Immunopathology of giardiasis: the role of lymphocytes in intestinal epithelial injury and malfunction

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T lymphocyte-mediated pathogenesis is common to a variety of enteropathies, including giardiasis, cryptosporidiosis, bacterial enteritis, celiac’s disease, food anaphylaxis, and Crohn’s disease. In giardiasis as well as in these other disorders, a diffuse loss of microvillous brush border, combined or not with villus atrophy, is responsible for disaccharidase insufficiencies and malabsorption of electrolytes, nutrients, and water, which ultimately cause diarrheal symptoms. Other mucosal changes may include crypt hyperplasia and increased infiltration of intra-epithelial lymphocytes. Recent studies using models of giardiasis have shed new light on the immune regulation of these abnormalities. Indeed, experiments using an athymic mouse model of infection have found that these epithelial injuries were T cell-dependent. Findings from further research indicate that the loss of brush border surface area, reduced disaccharidase activities, and increased crypt-villus ratios are mediated by CD8+ T cells, whereas both CD8+ and CD4+ small mesenteric lymph node T cells regulate the influx of intra-epithelial lymphocytes. Future investigations need to characterize the CD8+ T cell signaling cascades that ultimately lead to epithelial injury and malfunction in giardiasis and other malabsorptive disorders of the intestine.

Key words: giardiasis - lymphocytes - epithelial - malabsorption - intestinal disease

Giardia duodenalis (also referred to in the literature as G. lamblia or G. intestinalis) is an enteric Protozoan parasite responsible for diarrheal disease in a variety of host species, including humans. G. duodenalis is the most common human intestinal parasite worldwide and is ranked in the top 10 parasites of man (Schofield 1985, Wolfe 1992, Farthing 1997). Giardiasis may cause acute or chronic diarrhea, dehydration, abdominal discomfort, and weight loss (Wolfe 1992, Farthing 1994, 1997). Despite the great prevalence of the infection, the patho-physiological processes responsible for giardiasis remain incompletely understood. Enteric infection with Giardia spp. is responsible for decreased absorption of electrolytes, glucose and fluid, at least in part because of diffuse epithelial microvillus shortening, which may be combined or not with villus atrophy (Buret et al. 1991, 1992, Farthing 1993). Together these abnormalities lead to the malabsorption and malnutrition that ultimately cause diarrheal disease during giardiasis. Similar path-physiology associated with loss of epithelial brush border surface area is observed in bacterial enteritis (Buret et al. 1990, 1998), chronic food anaphylaxis (Curtis et al. 1990), celiac disease (Rubin et al. 1966), and Crohn’s disease (Dvorak 1988). Moreover, giardiasis has been reported to mimic inflammatory bowel disease in man (Gunasekaran & Hassall 1992). The fact that some of these disorders do not involve colonization by a microbial pathogen support the hypothesis that host immune factors are involved in the pathogenesis of these tissue abnormalities. The aim of this article is to review the role played by lymphocytes in the immuno-pathophysiology of giardiasis, in an attempt to shed new light on pathogenic mechanisms common to a variety of disorders of the intestinal tract.

Lymphocytes in giardiasis: friends or foes?

Gut associated lymphoid tissue-derived immunity is necessary to clear Giardia infections from the gut (Cevallos & Farthing 1992), and unlike immunocompetent mice, nude athymic (nu/nu) mice that are infected with G. muris fail to clear the infection and develop chronic giardiasis (Roberts-Thomson et al. 1978). Immunocompetent mice also become immune to re-infection, while nude mice gain no immunity to Giardia infection and are susceptible to secondary infections. In addition, reconstitution of athymic mice with T cells leads to decreased parasite load as well as further villus atrophy (Roberts-Thomson & Mitchell 1978). Villus atrophy can also be caused by activated T-cells in absence of Giardia infection (Farthing 1993) and T-lymphocytes have been implicated in the loss of villus height during other disorders (Ferguson 1976, da Cunha Ferreira et al. 1990, Lionetti et al. 1993). Together these observations imply a protective function for T-cells in giardiasis, as well as a role for T-cells in the pathogenesis of intestinal villus injury. In the absence of villus atrophy, it is the ultrastructural loss of brush border microvilli that represent the limiting factor to absorption and digestion in a number of intestinal disorders (Curtis et al. 1990, Buret et al. 1992, Farthing 1993). Recent findings indicate that in giardiasis this brush border injury and malfunction are mediated by CD8+ T lymphocytes (Scott et al. 2004).

