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Host Immunity and Pathogen Strain Contribute to Intestinal Disaccharidase Impairment following Gut Infection

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Infection or other inflammatory insults in the small intestine often result in reduced disaccharidase enzyme levels. Using a mouse model of giardiasis, we examined the role of host immunity and pathogen virulence in mediating disaccharidase deficiency post-infection (p.i.). C57BL/6J mice were infected with two strains, WB and GS, of the human parasite *Giardia duodenalis*. The levels of sucrase, maltase, and lactase decreased in wild-type mice p.i. with the GS strain but not with the WB strain. Both CD4-deficient and SCID mice failed to eliminate the infection and did not exhibit disaccharidase deficiency. β2-Microglobulin knockout animals controlled infections similar to wild-type mice but exhibited no decrease in disaccharidase activity. Analysis of cytokine production by spleen and mesenteric lymph node cells showed production of IL-4, IL-10, IL-13, IL-17, IL-22, TNF-α, and IFN-γ p.i. with both WB and GS, with IFN-γ being the dominant cytokine for both parasite strains. Mesenteric lymph node cells produced lower levels of cytokines compared with splenocytes in response to parasite extract, although the overall pattern was similar. These data suggest that T cell responses mediate parasite clearance whereas also contributing to pathogenesis. They also demonstrate that differences in pathogen strain can also determine the outcome of infection and further our understanding of the clinical variation seen in human giardiasis. *The Journal of Immunology*, 2011, 187: 3769–3775.

The brush border (BB) lining the surface of the small intestinal epithelium provides the major interface for nutrient absorption. Reduction in the surface area of the microvilli is linked with impaired levels of disaccharidase enzymes such as sucrase and lactate that are essential for proper digestion and absorption of sugars (1). Several infectious agents, such as rotavirus (2) and HIV (3, 4), as well as noninfectious causes including celiac disease (5), iron deficiency (6, 7), and vitamin A deficiency (8) are reported to be linked with decreased intestinal disaccharidase activity. Reduced BB surface area has been documented in human and murine giardiasis (9, 10). Reduced disaccharidase activity has been previously reported in murine, human, and gerbil models of infection with *Giardia muris* and *Giardia duodenalis* (11, 12); infected gerbils exhibited symptomatic disease, and reduced BB enzymes were evident from day 10 postinfection (p.i.) and onward.

*G. duodenalis* has eight distinctive genotypes, of which only two genotypes (assemblages A and B) infect humans (13). Several reports have demonstrated variations in symptoms and pathology resulting from infections with different *Giardia* isolates. It is controversy surrounding the impact of a given assemblage of *G. duodenalis* (i.e., assemblage A or B) on symptomatic disease and intestinal pathology. For example, Singh et al. (14) showed that 74% of isolates examined from symptomatic patients in Nepal were assemblage B, whereas 20 and 6% were assemblage A and mixed infections, respectively. As such, a strong correlation was found between assemblage B and symptomatic disease, whereas all asymptomatic individuals harbored assemblage A (15). In contrast, different studies found assemblage A to be predominantly associated with symptomatic disease in Bangladesh (16). However, some reports did not find any correlation between the parasite genotype and symptomatic disease. For instance, Kohli et al. (17) did not find any differences in the ability of either assemblage A or B to cause symptomatic infections in children in Brazil. One possibility is that the differences observed reflect variation within these genotypic classifications, especially the presence or absence of specific virulence factors. Alternatively, given that host immune responses are, in part, responsible for pathology induced by *G. duodenalis*, it is possible that different isolates may elicit different immune effectors of the host immune responses in different ways (18).

Studies in vitro and in vivo showed differences in the ability of different *Giardia* isolates to induce apoptosis and mucosal damage, whereas other strains did not affect the gut integrity. For instance, the NF and S2 strains of *G. duodenalis* induced enterocyte (EC) apoptosis in cell culture, whereas other strains such as WB or PB did not (19). Likewise, studies in a neonatal rat model validated mucosal damage to be strain dependent (20). This may support the idea that the pathology observed in human giardiasis is, in part, strain dependent.

