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ORIGINAL PAPER

Goblet cells: are they an unspecific barrier against *Giardia intestinalis* or a gate?

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Abstract Giardiosis is one of the major intestinal parasitic diseases of human beings as well as wild and domesticated animals. Several protective mechanisms against infection have been described. However, specific information about relationship between giardiosis and the increased proliferation of goblet cells (GC) in patients infected with *Giardia intestinalis* (Syn. *G. duodenalis*, *G. lamblia*) is scarce. In this work, we compare and quantify the number of GC, and have inferred their metabolic state in the small intestine of dogs parasitized with *Giardia intestinalis* compared to dogs without parasites. Small intestine segments were processed using routine methods for histology and electron microscopy; areas and cells were screened with an Axiovision Ver. 4.0 system. Data were analyzed by ANOVA and comparison of averages. Parasitized dogs showed higher GC numbers than nonparasitized ones. Averages were: 20± 0.81 GC/25 μm^2 with independent mucin granules and 11± 1.53 GC/25 μm^2 that were expelling mucus, compared to 11±0.94 GC/25 μm^2 and 1±0.27 GC/25 μm^2, respectively, in nonparasitized dogs (Tukey, $p<0.001$). The increases in GC number seem to be an unspecific defensive mechanism against *Giardia* trophozoites. However, we found some evidence supporting that GC hyperplasia could be a prejudicial to epithelial barrier that gives rise to gates allowing for *Giardia*-tissue invasion.

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Introduction

Giardiosis is a cosmopolitan re-emergent (Thompson 2000) parasitic disease that is highly prevalent in humans, especially children (Thompson 1994; Adam 2001), as well as domesticated and wild animals (Ponce-Macotela et al. 2005; Polley 2005). It may be asymptomatic or give rise to abdominal pain, explosive watery, foul-smelling diarrhea, intestinal malabsorption, and physical growth arrest to different degrees (Astiazarán-Garcia et al. 2000). Various pathogenic mechanisms have been described, such as: (1) mechanical damage of the intestinal epithelium caused by the interaction of the *Giardia intestinalis* ventral sucking disc, (2) lectin-mediated biochemical injury, due to the specific interaction between host intestinal cells and the parasite (Lev et al. 1986). (3) Possibly, G*iardia VSPs* with a LIM motif (Michelsen et al. 1993) or RING structures may modulate the protein–protein interaction between host and parasite cells (Singer and Nash 2000; Singer et al. 2002) by binding divalent metals such as zinc or iron (Luján et al. 1995), which are essential micro-nutriments for the host. (4) *Giardia* trophozoites secrete proteinases that can contribute to injury in several ways: (a) by breaking secreted IgA directed against *Giardia*, (b) by injuring epithelial cells (Parenti 1989), or (c) by acting like caspases to promote apoptosis (Chin et al. 2002). (5) There were alterations in permeability due to the increase of trans-epithelial resistance (Chávez et al. 1995) and the interaction with the tight-junction zonula-occludens (Buret et al. 2002). (6) One interesting and very explanatory, albeit little explored, hypothesis could be the existence of low molecular weight polypeptides with toxin activity (Chen et al. 1995; Upcroft and Upcroft 1998). All these stimuli modify the intestinal morphology and give rise to intestinal villi atrophy and a reduction in the enzymatic activity of isomaltase and other

disacharidases, alcaline phosphatase, ATPases, and others (Belosevic et al. 1989; Nain et al. 1991). On the other hand, the host develops specific (Faubert 2000) and unspecific self-defense mechanisms against parasites (Eckmann 2003; Roskens and Erlandsen 2002). Among specific mechanisms are immune responses based on B- and T-cell-dependent mechanisms (Singer and Nash 2000). The unspecific responses are partially supported by nitric oxide, antimicrobial peptides, and others (Eckmann 2003) as well as the mucus-secreting goblet cells (GC) that protect the gut by layering the epithelium (Ho et al. 1995). There are supporting evidences showing that mucus glycoproteins act by inhibiting the adherence of *Escherichia coli* (Mack and Sherman 1991) and *Entamoeba histolytica* (Chadee et al. 1990) to epithelial cells. In addition, there are data showing that some compounds present in the secretion of goblet cells impede rotaviruses' replication (Yolken et al. 1994). In the present work, we show a differential activity of goblet cells in dogs infected and uninfected with *Giardia intestinalis* and propose that goblet cell hyperplasia could generate gates allowing an unusual process: *Giardia* tissue invasion.

