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The Intersection of Immune Responses, Microbiota and Pathogenesis in Giardiasis

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Abstract

Giardia lamblia is one of the most common infectious protozoans in the world. *Giardia* rarely causes severe life-threatening diarrhea, and may even have a slight protective effect in this regard, but it is a major contributor to malnutrition and growth faltering in children in the developing world. *Giardia* infection also appears to be a significant risk factor for post-infectious irritable bowel and chronic fatigue syndromes. In this review we highlight recent work focused on the impact of giardiasis and the mechanisms that contribute to the various outcomes of this infection, including changes in the composition of the microbiota, activation of immune responses, and immunopathology.

Keywords

Giardia; Giardiasis; Immunity; Microbiota; Immunopathology

THE IMPACT OF GIARDIASIS

Giardia lamblia (syn. *G. duodenalis*, *G. intestinalis*) is a protozoan parasite that infects both humans and other mammals through the ingestion of parasite-contaminated water or food [1, 2]. This parasite is divided into 8 genetic groups, termed assemblages A-H. Assemblages A and B are the main assemblages found to infect humans [2], although a recent report has described human infections with assemblage E [3]. The *Giardia* lifecycle has two distinct phases: a vegetative trophozoite and an infective cyst that is resistant to harsh environmental conditions. Cysts are transmitted via the fecal-oral route and an excystation process begins after reaching the stomach. Each cyst will release an excyzoite, which can generate four trophozoites following two rounds of division. The trophozoites will colonize the host's intestinal tract, particularly the upper intestine or duodenum and replicate via asexual binary fission. Eventually, some trophozoites initiate the encystation process, migrating towards the lower intestine where they are shed from the hosts to the outside environment as infective cysts [1].

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Human *Giardia* infections (giardiasis) are found globally, with prevalence ranging between 20–30% in the developing world and 3–7% in industrialized nations [4, 5]. Unfortunately, no protective vaccines are available for human use and drug therapy options have varying efficacies [6, 7]. Although *Giardia* infections typically resolve within a few weeks, infections may also last for several months as chronic infections.

Symptoms can present as diarrhea, cramps, nausea, intestinal malabsorption, and reduction in brush-border dissacharidases, though asymptomatic infections appear to be very common. Several reports have noted an association between *Giardia* infections and food sensitivities and allergies, possibly because food antigens may be able to translocate outside of the intestinal lumen during infection [8, 9]. Additionally, *Giardia* exposure may lead to increased prevalence of perceived food intolerance in humans [10], although the cause for this reaction was not identified. The concept of food antigen translocation is supported by various reports of increased intestinal epithelial barrier permeability during infection [5, 11–14]. The Global Enteric Multicenter Study (GEMS) reported that *Giardia* infections appear to reduce the incidence of severe diarrheal disease in children in the developing world [15]. The reason for this is not well understood, though it could be attributed to several factors including development of cross-protective immune responses, reduced inflammatory response or altered composition of the microbiota. Nevertheless, *Giardia* was found to be the 4th most common pathogen found in stools of children younger than 1 year of age and the second most common pathogen among children between 1 and 2 years of age [16]. In the latest report from the Interactions of Malnutrition and Enteric Infections: Consequences for Child Health and Development Project (MAL-ED) project, *Giardia* detection was associated with reduced height and weight measurements by children of age 2 [17], though stunting and wasting were reported to be somewhat variable between infection studies [18].

Giardia infections can also lead to post-infectious syndromes. Recent work has shown that irritable bowel syndrome (IBS) and chronic fatigue syndrome (CFS) develop years after this parasite has been eliminated [19]. Recently a potential mechanism for post-infectious IBS has been examined using a neonatal rat infection model. Infections led to visceral hypersensitivity in the jejunum and rectum of rats 50 days post infection and were associated with villus atrophy, crypt hyperplasia and increased immune cell numbers. This study also reported the translocation of commensal bacteria across the epithelial barrier and increased expression of neuronal c-*fos*, which is also associated with post-infectious IBS [20].

The aim of this review is to highlight recent work that focus on the impact of giardiasis on the intestinal microbiota and examine mechanisms that may contribute to the development of immune responses and the various outcomes of this infection (Figure 1). We will first discuss the interactions between *Giardia* and the intestinal microbiota, reviewing recent reports that describe shifts in microbial composition and the effects on infection outcomes. Next, we describe the role of immune cells and protective host mechanisms that aid in *Giardia* protection and provide several directions of future research. Lastly, the mechanisms behind *Giardia*-induced immunopathology are discussed.

GIARDIA INTERACTIONS WITH INTESTINAL COMMENSAL MICROBIOTA

There are rare (and controversial) instances of invasive *Giardia* infections [21], however, *Giardia* parasites are typically extracellular organisms that do not invade cells lining the small intestine and instead remain attached to the microvilli within the intestinal lumen where they proliferate [1]. As such, it is not surprising that interactions occur between this parasite and commensal intestinal microbiota. Singer and Nash first reported that infection outcomes in the adult mouse model of giardiasis were dependent on the source of animals [22]. Mice purchased from Taconic Farms were resistant to *Giardia* infections while mice of the same strain from Jackson Laboratories were susceptible. Treatment with antibiotics made mice from either vendor susceptible to infection, while co-housing animals made them all resistant, indicating that the composition of microbes within the gut determined susceptibility to this parasitic infection [22].

