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

AG Buret

Department of Biological Sciences, Mucosal Inflammation Research Group, University of Calgary, 2500 University Drive NW Calgary, T2N 1N4 Alberta, Canada

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 that the loss of brush border surface area, reduced disaccharidase activities, and increase 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 villous atrophy (Buret et al. 1991, 1992, Farthing 1993). Together these abnormalities lead to the malabsorption and maldigestion that ultimately cause diarrheal disease during giardiasis. Similar patho-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.

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+Corresponding author. E-mail: aburet@ucalgary.ca
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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 immunocompetent 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 giardiasis 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 orogastric 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

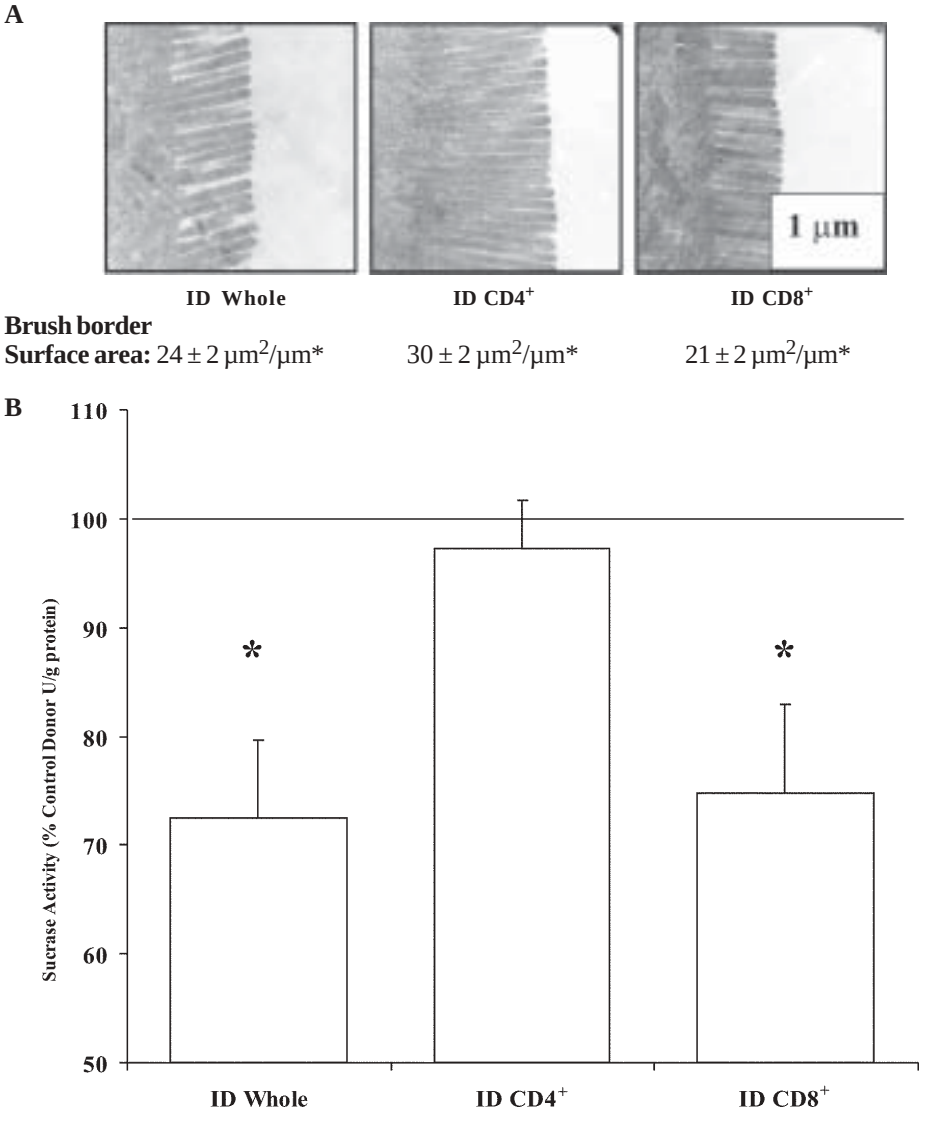
TABLE

Selected examples of *Giardia*-induced digestive enzyme deficiencies

Enzyme	Study model	Type of *Giardia* challenge
Brush border enzymes		
Maltase	Gerbils, mice, human patients, cattle, cells in vitro	Live parasites, soluble extracts
Sucrase	Gerbils, mice, humans	Live parasites, soluble extracts
Lactase	Gerbils, mice, humans, cattle, rats	Live parasites
Trehalase	Gerbils	Live parasites
Saccharase	Gerbils	Live parasites
Alkaline phosphatase	Gerbils, cells in vitro	Live parasites
Pancreatic enzymes		
Trypsin	Human patients, cells in vitro	Live parasites,soluble extracts
Chymotrypsin	Humans	Live parasites, soluble extracts

See the following references: Gupta et al. 1973, Chawla 1975, Ducombe et al. 1978, Gillon et al. 1982, Faubert 1988, Belosevic et al. 1989, Buret et al. 1991, 1992, Favennec et al. 1991, Katelaris et al. 1991, Cevallos et al. 1995, Mohammed et al. 1995, O'Handley et al. 2001.