Materials and methods

We retrieved the small intestines of 200 euthanized adult dogs from the Canine Control Centre "Culhuacan". Intestinal samples were taken by making small longitudinal incisions that were 3 cm apart. With a sterile bacteriological loop, mucus and intestinal contents were obtained. The materials were resuspended on a slide and examined with a Carl Zeiss microscope under phase contrast illumination (Ponce-Macotela et al. 2005).

We studied goblet cells in the jejunum mucosa from 11 adult dogs; 7 of them were infected with *Giardia intestinalis* trophozoites and 4 were devoid of parasites. We chose jejunal tissue since abundant *Giardia intestinalis* trophozoites were shown at this site in almost all infected dogs. Two transversal samples 0f 2.0 to 2.5 cm long were taken 3 cm below the Treitz ligament and fixed in 2.5% glutaraldehyde buffered in 0.1-M phosphate buffer saline (PBS) pH 7.4. The specimens were postfixed in 2% osmium tetroxide, dehydrated by ethanol series and included in the epoxy resin.

The morphologic analysis was performed on semi-thin segments (1.0 μm) stained with toluidine blue and on thin segments (60–90 nm) stained with led citrate/uranyl acetate. The samples were analyzed with a Carl Zeiss EM-109 transmission electronic microscope. We counted the GC population size in an average of 15 intestinal segments from each of the intestinal samples obtained from uninfected and *Giardia*-infected dogs. Morphometric analysis was performed with a Zeiss Axioscop-2 Plus microscope at ×10 magnification coupled with Image System Analysis, Axiovision Ver. 4.0. Surface area measurements and cell number counts were performed using the single-object manual counting function. We identified cells with single mucin inclusions by a deep magenta color (dark), and cells with fusing mucin granules, representing a release process by a hyaline color (clear). We defined the density of goblet cells as the total number of cells found in 25 μm^2.

Statistics

Variance analysis (ANOVA) and average comparison (Tukey method) were carried out using the Micro Cal Origin program.

Results

The histological analysis showed that parasitized dogs had a greater number of GC in comparison with nonparasitized dogs. On average, the intestines retrieved from giardiasic dogs contained 20±0.81 GC/25 μm^2 with single mucin granules and 11.53±1.53 GC/25 μm^2 in the mucus ejection process. In contrast, in dogs without *Giardia* infection

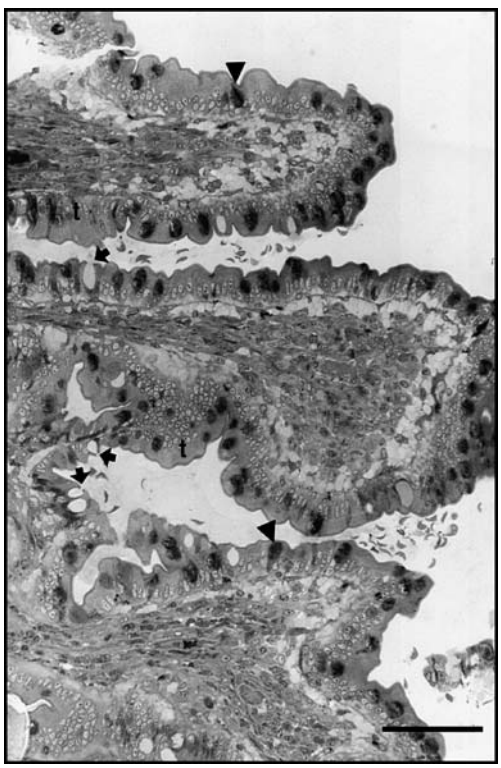

Fig. 1 Semi-thin jejunum sample retrieved from a giardiasic dog. The section shows goblet cells with packed mucin drops (*arrowhead*), goblet cells in the secretion process (*arrows*) and many *Giardia* trophozoites enclosed in the mucus (*t*). Bar is equal to 25 μm