We performed this study to address to what extent host immunity and parasite strain mediate the development of disaccharidase impairment in a murine model of gut infection. Analyses showed that murine hosts that lack a functional adaptive immune response did not exhibit impaired enzymatic activity despite considerable parasite burdens and the host’s inability to resolve infections with strain GS. Also of interest was the finding that mice infected with the WB strain of the parasite did not manifest any sign of intestinal enzyme deficiency, although spleen cell cytokine production was similar p.i. with either strain.

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Abbreviations used in this article: BB, brush border; EC, enterocyte; β2m, β2-microglobulin; MLN, mesenteric lymph node; p.i., postinfection; WT, wild-type.

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Materials and Methods

**Mice**

Six- to 8-week-old female C57BL/6J, SCID (B6.CB17-PrkaatSiz2m/Siz2, B6.CD4Min,B6.129S2-Cd4Min mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were housed at the Georgetown University Animal Care Facility, and all experiments were carried out in accordance with guidelines approved by the Georgetown University Animal Care and Use Committee in compliance with the National Institutes of Health guidelines.

**Parasites and infection protocols**

The GSAm-83-H7 (ATCC 50581) and the WB clone-C6 (ATCC 50803) strains of *G. duodenalis* were used in these experiments. Parasites were axenically grown in TYI-S-33 media supplemented with adult bovine bile, t-cysteine, ascorbic acid, and antibiotics (all from Sigma-Aldrich, St. Louis, MO). Forty-eight hours before infection, mice were given antibiotics in drinking water ad libitum: neomycin oral solution (1.4 mg/ml; Durvet, Blue Spring, MO), ampicillin (1 mg/ml; Sigma-Aldrich), and vancomycin (1 mg/ml; Hospira, Lake Forest, IL) (21). Mice were gavaged with 10^6 trophozoites in 0.1 ml PBS (pH 7.4). Antibiotic use was maintained for the duration of the infection. To quantify parasite loads at different times p.i., we euthanized mice and discarded the first 3 cm of the small intestine (pylorus to ligament of Treitz). The next 2-cm section of the duodenum was removed, opened longitudinally, and minced in 4 ml ice-cold PBS (pH 7.4). Tissues were kept on ice for 15 min, and the numbers of trophozoites were counted using a hemocytometer.

**Disaccharidase activity assay**

Intestinal disaccharidase activity was measured using the method originally developed by Dahlqvist (22) with some modifications (23, 24). In brief, jejunal segments of ~10 cm in length were removed, and homogenates were prepared in Milli-Q water supplemented with protease inhibitor mixture III (Calbiochem, La Jolla, CA). Protein concentrations for each sample were determined by the Bradford method (Bio-Rad Laboratories, Hercules, CA) with BSA as standard. The relevant substrates (56 mM) were prepared in maleic buffer (0.1 M; pH 6.0) as previously described (25). After initial incubation of the intestinal lysates with relevant substrates for 1 h at 37°C, 300 μl Tris-glucose oxidase-peroxidase was added to each well. The samples were incubated at 37°C for another hour. The glucose release was measured at 450 nm using a microplate reader (Bio-Rad Microplate Instrument, Winooski, VT) with t-glucose as standard. Disaccharidase activity was expressed as nanomole glucose produced per milligram of total protein per minute.

