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Epithelial dysfunction in giardiasis

Mechanisms of epithelial dysfunction in giardiasis

Andre G Buret

A better understanding of the pathophysiological processes of *Giardia* may lead to understanding the diseases it causes and to identifying new therapeutic agents

Infection with the ubiquitous intestinal parasite *Giardia lamblia* (synonymous *G duodenalis* or *G intestinalis*) may cause acute or chronic diarrhoea, dehydration, abdominal discomfort and weight loss.[1–4] Despite the prevalence of this disease, the pathophysiological features underlying intestinal disturbances in giardiasis remain incompletely understood. *Giardia* causes disease without penetrating the epithelium, invading the surrounding tissues or entering the blood stream. Interestingly, the epithelial abnormalities responsible for intestinal malabsorption and diarrhoea in giardiasis seem to share similarities with those observed in other enteric disorders, such as bacterial enteritis, chronic food anaphylaxis, Crohn's disease and coeliac disease.[5–9] Therefore, a better understanding of these pathophysiological processes may help identify new therapeutic targets for a variety of gastrointestinal diseases. In an attempt to unravel the mechanisms by which *Giardia* exerts its clinical effects, researchers have relied on a variety of cell systems and animal models. This issue of *Gut* presents data from an elegant human clinical study by Troeger *et al*[10] (***see page 328***) that sheds new light on these processes. In view of the limited space available, this commentary only highlights selected mechanisms whereby *Giardia*-induced epithelial dysfunction may contribute to disease development.

ELECTROLYTE TRANSPORT ABNORMALITIES

Previous studies using models in vivo and in vitro have established that *Giardia* causes malabsorption of glucose, sodium and water, and reduced disaccharidase activity, due to loss of epithelial absorptive surface area.[1 3 4 11–13] Observations from humans infected with this parasite now confirm these findings.[10] Recent reports have suggested that this parasite may also alter chloride secretory responses in human colonic cells in vitro, as well as in murine models.[14 15] The findings of Troeger *et al*[10] show for the first time that, in addition to malabsorption, chronic giardiasis may cause hypersecretion of chloride in humans. Therefore, a combination of malabsorption and secretion of electrolytes seems to be responsible for fluid accumulation in the intestinal lumen during this infection. The cascade of events ultimately responsible for these epithelial abnormalities remains incompletely understood. Findings to date imply that parasite products may break the epithelial barrier, after which activated T lymphocytes cause the brush border to retract, which in turn causes the disaccharidase deficiencies and epithelial malabsorption responsible for diarrhoea.[1 3 4] Epithelial brush-border injury and disaccharidase deficiencies in giardiasis seem to be mediated by CD8+ T cells, whereas CD4+ T cell activation contributes to parasite clearance.[2 16 17] Consistent with these observations, microvillus brush-border abnormalities and parasite clearance do not occur in hosts devoid of functional T lymphocytes.[2 17 18] The findings that athymic mice infected with *Giardia* do not exhibit microvillous injury and dysfunction despite the presence of live parasites refutes the hypothesis that intestinal malfunction solely results from trophozoite attachment or parasite virulence factors. In this issue of *Gut*, Troeger *et al*[10] confirm that increased numbers of intraepithelial lymphocytes are associated with the sodium/glucose malabsorption detected in their *Giardia*-infected patients.