Increased infiltration of intraepithelial lymphocytes (IEL) has been associated with giardiasis in a number of
reports (Gillon et al. 1982, Oberhuber et al. 1996, Wakelin 1997). Intriguingly, this increase was reported in immuno-competent as well as in athymic animals infected with *Giardia* (Scott et al. 2000), suggesting a role for extrathymic differentiation in this response. T-lymphocytes express the αβ T cell receptor (TCR) or the γδ TCR. αβ T lymphocytes primarily develop in the thymus and represent the majority of T-cells in systemic and mucosal lymphoid tissues of mice (Rocha et al. 1992). In contrast, γδ T-cells largely differentiate extra-thymically, are over-represented in the intestine, making up approximately one half of the IEL population of the murine intestine, and mostly express the CD8+ cytotoxic phenotype (Rocha et al. 1992). Microbial colonization of the gut markedly increases the αβ IEL population, while γδ IEL numbers are similar in germ-free and conventional mice (Banderia et al. 1990). Conversely, in intestinal disorders such as celiac disease there is a striking increase in γδ IELs versus αβ T-cells (Kagnoff 1998). Results from studies in giardiasis reveal a much more subtle, but significant, increase in IELs than that commonly seen in celiac disease (Heyworth et al. 1985, Oberhuber et al. 1996, Scott et al. 2000). More research is warranted to determine whether, unlike in celiac disease, the pathophysiology of giardiasis may be mediated by T-cells other than cytotoxic γδ IELs. Whether the findings suggest a role for extraepithelial αβ T-cells and/or suppressor CD8+ IELs in the pathogenesis of *Giardia* needs to be further investigated. The later hypothesis would be consistent with a recent report that showed that the acute phase of *G. duodenalis* infection in mice is accompanied by an increase of intraepithelial and lamina propria T-lymphocytes belonging to the CD8+ subset (Vinayak et al. 1991).

**The role of CD8+ lymphocytes in pathogenesis**

Intestinal epithelial brush border microvilli harbour various digestive enzymes and transporters for nutrients, and ions. As discussed previously, malabsorption of electrolytes, nutrients and water, in association with brush border injury, are responsible for the diarrheal symptoms seen in giardiasis (Buret et al. 1992). Numerous reports have also established that infections with *Giardia* significantly impair digestive enzyme function (Table). A number of laboratories have established that oro gastric inoculation of mice or gerbils with *G. muris* or *G. duodenalis* cysts or trophozoites provides a reproducible model of giardiasis despite the difficulty in demonstrating diarrheal symptoms in small rodents. The use of these models has significantly improved our understanding of the pathobiology of this parasite. Injury to small intestinal microvilli has been reported in mice. Mongolian gerbils, cattle, and goats infected with this parasite (Buret et al. 1991, 1992, Kudela et al. 1998, O’Handley et al. 2001). Together, these observations indicate that in giardiasis, loss of absorptive surface area coupled with defective glucose-stimulated electrolyte, fluid, and solute absorption, rather than hypersecretory processes such as those observed during cholera, are responsible for excessive loss of fluids in the stools. As mentioned above, a similar pathophysiological cascade has been reported in a number of other disorders, including cryptosporidiosis (Argenzio et al. 1990), Crohn’s disease (Dvorak 1988), bacterial enteritis (Buret et al. 1990, 1998), celiac disease (Rubin et al. 1966), and chronic intestinal anaphylaxis (Curtis et al. 1990). Recent studies have shed new light on the mechanisms whereby host immune factors may lead to these abnormalities in giardiasis. First, in immuno-competent but not in T-cell deficient animals, acute giardiasis causes a diffuse loss of epithelial brush border surface area and decreases sucrase and maltase activities (Scott et al. 2000). During the acute phase of the infection, epithelial abnormalities were not seen in the jejunum of