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 lymphoctes 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. Morever, 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



Representative transmission electron micrographs (A, at same magnification) and sucrase activities (B) from jejunal tissues of naïve mice that received lymphocytes from *Giardia*-infected donor (ID) mice. Brush border surface areas (A) were calculated and expressed as $\mu m^2/\mu m^2$ of epithelial surface. Animals received either whole small mesenteric lymphnode (SMLN) lymphocyte populations (ID Whole), enriched SMLN $CD4^+$ T cells (ID $CD4^+$), or enriched SMLN CD8+ T cells (ID $CD8^+$) from infected donors. Sucrase activity is compared with the average activity measured in unmanipulated control tissues (solid line). Whole SMLN and $CD8^+$ but not $CD4^+$ T cells from infected animals significantly reduce brush border height, surface area, and disaccharidase activity; * $P < 0.05$ vs control, n = 8-12 per group (modified from Scott et al. 2000).

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 $\alpha_X\beta_2$ (Huleatt et al. 1995). The expression of this integrin has been linked to that of $\alpha_E\beta_7$, 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 $\alpha_E\beta_7$ 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 $\alpha_E\beta_7$, 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 microvillous 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 microvillous length (Mooseker et al. 1982). Recent observations also suggest that the rapid upregulation of microvillous 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 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

Argenzio RA, Liacos JA, Levy ML, Meuten DJ, Lecce JG, Powell DW 1990. Villous atrophy, crypt hyperplasia, cellular infiltration, and impaired glucose-NA absorption in enteric cryptosporidiosis of pigs. *Gastroenterology 98:* 1129-1140.

Banderia A, Mota-Santos T, Itohara S, Degermann S, Heusser C, Tonegawa S, Coutinho A 1990. Localization of $\gamma\delta$ T cells to the intestinal epithelium is independent of normal microbial colonization. *J Exp Med 172:* 239-244.

Belosevic M, Faubert GM, MacLean JD 1989. Disaccharidase activity in the small intestine of gerbils (*Meriones unguiculatus*) during primary and challenge infections with *Giardia lamblia*. *Gut 30:* 1213-1219.

Buret A, Gall DG, Olson ME 1991. Growth, activities of enzymes in the small intestine, and ultrastructure of microvillous border in gerbils infected with *Giardia duodenalis*. *Parasitol Res 77:* 109-114.

Buret A, Hardin JA, Olson ME, Gall DG 1992. Pathophysiology of small intestinal malabsorption in gerbils infected with *Giardia lamblia*. *Gastroenterology 103:* 506-513.

Buret A, O'Loughlin O, Curtis G, Gall D 1990. Effect of acute *Yersinia enterocolitica* infection on small intestinal ultrastructure. *Gastroenterology 98:* 1401-1407.

Buret AG, Gall DG, Olson ME 1991. Growth, activities of enzymes in the small intestine, and ultrastructure of microvillous border in gerbils infected with *Giardia duodenalis*. *Parasitol Res 77:* 109-114.

Buret AG, Olson ME, Gall DG, Hardin JA 1998. Effects of orally administered epidermal growth factor on enteropthogenic *Escherichia coli* infection in rabbits. *Infect Immun 66:* 4917-4923.

Buschmann RJ 1983 Morphometry of the small intestinal enterocytes of the fasted rat and the effects of colchicine. *Cell Tiss Res 231:* 289-299.

Butcher EC, Picker LJ 1996 Lymphocyte homing and homeostasis. *Science 272:* 60-66.

Cevallos A, Carnaby S, Farthing MJ 1995. Small intestinal injury in a neonatal rat model of giardiasis is strain dependent. *Gastroenterology 109*: 766-773.

Cevallos AM, Farthing MJG 1992. Parasitic infections of the gastrointestinal tract. *Curr Opin Gastroenter 8:* 101-109.

Chawla LS, Sehgal AK, Broor SL, Verma RS, Chuttani PN 1975. Tryptic activity in the duodenal aspirate following a standard test meal in giardiasis. *Scand J Gastroenterol 10*: 445-447.