**Ex vivo restimulation of splenocytes and mesenteric lymph node cells**

*G. duodenalis* trophozoites were axenically grown in TYI-S-33 media as described earlier. Confluent tissue flasks were kept on ice for 30 min, and detached trophozoites were washed five times with ice-cold PBS (pH 7.4). Whole-cell lysate was prepared using repeated freeze–thaw cycles (5×), stored at 80°C, and protein concentration of each sample was measured by the Bradford method (Bio-Rad Laboratories, Hercules, CA). Spleens and mesenteric lymph node (MLNs) from uninfected and infected mice were removed aseptically, and cells were isolated by mechanical disruption. RBCs were lysed in cold isotonic NH4Cl lysis buffer (155 mM NH4Cl, 10 mM KHCO3, and 100 mM EDTA, pH 7.4), and the remaining cells were washed twice with ice-cold PBS. A total of 5 × 10^6 cells were cultured in duplicate in 1 ml RPMI 1640 (Invitrogen) supplemented with 10% FBS (HyClone, Logan, UT), 1 mM glutamine (Life Technologies-BRL), 50 μM 2-ME, and 100 μg/ml antibiotics/antimycotics (Sigma-Aldrich). Cultures were seeded in 24-well plates (Corning Costar Corporation, Cambridge, MA), were stimulated with 100 ng/ml relevant *Giardia* extract, and were incubated at 37°C containing 5% CO2. Culture supernatants were harvested 48 h after stimulation and kept at −20°C until further analysis. In some experiments, anti-mouse CD4 FITC (clone RM4-5; BioLegend, San Diego, CA) was added to cultures at a concentration of 20 μg/ml to block activation of CD4+ T cells. Control wells received PBS alone.

**Cytokine analysis by ELISA**

Culture supernatants from splenocytes were diluted with an appropriate sample diluent, and cytokines were measured in duplicate by a sandwich ELISA method. Supernatants from MLN cultures were measured undiluted. Mouse ELISA kits for TNF-α, IFN-γ, and IL-10 (BD-OptEIA, San Diego, CA), IL-4 (SouthernBiotech, Birmingham, AL), and IL-13 and IL-22 (eBioscience, San Diego, CA), as well as IL-17 (DuoSet; R&D Systems, Minneapolis, MN) were used.

**Flow cytometry**

For flow cytometry, MLNs and spleens were collected in HBSS supplemented with 5% FBS (HyClone) and 25 mM HEPES and strained through a 70-μm nylon membrane (BD Falcon). Live/dead cell labeling was performed using a LIVE/DEAD Fixable Yellow Dead Cell Stain Kit (Invitrogen) for 45 min at 4°C in the dark according to the manufacturer’s instructions. Cells were incubated in HBSS supplemented with 5% FBS and 25 mM HEPES to reduce nonspecific binding. Cells (10^6/sample) were stained with anti–CD3-PerCP (clone 145-2C11), anti-CD4-PE (clone RM4-5), and anti–CD8-PE (clone 53-6.6; all from BioLegend) for 45 min at 4°C and were fixed with 1% paraformaldehyde. Cells were analyzed using a Becton Dickinson FACSAria (BD Biosciences) and FACS Express Version 4.0 software (DeNovo Software, Los Angeles, CA).

**Statistical analyses**

Data were analyzed using GraphPad Prism 5 software (GraphPad, San Diego, CA) and expressed as mean ± SEM. For statistical analyses, a two-tailed Mann–Whitney *U* test was used, and *p* < 0.05 was considered statistically significant.

**Results**

**CD8+ T cells are required for disaccharidase deficiency but not control of infection**

To investigate the role of different T cell subsets in control of infection and in mediating intestinal pathology, we used a murine model system. We infected adult mice with the GS strain of *G. duodenalis* (26). Wild-type (WT) mice and mice lacking all adaptive immune responses (SCID), CD4+ T cells only (CD4−/−), or CD8+ T cells (β-2m−/−) were infected and parasite loads were enumerated at days 5 and 18 p.i. Results showed that all mice were heavily infected at day 5, and that WT mice eliminated almost all parasites by day 18 p.i. (Fig. 1A). In SCID and CD4−/− mice, however, parasite loads were even higher at day 5 p.i. and significant parasite elimination was not seen by day 18 (Fig. 1B, 1D), indicating the vital role of host immunity in clearing this infection. In contrast, β-2m−/− mice eliminated parasites with essentially the same kinetics as WT mice (Fig. 1C), indicating that although CD4+ T cells are required to control this infection, CD8+ T cells are not.