In addition to susceptibility to infection, intestinal microbiota may also affect pathogenesis [23]. A group in Brazil reported that duodenal microbes are able to stimulate *Giardia* pathogenicity. Conventional, germ-free, or germ-free mice that were reconstituted with duodenal microbes from five patients with symptomatic giardiasis were infected with *G. lamblia* trophozoites. Infected conventional mice showed the greatest signs of intestinal pathology among the three groups, with the reconstituted germ-free mice showing moderate pathology. Interestingly, infected germ-free mice did not develop intestinal pathology as compared to the other groups, suggesting a need for intestinal microbes to stimulate pathology [24]. Parasite genetics may also play a role in infection susceptibility and outcomes. Murine infections using the WB strain (assemblage A) of *Giardia* require the use of a pre-infection antibiotic treatment to render mice susceptible to infection, whereas the GS strain (assemblage B) of *Giardia* do not require antibiotic treatment and readily infect mice [25]. This report describes higher cytokines levels in mice infected with WB, when compared to GS-infected mice, and also the loss of disaccharidase impairment in WB-infected mice, though it is now known that intestinal microbiota are important for *Giardia*-induced disaccharidase deficiency *in vivo* [26].

Several recent studies have also highlighted the interactions between *Giardia* and intestinal microbes. Exposure of lab strains of *Escherichia coli* or human intestinal microbiota to *Giardia* was able to convert the bacteria to a toxic state that is deadly to *Caenorhabditis elegans* [27]. Moreover, the same group demonstrated that *Giardia* caused not only microbial dysbiosis in co-cultures with human mucosal microbial biofilms, but also increased virulence of commensal microbiota towards human epithelial cells *in vitro*; this report also described increased levels of inflammatory markers in humanized germ-free mice that were reconstituted with human intestinal microbes that had been exposed to *Giardia* products, but not the living parasite [28]. These *Giardia*-altered biofilms were capable of inducing immunopathology (intestinal permeability) that was dependent on microbial community disruption caused by *Giardia* secretory proteases. As nutrient malnourishment and *Giardia* infection in humans both likely contribute to decreased immune function [29], a recent report has examined the effects of protein malnourishment on murine *Giardia* infections. Mice fed a protein deficient diet presented with persistent infections, an alteration in the composition of duodenal microbes, and enhanced growth (weight) impairment when

infected with *Giardia* [30]. Taken together, these reports suggest that *Giardia* have significant interactions with the intestinal microbiota, which may lead to changes in infection outcome and immunopathology.

Non-invasive microbial probiotic therapies have been examined as a possible avenue to aid in the resolution of infections. It was found *in vitro* that the proliferative ability of the WB (assemblage A) strain of *Giardia* was inhibited when trophozoites were exposed to supernatants of *Lactobacillus johnsonii* culture medium [31] and confirmed *in vivo* in gerbils that evaded WB colonization when treated with *Lactobacilli* [32]. The cause for this toxicity to *Giardia* trophozoites was recently attributed to the generation of deconjugated bile salts produced by *L. johnsonii* [33]. Additionally, in mouse models of *G. lamblia* infections, probiotic administration of *Enterococcus faecium* and *Lactobacillus casei* both lessened parasite burdens and induced a stronger antibody immune response [34] and reduced parasite-mediated mucosal damage [35]. *Giardia*-mediated immunopathology may also be alleviated with probiotic treatments. *Lactobacillus rhamnosus* treatments in infected mice increased levels of anti-oxidants and brush-border disaccharidases, while decreasing levels of oxidants in the small intestine [36].

Since microbiota were shown to influence the outcome of infection, our lab and the Dawson group conducted the first culture-independent assessment of the intestinal microbiota during *Giardia* infections and found that infections influence both the architecture and abundance of the intestinal microbiota in the small intestine [37]. While this report emphasizes data using *Giardia*-infected mice that were pre-treated with antibiotics, and although antibiotic treatment itself causes a shift in the microbiota composition, the overall shift in microbial ecology was still present in infected mice that had not been treated with antibiotics. A decrease in the diversity and abundance of the obligate anaerobic *Firimicutes* taxa occurred, while an increase in the diversity of the taxa of aerobic *Rhodocyclaceae, Moraxellaceae*, *Flavobacteriales*, and *Comomonadaceae* was reported [37]. These enriched taxa are all considered to be metabolically flexible, and prosper with increased oxygen tension, lipid availability, and competition for arginine. Thus, *Giardia* colonization may lead to a metabolic shift among the intestinal microbial community, and this altered microbiota then could contribute to the symptoms present during giardiasis or aid in protection.

