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Leading article

Diarrhoea and malabsorption in giardiasis: A multifactorial process?

There have been major developments over the last decade in our understanding of the biology and pathophysiology of the protozoan parasite *Giardia lamblia*. As late as the mid 1970s there was not universal agreement that this parasite was indeed an important pathogen. Part of this confusion stemmed from the broad clinical spectrum seen with giardiasis, ranging from asymptomatic cyst passage to acute or chronic diarrhoea with malabsorption. The ability to axenically culture *Giardia* has greatly facilitated study of the organism. Recent developments have allowed the full life cycle of the parasite to be conducted in vitro.[1 2] In addition there are well characterised animal models of infection.[3 4] Despite these advances, information on the pathogenesis of human giardiasis remains controversial and incomplete.

Life cycle and biology of colonisation

Giardia is superbly adapted to parasitise the upper gut of man. The cyst form is resilient in the environment and only a small number are needed to initiate infection. In human volunteer studies as few as 10 cysts were capable of causing giardiasis.[5] In vitro, lowering the pH of the surrounding medium triggers excystation, a situation analogous to the acid milieu of the stomach.[6] Trophozoites preferentially localise to the upper small bowel. There is evidence suggesting that host proteases activate a specific lectin on the surface of *Giardia* that promotes attachment to enterocytes.[7] Avidity for attachment is maximal for jejunal cells and decreases distally.[8] *Giardia* have a ventral 'sucker' disc which is also important for attachment to surfaces. Lectin associated binding may serve to localise the organism to the upper gut before mechanical attachment with the disc occurs. Bile, which is plentiful in the proximal gut has a trophic effect on growth and replication of trophozoites in vitro,[9] while increasing the concentration of bile and raising the pH stimulates encystation,[2] events which both occur regularly in the upper small bowel. Once triggered, encystation probably occurs in small bowel and colon and cysts and trophozoites are shed in the stool.

Mechanisms of diarrhoea

The pathogenesis of the diarrhoea and malabsorption in giardiasis is multifactorial. Most attention has focused on functional and morphological damage to the small bowel mucosa. While some workers have observed a reasonable correlation between the extent of mucosal injury and absorptive impairment,[10 11] this is not universal.[12] Furthermore, the morphological abnormalities are often minor and not invariably present despite diarrhoea and malabsorption of fat and other nutrients.[13 14] Two thirds of infected patients in one study had some degree of steatorrhoea, mostly without significant morphological changes.[15] It has therefore been suggested that luminal as well as mucosal factors are important in the development of diarrhoea and malabsorption in giardiasis.

Luminal factors

Giardia in vitro avidly consume bile salts by an apparently carrier mediated active transport process.[16] The metabolic advantage of this for the organism is not known, but consumption of host bile salts in chronic infection could theoretically deplete the bile salt pool and thus contribute to fat malabsorption by impairing micellar solubilisation of ingested fats and decreasing the effectiveness of pancreatic lipase. *Giardia* may also deconjugate bile salts as increased concentrations have been found in small bowel aspirates from infected patients.[17] This finding has not been confirmed in other studies.[11 16 18] Reports that *Giardia* promotes secondary bacterial overgrowth,[19] which in turn leads to bile salt deconjugation are not uniformly supported by other workers[16 20] nor by the observation that symptomatic giardiasis does not improve with non-giardiacidal antibiotics.[11] The mucosal lesion of small intestinal overgrowth, however, is similar to that described in giardiasis[21] and the possibility that *Giardia* promotes coexisting infection needs further exploration.

Abnormal pancreatic exocrine function in patients with symptomatic giardiasis has been reported. Tryptic activity in duodenal aspirates was reduced in a group of infected subjects and returned to normal after eradication of the parasite.[22] Abnormal chymotryptic activity using the BT-PABA test in symptomatic adults has also been described.[23] Stimulated pancreatic lipase activity was reduced in infected children and correlated with faecal fat excretion.[24] Given the large pancreatic functional reserve, the magnitude of the reduction observed in these studies is not itself enough to account for malabsorption, but could contribute to a cascade of abnormalities that together impair the absorptive mechanism of the gut to cause diarrhoea with malabsorption. Possible mechanisms for these effects on pancreatic function have been suggested by in vitro studies. Trophozoites actively inhibit trypsin[24a] and inhibition of lipolysis has been shown with live trophozoites, independent of bile salt concentration,[25] and with intracellular sonicate of trophozoites.[18]

As abdominal cramps are common in symptomatic patients it has been suggested that *Giardia* may induce a motility disturbance with rapid transit and decreased absorptive time for nutrients. The evidence for this is scant, but trophozoites can increase monocyte prostaglandin E_2 production in vitro, a known stimulator of smooth muscle.[26]

