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Jejunal Brush Border Microvillous Alterations in *Giardia muris*-Infected Mice: Role of T Lymphocytes and Interleukin-6

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Intestinal colonization with the protozoan *Giardia* causes diffuse brush border microvillous alterations and disaccharidase deficiencies, which in turn are responsible for intestinal malabsorption and maldigestion. The role of T cells and/or cytokines in the pathogenesis of *Giardia*-induced microvillous injury remains unclear. The aim of this study was to assess the role of T cells and interleukin-6 (IL-6) in the brush border pathophysiology of acute murine giardiasis in vivo. Athymic nude (nu^-/nu^-) CD-1 mice and isogenic immunocompetent (nu^+/nu^+) CD-1 mice (4 weeks old) received an axenic *Giardia muris* trophozoite inoculum or vehicle (control) via orogastric gavage. Weight gain and food intake were assessed daily. On day 6, segments of jejunum were assessed for parasite load, brush border ultrastructure, IL-6 content, maltase and sucrase activities, villus-crypt architecture, and intraepithelial lymphocyte (IEL) infiltration. Despite similar parasitic loads on day 6, infected immunocompetent animals, but not infected nude mice, showed a diffuse loss of brush border microvillous surface area, which was correlated with a significant reduction in maltase and sucrase activities and a decrease in jejunal IL-6 concentration. In both athymic control and infected mice, jejunal brush border surface area and disaccharidases were high, but levels of tissue IL-6 were low and comparable to the concentration measured in immunocompetent infected animals. In both immunocompetent and nude mice, infection caused a small but significant increase in the numbers of IELs. These findings suggest that the enterocyte brush border injury and malfunction seen in giardiasis is, at least in part, mediated by thymus-derived T lymphocytes and that suppressed jejunal IL-6 does not necessarily accompany microvillous shortening.

Intestinal infection with *Giardia* spp. decreases electrolyte, glucose, and fluid absorption and causes disaccharidase deficiencies at least in part via diffuse brush border microvillus shortening (7, 8, 19). Together, these abnormalities lead to malabsorption and maldigestion. Similar pathophysiology, including loss of brush border surface area, is observed in bacterial enteritis (9, 10), chronic food anaphylaxis (14), celiac disease (47), and Crohn's disease (18). Moreover, giardiasis has been reported to mimic inflammatory bowel disease in humans (25). These similarities make experimental giardiasis a useful model for the study of the pathogenesis of epithelial brush border injury. The fact that some of the above-mentioned disorders do not involve colonization by a microbial pathogen suggests that host immune factors may be involved in pathogenesis.

Gut-associated lymphoid tissue-derived immunity is necessary to clear *Giardia* infections from the gut (12), and unlike immunocompetent mice, nude athymic (nu^-/nu^-) mice which are infected with *Giardia muris* fail to clear the infection and develop chronic giardiasis (45). Immunocompetent mice also become immune to reinfection, 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 (45). Villus atrophy can also be caused by activated T cells in the absence of *Giardia* infection (19), and T lymphocytes have been implicated in the manifestation of villous atrophy during other disorders (15, 22, 34). Together, these observations imply a protective function for T cells, as well as a role for T cells in the pathogenesis of intestinal villous injury. The role of T lymphocytes and their cytokines in the pathogenesis of the injury to brush border microvilli rather than villi remains unknown, and yet it is the ultrastructural changes of the latter that represent the limiting factor for absorption and digestion in a number of intestinal disorders (8, 14, 19).

Interleukin-6 (IL-6) is a prominent cytokine of the Th2 subset which is commonly associated with antibody-dependent immunity, as opposed to the leukocyte infiltration observed during Th1-dependent immunity. IL-6 is produced by a large number of cell types, including epithelial enterocytes, Th2 lymphocytes, monocytes, and macrophages (26, 31, 35, 42). Among its pleiotropic effects, IL-6 induces B-cell differentiation, immunoglobulin synthesis, T-cell proliferation, and enhancement of immunoglobulin A (IgA) responses (35, 42). IL-6 has been implicated as a possible pathogenic factor in diseases, such as Crohn's disease (39, 43). Previous studies have shown that excess IL-6 is associated with decreased activity of sucrase-isomaltase (57), a brush border enzyme which is also markedly decreased in patients with active Crohn's disease or giardiasis. Hosts infected with *Giardia* commonly mount a humoral immune response, and there is no noticeable granulocytic cell influx into the intestine during the acute phase of giardiasis although the immune response to *Giardia* unquestionably involves activation of T lymphocytes (20, 24, 27, 29, 49, 53, 54). The antibody response to *Giardia* is predominantly of the IgA and IgG isotypes (4, 27). The effects of giardiasis on intestinal IL-6 and the implications for pathogenesis have not been investigated.

