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Advances in understanding *Giardia*: determinants and mechanisms of chronic sequelae

Luther A. Bartelt[1]* and R. Balfour Sartor[2]

Addresses: [1]Division of Infectious Diseases and International Health, University of Virginia, Box 801340, Charlottesville, VA 22908, USA; [2]Division of Gastroenterology, Center for Gastrointestinal Biology and Disease, University of North Carolina at Chapel Hill, Campus Box 7032, Chapel Hill, NC 27599-7032, USA

* Corresponding author: Luther A. Bartelt (lab2za@virginia.edu)

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Abstract

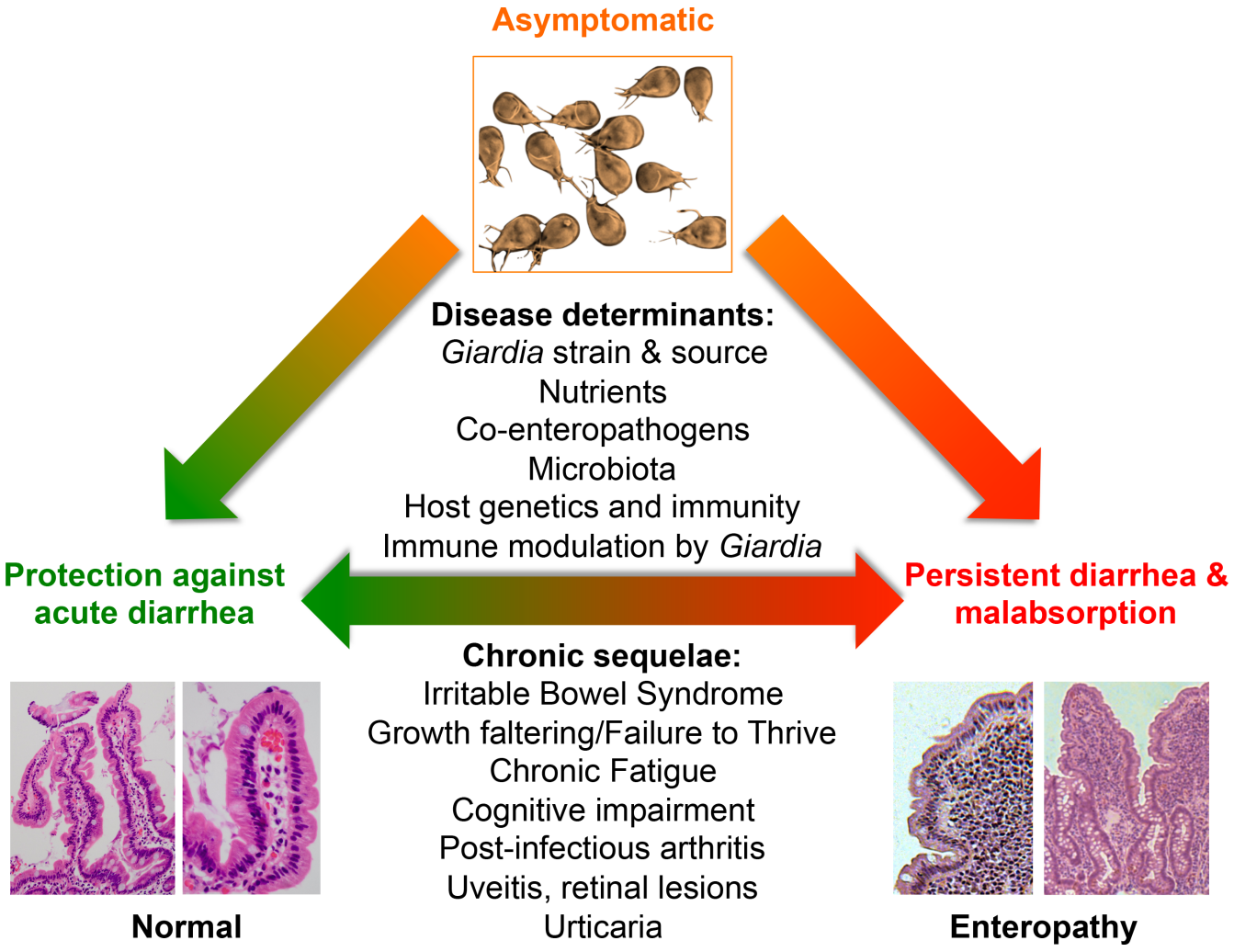
Giardia lamblia is a flagellated protozoan that is the most common cause of intestinal parasitic infection in children living in resource-limited settings. The pathogenicity of *Giardia* has been debated since the parasite was first identified, and clinical outcomes vary across studies. Among recent perplexing findings are diametrically opposed associations between *Giardia* and acute versus persistent diarrhea and a poorly understood potential for long-term sequelae, including impaired child growth and cognitive development. The mechanisms driving these protean clinical outcomes remain elusive, but recent advances suggest that variability in *Giardia* strains, host nutritional status, the composition of microbiota, co-infecting enteropathogens, host genetically determined mucosal immune responses, and immune modulation by *Giardia* are all relevant factors influencing disease manifestations after *Giardia* infection.