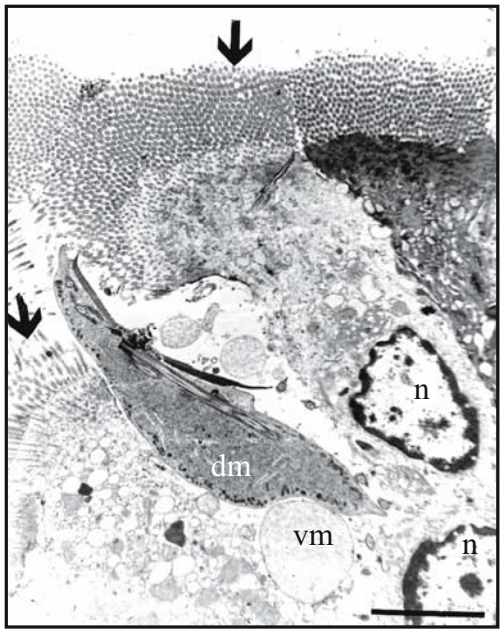

Fig. 2 Transmission electronic microphotography shows a discontinuity in the brush border and a trophozoite invasion process inside of the epithelium. The dorsal membrane (*dm*) of the *Giardia* trophozoite was in close contact with the epithelial cell and mucus vesicle (*vm*). Brush border (*arrows*), two nuclei (*n*) from epithelial cells. Bar is equal to 730 nm

there were 11±0.94 GC/25 μm^2 with single mucin inclusions and 1±0.27 GC/25 μm^2 with mucus in the releasing state. Comparison between the four groups using the Tukey test showed significant differences ($p<0.01$). Intestinal *villi* from dogs with *Giardia* (Fig. 1) showed goblet cells in different activity states, several still containing mucus and others in the secretion process; *Giardia* trophozoites were seen as inclusions within the mucus. In samples from dogs with no parasites, there were fewer goblet cells (photography not presented). At the ultrastructural level, we found some areas without and some areas with villous atrophy in samples from giardiasic dogs and, sporadically, *Giardia* trophozoites in the process of invasion as shown in Fig. 2. This opportunistic behavior of *Giardia* is remarkable, since it self-inserts between enterocyte gaps. Additionally, this trophozoite exhibits amazing morphological integrity; it is possible to identify an undamaged ventral disc, the glucocalix with electron-dense vacuoles and the dorsal membrane in close contact with mucus vesicles. The parasite can be seen within the enterocyte microvilli brush border. Figure 3 shows goblet cells in two metabolic stages: (a) GC-containing individual granules and (b) GC-expelling blended granules as mucus.

Discussion

In giardiosis, clinical manifestations represent the expression of host resistance and parasite virulence (Chester et al. 1985). While structural studies only show alterations in the intestinal architecture (Ceballos et al. 1995), in this series, we found changes in the intestinal structure as well as an increase in the GC population. Williamson et al. (2000) found that one *Giardia* isolate from birds produced a greater number of GC in mice than *Giardia* isolates from humans, suggesting that this avian isolate is highly virulent.

We found an increase in the metabolic activity of goblet cells, which was morphologically expressed by the coalescence of single granule inclusions and by mucin secretion. Intestinal goblet cells have several functions. One of these functions is to cover the intestinal epithelium with glycoproteins that protect it by neutralizing free fatty acids, which can injure the cellular membrane, and by shielding it against viruses, bacteria, and parasites. In giardiosis, the increase in GC may be responsible for at least two quite different processes: (a) During initial steps, *Giardia* trophozoites are privileged because the mucus neutralizes free fatty acids that are deleterious to *Giardia* trophozoites' cellular membranes (Rayan et al. 2005), a phenomena that is *Giardia*-boosted because the trophozoites release a lipase inhibitor (Gilling et al. 1983). (b) The trophozoites are self-protected against IgA contained in GC by the proteases that break down the chains CH2 and CH3 (Parenti 1989). (c) The parasites are adversely affected because mucin glycoproteins block the lectin receptors on trophozoites' surface