The aims of this study were (i) to assess the role played by T cells in the pathogenesis of *Giardia*-induced changes in jejunal brush border microvillous architecture and disaccharidase activities and (ii) to characterize IL-6 production in association with these alterations.

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MATERIALS AND METHODS

Animal model. Specific pathogen-free CD-1 mice (nu^+/nu^+) were obtained from a colony maintained by the Life and Environmental Sciences Animal Resource Centre at the University of Calgary. Isogenic athymic (nude) nu^-/nu^- mice were obtained from Charles River Laboratories (Wilmington, Mass.). Female mice aged 4 weeks and weighing 17 to 20 g were used in all experiments. Animals were housed in pairs and were given water and fed commercial mouse diet ad libitum. Cages were covered with microisolator lids to ensure disease containment. Room environment was maintained at 22°C and 40% rH with 12-h photoperiods. Maintenance of animals and experimental procedures were carried out in accordance with guidelines of the Canadian Council on Animal Care.

Experimental protocol. Animals of each strain were either infected with axenic *Giardia muris* trophozoites (see below) or given vehicle alone (control). Mice of the infected groups received 2×10^5 trophozoites suspended in 0.2 ml of sterile phosphate-buffered saline (PBS) by orogastric gavage. Mice of sham-treated control groups received 0.2 ml of sterile PBS. Food intake by each pair of mice was measured daily, and mean daily food intake per animal was calculated. Mean individual weight gain was also calculated for each group, and the percent increase in weight relative to that on day zero was calculated. Animals were killed by cervical dislocation 6 days after inoculation. This time point occurs during the peak of intestinal colonization and reproduces mucosal abnormalities reported in previous studies (6). The jejunum, 1 to 10 cm distal to the ligament of Treitz, was excised and prepared as described below. Blood was collected via cardiac puncture.

Axenic isolation. As bacterial and/or viral contaminants of the inoculum may affect the outcome of these studies, *G. muris* trophozoites were axenized as described previously (51), with a few modifications. Briefly, 3 days after mice received *G. muris* trophozoites by orogastric gavage, the duodenum and proximal jejunum of the mouse were removed and opened longitudinally. The 15-cm gut segment was placed in a tube containing chilled, sterile TYI-S33 *Giardia* medium containing 3.0 mg of piperacillin (Piperacil; Cyanamid Canada, Montreal, Quebec, Canada) and vortexed for 30 s. Tube contents were filtered through sterile gauze and centrifuged, and the pellet was suspended in new sterile TYI-S33 *Giardia* medium containing piperacillin in polystyrene centrifuge tubes and incubated on an orbital shaker at 150 rpm and 37°C for 2 h to allow for trophozoite adherence to the walls of the tube. The medium was decanted, and trophozoites were detached by cold shock with chilled, sterile PBS and centrifuged at $800 \times g$ for 10 min at 4°C. Pure *Giardia* trophozoites were diluted to 2×10^5 trophozoites/ml for use in further experiments.

The axenic status of the trophozoite suspension was confirmed by plating serial dilutions of the inoculum on Columbia blood agar and MacConkey agar (Bacto; Difco Laboratories, Detroit, Mich.) and incubating for 48 h both aerobically and anaerobically at 37°C. In addition, serum of control and infected mice was collected 6 and 35 days postinoculation and sent to Charles River Laboratories for a complete assessment of viral titers (Mouse Assessment Plus service package).

Trophozoite quantification. A 1-cm segment of jejunum was removed, placed in 1 ml of chilled sterile PBS, and vortexed for 30 s to release trophozoites from the intestinal wall. Trophozoites were counted in a hemocytometer, and colonization was expressed as the number ($\log_{10}$) of trophozoites per centimeter of jejunum.

Transmission electron microscopy. Jejunal enterocyte ultrastructure was assessed with transmission electron microscopy. Intestinal segments (1 cm) taken 2 cm distal to the ligament of Treitz were removed and fixed in 5% glutaraldehyde overnight at 4°C. Samples were cut into 1-mm squares and postfixed in 1% OsO_4 for 2 h, dehydrated in ethanol, cleared with propylene oxide, and embedded in Spurr low-viscosity medium (Sigma, St. Louis, Mo.). Semithin sections were stained with basic toluidine blue for light microscopy to select midvillus areas for thin sectioning for electron microscopy. Sections (80 nm) were double stained with saturated uranyl acetate in 50% ethanol and 0.4% lead citrate (52). Sections were examined at 75 kV on a Hitachi H7000 transmission electron microscope. Micrographs of midvillus enterocyte apical membranes were obtained at the same magnification (×12,000), and the height, width, and density of microvilli were calculated. Surface area of the epithelial brush border was determined as described previously (7). To avoid observer bias, micrographs were coded and observations were recorded in a blind fashion. For each group, 14 to 21 micrographs were obtained from a total of three animals in each group and microvillus brush border surface area was calculated. Previous studies have demonstrated that *Giardia*-induced microvillus injury in the midvillus area is representative of the changes along the entire villus axis (7, 8).