Introduction

Giardia lamblia (also known as *G. duodenalis* and *G. intestinalis*) is the most common and oftentimes earliest parasitic infection in children and the most common cause of diarrhea in travelers presenting to clinics [1–4]. Owing to an estimated 280 million infections every year [5,6], nearly universal infection among children living in resource-limited regions worldwide [6,7], the potential for persistent and recurrent infections with an average duration of *Giardia* carriage of 6.3 months among children in some populations [2], and limited affordable effective therapeutic options [8,9], the World Health Organization (WHO) considers *Giardia* a neglected tropical disease [10,11]. Because the parasite is environmentally ubiquitous, can persist for prolonged periods in the environment as hardy cysts, is capable of propagating through both anthroponotic [12] and zoonotic [13–19] reservoirs, and has an infectious dose as low as 10 cysts [20], *Giardia* exposures can also occur in resource-abundant settings [21–25]. In the United States, for example, infection is characterized by seasonal and recreational waterborne transmission [22,23] and clustered daycare outbreaks [26]. Transmission through food sources, such as leafy green vegetables and culinary bivalves harboring *G. lamblia* shed from coastal and marine life [27–29], is also possible.

Anton van Leeuwenhoek first identified *Giardia* in his own diarrheal stool in 1681, but the parasite did not become an officially WHO-recognized pathogen until 1981. Even after Theodore Nash and colleagues [30] fulfilled Robert Koch's postulates in human volunteers in 1987 [20], disease attributable to *Giardia* was debated. Complicating this clinical equipoise is the predominance of apparently asymptomatic hosts and as-of-yet elusive mechanisms explaining how some individuals develop the characteristic giardiasis syndrome: abdominal cramping and bloating, malabsorptive diarrhea (steatorrhea), and weight loss [18]. Furthermore, for unclear reasons, chronic sequelae, including post-infectious irritable bowel syndrome, chronic fatigue [31], malnutrition [32], cognitive impairment [33], and extra-intestinal manifestations (such as food allergy, urticaria, reactive arthritis, and inflammatory ocular manifestations), can develop and

Figure 1. The clinical spectrum of *Giardia* infection



The majority of individuals infected with *Giardia* are asymptomatic. Within the diarrhea spectrum of disease, *Giardia* paradoxically associates with both protection against acute diarrhea and persistent diarrhea with or without malabsorption and enteropathy. Hypothesized determinants of how disease will manifest on this spectrum include the virulence of the infecting strain(s) of *Giardia*, host nutritional intake, co-infecting enteropathogens, the composition and function of resident microbiota, immune modulation by *Giardia*, and host genetics and immunity. Changes in these dynamic variables may further skew disease manifestation. Histological changes vary from normal histopathology (bottom left) to villus shortening and chronic inflammatory infiltrate (enteropathy) (bottom right). Chronic sequelae associated with giardiasis include irritable bowel syndrome, chronic fatigue, childhood growth faltering, failure to thrive, cognitive impairment, and extra-intestinal manifestations presumed to be related to immunologic phenomena (reactive arthritis, inflammatory ocular manifestations, and urticaria) that are not necessarily dependent on severity of diarrheal manifestations and may persist even beyond detection of the parasite. *Giardia trophozoite* image courtesy of Joel Mancuso and Scott Dawson. Images of small-bowel biopsies from patients with giardiasis showing normal (left) and abnormal (right) histopathology courtesy of Leana Guerin (left) and Jörg-Dieter Schulzke (right).

possibly persist beyond detectable parasite shedding (Figure 1).

Global efforts to improve child health in resource-limited settings situate *Giardia* infections amidst the broader context of childhood malnutrition, a multitude of diverse and frequent enteric pathogen exposures [34], and new understandings of the role of environmental enteropathy (a form of chronic gastrointestinal dysfunction and inflammation possibly inclusive of the previously termed clinical condition 'tropical sprue') [11,35] and microbiota [36] on childhood development and growth. The most recent analyses, equipped with more rigorous surveillance methods and more sensitive molecular diagnostics [37–39], have not only reiterated the wide range of variability in pediatric *Giardia* infection

outcomes seen in prior studies [3] but unveiled a much greater burden of exposure than estimates based on microscopic techniques [39,40]. These and other studies have raised provocative considerations, including the potential for *Giardia* to be protective against acute diarrhea [41–43].

