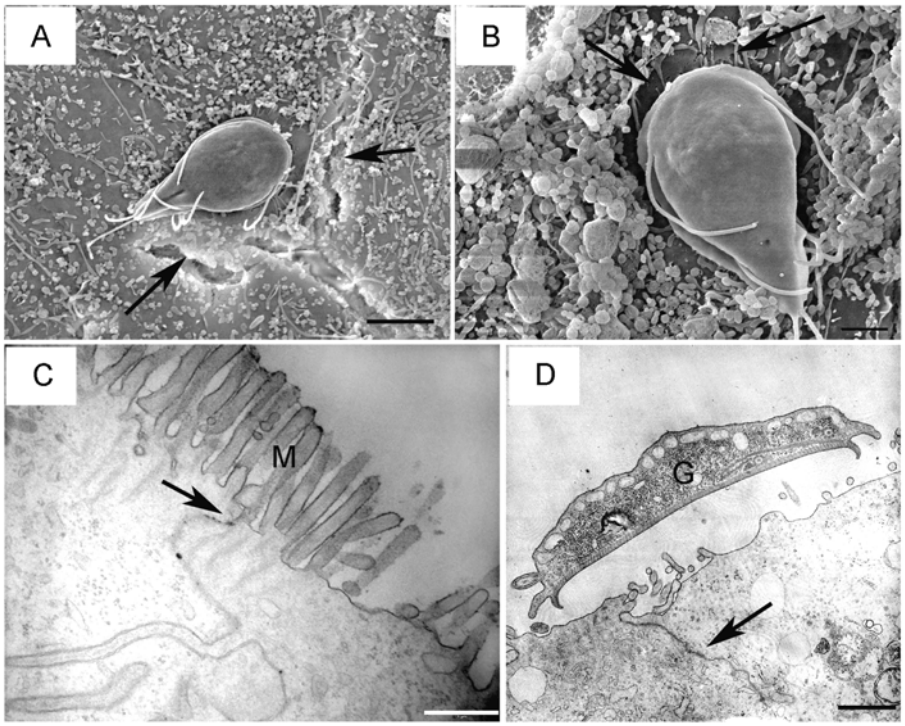

Figure 3. Changes on the surface of Caco-2 monolayers caused by the interaction with *G. lamblia* as seen by scanning electron microscopy. A: Breakdown of cellular contacts caused by the parasite (arrows); B: Retraction of microvilli at parasite adhesion site (arrows). Using transmission electron microscopy, it is possible to observe that in normal intestinal cells (C), ruthenium red permeation is blocked (arrow) at the cell-cell contacts (tight junctions). After interaction (D), the dye flowed through paracellular space, and the microvilli were reduced. M: Microvilli; G: *Giardia*; arrows: cell-cell contacts. A, B: bars 2 µm; C, D: bars 1 µm. Reprinted from Maia-Brigagão, C., Morgado-Díaz, J. A. and de Souza, W. 2012, Parasitol. Int., 61, 280, with permission from Elsevier.

still remains scarce, although there are some data showing changes in phosphorylation processes during encystment and excystment [51, 52]. Kamda and Singer [53] demonstrated that PI3K plays an important role in inhibiting immune responses of dendritic cells during interaction with *G. lamblia*, thus helping to control the infection.

3. Interaction *in vivo*

Both mice and gerbils have been used to study the *Giardia* interaction *in vivo*, although there are still very few published studies in this area. The gerbil model of giardiasis seems to better resemble human infections than do most mouse models [54, 55, 56]. Attachment, virulence, and immune response assays have already been performed successfully using gerbils as an animal model [20, 54, 57]. Furthermore, in an elegant study carried out by Troeger and collaborators [27], tissue was utilized from human biopsies to extensively analyze the host-parasite relationships. The authors confirmed some previous data obtained *in vivo* and *in vitro*, such as epithelial barrier dysfunctions, apoptosis, and alterations at the level of the tight junctions.