Disaccharidase activity. As a number of studies have established that disaccharidase impairment represents a reliable marker of *Giardia*-induced mucosal injury, sucrase and maltase activities were measured. Jejunal segments (12 cm) were blotted dry, weighed, homogenized in 2.5 mM EDTA (4 ml/1 g of tissue), frozen in liquid nitrogen, and stored at −70°C until needed. Sucrase and maltase activities were determined as previously described by Dahlqvist (16) and Beloševic et al. (5) and expressed as units per gram of protein. Total protein content was determined by the Bradford protein assay (Bio-Rad, Mississauga, Ontario, Canada).

IL-6. IL-6 concentrations were measured in the jejunal homogenates (dilution of 1:4 in 2.5 mM EDTA), and in neat serum samples by using a commercial sandwich enzyme-linked immunosorbent assay sensitive to 5 pg/ml and specific to IL-6 (no detectable cross-reactivity with IL-1α, -1β, -2, -4, -5, -7, or -10, leukemia inhibitory factor (LIF), tumor necrosis factor alpha, granulocyte macrophage-colony-stimulating factor, and gamma interferon) (Intertest-6x; Genzyme, Cambridge, Mass.). Jejunal protein concentration was determined by the Bio-Rad Bradford protein assay, and tissue IL-6 was expressed as picograms per milligram of protein. Serum IL-6 concentration was expressed as picograms per milliliter.

Intestinal histology. Jejunal samples were processed for light microscopy. Samples were fixed in 10% neutral buffered formalin (pH 7.3) and embedded in paraffin wax, and sections were stained with hematoxylin and eosin. Crypts and villi were measured by using a calibrated micrometer, and intraepithelial lymphocytes (IELs) were counted along villus units. IELs were expressed as number per 100 epithelial enterocytes.

Statistical analysis. All values were expressed as mean ± standard error (SE). Means were compared by one-way analysis of variance and Tukey's compromise test for multiple comparison when applicable. Significance levels were established at P values of <0.05 and <0.01.

TABLE 1. Results from assessments of anti-viral titers in serum samples of control or *G. muris*-infected mice[a]

Viral agent	Result for:	
	Day 6	Day 35
Epizootic diarrhea of infant mice virus	–	–
Murine hepatitis virus	–	–
Mouse thymic virus	–	–
Sendai virus	–	–
Pulmonary virus in mice	–	–
Minute virus of mice	–	–
Mouse poliovirus	–	–
Reovirus	–	–
Lymphocytic choriomeningitis virus	–	–
Ectromelia virus	–	–
Pneumonitis virus	–	–
Polyomavirus	–	–
Mouse adenovirus	–	–
Mouse cytomegalovirus	–	–
Hanta virus	–	–
Mouse pneumonia virus	–	–

[a] Serum was taken with axenic trophozoite suspension at 6 and 35 days postinoculation. All samples were negative for cilia-associated respiratory bacillus and *Mycoplasma pulmonis*. For each group, $n = 3$.

RESULTS

Axenic status of inoculum and parasitic colonization. No bacterial growth was observed after 24 or 48 h when the inoculum was streaked onto Columbia blood or MacConkey agar and grown aerobically or anaerobically at 37°C (not shown). The presence of viral pathogens of control mice and mice infected with the inoculum assessed 6 and 35 days postinoculation is illustrated in Table 1.

All animals gavaged with a *G. muris* trophozoite inoculum became infected. Parasitic load in immunocompetent (5.99 $\log_{10} \pm 0.11 \log_{10}$ [mean ± SE] trophozoites/cm, $n = 31$) and athymic (6.24 $\log_{10} \pm 0.05 \log_{10}$ trophozoites/cm, $n = 14$) mice infected with 2×10^5 axenic *G. muris* trophozoites 6 days postinoculation were not significantly different. No trophozoites were found in any sham-treated control animals.

Clinical observations. Infection with *Giardia* spp. is known to affect weight gain and food intake in some cases, but not in others. As previous studies have demonstrated that gut epithelial ultrastructure and function may be affected by malnutrition (6), experiments assessed whether *G. muris* infection altered food intake in the present model. In the present study, daily food intake of immunocompetent infected (3.61 to 4.35 g/day) or athymic infected (2.40 to 3.23 g/day) animals was not significantly lower than that of their respective controls (immunocompetent control [3.23 to 3.85 g/day] or athymic control