In light of the incompletely understood pathology attributable to *Giardia* infection, recent advances in parasite and gastrointestinal biology hope to clarify how and when *Giardia* causes disease. These advances address fundamental questions regarding how *Giardia* interacts within the complex ecology of microbial, nutritional, and host factors within the small intestine, the determinants of host susceptibility, and whether and how host inflammation contributes to pathogenesis (Figure 2).

Endemic pediatric giardiasis equipoise

In 1971, after charting weights of children in Guatemala, Leonardo J. Mata reported an association between arrested growth and frequent episodes of diarrhea [44]. A follow-up analysis of these children associated increased duration and frequency of *Giardia* episodes with a greater detrimental growth impact [45]. Since then, several studies have attempted to demonstrate how early *Giardia* infections promote diarrhea and developmental shortfalls. A 2012 meta-analysis found that, although *Giardia* infection was associated with persistent diarrhea (>14 days; odds ratio [OR] 3.2, 95% confidence interval [CI] 1.50-6.76; P <0.001), there was a negative association with moderate-severe acute diarrhea (OR 0.6, 95% CI 0.38-0.95; P <0.03) [3]. Subsequently, the Global Enteric Multicenter Study identified a similar paradox [41]. This finding was consistent regardless of diagnostic modality and quantitative burden in stool specimens [39]. Other prospective cohort studies in Tanzania [42] and Iran [43] also demonstrated a delay in first episode of diarrhea in children who were positive for *Giardia* at enrollment. Elsewhere, *Giardia* is positively associated with acute diarrhea [46], and younger children may be at greater risk for acute diarrhea with *Giardia* infection [3].

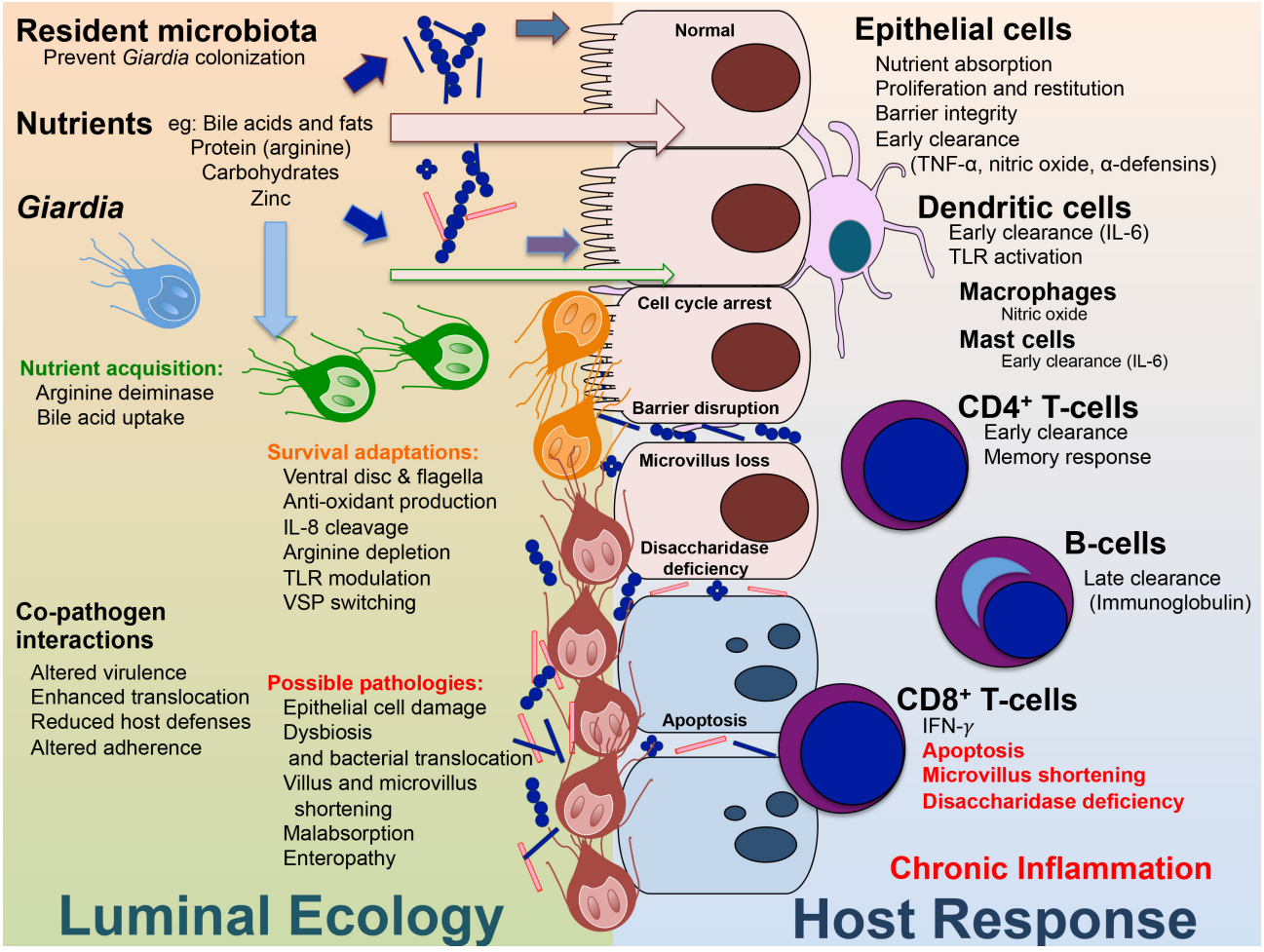
Associations between *Giardia* and childhood growth and developmental shortfalls have also been variable. Stunting (low height-for-age z score) has been documented in Brazil [47], Colombia [48], and Ecuador [49]; poor intestinal permeability has been seen in Nepali children [50], growth impairment in Rwandan children [51], wasting in Malaysia [32] and India [52], and low weight-for-age and height-for-age in Brazilian children with persistent symptoms [53,54]. In addition, cognitive impairment has been documented in children up to seven years after *Giardia* infection independent of physical growth [33]. Other equally valid studies, however, have shown no significant influence on developmental outcome or nutritional status [3,55–57]. The Malnutrition and Enteric Disease Study, to date the most comprehensive longitudinal cohort study aimed at determining environmental, nutritional, and enteric pathogenic influences on childhood development, is ongoing and will offer the most complete opportunity to ascribe associations between *Giardia*, growth, and gastrointestinal dysfunction [58]. There have been no randomized controlled trials evaluating the developmental impact of specific anti-giardial therapies in malnourished children. However, bovine fecal lactoferrin supplementation simultaneously decreases *Giardia* colonization and promotes child growth [59].

