Published in final edited form as:
Exp Parasitol. 2010 November ; 126(3): 292–297. doi:10.1016/j.exppara.2010.06.014.

Giardia duodenalis: The Double-edged Sword of Immune Responses in Giardiasis

Shahram Solaymani-Mohammadi and **Steven M. Singer**
Department of Biology and Center for Infectious Disease, Reiss Science Building, Room 406, Georgetown University, Washington, DC 20057.

Overview

Giardiasis is one of the most common intestinal protozoan infections worldwide. The etiological agent, *Giardia duodenalis* (syn. *G. lamblia*, *G. intestinalis*), is a flagellated, binucleated protozoan parasite which infects a wide array of mammalian hosts (Adam, 2001). The symptoms of giardiasis include abdominal cramps, nausea, and acute or chronic diarrhea, with malabsorption and failure of children to thrive occurring in both sub-clinical and symptomatic disease (Thompson et al., 1993). Infections are transmitted by cysts which are excreted in the feces of infected humans and animals. Human giardiasis is distributed worldwide, with rates of detection between 2-5% in the developed world and 20-30% in the developing nations (Farthing, 1994). There is significant variation in the outcome of *Giardia* infections. Most infections are self-limiting, although re-infection is common in endemic areas and chronic infections also occur. Moreover, some individuals suffer from severe cramps, nausea and diarrhea while others escape these overt symptoms. This review will describe recent advances in parasite genetics and host immunity that are helping to shed light on this variability.

Epidemiology

Recent studies of *Giardia* have identified eight distinct genotypes within *G. duodenalis*, only two of which, assemblages A and B, are capable of infecting humans (reviewed in Thompson, 2009). These studies have led to a reevaluation of the zoonotic potential of this organism. Although parasites with both A and B genotypes can infect numerous mammalian species in addition to humans, other genotypes appear to have more restricted host range. Assemblages C and D, for example, are commonly found in dogs, but have yet to be reported in humans. Thus, the idea that human transmission from dogs (and cats and livestock) to humans needs to be reevaluated. Fortunately, new data are becoming available indicating that most cases of giardiasis are due to anthroponotic spread, but zoonotic transmission can and does occur (Snel et al., 2009).

Better understanding of the molecular epidemiology of *Giardia* will also require reanalysis of studies of a commercially available vaccine for veterinary giardiasis (Olson et al., 2000). This vaccine is essentially a mixture of lyophilized trophozoites of four parasite strains. Since these strains can be grown in culture they are likely from assemblages A and B, but not the assemblages commonly found in and restricted to cats (F), dogs (C and D) or livestock (E).

sms3@georgetown.edu phone: 202-687-9884 fax: 202-687-5662.

Thus, while some studies have shown some protection against experimental infections (Olson et al., 1996; Olson et al., 1997; Olson et al., 2001), others failed to show such a protection against the parasite (Stein et al., 2003; Uehlinger et al., 2007; Anderson et al., 2004). For example, in one study vaccinated kittens had abnormal stools on fewer days, secreted fewer cysts, and had a significantly higher weight gain in the post-challenge period (Olson et al., 1996). Conversely, Stein and coworkers (2003) did not find any correlation between cats receiving 3 doses of a *Giardia* vaccine and reduction in cyst shedding compared to unvaccinated kittens. New veterinary vaccines will need to take into account the restricted host ranges of the different genotypes and work around our inability to culture those other than types A and B. Potential human vaccines will need to address the role of immune responses in contributing to pathology and determining which responses are protective, as opposed to those which are merely present.

The factors determining the variability in clinical outcome in giardiasis are still poorly understood (Buret, 2007). However, host factors (such as immune status, nutritional status and age), as well as differences in virulence and pathogenicity of *Giardia* strains are recognized as important determinants for the severity of infection (Haque et al., 2005). Numerous studies have attempted to correlate the development of symptoms to the presence of either assemblage A or B parasites. While individual studies often find a strong correlation between parasite genotype and virulence, the answer comparing across studies is very unclear. For example, one study in Dutch patients found assemblage A isolates solely in patients with intermittent diarrhea, while assemblage B isolates were present in patients with persistent diarrhea (Homan and Mank, 2001). In contrast, Guerden et al. (2009) found that infections with assemblage B parasites were commonly found in diarrhea patients, but that a high proportion of infections were with mixed assemblages that might have interfered with previous analyses. This may be due to the fact that assigning parasites to specific genotypes usually reflects alleles at loci such as glutamate dehydrogenase, 18S RNA and triose phoshate isomerase (TPI) which are unlikely to be directly associated with virulence. More effort, however, should be directed to understanding mechanisms of virulence and identifying specific parasite virulence factors in order to understand the relative contributions of both the host and the parasite to disease.