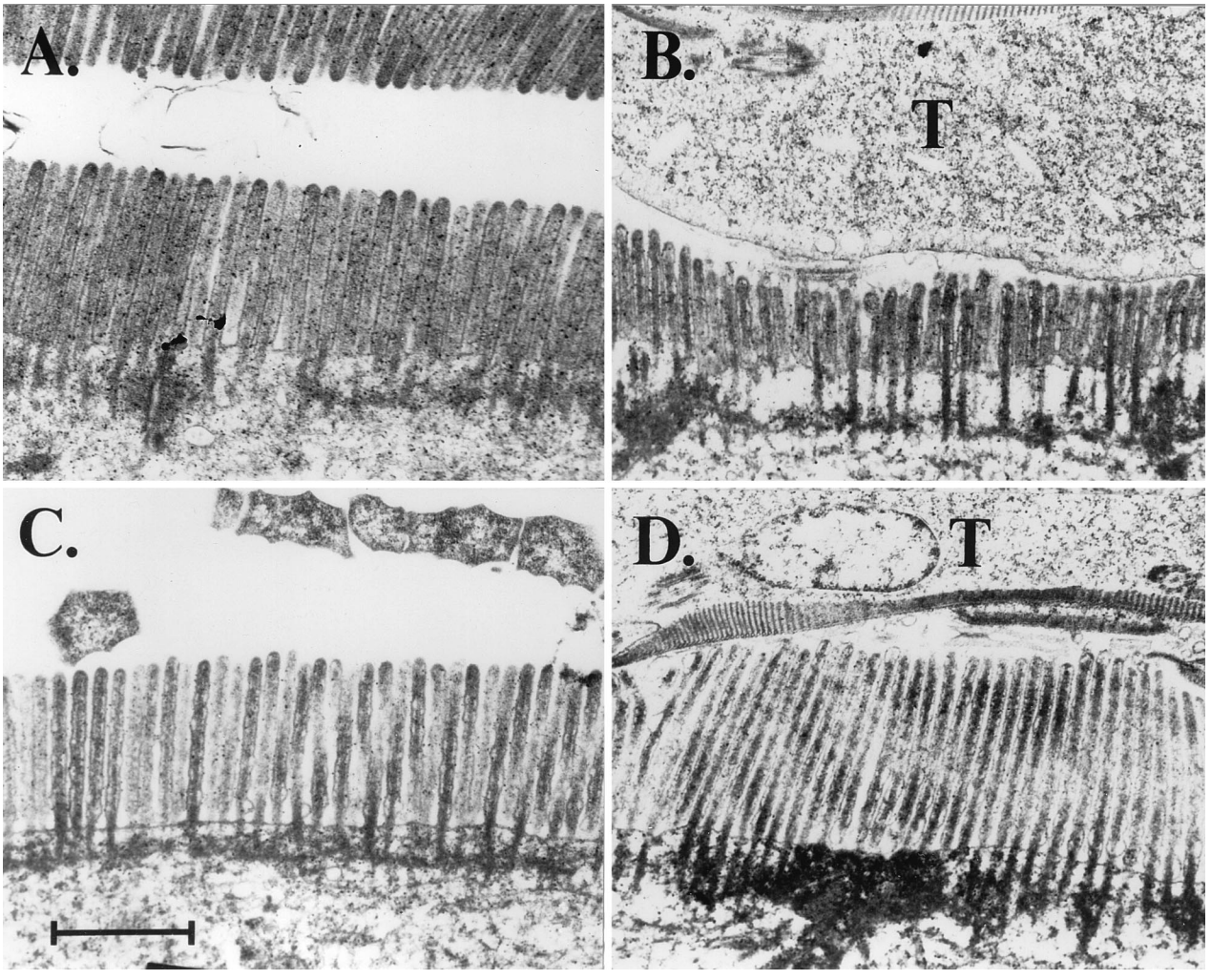

FIG. 1. Representative transmission electron micrographs, obtained at the same magnification, of the apical membrane of midvillus enterocytes from the jejunum of immunocompetent control (A), immunocompetent infected (B), athymic control (C), and athymic infected (D) mice 6 days postinoculation. Trophozoites (T) can be seen in two of the micrographs. Microvillous characteristics were consistent along the entire epithelium, at sites of trophozoite colonization as well as in other areas. Bar, 1 μm.

[2.60 to 3.48 g/day]) throughout the study. Similarly, weight gain was not significantly different between either infected group and its respective control group at any time during the study (data not shown).

Brush border ultrastructure. Brush border ultrastructure has been shown to correlate with enterocyte function in health and disease. Figure 1 presents representative transmission electron micrographs of enterocyte brush border membranes in each experimental group. In immunocompetent mice, but not in athymic animals, infection with *G. muris* caused a significant shortening of brush border microvilli along the entire epithelium, at sites of trophozoite attachment as well as in other areas (Fig. 1). Table 2 describes the different physical attributes of the microvilli and total brush border surface area

TABLE 2. Brush border microvillus characteristics of midvillus enterocytes in the jejunum of mice[a]

Test group	Characteristics of brush border microvillus			
	Height (μm)	Width (μm)	Density (no./μm^2 of epithelium)	Surface area (μm^2/μm^2 of epithelial surface)
Immunocompetent				
Control (n = 20)	1.061 ± 0.028	0.108 ± 0.0023	77.09 ± 4.77	28.21 ± 1.38
Infected (n = 21)	0.578 ± 0.039*	0.107 ± 0.0023	58.05 ± 5.25*	12.17 ± 1.32*
Athymic				
Control (n = 14)	1.141 ± 0.082	0.101 ± 0.0041	78.45 ± 4.72	29.14 ± 2.72
Infected (n = 21)	1.05 ± 0.057	0.107 ± 0.0019	74.29 ± 3.25	27.41 ± 1.95

[a] Measurements were made 6 days postinoculation. Values are means ± SE. *, P value of <0.01 when compared with respective control.