Whether *Giardia* infection initiates or promotes childhood malnutrition has major implications for the nearly 20% of undernourished children worldwide [58]. Malnutrition substantially increases mortality and morbidities to both infectious and non-infectious conditions, and undernourished children may not demonstrate expected restorative gains with nutritional supplementation. Indeed, protein energy malnutrition [60], zinc deficiency, and vitamin A deficiency [61] may increase susceptibility to *Giardia*. Conversely, *Giardia* infection may reduce supplemental zinc uptake [62–64] and influence dose response to vitamin A supplementation [65]. Further confounding nutritional determinants in *Giardia* outcomes are the findings in a study of multi-nutrient therapy for childhood diarrhea that *Giardia*-infected subjects in the placebo arm were protected against diarrhea but that *Giardia*-infected subjects in the multi-nutrient intervention arm were not [42]. Proposed mechanisms explaining such paradoxical findings suggest that specific nutrients may promote *Giardia* growth or otherwise directly mediate *Giardia* pathogenicity, alter host microbiota and other defenses, or interfere with the parasite's influence on co-enteropathogens and host immune responses.

Determining how *Giardia* causes disease

Infection with *Giardia* occurs after ingestion of the environmental cyst stage of the parasite, which upon gastric passage excysts to release two trophozoites into the less acidic and bile-enriched environment of the upper small bowel. Trophozoites adhere to epithelial cells and replicate but typically do not invade the intestinal mucosa or cause ulceration [6,66]. If symptoms develop, onset typically begins after a 6- to 15-day incubation period that oftentimes precedes detection of parasites in stool [5]. The type of diarrhea experienced in giardiasis is classically greasy and foul-smelling (steatorrhea) with findings of malabsorption of vitamins A and B12, d-xylose, iron, and zinc [3,67–69] as well as lactase deficiency in 20-40% of

Figure 2. Proposed determinants and mechanisms of *Giardia* infection outcomes



Complex interactions between microbiota, nutrients, *Giardia* strain, co-enteropathogens, and host molecular responses in the luminal and mucosal environment likely influence *Giardia* infectivity and disease outcomes. (Left) Resident microbiota maintain resiliency to colonization. *Giardia* uses, and potentially sequesters, nutrients such as bile, arginine, and zinc in order to survive, replicate, and evade microbiota and host defenses. Flagella and the ventral disc are structures of trophozoites that aid attachment and adherence to intestinal epithelial cells (IECs). *Giardia* uses functional virulence factors to evade host inflammatory responses through antioxidant production, cleavage of interleukin-8 (IL-8), arginine depletion via arginine deiminase (ADI), and shifts in variant surface protein (VSP) expression. Effects of *Giardia* on epithelial cells (that is cell-cycle arrest, impaired proliferation, tight-junction disruption, and apoptosis) may be strain dependent and either direct or indirect. Subsequent changes in nutrient availability, microbiota composition, inflammatory defenses, and epithelial cell pathogen attachment sites may secondarily alter disease manifestations of co-infecting enteropathogens. (Right) Redundant mucosal immune responses promote *Giardia* clearance early (epithelial cell nutrient uptake for host fitness, barrier function maintenance, and pro-inflammatory molecules; IL-6 derived from dendritic cells and mast cells; and $CD4^+$ and $CD8^+$ T cells) and late in disease ($CD4^+$ T-cell memory and B cells). $CD4^+$ T cells induce memory responses but also contribute to chronic inflammation and may promote disaccharidase deficiency. $CD8^+$ T cells mediate apoptosis, microvillus shortening, and disaccharidase deficiency. Epithelial cell damage may persist beyond parasite clearance, allowing sustained translocation of microbiota and microbial products. The altered mucosal homeostasis and inflammation (enteropathy) and microbiota composition may further impede nutrient uptake and contribute to prolonged sequelae, including impaired growth and cognitive development.