Chung BM, Wong JK, Hardin JA, Gall DG 1999. Role of actin in EGF-induced alterations in enterocyte SGLT1 expression. *Am J Phys 276:* G463-G469.

Corazza N, Muller S, Brunner T, Kagi D, Mueller C 2000. Differential contribution of Fas- and perforin-mediated mechanisms to the cell-mediated cytotoxic activity of naive and in vivo-primed intestinal intraepithelial lymphocytes. *J Immunol 164:* 398-403.

Curtis G, Patrick M, Catto-Smith A, Gall D 1990. Intestinal anaphylaxis in the rat: effect of chronic antigen exposure. *Gastroenterology 98*: 1558-1566.

da Cunha Ferreira R, Forsyth LE, Richman PI, Wells C, Spencer J, MacDonald TT 1990. Changes in the rate of crypt epithelial cell proliferation and mucosal morphology induced by a T-cell-mediated response in human small intestine. *Gastroenterology 98*: 1255-1263.

Duncombe VM, Bolin TD, Davis AE, Cummins AG, Crouch RL 1978. Histopathology in giardiasis: a correlation with diarrhea. *Aust NZ J Med 8:* 392-396.

Dvorak A 1988. Ultrastructural pathology of Crohn's disease. In H Goebell, B Peskar, H Malchow (eds), *Inflammatory Bowel Diseases - Basic Research and Clinical Implications*, MTP, Lancaster, England, p. 3-42.

Farthing M 1997. The molecular pathogenesis of giardiasis. *J Pediatr Gastroenterol Nutr 24*: 79-88.

Farthing MJG 1993. Diarrhoeal disease: current concepts and future challenges. *Trans R Soc Trop Med Hyg 87:* 17-21.

Farthing MJG 1994. Giardiasis as a disease. In RCA Thompson, JA Reynoldson, AL Lymberry (eds), *Giardia: from Molecules to Disease*, CAB International, Wallingford, Oxon UK, p. 15-39.

Faubert G 1988. *Giardia* and giardiasis. *Bull Can Soc Zool 19:* 6-8.

Favennec L, Magne D, Gobert J-G 1991. Cytopathogenic effect of *Giardia intestinalis* in vitro. *Parasitol Today 7*: 141.

Feng Y, Wang D, Yuan R, Parker CM, Farber DL, Hadley GA 2002. CD 103 expression is required for destruction of pancreatic islet allografts by $CD8^+$ T cells. *J Exp Med 196*: 877-886.

Ferguson A 1976. Models of intestinal hypersensitivity. *Clin Gastroenterol 5*: 271-288.

Finlay BB, Cossart P 1997. Exploitation of mammalian host cell functions by bacterial pathogens. *Science 276*: 718-725.

Gillon J, Thamery DA, Ferguson A 1982. Features of small intestinal pathology (epithelial cell kinetics, intraepithelial lymphocytes, disaccharidases) in a primary *Giardia muris* infection. *Gut 23*: 498-506.

Gunasekaran TS, Hassall E 1992. Giardiasis mimicking inflammatory bowel disease. *J Pediat 120*: 424-426.

Gupta RK, Mehta S 1973. Giardiasis in children: a study of pancreatic functions. *Ind J Med Res 61*: 743-748.

Heyworth MF, Carlson JR, Ermak TH 1987. Clearance of *Giardia muris* infection requires helper/inducer T lymphocytes. *J Exp Med 165*: 1743-1748.

Heyworth MF, Owen RL, Jones AL 1985. Comparison of leukocytes obtained from the intestinal lumen of *Giardia*-infected immunocompetent mice and nude mice. *Gastroenterology 89*: 1360-1365.

Holmes R, Lobley RW 1989. Intestinal brush border revisited. *Gut 30*: 1667-1678.

Honma J, Mitomi H, Murakami K, Igarashi M, Saigenji K, Toyama K 2001. Nodular duodenitis involving $CD8^+$ cell infiltration in patients with ulcerative colitis. *Hepatogastroenterology 48*: 1604-1610.

Huleatt JW, LeFrancois L 1995. Antigen-driven indcution of CD11 on intestinal IEL and $CD8^+$ T cells. *J Immunol 154*: 5684-5693.

Kagnoff MF 1998. Current concepts in mucosal immunity III. Ontogeny and function of gd T cells in the intestine. *Am J Physiol 274*: G455-G458.

Katelaris PH, Seow FS, Ngu MC 1991. Inhibition of trypsin by *Giardia lamblia* in vitro. *Gastroenterology 100*: A220 (abstract).