Immune responses that control infection

The immune response to microbial pathogens, including *Giardia* sp., relies on both innate and adaptive components. Although the actual host defense mechanisms responsible for controlling *Giardia* infections are poorly understood, many studies have demonstrated the development of adaptive immune responses as well as innate mechanisms in humans and other animals (Roxström-Lindquist et al., 2006). Understanding the complex network of immune responses and host-parasite cross-talk should assist us in identifying novel and common targets for the therapeutic intervention of the infection (Solaymani-Mohammadi et al., 2010).

Epidemiological studies suggest that previous infection with *Giardia* leads to a reduced risk of re-infection and to reduced development of overt symptoms in secondary infections. Analysis of cases in an outbreak at a ski resort in Colorado showed that individuals residing in the community for more than 2 years had a much lower risk of being affected than new residents (Istre et al., 1984). Similarly, a community in British Columbia experienced two outbreaks five years apart and individuals affected in the first outbreak were much less likely to be ill during the second outbreak (Isaac-Renton et al., 1994). Both studies suggest that previous exposure to *Giardia* produces an immunity to disease. It is unclear in these studies whether prior exposure actually prevented infection, or only if severe symptoms were avoided the second time. Nonetheless, these findings suggest that development of an effective vaccine could be feasible. A recent study in Brazilian children suggests that symptoms are less severe during re-infection, consistent with the idea that previous exposure doesn't prevent infection,

but does reduce the pathology which can occur (Kohli, 2008). Additional studies in humans and animal models are, however, needed to determine what types of immune responses mediate this protection.

Studies in animal models require careful interpretation. First, animal immune responses are not always equivalent to that seen in humans. Additionally, many studies have utilized *G. muris*, a rodent parasite that cannot be cultured, to analyze immune responses in mice. As described below, however, *G. muris* may be resistant to immune mechanisms capable of killing *G. duodenalis.* Studies of *G. duodenalis* are restricted by the inability of many strains of *G. duodenalis* to colonize adult mice. Experiments with *G. duodenalis* in gerbils and neonatal mice have also been performed, although immunologic analysis of these animals is difficult. In some cases, understanding the host restriction of *G. duodenalis* may provide insights into the biology of the host-parasite interaction. For example, certain bacterial flora have been shown to render mice resistant to colonization (Singer and Nash, 2000b). Bacterial flora could inhibit *Giardia* through direct action against the parasite. Studies *in vitro* showed that culture supernatants from *Lactobacillus johnsonii* La1 significantly inhibited the proliferation of *G. duodenalis* trophozoites in a strain-dependent manner (Pérez et al., 2007). Diverse animal species (and even individuals) differ greatly in the dominant microbiota colonizing their intestinal mucosal surfaces. Thus, the different microbiota could explain why some hosts are resistant to specific parasitic infections. Host immune mechanisms play a key role in regulating the microbiota, e.g. through expression of diverse sets of antimicrobial defensins by Paneth cells (Salzman et al., 2007). Indeed, some Paneth cell defensins have also been show to kill *G. duodenalis in vitro* (Aley, 1994). However, the effects of defensins on intestinal flora is bidirectional, and changes in flora can also effect production of defensins (Ayabe, 2004). Further analysis of the host-parasite-environment three-way interaction in giardiasis may provide novel means to prevent this infection.

Several lines of evidence suggest that IgA antibodies contribute to protective immunity against giardiasis. While most chronic infections have been reported in patients with no underlying immune abnormality, patients with common variable immunodeficiency (CVID) and Bruton's X-linked aggamaglobulinemia (XLA) are clearly prone to chronic giardiasis (Stark et al., 2009). Patients with these syndromes both lack normal B cell function and reduced production of IgG, suggesting the necessity of antibodies for control of the infection. However, both CVID and XLA have additional defects in immune function and chronic *Giardia* infection is not common in selective IgA deficiency, suggesting additional layers of complexity in host immunity. As such, the rates of giardiasis in HIV-infected patients are higher than controls in some studies (e.g. Angarano et. al, 1997; Feitosa et. al, 2001; Bachur et al., 2008), although it is still unclear whether epidemiological factors rather than immunosuppression are responsible for these differences (Stark et al., 2009).