symptomatic cases [70]. Histological biopsies of small-bowel mucosa are often without any apparent abnormality [71]. However, increased lymphocytic infiltrates and decreased villus-to-crypt ratios have been described in some children [72], and inflammation has been reported in some adults with either acute [73] or chronic [74] infection.

Experimental as well as clinical observations indicate that the primary cause of diarrhea in giardiasis is malabsorption and that hypersecretion during chronic infection possibly contributes [74,75]. Malabsorption is presumed to contribute to growth impairment but has not been causally linked to it. Multiple investigations

suggest both direct and indirect mechanisms of disease, including luminal competition for nutrients, *Giardia*-induced epithelial cell damage (apoptosis [76,77], arrested proliferation [78], and tight-junction abnormalities [76,77,79]); T cell-mediated epithelial cell injury [80,81], microvilli loss [75,80–82], and disaccharidase deficiency [83,84]; nutrient-dependent growth impairment and decreased villus-to-crypt ratios [85]; and translocation of microbiota across the mucosa [86] (Figure 2).

Unscrambling strain virulence and pathogenicity

Of at least six recognized species of *Giardia*, only *G. lamblia* causes infections in humans. *G. lamblia* isolates are further divided into eight assemblages, designated A to H. Among the assemblages, only assemblages A and B and their respective subtypes cause human infection. The relative proportion of assemblage A to assemblage B infection varies both temporally and spatially, with a predilection for more assemblage B infections in endemic settings [87–90].

Whole-genome sequencing has revealed that assemblage A and assemblage B laboratory isolates are quite dissimilar and may be better categorized as separate species [91]. Despite this genetic divergence, attempts to ascribe clinical variability and pathogenicity based on assemblage designation have been inconclusive. In separate studies, either assemblage A [92,93] or assemblage B [94,95] more strongly associates with diarrhea. A limitation in assessing assemblage-specific pathogenicity in naturally acquired infection is that targets used to differentiate between assemblages A and B—β-giardin (*bg*), glutamate dehydrogenase (*gdh*), triose phosphate isomerase (*tpi*), and the small subunit 18S rRNA genes—are not known virulence factors. Furthermore, studies that incorporate multilocus genotyping demonstrate a substantial proportion of specimens with mixed results [96,97], raising the possibility of heterologous infections or even inter-assemblage recombination events [98,99]. Expanded comparative genomics studies investigating several assemblage A and assemblage B isolates may further clarify strain-specific pathogenicity.

In experimental conditions, *Giardia* strain is the strongest predictor of disease outcome. In initial volunteer studies, the axenized assemblage B isolate GS/M caused more symptoms than other isolates, a finding that was reproduced in murine models [30,100]. Laboratory assemblage A and assemblage B isolates have different growth characteristics in standard media conditions, and the former are more readily recoverable; however, assemblage B isolates are more infectious [84,85]. Historically, there has been significant experimental heterogeneity in laboratory studies [101], and this limits comparisons between investigators. When tested under identical conditions, however, there are apparent assemblage-dependent differences in induction or disruption of interleukin-8 (IL-8) from epithelial cells *in vitro* [102,103] and *ex vivo* biopsies from patients with inflammatory bowel disease [104] and immune-mediated disaccharidase deficiency in murine models [84]. Furthermore, *in vitro* models demonstrate that strain-dependent changes occur in epithelial cells [77] and that mixed assemblage co-cultivation, or cultivation with a clone expressing mixed assemblage traits, induces more epithelial cell apoptosis and tight-junction disruption than mono-infection with either assemblage A or B [105]. Finally, proximity to a natural source may also be relevant given that recently human-passaged strains were more infectious in an animal model than laboratory strains were [106] and that gerbil-passaged cysts achieve prolonged infections [85].