Much of what we know about the human intestinal microbiota comes from fecal samples, which limits identification of microbial composition in a specific region of the intestine. Furthermore, if biopsy samples are used, they were most likely extracted from patients with gastrointestinal diseases that may already contain perturbed microbiota communities. One study comparing the intestinal microbial composition of obese humans with normal weighted individuals described the dominance of *Firmicutes* and *Actinobacteria* in all samples taken from gastric tubes inserted in the gut [38]. However, this result must be further examined as the data was provided from 12 year-frozen samples and were taken from a study that was not designed to examine the intestinal microbiota composition [38]. The two latest studies identifying the taxa of duodenal microbiota during murine *Giardia* infections both use samples extracted from intestinal segments for sequencing rather than fecal samples [30, 37]. However, bacterial community analysis derived from fecal samples of non-antibiotic treated, infected mice were indistinguishable when compared to uninfected

control mice [30]. Accordingly, if possible, future microbial studies should use samples isolated from intestinal segments rather than fecal samples to provide accurate results.

PROTECTIVE IMMUNITY TOWARDS *GIARDIA*

Innate Immunity

Intestinal Epithelial Cells—During infections, the immune system's first line of defense is the natural surface barrier (i.e. skin and mucous membranes) (Figure 2). Intestinal epithelial cells (IECs) express pattern-recognition receptors (PRRs) that enable them to sense the environment within the intestines and are important in directing immune responses when pathogens are detected. In Toll-like receptor (TLR) signaling-deficient mice or antibiotic-treated mice, intestinal inflammation was severely exacerbated during dextran sulfate sodium (DSS) induced colitis, thus implicating TLRs and microbes in maintaining intestinal homeostasis [39]. However, we are unaware of any reports describing the phenotype of *Giardia* infected MyD88-deficient mice which lack signaling via TLRs. Aley *et al*. [40] were the first to report that antimicrobial peptides produced by specialized IECs, specifically the α-defensins cryptdin-2 and -3, reduced *Giardia* viability *in vitro* [40]. However, *in vivo* infection studies suggest redundant host mechanisms are active during colonization, as metalloproteinase-7 (MMP-7) deficient mice, which do not produce any active α-defensins, have only minor deficits in control of *Giardia muris* [41] or *G. lamblia* infections [42]. However, mice lacking both MMP-7 and inducible nitric oxide synthase (NOS2), exhibited a significant defect in infection control [42].

Nitric oxide (NO) is produced from L-arginine by the three forms of nitric oxide synthase (NOS): neuronal NOS (NOS1), inducible NOS (NOS2), and endothelial NOS (NOS3). NO has been reported to have inhibitory effects on *Giardia* growth, differentiation, encystation, and excystation *in vitro* [43]. However, arginine consumption by *Giardia in vitro* was able to reduce NO production by differentiated CaCo-2 IECs [44]. Furthermore, NOS2 is not uniquely required for parasite clearance *in vivo* [42]. Instead, NOS1 is more important than NOS2 and NOS3 for *Giardia* immunity as it contributes to parasite clearance and intestinal motility changes during infection [45, 46].

Several studies have examined IEC responses to *Giardia* and parasite mechanisms to subvert these responses. IECs may help initiate immune responses against *Giardia* through production of chemokines. Microarray analysis revealed that exposure of human colon carcinoma, CaCo2 cells, to *Giardia* resulted in increased expression of chemokines, including CXCL1-3, CCL2, and CCL20 [47]. In contrast, a cathepsin B-like cysteine protease secreted by *Giardia* can modulate immune responses by degrading CXCL8, a neutrophil attractant [48]. In addition to reducing NO production, consumption of arginine by *Giardia* was shown to reduce IEC proliferation [44], and infection reduced intestinal epithelial barrier function [11, 12, 14, 49]. It is reasonable to assume that this disruption is partly mediated by *Giardia* proteins and enzymes through excretory/secretory pathways [50]. One report suggested that parasite proteases aid in parasite adhesion to IECs in a rat intestinal cell culture model [51]. Additionally, brief *Giardia* exposure to human epithelial cell cultures also triggers the release of three enzymes that participate in host immunoregulation and *Giardia* metabolism: arginine deiminase (ADI), ornithine carbamoyl

transferase (OCT) and enolase [52]. Taken together, it is becoming increasingly clear that the interactions between host and parasite dictate colonization outcomes during infection and is a topic that should be studied further.

Complement—The first role for the complement system in protective *Giardia* immunity was described by Hill *et al*. [53] when trophozoites were exposed to human sera extracted from patients with no history of giardiasis. Parasite killing was greater in groups exposed to sera rather than controls, and killing was lost when complement components were blocked, suggesting parasite killing was complement-dependent. Furthermore, this killing was dependent on structures found on the parasite's surface, as trophozoites treated with neuraminidase or trypsin did not activate the complement pathway [53]. While this paper attributed complement activation to the alternative pathway, the lectin pathway had not yet been identified. The lectin pathway is initiated through the binding of mannose binding lectin (MBL), a C-type lectin, to carbohydrates such as mannose and N-acetylglucosamine (GlcNAc) that are commonly found on pathogens [54], including surface proteins of *Giardia* trophozoites [55, 56]. It was shown *in vitro* that MBL is able to bind to trophozoites and cause cell lysis through the complement system [57]. More recently, Li *et al*. [58] reported a delay in the clearance of *Giardia* parasites and recruitment of mast cells to the small intestine in mice lacking MBL or complement component C3a receptor (C3aR). Furthermore, mice lacking C3aR also exhibited significantly reduced T-cell responses against parasite antigens following infection but IgA levels within the small intestine were unaffected [58]. Taken together, these results indicate a role for the complement system in not only activating but also mediating downstream immune responses during infection.