4. PERSPECTIVES

Regarding giardiasis, it is widely accepted that the clinical symptoms of this disease occur due to a combination of both host and parasitic factors that have not been fully elucidated. Studies concerning host-parasite interactions also require improvements in the models utilized to mimic the intestinal environment of the host. For this, 3D tissue engineering and human organoid production may be examples of more complex models to perform the assays, affording the option to work without using animals. Studies of the adhesion molecules

involved in the interaction of *Giardia* with intestinal cells, as well as products of secretion/excretion and cell signaling, require further investigation.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

REFERENCES

1. Adam, R. 2001, Clin. Microbiol. Rev., 14, 447.
2. Yoshida, N., Tyler, K. M. and Llewellyn, M. S. 2011, Trends Parasitol., 27, 459.
3. Cotton, J. A., Beatty, J. K. and Buret, A. G. 2011, Int. J. Parasitol., 41, 925.
4. Sambuy, Y., De Angelis, I., Ranaldi, G., Scarino, M. L., Stammati, A. and Zucco, F. 2005, Cell Biol. Toxicol., 21, 1.
5. Buret, A. G. 2007, Gut, 56, 316.
6. Ringqvist, E., Avesson, L., Söderbom, F. and Svärd S. G. 2011, Int. J. Parasitol., 41, 277.
7. Sousa, M. C., Gonçalves, C. A., Bairos, V. A. and Poiares-Da-Silva, J. 2001, Clin. Diagn. Lab. Immunol., 8, 258. Erratum in: Clin. Diagn. Lab. Immunol., 8, 669.
8. Klotz, C., Aebischer, T. and Seeber, F. 2012, Int. J. Med. Microbiol., 302, 203.
9. Ootani, A., Li, X., Sangiorgi, E., Ho, Q. T., Ueno, H., Toda, S., Sugihara, H., Fujimoto, K., Weissman, I. L., Capecchi, M. R. and Kuo, C. J. 2009, Nat. Med., 15, 701.
10. Sato, T., Vries, R. G., Snippert, H. J., van de Wetering, M., Barker, N., Stange, D. E., van Es, J. H., Abo, A., Kujala, P., Peters, P. J. and Clevers, H. 2009, Nature, 459, 262.
11. Maia-Brigagão, C. and de Souza, W. 2012, Micron, 43, 494.
12. Hansen, W. R., Tulyathan, O., Dawson, S. C., Cande, W. Z. and Fletcher, D. A. 2006, Eukaryot. Cell., 5, 781.
13. Weiland, M. E., McArthur, A. G., Morrison, H. G., Sogin, M. L. and Svärd, S. G. 2005, Int. J. Parasitol., 35, 617.
14. Jenkins, M. C., O'Brien, C. N., Murphy, C., Schwarz, R., Miska, K., Rosenthal, B. and Trout, J. M. 2009, J. Parasitol., 95, 895.
15. Jiménez-Cardoso, E., Eligio-García, L., Cortés-Campos, A., Flores-Luna, A., Valencia-Mayoral, P. and Lozada-Chávez, I. 2009, Parasitol. Res., 105, 25.
16. Pegado, M. G. and de Souza, W. 1994, Parasitol. Res., 80, 320.
17. Sousa, M. C., Gonçalves, C. A., Bairos, V. A. and Poiares-Da-Silva, J. 2001, Clin. Diagn. Lab. Immunol., 8, 258.
18. Ward, H. D., Lev, B. I., Kane, A. V., Keusch, G. T. and Pereira, M. E. A. 1987, Biochemistry, 26, 8869.
19. Farthing, M. J., Pereira, M. E. A. and Keusch, G. T. 1986, Infect. Immun., 51, 661.
20. Hernández-Sánchez, J., Liñan, R. F., Salinas-Tobón Mdel, R. and Ortega-Pierres, G. 2008, Exp. Parasitol., 119, 364.
21. de Carvalho, T. B., David, E. B., Coradi, S. T. and Guimarães, S. 2008, Parasitol. Res., 104, 185.
22. Ringqvist, E., Palm, J. E., Skarin, H., Hehl, A. B., Weiland, M., Davids, B. J., Reiner, D. S., Griffiths, W. J., Eckmann, L., Gillin, F. D. and Svärd, S. G. 2008, Mol. Biochem. Parasitol., 159, 85.
23. Ma'ayeh, S. Y. and Brook-Carter, P. T. 2012, Int. J. Parasitol., 42, 501.
24. Eckmann, L., Laurent, F., Langford, T. D., Hetsko, M. L., Smith, J. R., Kagnoff, M. F. and Gillin, F. D. 2000, J. Immunol., 164, 1478.
25. Stadelmann, B., Merino, M. C., Persson, L. and Svärd, S. G. 2012, PLoS One, 7, e45325.
26. Philip, R., Campbell, E. and Wheatley, D. N. 2003, Br. J. Cancer, 88, 613.
27. Troeger, H., Epple, H. J., Schneider, T., Wahnschaffe, U., Ullrich, R., Burchard, G. D., Jelinek, T., Zeitz, M., Fromm, M. and Schulzke, J. D. 2007, Gut, 56, 328.
28. Prucca C. G. and Luján, H. D. 2009, Cell Microbiol., 11, 1706.
29. Touz, M. C., Rópolo, A. S., Rivero, M. R., Vranych, C. V., Conrad, J. T., Svard, S. G. and Nash, T. E. 2008, J. Cell Sci., 121, 2930.
30. Suzuki, T. 2013, Cell Mol. Life Sci., 70, 631.
31. Groschwitz, K. R. and Hogan, S. P. 2009, J. Allergy Clin. Immunol., 124, 3.
32. Hollande, F., Shulkes, A. and Baldwin, G. S. 2005, Sci. STKE., 29, pe13.
33. Koh, W. H., Geurden, T., Paget, T., O'Handley, R., Steuart, R. F., Thompson, R. C. and Buret, A. G. 2013, J. Parasitol., 99, 353.