Advances in *Giardia* biology and gene regulation using transcriptomic and proteomic approaches aim to identify new virulence traits that could be confirmed in human infections. For example, as a possible means of evading host humoral and innate defenses, *Giardia* has a repertoire of 20 to 200 kDa cysteine-rich proteins that densely coat the surface of trophozoites, termed variant surface proteins (VSPs). Although only a single VSP is expressed on an individual trophozoite, the breadth of potential VSPs (73 to 270 or more) [91,107] in a given strain is associated with enhanced virulence [108]. How VSP switching occurs every 6-13 generations and what events trigger post-transcriptional VSP regulation [107,109] and trafficking may help unravel parasite determinants of transient colonization opposed to chronic infection. The role of *Giardia* cathepsins as disease determinants also warrants further consideration [102,103].

Nutritional determinants of disease

It is increasingly recognized that, within the luminal ecology of the human intestine, resident microbiota modify ingested nutrients and their metabolites, influencing nutrient availability and uptake. *Giardia* lacks fundamental enzymes necessary for generating critical biomolecules such as cholesterol and therefore must rely on acquisition of these materials from the luminal environment [110–112]. The parasite acquires nutrients primarily via bulk-phase uptake in endocytic vacuoles localized to the exposed dorsal (non-adherent) side [113], adheres to epithelial cells using the ventral disc [114,115], and may direct fluid flow toward adherent parasites via coordinated flagellar motion [116]. Arginine utilization represents an example of how shunting of key nutrients may provide an advantage to the parasite at a detriment to

the host. Stadelmann and colleagues [117] demonstrated that *Giardia* isolates expressing arginine deiminase rapidly deplete arginine, resulting in epithelial cell cell-cycle arrest. Since both *Giardia* and epithelial cells use arginine for growth, an arginine-depleting mechanism could explain why, under conditions of arginine scarcity such as severe protein malnutrition, there is increased susceptibility to villus shortening during *Giardia* infection [85,118]. For the host, decreased availability of arginine also impairs inducible nitric oxide production by epithelial cells and may alter cellular immune responses [119,120], whereas *Giardia* uses the sequestered arginine primarily for energy production via the arginine dehydrolase pathway. Host genetically determined pathways that facilitate arginine acquisition or utilization despite *Giardia* infection may be important for maintaining host resiliency during infection. For example, cellular arginine uptake occurs through the cationic transporter (CAT), and CAT expression is increased in some cell types in the presence of *APO-E 4/4* allelotype [121]. The *APO-E 4/4* allelotype confers protection against diarrhea in children [122,123] and *Cryptosporidium* infection in mice [121], and may also associate with better cognitive outcomes in *Giardia*-infected children [124].

There is mounting recognition of an intricate relationship between host nutrition and mucosal immune responses. Malnourished children, for example, have diminished seroconversion to some components of the oral polio vaccine [125]. In experimental models, malnutrition confers enhanced susceptibility to *Cryptosporidium* [126–128] and enteroaggregative *Escherichia coli* (EAEC) [129]. Zinc deficiency enhances susceptibility to *Giardia* [130] and diminishes inflammatory responses to EAEC [131]. In a chronic giardiasis model, protein malnutrition led to decreased expression of IL-4 and IL-5 and diminished B-cell populations in intestinal tissues, villus blunting, and growth failure despite a parasite burden similar to that of well-nourished infected controls [85]. These findings suggest that, in children with chronic giardiasis, nutritional status could similarly determine disease severity.

Microbiota and co-pathogen interactions

There is increasing recognition of the role of the nearly 10^{14} resident bacteria in regulating host metabolism during states of both obesity and undernutrition [36,132] as well as immunity. In experimental giardiasis, the microbiota composition was shown to have more influence over susceptibility to infection than the absence of $CD4^+$ T cells [133]. Thus, several experimental models rely on manipulating host microbiota through the use of antibiotic-containing water to promote infection [84,85,103]. Though sometimes necessary, the depletion of microbiota in these models may exclude important *Giardia* effects on microbiota that indirectly influence pathogenesis. Although attempts to identify small-bowel bacterial overgrowth in children with giardiasis have failed to show an association [134], microbiota from duodenal contents of *Giardia*-infected patients caused more inflammation than axenized *Giardia* trophozoites in germ-free mice [135]. Chen and colleagues [86] also observed that diverse populations of mucosally associated bacteria, including *Lactobacillus*, *Streptococcus*, and *Staphylococcus* species, are increased during experimental *Giardia* infection and after clearance of the parasite. Associated increases in the pro-inflammatory cytokines interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), and myeloperoxidase were also seen [86]. The persistent translocation of mucosally associated bacterial microbiota has been proposed to explain post-infection irritable bowel syndrome and chronic fatigue [31], sequelae present in up to 5% of individuals with prior giardiasis [136], particularly in those whose initial course was more protracted [137]. Mechanisms by which *Giardia* could influence microbiota composition include alterations in the luminal nutritional environment as previously stated, as well as dampening the epithelial cell inflammatory responses through post-translational reductions in IL-8 and decreased nitric oxide synthesis [102,120,138–140].