ENTEROCYTE APOPTOSIS AND LOSS OF EPITHELIAL BARRIER FUNCTION

Observations from models in vitro and in vivo have established that *Giardia* parasites increase intestinal permeability.[19 20] Moreover, infection with *G lamblia* in gerbils has been associated with increased macromolecular uptake in the jejunum during the period of peak trophozoite colonisation, but not during the parasite clearance phase.[21] Using impedance spectroscopy, Troeger *et al*[10] now demonstrate that chronic giardiasis is also responsible for a loss of epithelial barrier function in human infections.[10] Infection-associated loss of epithelial barrier function allows luminal antigens to activate host immune-dependent pathological pathways. Therefore, such events may be of great clinical relevance. Not surprisingly, intense research efforts are trying to identify the molecular events regulating epithelial tight-junctional function in gastrointestinal health and disease.[22 23] In giardiasis, disruptions of cellular F-actin and tight junctional ZO-1, as well as the resulting increase in transepithelial permeability, seem to be modulated at least in part by myosin-light-chain kinase and pro-apoptotic caspase-3.[20 24] Using TUNEL labelling, Troeger *et al*[10] report that epithelial barrier dysfunction in patients with chronic giardiasis is associated with increased rates of enterocyte apoptosis. Consistent with these observations, recent findings from microarray analyses on the effects of *G duodenalis* on human CaCo2 cells found that the parasite–host interactions lead to a pronounced up-regulation of genes implicated in the apoptotic cascade and the formation of reactive oxygen species.[25] *Giardia* can also prevent the formation of nitric oxide, a compound known to inhibit giardial growth, by consuming local arginine, which effectively removes the substrate needed by enterocytes to produce nitric oxide.[26] This mechanism may contribute to

Giardia-induced enterocyte apoptosis, as arginine starvation in these cells is known to cause programmed cell death.[27] Other studies also found that *Giardia* disrupts enterocyte α-actinin, a component of the actomyosin ring that regulates paracellular flow across intestinal epithelia.[28] In their study of human giardiasis, Troeger *et al*[10] demonstrate that the parasite may also alter members of the claudin protein family, a critical component of the sealing properties of tight junctions. Together, the findings indicate that in giardiasis, changes in enterocyte ultrastructure and function are associated with a loss of intestinal epithelial barrier function, and ultimately lead to diarrhoea. More research is needed to establish whether or not epithelial dysfunction in giardiasis also directly contributes to the symptoms of irritable bowel syndrome that may be elicited by the infection.[29]

ROLE OF PARASITE VIRULENCE FACTORS

Strain-dependent activation of enterocyte apoptosis as well as loss of epithelial barrier function induced by *Giardia* may occur in the absence of any other cell type, and small-intestinal permeability returns to baseline on parasite clearance in murine giardiasis.[12 20 24 28] *Giardia* virulence products that may instigate these cascades of events are topics of intensive research. In addition to expressing surface glycoproteins able to induce fluid accumulation in the intestine, *Giardia* is known to contain and/or release a variety of potentially ''toxic'' substances, such as proteinases and lectins that may be responsible for direct epithelial injury.[30–34] Recent findings suggest that a 58 kDa *Giardia* ''enterotoxin'' may induce chloride secretion in a model of murine giardiasis.[30 35] Whether or not such a product may be implicated in the secretory response seen in humans [10] needs to be clarified. Also, proteinases have long been recognised as important virulence factors in a variety of microbial pathogens, including *Giardia*.[36 37] Proteinase-Activated Receptors are members of a unique class of G-protein-coupled signalling receptors that can modulate enterocyte apoptosis and increase intestinal epithelial permeability in a caspase-3-dependent fashion.[38] Much remains to be learnt of the ability of *Giardia* proteinases to activate host proteinase-activated receptors in the gastrointestinal tract. Full characterisation of the *Giardia* genome should facilitate the identification of putative *Giardia* enterotoxins (see *Giardia* genome project database at http://gmod.mbl.edu/perl/site/giardia?page = intro for updates).[39] Such advances may help identify novel pharmacological targets for the treatment of giardiasis.

CONCLUSION

The article by Troeger *et al* offers important confirmatory and new evidence on several critical events implicated in the pathophysiology of giardiasis. As these data emanate from humans with chronic infections, they bear direct clinical relevance. A better knowledge of how *Giardia* parasites alter epithelial structure and function may provide the key to understanding the diseases caused by giardiasis, and possibly a number of other gastrointestinal disorders.

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