Disaccharidase deficiency has been documented p.i. and after other inflammatory conditions (7, 27). We hypothesized that...

**FIGURE 1.** CD4+ T cells are required for the clearance of infections in a mouse model of disaccharidase deficiency. C57BL/6J (A), SCID (B), β-2m−/− (C), and CD4−/− (D) mice were infected on day 0 with the GS strain of *G. duodenalis*. Mice were euthanized on the indicated days p.i., and parasite loads in the small intestine were enumerated as described in Materials and Methods. Each bar indicates the mean ± SEM of four mice per time point. Data are representative of two independent experiments.
infection of WT C57BL/6J mice with *G. duodenalis* would result in disaccharidase deficiency as previously shown for the murine species of *Giardia, G. muris* (11). To test this hypothesis, we measured disaccharidase enzyme activity in the jejunum of infected mice. We found that the levels of sucrase, maltase, and lactase, decreased 40, 29, and 37%, respectively, on day 5 p.i. with the GS strain of the parasite (Fig. 2A–C). The decrease in trehalase levels was not statistically significant (Fig. 2D). All enzyme levels returned to normal by day 18 when the hosts had cleared the infections.

We next investigated whether host immunity could mediate the depression of intestinal enzyme activity after gut infections. To test this, we infected SCID mice with the GS strain of the parasite and measured disaccharidase activities in the jejunum. Although infection in SCID mice resulted in increased parasite burdens and failure to eliminate the infection by the host (Fig. 1B), no significant impairment of intestinal enzymes was observed (*p* < 0.05; Fig. 3). These findings suggest that disaccharidase impairment is mediated by host immunity.

Having established that disaccharidase impairment is immune mediated, we examined specific immune cell populations that could contribute to disaccharidase impairment during *Giardia* infection. In β2m<sup>−/−</sup> mice infected with *Giardia*, there were no significant changes in the levels of sucrase activity at day 5 p.i. (*p* < 0.05; Fig. 4A). Similarly, no significant changes were observed in the activity levels of maltase, lactase, and trehalase 5 d p.i. compared with uninfected animals (Fig. 4B–D). Results showed that the absence of CD4<sup>+</sup> T cells in infected mice did not induce lower enzymatic levels compared with uninfected controls (Fig. 4). These findings clearly suggest that the pathology seen in the intestine during the *Giardia* infections is T cell dependent.

Cytokine responses by spleen and MLN cells p.i.

Our results suggest that T cell responses are required for elimination of infection and induction of disaccharidase deficiency. We were therefore interested to determine whether the proportion of CD4<sup>+</sup> and CD8<sup>+</sup> T cells would change p.i. and to determine the cytokines being produced by T cells. Flow cytometry analysis showed that the composition of CD4<sup>+</sup> and CD8<sup>+</sup> T cells in the spleen and MLNs remained constant in mice infected with the GS strain of *G. duodenalis* 5 and 7 d p.i. compared with uninfected mice (Fig. 5A,5B). Analysis of cytokine production by cells from spleens and MLNs of mice infected with the GS strain showed that the peak of cytokine production occurred at day 7 p.i., and that the levels of cytokines produced by splenocytes were generally higher than the levels of cytokines produced by MLN cells (Fig. 5C–H). IFN-γ was the dominant cytokine produced by both splenocytes and MLN cells in response to *Giardia* infection.

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MLN cells in response to parasite Ags, followed by IL-10. Lesser amounts of TNF-α, IL-4, IL-17, and IL-22 were also detected.