Koudela B, Vitovec J 1998. Experimental giardiasis in goat kids. *Vet Parasitol 74*: 9-18.

LeCount T, Gray R 1972. Transient shortening of microvilli induced by cycloheximide in the duodenal epithelium of the chicken. *J Cell Biol 53*: 601-605.

Lionetti P, Breese E, Braegger CP, Murch SH, Taylor J, MacDonald TT 1993. T-cell activation can induce either mucosal destruction or adaptation in cultured human fetal small intestine. *Gastroenterology 105:* 373-381.

Melgar S, Bas A, Hammarstrom S, Hammarstrom ML 2002. Human small intestinal mucosa harbours a small population of cytolytically active $CD8^+$ alphabeta T lymphocytes. *Immunology 106*: 476-485.

Mohammed SR, Faubert GM 1995. Purification of a fraction of *Giardia lamblia* trophozoites extract associated with disaccharidase deficiencies in immune Mongolian gerbils (*M. unguiculatus*). *Parasite 2*: 31-39.

Mooseker M, Pollard T, Wharton K 1982. Nucleated polymerization of actin from the membrane associated ends of microvillar filaments in the intestinal brush border. *J Cell Biol 85*: 223-233.

Nussler NC, Stange B, Hoffman RA, Schraut WH, Bauer AJ, Neuhaus P 2000. Enhanced cytolytic activity of intestinal intraepithelial lymphocytes in patients with Crohn's disease. *Langenbecks Arch Surg 385*: 218-224.

O'Handley R, Buret AG, McAllister TA, Jelinski M, Olson ME 2001. Giardiasis in dairy calves: Effects of fenbendazole treatment on intestinal structure and function. *Int J Parasitol 31*: 73-79.

Oberhuber GH, Vogelsang M, Stolte S, Muthenthaler AJ, Kummer T, Radaszkiewicz T 1996. Evidence that intestinal intraepithelial lymphocytes are activated cytotoxic T cells in Celiac disease but not giardiasis. *Am J Pathol 148*: 1351-1357.

Roberts-Thomson IC, Mitchell GF 1978. Giardiasis in mice. I. Prolonged infections in certain mouse strains and hypothymic (nude) mice. *Gastroenterology 75*: 42-46.

Rocha B, Vasalli P, Guy-Grand D 1992. The extrathymic T-cell development pathway. *Immunol Today 13*: 449-454.

Rubin W, Ross LL, Sleisenger MH, Weser E 1966. An electron microscopic study of adult celiac disease. *Lab Invest 1*5: 1720-1747.

Schofield CJ 1985. Parasitology today: an ambitious project. *Parasitol Today 1*: 2.

Scott KGE, Logan MR, Klammer GM, Teoh DA, Buret AG 2000. Jejunal brush border microvillous alterations in *Giardia-muris*-infected mice: role of T lymphocytes and interleukin-6. *Infect Immun 68*: 3412-3418.

Scott KGE, Meddings JB, Kirk DR, Lees-miller SP, Buret AG 2002. Intestinal infection with *Giardia* spp. reduces epithelial barrier function in a myosin light chain kinase-dependent fashion. *Gastroenterology 123*: 1179-1190.

Scott KGE, Yu LCH, Buret AG 2004. Role of $CD8^+$ and $CD4^+$ T lymphocytes in jejunal mucosal injury during murine giardiasis. *Infect Immun 72*: 3536-3542.

Shibahara T, Si-Tahar M, Madara JL 2000 Adhesion molecules expressed on homing lymphocytes in model intestinal epithelia. *Gastroenterology 118*: 289-298.

Shibahara T, Wilcox JN, Couse T, Madara JL 2001. Characterization of epithelial chemoattractant for human intestinal IEL. *Gastroenterology 120*: 60-70.

Singer SM, Nash TE 2000. T-cell-dependent control of acute *Giardia lamblia* infections in mice. *Infect Immun 68*: 170-175.

Teoh DA, Kamieniecki D, Pang G, Buret AG 2000. *Giardia lamblia* rearranges F-actin and α-actinin in human colonic and duodenal monolayers, and reduces transepithelial electrical resistance. *J Parasitol 86*: 800-806.

Vinayak VK, Khanna R, Kum K 1991. Kinetics of intraepithelium and lamina propria lymphocyte responses during *Giardia lamblia* infection in mice. *Microbial Pathogen 10*: 343-350.

Wakelin D 1997. Immune responses to intestinal parasites: protection, pathology and prophylaxis. *Parasitologia 39*: 269-274.

Wolfe MS 1992. Giardiasis. *Clin Microbiol Rev 5*: 93-100.