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athymic mice, despite comparable parasitic loads to those seen in immuno-competent animals, implying that the lack of microvillous injury could not be attributed to a reduction in trophozoite numbers. Second, experiments using in vivo T lymphocyte transfer into naïve animals demonstrate that brush border injury and malfunction in giardiasis are mediated by CD8+ T lymphocytes, while CD4+ T lymphocytes are responsible for parasite clearance (Scott et al. 2004). Indeed, transfer of whole populations of small mesenteric lymph node lymphocytes as well as purified CD8+, but not CD4+ cells, from previously infected mice, are able to induce microvillous shortening and sucrase deficiencies in the jejunum of naïve recipients (Figure). Transfer of lymphocytes from control donors had no effect. Moreover, increased crypt/villus ratios were reported in the jejunum of naïve mice that received whole SMLN lymphocytes and CD8+ T cells from infected donors compared to their paired control donor groups. In contrast, crypt/villus ratios remained unchanged in mice that received SMLN CD4+ T cells from infected and control donors (Scott et al. 2004). Data published to date support a role for CD4+ T lymphocytes in protective immunity, while CD8+ cells are implicated in pathophysiology. In keeping with these observations, depletion of CD4+ helper/inducer T lymphocytes in G. muris-infected mice results in chronic infection (Heyworth et al. 1987, Singer & Nash 2000). In contrast, infected mice depleted of CD8+ suppressor/cytotoxic T cell subtypes show normal parasite eradication (Heyworth et al. 1987). In addition, significant infiltration of CD8+ T cells has also been documented in the small
intestinal mucosa of Crohn’s disease patients, and it has been suggested that increased cytolytic activity of CD8+ T cells in the intraepithelial compartment of the patients may be involved in the induction of epithelial tissue damage (Nussler et al. 2000, Honma et al. 2001, Melgar et al. 2002). The findings from studies in giardiasis demonstrating that CD8+ lymphocytes are implicated in brush border injury and malfunction are consistent with this hypothesis. Immunostaining of Giardia-infected human intestinal tissues has demonstrated that these specimen’s intraepithelial lymphocytes, which are mostly CD8+ T cells, are positive for T-cell restricted intracellular antigen (TIA)-1, but negative of Granzyme B, implying that these CD8+ T cells are resting cytotoxic cells (Oberhuber et al. 1996). Furthermore, CD8+ IEL isolated from TCR transgenic mice exhibit antigen-specific perforin- and FasL-mediated cytotoxic activity toward intestinal epithelial cells and T cells (Corazza et al. 2000, Melgar et al. 2002). Potent FasL-dependent cytolytic activity of CD8+ IEL toward enterocytes was also reported in graft-versus-host disease, which shares a number of the pathophysiological features of giardiasis (Corazza et al. 2000). In graft versus host disease-associated intestinal inflammation, or upon intestinal bacterial infection, IEL express a high level of the integrin α Xβ 2 (Huleatt et al. 1995). The expression of this integrin has been linked to that of α Eβ 7, which may be upregulated by epithelial-derived TGFβ (Shibahara et al. 2000, 2001). Recent studies have found that CD8+ lymphocyte adhesion to epithelial cells via α Eβ 7 contributes to the destruction of pancreatic islet cells (Butcher et al. 1996, Feng et al. 2002). Previous reports have also shown that secondary challenge with fractions of G. duodenalis trophozoites may cause disaccharidase deficiencies in the absence of live parasites (Belosevic et al. 1989). The signaling events implicating CD8+ T cells, integrins like α Eβ 7, and possibly TGFβ, in the brush border injury during giardiasis and other disorders of the intestine warrant further investigations. Whether various degrees of severity in brush border injury during giardiasis may contribute to the broad range of clinical symptoms reported in this disease also needs to be investigated.

Mechanisms of microvillus shortening

Brush border microvilli contain a core of actin filaments that are anchored in the apical terminal web of enterocytes (Holmes & Lobley 1989). Whether the T-lymphocyte mediated brush border injury reported in giardiasis correlates with reorganization of cytoskeletal actin in vivo has yet to be determined. Administration of cycloheximide (LeCount & Gray 1972) or colchicine (Buschmann 1983) causes a transient shortening of microvilli in vivo. Conversely, addition of actin monomers to membrane-associated ends of brush border microvillar filaments increases microvillus length (Mooseker et al. 1982). Recent observations also suggest that the rapid upregulation of microvillus length by epidermal growth factor may involve an apical translocation of intracellular pools of membrane constituents via actin polymerization (Chung et al. 1999). In addition, bacterial pathogens such as Yersinia or enteropathogenic E. coli are known to affect apical distribution of F-actin in enterocytes (Finlay & Cossart 1997), and intestinal infection with these microorganisms causes a diffuse shortening of brush border microvilli (Buret et al. 1990, 1998).

Together these reports indicate that the alterations of brush border surface area may accompany reorganization of cytoskeletal actin. Findings from studies in vitro have demonstrated that G. duodenalis rearranges F-actin an actin and a-actinin in intestinal epithelia (Teoh et al. 2000, Scott et al. 2002). Further studies are needed to assess the effects of T-cells on enterocyte F-actin during giardiasis in vivo, and to unequivocally determine whether these effects directly correlate with shortening of brush border microvilli.

Conclusion

Together, the observations discussed in this review suggest that the reduction of microvillous brush border surface area may represent a host mucosal response common to a variety of stimuli, and that this response is mediated at least in part by thymus-derived CD8+ T-lymphocytes. In giardiasis, epithelial brush border injury, disaccharidase deficiencies, and increased crypt/villus ratio, which are the cause of malabsorptive diarrhea in this infection as well as in other intestinal diseases, are dependent on SMLN-derived CD8+ T-cells, but not CD4+ T cells. Moreover, transfer of either CD4+ or CD8+ T cells from Giardia-infected donors increases the numbers of IELs in recipient mice (Scott et al. 2004), suggesting that both subpopulations of T cells may regulate the influx of IELs in giardiasis. Future investigations will help characterize the cascade of events allowing CD8+ T cells to signal for epithelial brush border injury and malfunction from within the IEL compartment. Such research may help establish a rational basis for the development of new therapeutic approaches in giardiasis, as well as in other disorders of the intestinal tract.

REFERENCES