Mast Cells—Mast cells have been found to play a significant role during the immune response towards *Giardia* and are recruited to the small intestine in significant numbers during infection [59, 60]. Both mast cell-deficient mice (c-kit$^{w/wv}$) and mice treated with c-kit blocking antibody failed to control *Giardia*. Furthermore, IgA production was also effected as c-kit-deficient mice failed to make parasite-specific IgA [60]. These cells have also been shown to contribute to pathological condition of intestinal hypermotility, which will be discussed in the immunopathology section.

Neutrophils and Eosinophils—Neutrophils are phagocytic cells that are often the first immune cells recruited to the site of infection. Currently, it is uncertain if neutrophils provide significant contributions to parasite clearance during *Giardia* infections. One report noted an increase in neutrophil numbers in the intestinal lamina propria during infection *in vivo*, due to an influx of microbes. However, the staining method used to detect neutrophils (chloroacetate esterase activity) may also identify mast cells [14]. Functionally, human peripheral blood neutrophils that were incubated *in vitro* with *Giardia* trophozoites and human hyperimmune serum increased production of reactive oxygen species (ROS) suggesting an oxidative response occurred [61]. Interestingly, this oxidative response decreased with the addition of anti-CR1 and anti-CR3 blocking antibody suggesting an important role for complement in this cellular response [61]. *Giardia* is capable of using NADH oxidase and NADH peroxidase to render ROS less harmful [62]. It is unclear if ROS

is effective in parasite killing *in vivo*, although a recent *in vitro* study reported that *Giardia* was susceptible to IEC-mediated ROS [63].

Several groups reported an increase in the number of eosinophils, granulocytes involved in host protection against parasites, during *Giardia* infection [29, 64,65]. Recurrent giardiasis has been associated to chronic eosinophilia [66], along with increased IL-5 concentrations within blood serum in humans [67]. One report described a critical role for eosinophils in the production of IgA in an IL-1β dependent manner [68]. Thus, during *Giardia* infections, it is possible that these cells are not only aiding in the production of IgA but also aiding the in the activation of other IL-1β-sensitive cells, such as group 3 innate lymphoid cells (ILC3s), Th17 cells, [69] or γδ T-cells [70].

Mononuclear Phagocytic Cells (Macrophages and Dendritic Cells)—Intestinal macrophages and dendritic cells are antigen presenting cells that are found throughout the intestine and are known for their phagocytic activity. These cells must be able to distinguish very rapidly between invasive pathogens or innocuous commensals or food, and are partly responsible for maintaining a balance between inflammation and tolerance within the gut. Recently, our lab has identified an increase in a population of intestinal macrophages, following infection, that expresses both NOS2 and arginase 1 (ARG1) [71]. While NOS2 is normally a marker for inflammatory M1 macrophages and ARG1 is a marker for alternatively activated M2 macrophages, expression of both is more similar to myeloid derived suppressor cells (MDSCs) associated with certain tumor microenvironments [72]. Recruitment of MDSCs in other infections is thought to reduce inflammatory responses and may contribute to the development of chronic infections [73–75]

Dendritic cells (DCs) are necessary for proper parasite clearance during infection. DCs have been shown to be a significant source of IL-6 during infection and IL-6 deficient mice have a defect in parasite control [76–78]. However, it is unclear how DCs recognize *Giardia* parasites directly and how DC activation contributes to development of adaptive responses against this parasite. One study, using recombinant *Giardia* immunoglobulin binding protein (BiP) suggests that DCs are activated by TLR4, but the relevance of this *in vivo* is unclear [79]. Total *Giardia* extract increased expression of the costimulatory molecules, CD80 and CD86, on murine bone marrow derived DCs, yet it did not invoke a strong cytokine response [80]. This weak cytokine response was also seen in human monocyte-derived DCs [81] exposed to *Giardia*. Interestingly, both studies [80, 81] showed that DC exposure to lipopolysaccharide (LPS) and *Giardia* resulted in an increase in IL-10 production; Kamda and Singer [80] further showed that blocking IL-10R and phosphoinositide-3 kinase (PI-3K) restores production of IL-12, which suggests that *Giardia* may activate PI-3K in DCs. This reduced production of IL-12 may restrict development of Th1 cells.

Adaptive Immunity

T cells—It is well established that $CD4^+$ T cells are essential for the clearance of *Giardia* since T cell-deficient mice cannot control infections, whereas B cell-deficient mice can eliminate parasites [82, 83]. T cells were found to provide distinct antibody-independent, but T cell-dependent, immune mechanisms that lead to elimination of the parasite [83]. $CD8^+$ T

cells are not required for parasite clearance, as β_2m-deficent mice which lack class I MHC expression and are devoid of $CD8^+$ T cells are able to rapidly control infection [83]; however, it appears that $CD8^+$ cells are required for reduction of intestinal disaccharidase activity [84], as will be discussed in the immunopathology section.