Disaccharidase deficiency in WT mice is strain dependent

Human giardiasis can cause a wide range of clinical symptoms ranging from asymptomatic infections to severe diarrhea and cramps, which can result in maldigestion and stunted growth, especially in pediatric patients (1). It has been suggested that distinct assemblages of *G. duodenalis* differ in their capacity to cause symptomatic disease. We therefore asked whether the changes we observed in C57BL/6J mice infected with the GS strain of the parasite, the prototype for assemblage B, would occur during infection with the prototype strain for assemblage A. Because initial studies indicated that mouse infections with strain WB, the prototype for assemblage A, were unsuccessful (26), we developed a revised protocol for mouse infections to allow us to work with this strain. To determine the kinetics of infection and disaccharidase activity p.i. in mice with both the GS and WB strains, we infected WT mice with each strain

![Figure 5](http://www.jimmunol.org/)

**FIGURE 5.** Cytokine production by cells from spleens and MLNs of WT mice infected with the GS strain of *G. duodenalis*. WT mice were infected with the GS strain of *G. duodenalis* and euthanized at days 5 and 7 p.i. The proportions of CD4+ and CD8+ cells in spleens (A) and MLNs (B) were determined in uninfected and infected mice during the course of infection. Production of IFN-γ (C), TNF-α (D), IL-4 (E), IL-10 (F), IL-17 (G), and IL-22 (H) by spleens and MLNs of infected mice was determined using ELISA. Cell viability after 48 h of stimulation was ~50 and 70% in stimulated and unstimulated preparations, respectively. Data presented are means and SEM for four mice per time point.

![Figure 6](http://www.jimmunol.org/)

**FIGURE 6.** Infection and disaccharidase kinetics and cytokine production profile in WT mice infected with WB and GS strain of *G. duodenalis*. WT C57BL/6J mice were infected with 10⁶ trophozoites of either the WB or GS strain. Mice were euthanized at 5, 7, 10, 14, and 18 d p.i. Parasite burdens (A) and disaccharidase activities (B) were determined at each time point for both strains (A). Spleen cells were restimulated in vitro with *Giardia* extracts prepared from the same strain used for infection, and supernatants were collected after 48 h. Levels of IFN-γ (C), TNF-α (D), IL-4 (E), IL-10 (F), IL-13 (G), IL-17 (H), and IL-22 (I) were measured by ELISA. Intestines and splenocytes from individual mice were assayed in duplicate, and the data presented are means and SEM for four mice per time point (*p < 0.05, **p < 0.005, compared with uninfected mice). Data are representative of three independent experiments.
of *G. duodenalis* separately, and the infection and disaccharidase activity kinetics were measured at days 5, 7, 10, 14, and 18 p.i. Parasitological examinations revealed substantial colonization of the C57BL/6J mouse intestine by the WB strain of *G. duodenalis*. Although parasite burdens in the intestines of mice infected with the WB strain were generally slightly lower compared with mice infected with the GS strain of the parasite, the differences in parasite burdens between these strains were not statistically significant (Fig. 6A). In contrast, our analyses showed that the WB strain of *G. duodenalis* was not able to induce disaccharidase deficiency, whereas WT mice infected with the GS strain exhibited enzyme impairment p.i.; the reduced enzyme activity in the GS-infected mice started 5 d p.i., remained low 7 d p.i., and returned to normal levels by day 18 p.i. (Fig. 6B). These data suggest a strain-dependent basis for disaccharidase impairment.

**Cytokine production by T cells p.i.**

We next sought to determine whether T cell cytokine responses were significantly different p.i. between these two strains. Cytokine production by spleen cells from mice infected with each strain of the parasite was measured ex vivo at six different time points (i.e., days 0, 5, 7, 10, 14, 18). Our analysis showed that both strains of the parasite elicited robust cytokine production in response to parasite extract, with detectable levels of IFN-γ, TNF-α, IL-4, IL-10, IL-13, IL-17, and IL-22 being produced at day 7 p.i. with either strain (Fig. 6C–I). IFN-γ was the dominant cytokine secreted p.i. with both parasite strains. An average of 5000 and 2500 pg/ml was observed at 7 d p.i. in mice infected with the WB and GS strains, respectively (Fig. 6C). Interestingly, the levels of all cytokines assayed in mice infected with the WB strain of *G. duodenalis* were somewhat higher than those infected with the GS strain of the parasite.