Mucosal injury

Despite these proposed luminal mechanisms for diarrhoea, the mucosal injury induced by *Giardia* is likely to be the major lesion in giardiasis. These mucosal abnormalities have been studied in detail in vitro and in animal models, although less in human infection. The earliest suggestion, that *Giardia* formed a mechanical barrier to absorption by covering a vast surface area of the small intestine,[27] is no longer tenable. A broad spectrum of mucosal appearances ranging from normal

to subtotal villous atrophy have been described.[14 15 28] Ultrastructural studies often reveal minor shortening and distortion of the microvillus membrane although normal appearances are seen even in symptomatic patients.[13 14 29] Several studies have shown functional impairment of enterocytes.[11 15 30] Reduced brush border disaccharidase activity is a common finding.[10 31 32] A diffuse defect in sodium mediated glucose transport has recently been described.[33] These descriptive studies documenting the range and time course of functional and structural derangements do not identify their pathogenesis. This remains largely speculative. *Giardia* do not invade the mucosa except in exceptional circumstances. They move over the surface of the epithelium and attach to the base of villi. Trophozoites can be shown in all orientations with respect to enterocytes but are frequently seen attached by their ventral disc. Whether this is a mechanical attachment brought about by contractile proteins in the disc or a hydrodynamic attachment by suction is not clear. Nonetheless electron microscopy in some studies has clearly shown the disc embedded into the microvilli and circular suction 'marks' imprinted onto the brush border surface, sometimes with deformed microvilli within[34 35] It seems unlikely that this relatively mild insult to the surface epithelium is sufficient in itself to cause diarrhoea.

The possibility that *Giardia* elaborate specific toxins or have products of metabolism that are toxic to the small intestinal mucosa is an attractive hypothesis. No secretion inducing toxin has been demonstrated[36] although culture filtrates of *Giardia* damaged fibroblasts in culture and reduced salt and water absorption from perfused loops of rats in preliminary studies.[37 38] *Giardia* elaborate excretory secretory products into culture medium[39] but the role of these substances in the pathogenesis of giardiasis is unknown.

The lectin activity exhibited by *Giardia* merits investigation in the study of putative toxic mechanisms. Lectins may directly or indirectly contribute to microvillus damage. Plant lectins are capable of directly damaging enterocytes in animal models producing brush border abnormalities similar to those seen in giardiasis.[40] Alternatively, lectins may facilitate the action of toxins. As shown with *Giardia* and some bacteria, lectins promote adherence of the organism to the mucosa.[8 41 42] In certain bacterial infections, lectin mediated adherence to oligosaccharides of the microvillus membrane may precede a reduction in brush border enzyme concentrations caused by the action of bacterial proteases.[43 44] *Giardia* trophozoites also possess proteinase activity[45] although whether this functions in cell regulation or contributes to virulence is unknown.

Much attention has focused on immune and inflammatory mechanisms as the likely mode of epithelial damage in giardiasis. An inflammatory response in the lamina propria is frequent in giardiasis and is similar to that seen in other small intestinal disorders where there is villus shortening and crypt elongation. Analogies have been drawn with the mucosal lesion of coeliac disease with the suggestion that *Giardia* may elaborate a 'toxic' protein which contributes to mucosal damage by eliciting an adverse immune response. There is evidence that T cells activated by lectin stimulation play a role in the pathogenesis of villous atrophy in human enterocytes in vitro.[46] Evidence for the role of the immune system in the production of the mucosal injury in giardiasis has been implied from the study of experimental *G muris* infection in immunodeficient athymic mice. Despite prolongation of infection in these animals, abnormalities of villus architecture were less severe than in immunocompetent mice. When lymphocytes from the spleens of immunologically intact mice were injected into athymic infected mice, histological abnormalities developed in the mucosa.[47] Mucosal damage in man, however, is more pronounced in immunocompromised hosts.[15 48] Furthermore, although intraepithelial lymphocytes are frequently increased in giardiasis and have been incriminated in contributing to immune mediated tissue damage, in a careful study of the time course of infection in primary *G muris* infection, intraepithelial lymphocytes increased after villus shortening and a decrease in brush border disaccharidases had already occurred.[31]

Increased epithelial turnover of the small intestine with increased crypt cell production rates has been observed in *G muris* infection of mice suggesting that such a population of immature enterocytes may result in an absorptive defect and contribute to diarrhoea. It is unlikely, however, that this mechanism is important as the fall in brush border disaccharidase activity precedes the changes in the crypts and returns to normal by the time the crypt changes are maximal.[31]

Conclusions

Much has been learned in recent years about the biology of this remarkable parasite. Relatively little is known about the pathogenesis of the illness it causes despite abundant observations of the infection. It seems likely that *Giardia* causes diarrhoea and malabsorption by a number of mechanisms although the relative importance and relevance of each is unclear. At present, speculation exceeds knowledge regarding pathogenic mechanisms in giardiasis. Exploration of the mechanisms of the mucosal and luminal abnormalities induced by *Giardia* will increase our understanding of both the parasite and its host and of the interaction between the two.

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