$CD4^+$ T cells typically specialize in the types of cytokines that they produce. For example, Th1 cells produce interferon (IFN)-γ, while Th2 cells produce IL-4, among others. Though both IFN-γ and IL-4 are increased in sera from humans infected with *Giardia* [67, 85], there is no substantial defect in parasite clearance when IFN-γ and IL-4-deficient female mice are infected [83]. It is becoming increasingly evident that IL-17, within the context of a *Giardia* infection, is essential for protective immunity [86]. This pro-inflammatory cytokine is produced by Th17 cells that require both IL-6 and TGF-β for their development. IL-6-deficent mice fail to clear infections and also lack IL-17 expression [87], which suggests Th17 cells are required for parasite clearance in an IL-6 dependent manner. Additionally, it would be interesting to understand if mast cell-derived IL-6 is critical to Th17 cell development. Li et al. [88] found a reduction in IL-17 responses in the absence of signaling via C3aR, which suggest that a decrease in mast cell recruitment also correlates with decreased IL-17 production. In human patients that have recovered from *Giardia*, IL-17 has been found to be upregulated when effector memory $CD4^+$ T-cell are restimulated with *Giardia* antigen [89]; in addition, several studies [25, 87, 90, 91] reported the upregulation of IL-17 during murine or bovine infections, and delayed parasite clearance in IL-17 knockout mice has also been reported [87, 90]. Moving forward it will be interesting to understand the effector mechanisms triggered by IL-17 and the signaling mechanisms leading to activation of IL-17 production during giardiasis. For example, are the activation signals for Th17 development dependent on direct recognition of parasite molecules or do defects in the epithelial barrier permit components of the intestinal microbiome to activate immune responses? Additionally, it would be important to understand if Th17 cells are involved in any immunopathology or long lasting post-infectious diseases such as, irritable bowel syndrome or chronic fatigue syndrome [19].

IgA—Humans with immunoglobulin deficiencies develop chronic giardiasis and it is well known that *Giardia* generate large amounts of IgA in response to infection [92–94]. As such, IgA-mediated protection has been examined to elucidate mechanisms that control parasite infections. B-cells within the intestinal lamina propria (LP) are responsible for the production of IgA – the most abundant antibody within mucosal secretions [95]. IECs transport IgA into the lumen using a polymeric immunoglobulin receptor (pIgR) where it can then bind to various microbial (commensal or pathogenic) surfaces to prevent adhesion to IECs [96]. Langford *et al.* [97]reported a defect in parasite clearance when IgA-deficient mice were infected with *G. muris*; furthermore plgR-deficient mice, which fail to transport IgA into the intestinal lumen, also developed chronic infections when infected with *G. muris* [97]. However, *G. lamblia* infections do not seem to be as sensitive to IgA as no difference in parasite clearance was detected in pIgR-deficient [98] or B-cell deficient mice [83]. Recently, a report linked IL-17 signaling to the transport of IgA into the lumen of the intestine, as fecal, but not serum, IgA diminished in infected IL-17-deficient mice [87]. This difference in sensitivity to IgA between *Giardia* species is not fully understood and may be

dependent on several factors such as antigenic surface variation, susceptibility to other antimicrobial factors (e.g., complement or NO), and *Giardia* infection kinetics (*G. muris* infections typically peak one to two weeks later than *G. lamblia* in adult mice).

IMMUNOPATHOLOGY

Mast Cells and Hypermotility

Severe cramping is a commonly reported symptom of giardiasis in humans. Similarly, infections with *Giardia* in mice lead to changes in intestinal motility [45], and subsequent reports linked enhanced smooth muscle contractility to mast cell responses [99]. The neurotransmitter cholecystokinin (CCK) was found to significantly increase muscle contractions when longitudinal muscle from infected mice was exposed to CCK *ex vivo*. This effect could be blocked with either the addition of ketotifen, which inhibits mast cell degranulation, or pre-treatment of tissues with compound 48/80, which depletes mast cells of their granule contents, indicating that CCK increases muscle contractility through mast cell degranulation. Enhanced muscle contractility in conjunction with NOS1-mediated muscle relaxation leads to the promotion of intestinal propulsion and hypermotility [99]. Hsu *et al.*, [100] also describe an increase in CCK levels in the colon of *Giardia* infected mice, although blockade of CCK receptors did not ameliorate the visceral hypersensitivity induced by infection [101]. In human *Giardia* infections, CCK was found to be elevated among a small number of patients with symptomatic giardiasis [102]. CCK is released by enteroendocrine cells located in the small intestine and signals the presence of fat in the lumen. This CCK normally leads to luminal bile release to aid in fat solubilization and absorption, although bile is readily used by *Giardia* trophozoites during growth. As such, parasite-mediated CCK release should be advantageous for the parasite during infection.