Whether resident microbiota in the small intestine are protective against *Giardia* or promote chronic gastrointestinal disease may be conditional on their composition. When used as probiotics in experimental models, *Lactobacillus* species (*L. johnsonii*, *L. casei*, and *L. rahmnosus* GG) have been shown to promote *Giardia* clearance [141]. Concurrently, these *Lactobacillus* species enhance pro-inflammatory responses and improve histopathological parameters [142–144]. Furthermore, bacteriocins produced by *L. acidophilus* (P106) and *L. plantarum* (P164) reduced parasite adhesion [145,146]. The fermented milk product kefir, which contains a combination of lactic acid bacteria (lactobacillus, lactococci, and *Leuconostoc*), acetic acid bacteria, and yeast, protected mice against *G. lamblia* trophozoite infection and enhanced pro-inflammatory responses, including IFN-γ and TNF-α, with a decrease in $CD4^+$ T-cell expansion [147]. Interestingly, activation of Toll-like receptor 2 (TLR2) signaling in murine dendritic cells, as occurs in response to some *Lactobacillus* species, in combination with TLR1 or TLR6, leads to increased IL-12/23p40, IL-23, and IL-10 secretion in the presence of *Giardia* lysate [148], whereas *Giardia* lysates decrease IL-12p70 and IL-23 but increase IL-10 responses to TLR4 agonists [148]. The down-regulatory effect of *Giardia* lysate on dendritic cell responses to TLR4 agonists has been shown to be dependent on phosphoinositide 3-kinase

inhibition and may depend on *Giardia* arginine deiminase [78,149]. In contrast, *Giardia* lysates have no regulatory effect on bovine monocyte-derived dendritic cells [150].

The influence of *Giardia*-mediated changes in the luminal ecology may also alter susceptibility to other co-infecting enteropathogens, which are now recognized to be more common than mono-infections in children in resource-limited settings [34]. Furthermore, only a narrow margin separates the mean number of co-enteropathogens found in non-diarrheal specimens (4.3 ± 0.1) from the number in diarrheal specimens (5.6 ± 0.1) [34]. In some populations, up to 75% of *Giardia* infections include co-enteropathogens, most commonly with *Vibrio cholerae* and rotavirus [151] and elsewhere with norovirus and enteropathogenic *E. coli* [46]. Cases of co-infections with *Tropheryma whipplei* have also been reported [152]. In rural Ecuador, the OR for diarrhea with rotavirus infection was 6-14.8, but *G. lamblia* with rotavirus increased the OR to 11-24.13. However, *Giardia* co-infection with enterotoxigenic *Escherichia coli*, *Campylobacter*, *Cryptosporidium*, and enteroinvasive *Escherichia coli* did not demonstrate an increased risk of diarrhea, suggesting specificity to *Giardia* co-pathogen interactions [153]. On the other hand, associations between decreased risk of acute diarrhea in *Giardia*-infected cohorts raise questions that require additional investigation of whether *Giardia* decreases susceptibility to some pathogens or attenuates their severity. Cotton and colleagues [103], for example, demonstrated that *Giardia* infection diminished neutrophil chemotaxis and inflammation in a model of *Clostridium difficile* toxin-induced colitis.

Host responses and disease pathogenesis

The duality of host immune response in *Giardia* pathogenesis is complex and has been reviewed extensively [83,154]. Increasingly, redundant mechanisms of immunologic control are being identified, including chemokines (CCL2, CCL20, CXCL1, CXCL2, and CXCL3) from epithelial cells [155], IL-6 derived from dendritic cells and possibly mast cells [156–158], TNF-α [159], α-defensins activated by matrix metalloprotease 7 (Mmp7) [159], and nitric oxide generated from either nitric oxide synthase 1 (NOS1) or NOS2 [159–161]. Data from isolated outbreaks and experimental models demonstrate a mixed immune response consisting of both antibody production [162,163] and mucosal and systemic Th1-type $CD4^+$ T-cell responses [84,164,165]. Studies in populations with serial outbreaks demonstrate that exposure to *Giardia* can promote protective immunity [166], which is characterized by a predominance of circulating T cells with a Th1-memory phenotype ($CD25^+CD26^{bright}$ cells that produce IFN-γ) [165]. Also, the absence of $CD4^+$ T cells leads to more prolonged infection in experimental models [84].

Despite evidence for the role of T cells in protective immunity, clinical observations suggest that humoral responses are critical for immunity. These studies demonstrate greater prevalence of *Giardia* cysts and more symptomatology in patients with hypogammaglobulinemia (that is, x-linked agammaglobulinemia [Bruton's disease] and common variable immunodeficiency) [167–169] than those with T-cell deficits (advanced AIDS or thymic aplasia). Decreased secretory IgA variably associates with increased risk [170] and impaired IgA responses are seen in children with persistent *Giardia* infection [171]. In animal models, the majority of genes expressed in experimental murine infections are related to antibody production [159], and IgA-deficient knockout mice have difficulty clearing *Giardia* [172]. The polymeric Ig receptor, which transports IgA and IgM across the epithelium, is essential for eliminating *G. muris* from murine hosts, further supporting possible dependence on mucosal secretory antibody responses for eradication of infection [173].