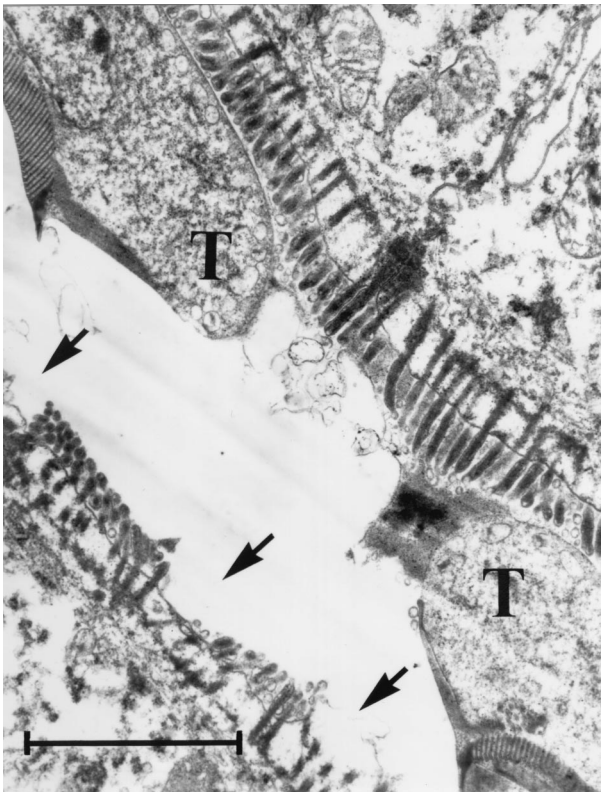

FIG. 2. Transmission electron micrograph of the jejunal epithelium from an infected immunocompetent mouse demonstrating localized microvillus effacement (arrowheads). Two trophozoites (T) can be seen in the micrograph. Bar, 2 μm.

for all groups. In immunocompetent mice, but not in athymic mice, infection caused a twofold decrease in microvillus height compared to that of controls, as well as a significant reduction in microvillus density per square micrometer of epithelium (Table 2). Localized effacement of epithelial microvilli in immunocompetent infected mice is illustrated in Fig. 2. The widths of individual microvilli were not different between any groups. Reduction of microvillus height and density in immunocompetent animals lead to a significant ($P < 0.01$) reduction of microvillous surface area per square micrometer of epithelial surface, which was not observed in athymic animals. Ultrastructural characteristics of jejunal microvilli in athymic infected mice were not different from those in their respective controls, nor were they different between control groups (Table 2).

Disaccharidase activity. As the results indicated that the *Giardia*-induced microvillous shortening is not observed in athymic animals, a functional marker needed to be assessed to confer physiological relevance to this observation. Disaccharidase activities were measured as a marker of epithelial digestive function. In immunocompetent mice, but not in athymic animals, infection caused a significant ($P < 0.01$) decrease in sucrase and maltase activities (Fig. 3). There was no significant difference in sucrase or maltase activities between athymic control mice and immunocompetent control mice (Fig. 3). Overall, the differences in disaccharidase activities between groups paralleled those measured in the ultrastructural characteristics of the brush border.

IL-6. To determine whether jejunal abnormalities associated with giardiasis involved changes in IL-6 production, IL-6 was

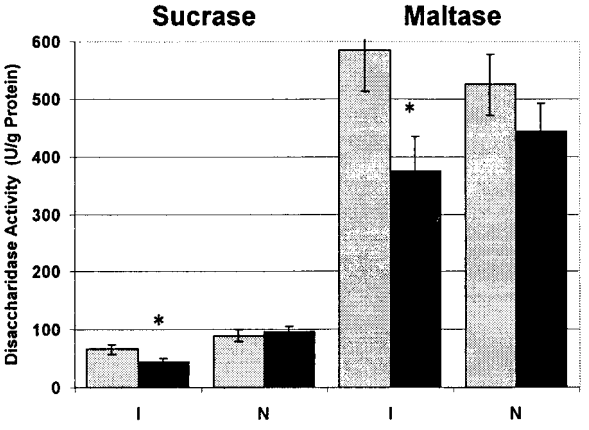

FIG. 3. Sucrase and maltase activities in the jejunum of immunocompetent (I) or athymic nude (N) mice. Animals were sham-treated controls (▨) or infected with *G. muris* (■) (n = 16 to 24 per group). Values are expressed as means (± SE) of units/gram of protein. *, P value of <0.05 versus paired control.