$CD8^+$ T-cells and Intestinal Malabsorption

Another hallmark symptom of giardiasis is the shortening of microvilli lining the gut, which results in a reduction in dissacharidase activity [103, 104]. This pathological disruption has been reported in both human infections and in animal infection models [105, 106] and leads to reduced nutrient absorption. Scott et al. [84] showed that athymic nude mice, lacking T cells, failed to exhibit microvilli shortening or reduced dissacharidase activity after infection. Moreover, adoptive transfers of $CD8^+$ T cells, but not $CD4^+$ T cells, from infected donors into uninfected nude mice produced both microvilli shortening and reduced dissacharidase activity [84]. Furthermore, neither infected $CD4^{-/-}$ nor $\beta_2m^{-/-}$ mice (which lack surface expression of major histocompatibility complex I (MHC I) and are deficient in $CD8^+$ T cells) exhibited reduced dissacharidase activity [25]. This is consistent with $CD8^+$ T cells mediating this pathology and $CD4^+$ T cells being important for development of the $CD8^+$ T cell response.

While $CD8^+$ T-cells are responsible for this immunopathology, events leading to the activation of these cells are not well understood. Recently, we demonstrated that this effect is dependent on intestinal microbiota as antibiotic treatments reduced activation of $CD8^+$ T cells and dissacharidase deficiency during infections [26]. Furthermore, this effect also appears to be *Giardia* strain dependent [25]. As *Giardia* can alter intestinal microbial

communities [37] it would be interesting to understand the link between intestinal microbial diversity, *Giardia*, and immune cells leading to subsequent immunopathology.

Malnutrition Cycle

Chronic giardiasis (recurring incidents of diarrhea) appears to be dependent on several factors that lead to a cyclical system. In low and middle-income countries, malnourishment and *Giardia* infections in humans both likely contribute to decreased immune function [29] and further nutrient malabsorption due to intestinal damage [107]. In a murine malnutrition model, protective *Giardia* cytokines and eosinophil recruitment were reduced in mice that were fed a low protein diet prior to infection with *Giardia* cysts [29]. Micronutrients appear to be important in *Giardia* protection as children with higher concentrations of vitamin A presented with fewer detectable parasites and zinc deficiency led to increased *Giardia* susceptibility [108]; additionally, vitamin A supplementation in Brazilian children decreased *Giardia* susceptibility [109].

Past studies examining an association between *Giardia* and human growth seem to vary [18, 110–114]. Yet in the past several years, it has become apparent that *Giardia* infections that occur early in childhood (earlier than age 2) are associated with reduced height and weight attainment [17, 115], with a critical window of preventing infection between birth and 6 months of life having the greatest impact on growth faltering [115]. This window may be critical due to the establishment and shaping of the composition of intestinal microbiota that provide essential amino acids and vitamins used for growth [116]. As such, these two recent studies suggest that the prevention of exposure to this parasite is more important in preventing long-term growth deficiencies rather than early intervention therapies, such as the use of metronidazole [117]. Moreover, the cause for the variation in studies examining the association between *Giardia* and growth development may be due to the presence (or absence) of very early childhood *Giardia* infections since acquired childhood protection may only limit the severity of giardiasis rather than provide full protection [115].

Concluding Remarks: Looking Ahead

Future research highlighting the interaction between the intestinal microbiota and immune system will be of great importance to the field of *Giardia* pathogenesis (see Outstanding Questions). Several studies have identified mechanisms whereby intestinal bacteria can influence the development of Th17 and Treg immune responses [118][119][120][121]. Intestinal microbiota clearly impact colonization and pathogenesis of *Giardia* and recent work highlights potential influences on the anti-*Giardia* immune response as well. At steady state, there is complex crosstalk between microbes and host cells and it is becoming increasingly evident that *Giardia* infections have the capability to perturb microbial homeostasis, causing microbial dysbiosis. Since *Giardia* infections can lead to post-infectious IBS and CFS, it will be interesting to understand the long-lasting effects of *Giardia*-induced microbial dysbiosis in promoting these postinfectious disease syndromes. Finally, since many advances in our understanding of protective *Giardia* immunity have been discovered using murine infection models or human cell culture models, additional emphasis on studies of immune responses in human infections are needed. The greater challenge for

our field resides in translating these advances towards humans systems to generate novel therapeutics that promote human health.

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TRENDS BOX

- *Giardia* infections can perturb intestinal microbial diversity and lead to an increase in the abundance of aerobic microbial taxa
- Giardiasis immunopathology is driven by the presence of intestinal microbiota and activation of immune responses
- Interleukin-17 is a cytokine that appears to be essential for protective immunity during giardiasis
- *Giardia* infections can lead to post-infectious syndromes, including malnutrition, irritable bowel syndrome and chronic fatigue syndrome.

OUTSTANDING QUESTIONS BOX

- What are the main drivers of protective immunity during *Giardia* infections? Specifically what drives production of IL-17 and how does this cytokine exert its protective effects in humans?
- What are the effects of co-infections on *Giardia* pathogenesis in humans? And on the pathogenesis of other enteric pathogens?
- What are the long-term consequences of *Giardia*-induced microbial dysbiosis?
- How can current advances in *Giardia* research be translated to human systems?