In addition to facilitating parasite clearance, the secondary host immune response to *Giardia* may promote pathologic changes [84,174]. $CD8^+$ T cells may contribute to villus [85] and microvillus [175] shortening in *G. lamblia* infection as demonstrated in models of murine giardiasis (*G. muris*) [80,81,84]. In addition, brush border actin cytoskeletal changes in ezrin and villin and disaccharidase deficiency after *G. lamblia* infection are dependent on T-cell responses [174]. Clinical findings also demonstrate that subsets of infected symptomatic adults [74] and some children show increased lymphocytic infiltrates as well as decreased villus-to-crypt ratios [72] on histological biopsy. Decreased villus surface area in patients with chronic giardiasis coincides with increased T-cell populations and epithelial cell apoptosis [74], and may similarly suggest immune-mediated diffuse microvillus shortening seen in some patients with giardiasis [82]. A murine model using freshly acquired gerbil-passaged *G. lamblia* cysts in combination with antibiotic-containing water to reduce resident microbiota similarly demonstrated persistent parasitism, with increased mucosal T cells and epithelial cell apoptosis through 9 weeks in immunocompetent hosts, suggesting that some *Giardia* strains that evade host responses can promote chronic mucosal inflammation [85].

Such chronic and repeated *Giardia* exposures are common in children in low-income settings who also have a high prevalence of malnutrition and an increasingly recognized gut dysfunction with chronic intestinal inflammation, increased gut permeability, and reduced

villus length, termed environmental enteropathy [11]. Few studies have characterized immune responses to *Giardia* in these children. Kohli and colleagues [87] identified inflammatory diarrhea by using fecal lactoferrin during the first case (but not subsequent cases) of *Giardia*, and Long and colleagues [176] demonstrated increased IL-4, IL-5, monocyte chemoattractant protein-1 (MCP-1), and IFN-γ but decreased IL-8 in children with more prolonged episodes of *Giardia*. Thus, relationships between intestinal inflammatory response and *Giardia* infection may influence disease outcomes in these children.

Elucidation of immune-protective responses in contrast to those that might directly promote enteropathy in these children could also help guide vaccine development. Currently, the only licensed vaccine, GiardiaVax, is for use only in canines and has modest efficacy [177]. Attempts to identify correlates of protection in children in low-resource endemic settings are inconclusive. For example, data from Bangladesh demonstrated that, although human breast milk has direct anti-*Giardia* properties and may contain high concentrations of anti-*Giardia* antibodies [18], there is no correlation between the presence of *Giardia*-specific antibodies in breast milk and incidence, severity, or recurrence of *Giardia* in these children [178]. Potential approaches to vaccine development include targeting the repertoire of *G. lamblia* VSPs, since purified VSPs can protect against primary infection in experimental models [179]. Another vaccine candidate, α1-giardin expressed in an attenuated *Salmonella* vector, showed efficacy in mice, but α-enolase and ornithine carbamoyl transferase did not [180]. Challenging field vaccine application is the recognition that some mucosal vaccines are less effective in children in low-resource settings, with an as-of-yet unidentified but hypothesized immunologic hurdle in the setting of malnutrition [125], adding another layer of complexity to this preventive strategy.

Conclusions

G. lamblia remains an enigmatic parasite with potential influence over health and development in children in endemic resource-limited settings where infection is nearly universal. One conclusion explaining the variable outcomes in both field studies and experimental models is that *Giardia* may not conform to traditional concepts of the 'commensal' or 'pathogenic' microbe and at times may promote states of either mucosal protection or disease. Therefore, discerning the direct effects of various *Giardia* strains from indirect effects mediated through the parasite's influence on a complex and dynamic luminal ecology and host mucosal homeostasis will be a critical step toward translating basic laboratory findings to outcomes in human disease. Such understanding could lead to improved interventions for chronic giardiasis, as well as environmental enteropathy and childhood malnutrition, while also identifying properties of the organism that could be exploited for the promotion of human health.

Abbreviations

APO-E, apolipoprotein E; CAT, cationic transporter; CI, confidence interval; EAEC, enteroaggregative *Escherichia coli*; IFN-γ, interferon-gamma; IL, interleukin; NOS, nitric oxide synthase; OR, odds ratio; TLR, Toll-like receptor; TNF-α, tumor necrosis factor-alpha; VSP, variant surface protein; WHO, World Health Organization.

Disclosures

The authors declare that they have no disclosures.

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