measured in tissue and serum samples. Infection with *G. muris* caused a significant ($P < 0.05$) decrease in the level of jejunal tissue IL-6 (Fig. 4A). This decrease was not seen in the jejunum of nude mice, and IL-6 concentrations found in both infected and control athymic mice were comparable to those in immunocompetent infected mice (Fig. 4A.). Serum IL-6 levels were assessed in order to determine mucosal versus systemic cytokine concentrations. There was no significant difference in the levels of serum IL-6 between any experimental groups (Fig. 4B).

Intestinal histology. While several host species have been shown to exhibit intestinal villous atrophy and crypt hyperplasia in response to giardiasis, clinical signs have also been reported in infected humans and rodents in the absence of villous atrophy (8, 19). In the present study, villus height was not significantly different between any group (Fig. 5). Crypt depth was increased in athymic infected mice compared with that in athymic controls ($P < 0.05$) but did not differ between the other groups (Fig. 5). Giardiasis is known to increase the

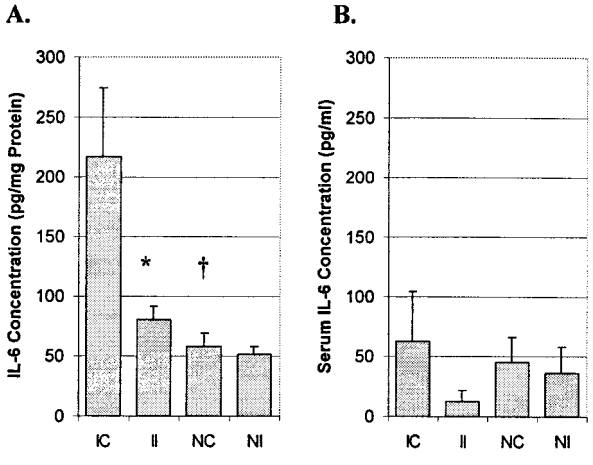

FIG. 4. IL-6 concentrations in jejunal homogenates (A) and serum (B) of immunocompetent control (IC, n = 14), immunocompetent infected (II, n = 14), athymic control (NC, n = 4), and athymic infected (NI, n = 9) mice 6 days postinoculation. Values are means (± SE) of picograms/milligram of protein (A) or picograms/milliliter (B). *, P value of <0.05 versus paired control; †, P value of <0.05 for nude control versus immunocompetent control.

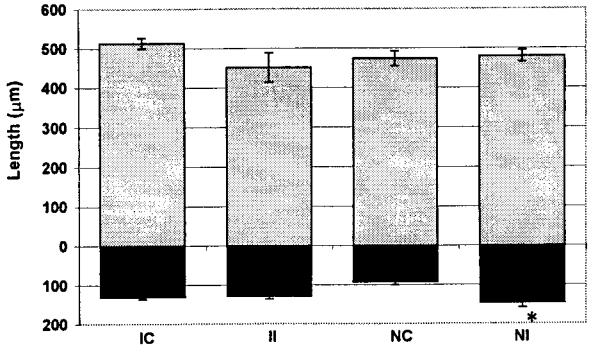

FIG. 5. Jejunal villus height (▨) and crypt depth (■) of immunocompetent control (IC, $n = 7$), immunocompetent infected (II, $n = 7$), athymic control (NC, $n = 4$), and athymic infected (NI, $n = 9$) mice 6 days postinoculation. 0 represents the villus-crypt junction. Values are expressed as mean (± SE) micrometers. *, P value of <0.05 versus paired control.

numbers of intraepithelial lymphocytes, particularly during the clearance phase of the infection. In an attempt to verify whether the changes of IL-6 concentration correlated with IEL infiltration, IELs were counted in all tissue samples. Upon infection, there was a small but significant increase in the number of IELs in both immunocompetent ($P < 0.05$) and nude ($P < 0.01$) animals (8.71 ± 0.49 [mean ± SE] and 4.08 ± 0.39 IELs/100 epithelial cells, respectively) when compared to their respective controls (10.93 ± 0.67 and 8.58 ± 0.34 IELs/100 epithelial cells for immunocompetent and athymic mice, respectively). IEL numbers were significantly ($P < 0.01$) lower in athymic control mice than in immunocompetent control mice.

DISCUSSION

Results from this study indicate that, in immunocompetent but not in T-cell-deficient animals, acute giardiasis causes a diffuse loss of epithelial brush border surface area, decreases sucrase and maltase activities, and reduces tissue IL-6 concentration. Inhibition of these changes in the jejunum of athymic mice was observed despite comparable parasitic loads 6 days postinoculation. The findings imply a role for thymus-derived T lymphocytes in the pathogenesis of epithelial microvillous injury and malfunction in giardiasis.