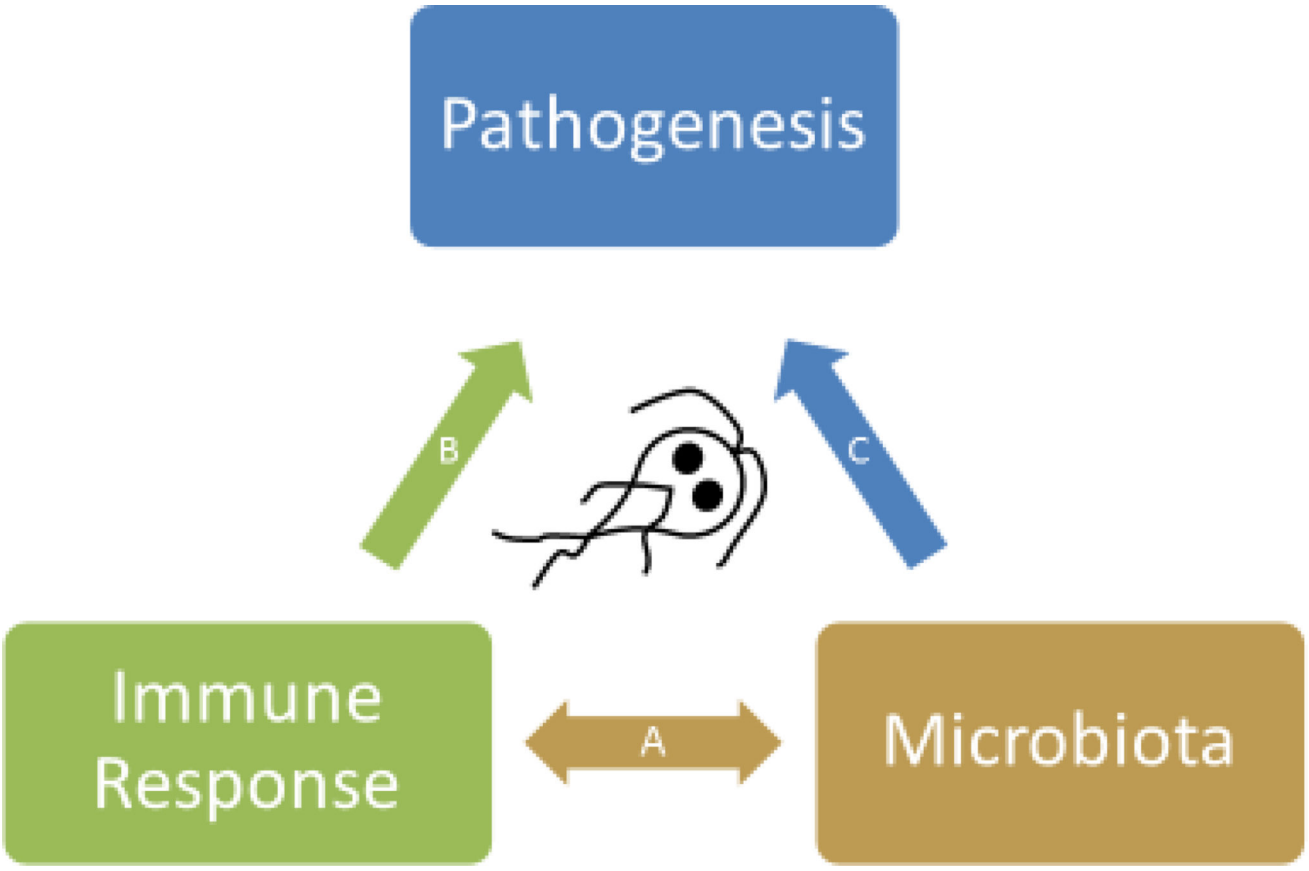

Figure 1. A model for the intersection between Immune Response, Microbiota, and Pathogenesis during *Giardia* infections

A) Immune response and composition of the intestinal microbiota are highly linked to each other. The composition of the microbiota influences T cell development (e.g. segmented filamentous bacteria promote Th17 responses while *Clostridia* promote regulatory T cell development). Conversely, immune responses modulate the microbiota composition through numerous effector mechanisms such as anti-microbial peptides, mucous, nitric oxide (NO) and IgA.

B) Several elements of *Giardia* pathogenesis have been shown to depend on immune responses activated by infection. These include anti-parasitic effects of anti-microbial peptides, NO and IgA. Smooth muscle hypercontractility and intestinal hypermotility also depend on adaptive immune responses as well as mast cell responses, and disaccahridase deficiency is driven by activated CD8+ T cells.

C) Commensal microbiota also contribute to the pathology observed during, and possibly after, *Giardia* infections. In addition to contributing to activation of the immune response during infections, dysbiosis of the gut microbiome may also directly mediate some of the nutrient malabsorption observed during infections and also the long-term sequelae, such as post-infectious irritable bowel syndrome.