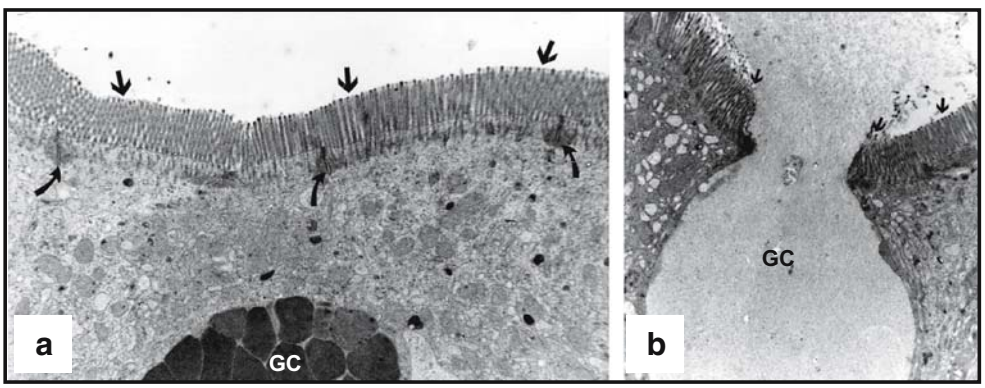

Fig. 3 Transmission electronic microphotography shows the intact brush border (*arrows*) and tight junctions (*curved arrows*): **a** individualized mucine granules in a goblet cell, **b** a goblet cell in the discharge process (*GC*)

in a potentially similar way as that observed in amoeba (Chadee et al. 1990), thus impairing the adherence of *Giardia* to epithelial cells or, alternatively, as observed for *Nippostrongylus brasiliensis* (Ishikawa et al. 1993), via modification of the terminal sugar of the mucin.

So far, information on this subject appears to be contradictory, since there is at least one work supporting the beneficial effect of human mucus on *Giardia* growth, while other observations (Roskens and Erlandsen 2002) show the opposite effect, as reviewed by Müller and von Allmen (2005). From our morphological data, we can speculate that mucus can participate in a dual process, by first protecting *Giardia* trophozoites against fatty free acids and, in a second step, probably in response to an increase in the parasite population, by modifying adherence by enhancement of viscosity or by changing the structure of glycoproteins, blocking receptors on *Giardia* trophozoites and/or receptors on the surface of the epithelial cells.

To date, it is paradigmatic that *Giardia* trophozoites cannot invade the intestinal epithelia. However, goblet cell hyperplasia implies a disturbance in the epithelial continuity, causing a weakening of the intercellular unions and thus increasing the opportunity for trophozoites to penetrate through cells, as shown in Fig. 2. Tissue-invasion by *Giardia* trophozoites is an unusual process but one that undoubtedly exists. It could very well explain unsuccessful treatments, the high re-infection rate in some patients, as well as cases of hypersensibility or allergy. The invasion mechanism is as yet unknown. It may be of interest in this regard that *Giardia* provokes a weakening of the tight-junctions (Buret et al. 2002; Troeger et al. 2007) due to induction of apoptosis and disruption of the epithelial barrier (Chin et al. 2002; Troeger et al. 2007). GC hyperplasia may be unfavorable to epithelial stability by causing gaps that may facilitate invasion by *Giardia* trophozoites. By itself, epithelial discontinuity may produce membrane depolarization, electrolytic imbalance, hyperperistalsis and, obviously, diarrhea, one of the main symptoms of symptomatic giardiosis.

Conclusion

Interaction between *G. intestinalis* trophozoites and epithelial cells produces both an increase in the number of goblet cells and in the mucus secretion that protects the gut epithelium against the aggression of parasitic cells. Although it protects *Giardia* trophozoites in the earliest steps of infection, thereafter the process interferes with trophozoites attachment to epithelial cells. Whether mucins have any specific effect against *Giardia* or are acting through nonspecific inhibition by blocking receptors on both trophozoites and enteric cells, is a matter of future research. *Giardia* tissue invasion is a rare event. This is because once *Giardia* infection is diagnosed; a duodenal biopsy is rarely justified, which nevertheless may be necessary in cases with severe intestinal malabsorption and consequent steatorrhea. In such instances, intraepithelial parasites ought to be carefully examined. What are the implicated variables in *Giardia* tissue invasion? There are still many ignored facts with regard to the host–parasite interaction: The field is wide and greater understanding is forthcoming.

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