A number of studies have used animal models of infection to help clarify the role of antibodies in controlling *Giardia* infections. Snider et al. (1988) first showed that xid mice (hypogammaglobulinemic mice with a defect in the same kinase that is altered in human XLA) and wild-type mice treated with anti-IgM to deplete B cells developed chronic infections with *G. muris*. However, when we used gene-targeted B cell deficient mice and *G. duodenalis* for infections, no defect was apparent, suggesting alternate mechanisms exist to eradicate the infection (Singer and Nash, 2000a). This contradiction has been resolved by studies directly comparing *G. muris* and *G. duodenalis* infections in mice lacking the polyIg receptor that cannot transport IgA or IgM into the intestinal lumen (Davids et al., 2006). *G. duodenalis* infections were controlled in the absence of antibodies, while *G. muris* infections became chronic. This suggests that mice have additional mechanisms able to kill the human pathogen to which the mouse species is resistant. Identification of these mechanisms in mice and their

human counterparts would therefore greatly facilitate development of effective vaccines or immunotherapeutics.

The existence of antibody-independent mechanisms for *Giardia* elimination does not necessarily mean that antibodies have no role. However, the ability of *G. duodenalis* to undergo extensive variation of the surface coat antigens, called variant-specific surface proteins (VSPs), likely delays the effectiveness of the antibody response (Nash, 1997). Mice deficient in the cytokine interleukin (IL)-6 develop chronic infections with *G. duodenalis* (Bienz et al., 2003; Zhou et al., 2003). In the first 2 weeks following infection, these mice make a strong IgA response which reacts with only a small subset of parasites growing *in vitro*, suggesting a response to a limited subset of parasite VSPs. In contrast, eight weeks after infection IL-6 deficient mice produce IgA reactive with all parasites from *in vitro* cultures, suggesting that the IgA response now recognizes all possible VSPs, common epitopes on VSPs, or invariant antigens on the parasite. The IL-6 deficient mice cleared their infections at this time point, indicating that such broadly reactive antibodies could indeed confer protection (Zhou et al., 2007).

In contrast to B cells, T cells appear to be required for control of *Giardia* at all time points post-infection. Nude mice, mice treated with anti-CD4 and mice lacking the T cell receptor β gene all develop chronic infections with *G. muris* and/or *G. duodenalis* (Roberts-Thomson and Mitchell, 1978; Stevens et al., 1978; Heyworth et al., 1987; Singer and Nash, 2000a). One role of T cells is to provide help for production of antibodies. These cells also provide help for other aspects of the immune response, although it is unclear which of these are essential for parasite control. Human studies have provided limited information about cytokine levels in sera of patients. In one study, peripheral blood lymphocytes from naïve individuals produced interferon (IFN)-γ in response to trophozoites (Ebert, 1999), and sera from infected adults contained elevated levels of IL-5, IL-6 and IFN-γ (Matowicka-Karna, et al., 2009). Mice lacking either IL-4 or IFN-γ are able to control infections with kinetics similar to wild-type mice (Singer and Nash, 2000a). We have recently shown that mice lacking TNF are delayed in parasite elimination (Zhou et al., 2007). This is consistent with elevated TNF levels in sera of infected children (Bayraktar et al., 2005). Altogether, additional studies are warranted to help us better understand regulation of T cell development and the roles of these immune cells during giardiasis.

Several recent studies have begun to examine how the innate immune system responds to *Giardia* infection and how this might influence development of T cell responses. An early study from the Kagnoff group examined the response of Caco2 cells to *G. duodenalis* parasites and saw no change in the expression of numerous cytokines that were induced by other intestinal pathogens (Jung et al., 1995). Roxström-Lindquist et al. (2005) used microarrays to reexamine this interaction and discovered a strong response by a limited number of genes, including several chemokines that might be expected to recruit dendritic cells and lymphocytes to the intestinal mucosa. Unfortunately, studies *in vivo* have not determined the importance of these factors during infection. Our lab has recently examined the interaction of dendritic cells with *Giardia* and found that live parasites and parasite extracts activate these cells for antigen presentation, but inhibit secretion of IL-12 while enhancing production of IL-10 (Kamda and Singer, 2009). Inhibition of IL-12 production was partially mediated via IL-10, but could be more completely reversed by inhibiting phosphoinositide 2-kinase with wortmannin. The host receptors and parasite ligands involved in this inhibition remain to be identified. Interestingly, this inhibition of IL-12 production could suggest that the parasite actively restricts the development of severe inflammation and may contribute to the lack of pathology often seen during infections. How dendritic cells and epithelial cells interact to determine the development of T cell responses needs to be determined.