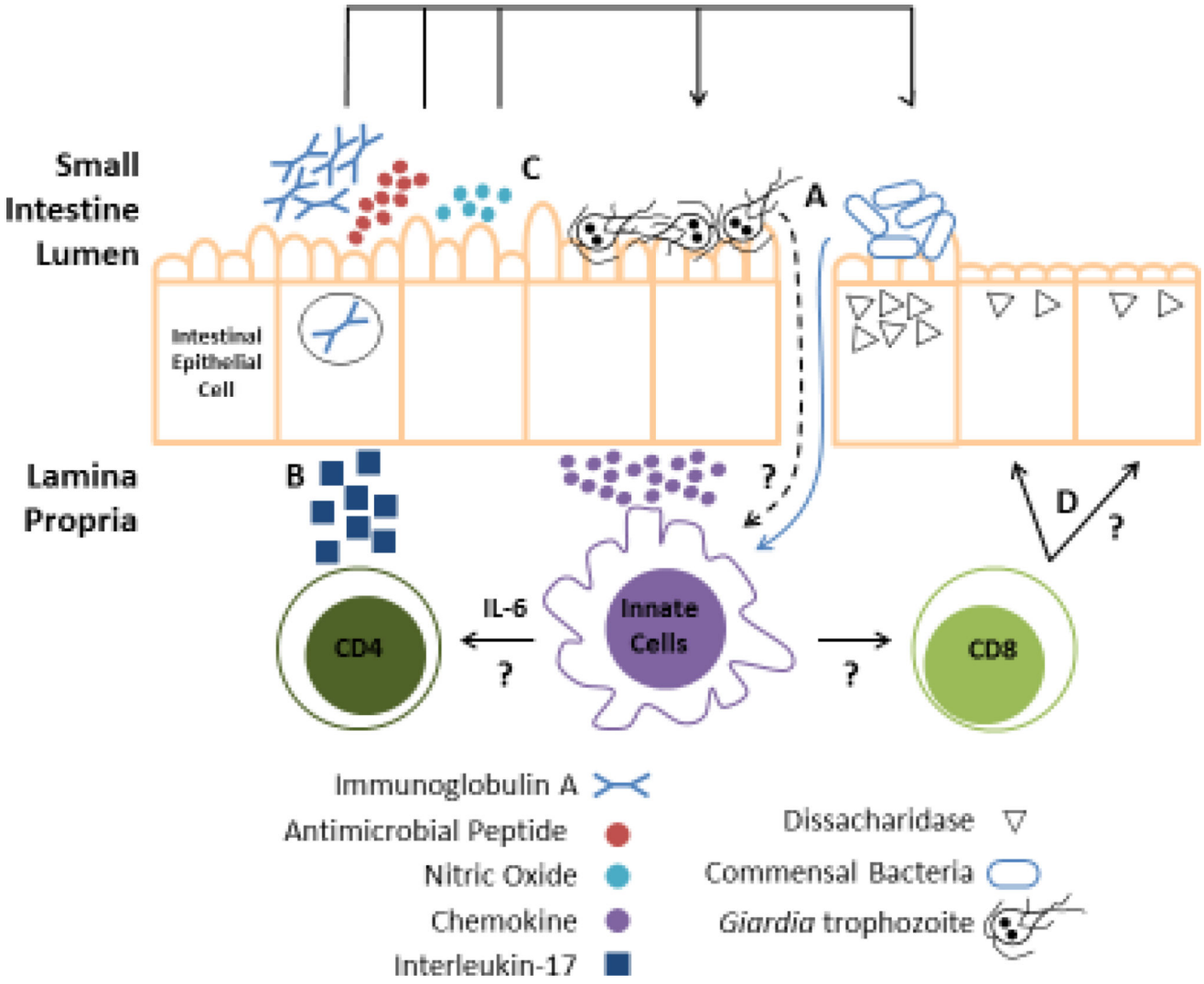

Figure 2. The multiple roles of intestinal epithelial cells during *Giardia* infections

The intestinal epithelium normally provides a secure barrier against invading microbes. (A) During *Giardia* colonization of the small intestine, permeability increases resulting in the translocation of intact microbes and/or microbial antigens into the lamina propria. Innate immune cells (e.g., macrophages and dendritic cells) become activated and initiate the downstream adaptive responses. Which host receptors and parasite ligands serve to activate these responses are unclear, and other than IL-6, the cytokines which drive development of adaptive responses are unknown. (B) Adaptive responses include IgA production by B cells, IL-17 production by CD4+ Th17 cells and activation of $CD8^+$ T cells, which contribute to protection or immunopathology as described in the text. In response to IL-17, intestinal epithelial cells (IECs) secrete antimicrobial peptides and transport IgA into the lumen where they can aid in *Giardia* protection, but also impact the commensal microbes. (C) Additionally, in response to *Giardia*, IECs can produce nitric oxide to aid in protective immunity and also release chemokines to recruit innate immune cells. (D) Activation of

$CD8^+$ T cells induces pathological symptoms including microvilli shortening and reduced dissacharidase activity through an unknown mechanism.