A number of laboratories, including our own, have established that orogastric inoculation of mice with *G. muris* cysts provides a reproducible model of giardiasis despite the difficulty in demonstrating diarrheal symptoms in the host (6, 37, 44). While the use of these models has significantly improved our understanding of the pathobiology of this parasite, the possibility of concurrent infections induced by inocula containing contaminated cysts has raised concerns in the recent scientific literature. Indeed, the presence of bacterial endosymbionts has been reported in *Giardia* cysts (21, 41) and these organisms may have *Shigella*-like characteristics. In addition, other observations have revealed that viral endosymbionts can be found within *Giardia* trophozoites (21). Therefore, in order to be able to attribute immunopathological factors to *Giardia* alone, and in an attempt to determine whether T lymphocytes are implicated in the pathogenesis of epithelial brush border injury during giardiasis, a new model, using an inoculum containing axenic (i.e., devoid of contaminating microorganisms) *G. muris* trophozoites, was developed and the absence of concurrent bacterial or viral inoculation was verified. The results suggest that the methods used in the present study allow for the preparation of an inoculum free of bacterial or viral contaminants and that mice infected with this inoculum develop a reproducible infection. In this model, infection causes mucosal abnormalities similar to those reported previously, including brush border injury and disaccharidase deficiencies. Consistent with previous observations (8), this study correlates mucosal disaccharidase deficiencies in giardiasis with a diffuse loss of epithelial brush border microvillous surface area rather than with villous atrophy. This injury to small intestinal microvilli has been previously reported for mice and Mongolian gerbils infected with *G. muris* and *Giardia lamblia*, respectively (6–8). The later studies have established that diffuse brush border abnormality is responsible for defective glucose-stimulated electrolyte, fluid, and solute absorption in giardiasis. A similar pathophysiological cascade has been reported in a number of other disorders, including cryptosporidiosis (2), Crohn's disease (18), bacterial enteritis (9, 10), celiac disease (47), and chronic intestinal anaphylaxis (14).

Findings from the present study indicate for the first time that enterocyte microvillous injury may be mediated by T lymphocytes. Indeed, results showed that infection caused a diffuse shortening of microvilli and reduced overall brush border surface area at sites of trophozoite attachment as well as in other areas only in immunocompetent animals. This injury was accompanied by decreased maltase and sucrase activities. In infected athymic mice, the absence of microvillous shortening was paralleled by disaccharidase activities similar to those measured in uninfected control animals. These observations further support the hypothesis that loss of brush border surface area represents a primary limiting factor in epithelial malfunction. Interestingly, electron microscopy revealed that brush border injury in infected immunocompetent mice also involved a reduction in microvillous density over the jejunal epithelial surface. These findings suggest that in addition to brush border shortening, under appropriate conditions, *Giardia* organisms are also capable of inflicting microvillous effacement on small intestinal enterocytes. Whether various degrees of severity in brush border injury during giardiasis may contribute to the broad range of clinical symptoms reported in this disease deserves further investigation.

Brush border microvilli contain a core of actin filaments which are anchored in the apical terminal web of enterocytes (30). Whether the T-lymphocyte-mediated brush border injury reported here correlates with reorganization of cytoskeletal actin in vivo has yet to be determined. Administration of cycloheximide (33) or colchicine (11) has been shown to cause a transient shortening of microvilli in vivo. Conversely, addition of actin monomers to membrane-associated ends of brush border microvillar filaments increases microvillous length (38), and recent observations suggest that the rapid upregulation of microvillous length by epidermal growth factor may implicate a redistribution of intracellular pools of membrane constituents via actin polymerization (13). In addition, bacterial pathogens, such as *Yersinia* or enteropathogenic *Escherichia coli*, are known to affect apical distribution of F actin in enterocytes (23), and intestinal infection with these microorganisms causes a diffuse shortening of brush border microvilli (9, 10). Together, these reports suggest that the alterations of brush border surface area may accompany reorganization of cytoskeletal actin. Interestingly, we recently demonstrated that *G. lamblia* rearranges F actin and α-actinin in human intestinal epithelia (50). 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.

Findings from the present study clearly illustrate that the

Giardia-induced loss of brush border surface area and subsequent disaccharidase impairment do not occur in the jejunum of athymic T-cell-deficient animals, hence implicating T lymphocytes in the pathogenesis of this injury. As jejunal trophozoite colonization was not different between immunocompetent and athymic mice in these experiments, the lack of microvillous injury cannot be attributed to differences in parasitic loads. The observations described here are consistent with previous reports suggesting that the severity of disaccharidase impairment in giardiasis may be related to the local host immune response to the parasite (5, 17). A number of studies have implicated $CD4^+$ T lymphocytes in the immune response to cryptosporidiosis (1, 56), and other reports have clearly established a role for T cells in the pathogenesis of Crohn's and celiac diseases (22, 32, 34). Together, with the results presented here, these observations suggest that the reduction of microvillous brush border surface area may represent a common host mucosal response to a variety of stimuli and that this response is mediated at least in part by thymus-derived T-lymphocytes. Additional studies are warranted to identify the source of these lymphocytes within the gut-associated lymphoid tissue and to determine which T lymphocyte subset they belong to.