Several effector mechanisms have been proposed to eradicate *Giardia* infections (reviewed in Eckmann, 2003). These include phagocytosis by macrophages, secretion of defensins, nitric oxide (NO) and mucins by epithelial cells as well as the recruitment and activation of mucosal mast cells. Macrophages have been shown to ingest and kill *Giardia* trophozoites *in vitro*, but a role *in vivo* has not been demonstrated (reviewed by Smith, 1985). Likewise, while some α-defensins can lyse trophozoites *in vitro* (Aley, et al., 1994), Eckmann (2003) reported that mice lacking MMP-7, the protease which converts pro-defensin peptides to active defensins, had no deficit in controlling G. *muris* infections. NO is cytostatic for *G. duodenalis in vitro*, but mice lacking inducible nitric oxide synthase (iNOS) eradicate *G. duodenalis* infections normally (Eckmann et al., 2000; Li et al., 2006; Andersen et al., 2006). Interestingly, the neuronal NOS isoform did have a defect in parasite control, but this was more likely due to effects on intestinal motility and not a direct effect on the parasite. The activity of mucins against the parasite has also only been shown *in vitro* (Roskens and Erlandsen, 2002).

In contrast to these other effector mechanisms, a significant role for mast cells in controlling *Giardia* has been demonstrated *in vivo* in several animal models. In an early study, mast cell deficient (wf/wf) mice, unlike their wild-type littermates, developed chronic giardiasis after oral administration of *G. muris*. Furthermore, the susceptible BALB/c mice injected with the anti-histamine and anti-serotonin drug, cyproheptadine, also showed prolonged infections with *G. muris*, implying some roles for mast cells in host's ability to eliminate the parasite (Erlich et al., 1983). A potential role for mast cells was suggested by Hardin et al. (1997), who examined jejunal macromolecular transport, epithelial permeability, and mucosal and connective tissue mast cell counts in Mongolian gerbils infected with *G. duodenalis*. In this study, mast cell counts were correlated with epithelial permeability and antigen uptake, suggesting a link between development of other immune responses and mast cell recruitment. This was further supported by data from our laboratory showing that c-kit$^{w/wv}$ mice infected with *G. duodenalis* failed to make parasite-specific IgA, mount a mast cell response, or to eliminate the infection (Li et al., 2004). Anti-c-kit-treated C57BL/6 mice had normal IgA responses, lacked mast cell responses, and failed to control the infection within 10 days. Hence, while the requirement for mast cells is clear, their role in development of antibody responses is not. A recent study, however, found that mast cells can help drive B cell switching to production of IgA (Merluzzi et al., 2010). We also recently described an additional role for mast cells in mediating smooth muscle contractions and contributing to changes in intestinal motility following infection (see below). It is likely that this contributes to both protection and pathology in giardiasis.

If mast cells are needed for elimination of *Giardia* infection, then prior or concurrent infection with intestinal helminthes that induce mastocytosis might be expected to prevent infection. However, pre-infection with *Trichinella spiralis* led to an increase in the number of trophozoites in the intestine and a delay in *Giardia* elimination, despite a large increase in mast cell numbers and normal production of anti-*Giardia* IgA (von Allmen et al., 2006). Thus, while mast cells are necessary for *Giardia* elimination, they are clearly not sufficient on their own. Indeed, the strong Th2 environment created by *T. spiralis* infection may make the intestinal tract more conducive to *Giardia* survival. Given that in most endemic areas where giardiasis is endemic, the overwhelming majority of inhabitants are infected with other parasitic protozoa and helminths as well, additional study of the interactions between *G. duodenalis* and other infections is clearly needed.