**CD4+ cells are the main cytokine-secreting T cells in mice infected with WB and GS strains**

Considering the essential roles of CD4+ T cells in resolving the intestinal infection in our model, we were interested in finding to what extent CD4+ cells contributed to the production of cytokines. WT mice were infected with either WB or GS and euthanized on day 7. Parasite burdens were similar to what was observed in previous experiments (Fig. 7A). Splenocytes were stimulated in vitro with *Giardia* extract, and CD4+ responses were blocked with an anti-CD4 IgG. Analyses of supernatants showed that CD4+ T cells were the major cytokine-secreting cells p.i. with either strain WB or GS. Blocking CD4+ significantly reduced the production of IFN-γ, IL-4, IL-13, IL-17, and IL-22 (Fig. 7B, 7D, 7F–H). CD4+ T cell involvement in the production of TNF-α was only 30 and 34% for WB and GS strains, respectively (Fig. 7C), consistent with our previous findings that dendritic cells contributed to the secretion of TNF-α during infection with *G. duodenalis* (28). The production of IL-10 in mice infected with each strain was reduced drastically (87.5 and 37.5% for WB and GS strains, respectively), and it seemed that non-CD4 sources continued to secrete IL-10 in mice infected with the GS strain of the infection despite CD4 blockade (Fig. 7E).

**Discussion**

The major findings of this study are that levels of disaccharidase enzymes such as sucrase, maltase, and lactase decline significantly after murine infections with the GS strain of the human parasite *G. duodenalis*. We also showed that in the absence of adaptive immunity in SCID mice, there was no reduction of intestinal enzyme activity. Infections in CD4+− and β2m−− mice further showed that this impairment was an immune-based event requiring both CD4+ and CD8+ T cells. The results of this study also revealed an important difference in the ability of different pathogen strains to induce intestinal enzymatic deficiency in the mouse model of human giardiasis; C57BL/6J mice infected with the GS strain of the parasite displayed enzymatic deficiency, whereas mice infected with the WB strain of the parasite did not.

Similar to results from previous studies, our results indicate that CD4+ T cells are required for parasite elimination, whereas CD8+ T cells are not required. Interestingly, although β2m−− eliminate infections normally, they fail to exhibit disaccharidase deficiency. Scott et al. (10) showed that CD8+ T cells from MLNs of *G. muris*-infected mice could induce disaccharidase deficiency after adoptive transfer into nude recipients. Our results are consistent with an important role for CD8+ T cells in causing disaccharidase deficiency. Our results further suggest that CD4+ T cells have a role in inducing disaccharidase deficiency because no disaccharidase deficiency was seen in CD4+− mice. This role may be indirect, however; for example, CD4+ T cells may be necessary to generate an effector CD8+ T cell population. In summary, these results suggest that immunotherapies or vaccines that promote CD4+ T cell responses without generating CD8+ T cell responses could lead to protection without resulting in disaccharidase deficiency and contributing to nutrient malabsorption.

Different mechanisms have been regarded as the cause of disaccharidase deficiency p.i. and after other intestinal insults. These include direct damage to the epithelium caused by the parasite,
changes in gene expression within epithelial cells, and altered rates of EC maturation p.i. (1). Our results clearly exclude direct damage as a cause of disaccharidase deficiency in this model because no change in disaccharidase levels were seen in either SCID or CD4<sup>−/−</sup> mice despite having prolonged infections with very heavy parasite burdens. Although our data suggest that CD8<sup>+</sup> T cells are important in mediating pathology, they do not discriminate between specific changes in gene expression (i.e., reduced transcription of the sucrase/isoamylase or lactase/phlorizin hydrolase genes) within epithelial cells or more global changes in epithelial cell biology (i.e., an increased proportion of immature ECs). Indeed, changes in the maturation of epithelial cells can involve both transcriptional and posttranscriptional regulation of disaccharidase enzymes (8, 29).