Additional experiments investigated whether the T-cell-mediated epithelial injury and malfunction described in the present study were associated with changes in jejunal IL-6. The results demonstrate that infection with *G. muris* reduces mucosal IL-6 concentration in the jejunum of immunocompetent mice, while tissues of athymic mice show no change in IL-6 concentrations between infected and control animals. The decrease in IL-6 measured in jejunal homogenates of immunocompetent mice was tissue dependent rather than systemic, as there was no significant difference in serum IL-6 concentrations between any experimental groups. Together, the findings suggest that thymus derived T lymphocytes may suppress jejunal IL-6 in murine giardiasis rather than cause a Th2-like increase in mucosal IL-6. Jejunal tissues of athymic control mice also contained significantly less IL-6 than the jejunum of immunocompetent controls. This difference likely reflects the deficiency of IL-6-containing T cells in nude mice, as T lymphocytes are an important source of IL-6 (31, 42). As stimulated epithelial enterocytes are also known to synthesize IL-6 (36), additional experiments need to assess whether the T-cell-mediated IL-6 reduction seen in murine giardiasis reflects a decrease in IL-6 production by epithelial enterocytes. Regardless, the pathophysiological significance, if any, of intestinal IL-6 suppression in giardiasis remains questionable. Indeed, excess IL-6, not reduction of IL-6 as reported herein, is known to impair epithelial sucrase-isomaltase (57). Consistent with this observation, pathogenesis in Crohn's disease is associated with increased tissue IL-6 (48). Moreover, results from the present study show that lower levels of IL-6 in athymic control mice versus those in immunocompetent controls were not paralleled by reduced brush border surface area and disaccharidase impairment in these mice, further dissociating changed IL-6 concentrations from brush border injury.

Increased infiltration of intraepithelial lymphocytes has been associated with giardiasis in a number of reports (24, 40, 56). As the findings reported here implicate T lymphocytes in the brush border abnormalities caused by the disease, and as epithelial injury and malfunction were associated with reduced jejunal IL-6, additional experiments assessed whether these changes paralleled alterations in IEL infiltration in this model. The findings indicate that acute *G. muris* infection may be accompanied by a small, albeit significant, increase in IEL numbers. The increase could be observed in immunocompetent as well as in athymic animals, suggesting a role for extrathymic differentiation in this response. T lymphocytes are known to express the αβ T-cell receptor or the γδ T-cell receptor. αβ T lymphocytes primarily develop in the thymus and represent the majority of T cells in systemic and mucosal lymphoid tissues of mice (46). In contrast, γδ T cells largely differentiate extrathymically, are overrepresented in the intestine (making up approximately one-half of the IEL population of the murine intestine) and commonly express a $CD8^+$ cytotoxic phenotype (46). The αβ IEL population is markedly increased by the presence of microorganisms, while γδ IEL numbers are similar in germ-free and conventional mice (3). Conversely, intestinal disorders such as celiac disease are characterized by a striking increase in γδ IELs versus αβ T cells (32). Results from the present study revealed a much more subtle increase in IELs than that commonly seen in celiac disease. This observation is consistent with previous reports (28, 40) and may imply that unlike that of celiac disease, the pathophysiology of giardiasis is mediated by T cells other than cytotoxic γδ IELs. Whether this suggests a role for extraepithelial αβ T cells and/or suppressor $CD8^+$ IELs in the pathogenesis of giardiasis, which may be consistent with the *Giardia*-induced reduction of intestinal IL-6 reported in this study, needs to be further investigated. The latter hypothesis would be consistent with a recent report that showed that the acute phase of *G. lamblia* infection in mice is accompanied by an increase of intraepithelial and lamina propria T lymphocytes belonging to the $CD8^+$ subset (55). T-cell transfer studies using the model described herein will help in understanding how lymphocytes contribute to *Giardia*-induced epithelial injury.

In conclusion, results from this study indicate that in acute murine giardiasis, loss of brush border surface area, disaccharidase deficiencies, and decreased IL-6 in the jejunum are all abolished by T-cell deficiencies. These findings clearly implicate T cells in the pathogenesis of epithelial microvillous injury and malfunction during giardiasis. Therefore, while previous reports have shown that T lymphocytes could mediate small intestinal villous atrophy (15, 19, 22, 34, 45), findings from the present study indicate for the first time that activated T cells may also be responsible for the ultrastructural and functional injury to brush border microvilli associated with intestinal maldigestion and malabsorption.

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