34. Maia-Brigagão, C., Morgado-Díaz, J. A. and de Souza, W. 2012, Parasitol. Int., 61, 280.
35. Buret, A. G., Mitchell, K., Muench, D. G. and Scott, K. G. 2002, Parasitology, 125, 11.
36. Chin, A. C., Teoh, D. A., Scott, K. G., Meddings, J. B., Macnaughton, W. K. and Buret, A. G. 2002, Infection and Immunity, 70, 3673.
37. Scott, K. G., Meddings, J. B., Kirk, D. R., Lees-Miller, S. P. and Buret, A. G. 2002, Gastroenterology, 123, 1179.
38. Teoh, D. A., Kamieniecki, D., Pang, G. and Buret, A. G. 2000, The Journal of Parasitology, 86, 800.
39. Chávez, B., Knaippe, F., Gonzalez-Mariscal, L. and Martínez-Palomo, A. 1986, Experimental Parasitology, 61, 379.
40. Humen, M. A., Pérez, P. F. and Liévin-Le Moal, V. 2011, Cell Microbiology, 13, 1683.
41. O'Hara, J. R. and Buret, A. G. 2008, Frontiers in Bioscience, 13, 7008.
42. Roxström-Lindquist, K., Ringqvist, E., Palm, D. and Svärd, S. 2005, Infection and Immunity, 73, 8204.
43. Madara, J. L. 1988, Cell, 53, 497
44. Citi, S., Volberg, T., Bershadsky, A. D., Denisenko, N. and Geiger, B. 1994, J. Cell Sci., 107, 683.
45. Stevenson, B. R. and Begg, D. A. 1994, J. Cell Sci., 107, 367.
46. Abe, K. and Takeichi, M. 2008, Proc. Natl. Acad. Sci. USA, 105, 13.
47. Turner, J. R. 2000, Semin. Cell Dev. Biol., 11, 301.
48. Hall, P. A., Coates, P. J., Ansari, B. and Hopwood, D. 1994, J. Cell Sci., 107, 3569.
49. Bartelt, L. A, Roche, J., Kolling, G., Bolick, D., Noronha, F., Naylor, C., Hoffman, P., Warren, C., Singer, S. and Guerrant, R. 2013, J. Clin. Invest., 123, 2672.
50. Panaro, M. A., Cianciulli, A., Mitolo, V., Mitolo, C. I., Acquafredda, A., Brandonisio, O. and Cavallo, P. 2007, FEMS Immunol. Med. Microbiol., 51, 302.
51. Smith, A. J., Lauwaet, T., Davids, B. J. and Gillin, F. D. 2012, Int. J. Parasitol., 42, 411.
52. Bittencourt-Silvestre, J., Lemgruber, L. and de Souza, W. 2010, Arch. Microbiol., 192, 259.
53. Kamda, J. D. and Singer, S. M. 2009, Infect. Immun., 77, 685.
54. Rivero, F. D., Saura, A., Prucca, C. G., Carranza, P. G., Torri, A. and Lujan, H. D. 2010, Nat. Med., 16, 551.
55. Deselliers, L. P., Tan, D. T., Scott, R. B. and Olson, M. E. 1997, Dig. Dis. Sci., 42, 2411.
56. Buret, A., Hardin, J. A., Olson, M. E. and Gall, D. G. 1992, Gastroenterology, 103, 506.
57. Bénéré, E., Van Assche, T., Van Ginneken, C., Peulen, O., Cos, P. and Maes, L. 2012, Parasitology, 139, 424.