Different isolates of G. duodenalis have been shown to differ in their abilities to trigger pathological changes in vitro. For example, some strains of the parasite (i.e., NF and S2) were able to induce apoptosis in a nontransformed epithelial cell line (SCBN), whereas other strains (i.e., WB and PB) could not (19). A more recent study showed that the WB strain could actually facilitate caspase-dependent apoptosis in the epithelial cell line HCT-8 (30). Consistent with previous reports describing important biological differences between WB and GS (18), we found that the induction of disaccharidase deficiency and the dynamics and kinetics of cytokine production in mice infected with each strain were distinct. Whether these differences in cytokine response are connected with the absence of disaccharidase deficiency p.i. with strain WB, however, remains to be determined.

To our knowledge, this study is the first to describe the cytokines produced by CD4<sup>+</sup> T cells in response to Giardia infection. Spleen cells and MLNs stimulated ex vivo with Giardia extracts produced IFN-γ, TNF-α, IL-4, IL-10, IL-13, IL-17, and IL-22. Blocking CD4<sup>+</sup> eliminated production of almost all of these cytokines, indicating that CD4<sup>+</sup> T cells are the likely source of these cytokines. The lone exception was TNF-α, which we have previously shown can be produced in small amounts by dendritic cells stimulated with Giardia extracts (28). Previous work in the G. muris model was unable to demonstrate cytokine production in response to parasite Ags, although some changes in mitogen-driven cytokine production were noted (31).

Among the cytokines produced, IFN-γ was the most abundant. However, previous work using GS infection in adult mice lacking IFN-γ suggested that parasite elimination could occur normally in the absence of this cytokine. In contrast, anti–IFN-γ treatment of C57BL/10 mice resulted in prolonged infections with G. muris (31). There may be differences in the effector mechanisms that are needed to eliminate the different pathogens. For example, NO, antimicrobial peptides, IgA, bile salts, and intestinal hypermotility have all been shown to participate in parasite clearance, and it is likely that significant redundancy results in the absence of strong effects after elimination of single cytokines or pathways.

Interestingly, WB-infected mice exhibited two distinct cytokine peaks, at 7 and 14 d p.i. Furthermore, all of the cytokines tested were produced at higher levels in mice infected with the WB strain of G. duodenalis than in mice infected with the GS strain. This may explain why mice infected with the WB strain of G. duodenalis showed consistently lower parasite burdens (Fig. 6A). Alternately, the differences in levels of cytokines produced could reflect different levels of immunogenicity associated with the two strains. In mice infected with either strain, marked cytokine production at day 7 p.i. seems to correlate with the peak of parasite burden within the small intestine. In mice infected with the GS strain of G. duodenalis, cytokine production by spleen T cells was observed at high levels on day 7 p.i. and at lower levels on day 10 p.i., whereas spleen cells from the WB-infected mice exhibited two distinct peaks of cytokine production. Potential explanations for the differences in cytokine production kinetics could be attributed to different patterns of antigenic variation between the strains or to different patterns of T cell migration into and out of the spleen p.i.

Disaccharidase deficiency can result from numerous situations such as intestinal infection or inflammation. Disaccharidase deficiency can contribute to diarrhea and nutrient malabsorption in these situations. Our results clearly indicate an important role for host T cell responses in contributing to this pathology. This study also marks important advances in our understanding of giardiasis. To our knowledge, it is the first report to directly show cytokine production by CD4<sup>+</sup> T cells p.i. It also establishes a model for direct, controlled comparisons among different parasite strains to examine their ability to stimulate immune responses and to reproduce aspects of the clinical syndrome seen in human disease.

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Disclosures
The authors have no financial interests of interest.

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