Murine models of infection have also been used to study possible new vaccine strategies for giardiasis. A recombinant live attenuated vaccine, based on a gene segment of the VSP (VSPH7) expressed in a *Salmonella typhimurium* vaccine strain, was evaluated for its potential to induce local and systemic immune responses (Stager et al., 1997). The recombinant vaccine elicited synthesis of serum IgG as well secretory IgA (sIgA) against both bacterial antigens

and the VSP. Vaccinated mice were never challenged with live parasites; however, several experiments have examined the possibility of transmission-blocking vaccines that target cyst antigens. Larocque et al. (2003) showed that oral immunization with recombinant cyst wall protein (CWP2) could elicit IgA and mixed Th1/Th2 responses and reduce cyst shedding after live challenge with *G. muris* cysts. Similarly, delivery of CWP2 expressed in *Streptococcus gordonii* or *Lactococcus lactis* elicited high levels of antibodies in the vaccinated mice and also reduced cyst shedding after challenge (Lee and Faubert, 2006 a,b). Most recently, a DNA vaccine encoding CWP2 delivered to the intestinal mucosa via *S. typhimurium* led to production of antibody, a mixed Th1/Th2 response and reduced cyst shedding compared to control mice (Abdul-Wahid and Faubert, 2007). Analysis of vaccine efficacy in mice lacking elements of the immune system, e.g. IL-4 deficiency, would be useful in determining which of the observed responses actually contribute to the observed protection.

Pathology and the Immune Response

Pathology in giardiasis is understood to arise in several ways. These include breakdown of the epithelial barrier, defects in the epithelial brush border, secretion of chloride ions and hypermotility of the intestinal smooth muscles. The role of parasite virulence factors and host immune responses in contributing to these mechanisms is beginning to be clarified, although much more remains to be understood.

Breakdown of the epithelial barrier has been shown to occur in human disease and animal models of giardiasis (Hardin et al., 1997; Troeger et al., 2007; Zhou et al., 2007). This allows for macromolecules and electrolytes to pass into the sub-mucosa, bypassing normal uptake by epithelial cells. The paracellular flow of nutrients and electrolytes can contribute to nutrient malabosrption by reducing electrochemical gradients needed for proper uptake and can cause inflammation in some individuals, probably through activation of innate immune effectors like macrophages. However, inflammation is often not observed in human giardiasis and does not correlate well to symptomatic disease (Oberhuber et al., 1997). In humans, epithelial barrier defects correlated with alteration in the level of expression of claudin-1, a component of the tight junctions (Troeger et al., 2007). In tissue culture models, changes in another tight junction protein, zona occludens (ZO)-1, as well as the cytoskeletal proteins actin and α-actinin have also been demonstrated (Teoh et al., 2000; Buret et al., 2002). *Giardia* has also been shown to increase epithelial permeability by inducing apoptosis in epithelial cells both *in vivo* and *in vitro* (Chin et al., 2002; Troeger et al., 2007). Interestingly, the magnitude of the apoptotic response *in vitro* is highly strain-dependent, although no specific parasite markers have been identified yet which can induce apoptosis (Chin et al., 2002). Finally, excess ion secretion was recently demonstrated *ex vivo* in biopsy material from giardiasis patients, suggesting that this too may contribute to pathology during this infection (Troeger et al., 2007).

The excretion and/or secretion of virulence factors by the parasite has been speculated to be responsible in part for the pathology induced during human giardiasis. Jiménez et al. (2004) demonstrated that oral administration of excretory/secretory antigens from *G. intestinalis* elicited a robust Th2 response (i.e. IgG1, IgG2, IgE) as well as moderate to profound histological changes. They tentatively attributed these changes to be potentially due to the parasite's proteinase activity. Ringqvist et al. (2008) identified three major secreted proteins (ornithine carbamoyl transferase, arginine deiminase, and enolase) released by *Giardia.* The release of these proteins was trigged upon contact with host epithelial cells, and these secreted proteins were able to impair host innate immune mechanisms, including the production of nitric oxide.

Another group has reported the presence of an excretory/secretory molecule in the parasite that can induce fluid accumulation in ligated loops of rabbit ileum (Shant et al., 2002). A 58 kDa

enterotoxin purified from the excretory-secretory product of the parasite was able to decrease host GTPase activity (Shant et al., 2004). In this study, activation of protein kinase A (PKA) as well an increase in intracellular calcium ions were also observed after enterotoxin treatment of mouse enterocytes. Subsequently, these authors demonstrated that purified 58 kDa enterotoxin induced increased levels of phospholipase C gamma1, inositol triphosphate, and an upregulation in the protein kinase C (PKC; Shant et al., 2005). An N-terminal peptide sequence for the putative toxin has been reported. However, this peptide sequence is not represented in the virtual proteome of the prototype assemblage A strain WB (Morrison et al., 2007). It is possible that this peptide is encoded in a portion of the genome which has not been assembled, or that the WB strain lacks this molecule. Further molecular characterization of this toxin would provide a significant advance in developing tools to combat this disease.

The protective roles of intraepithelial lymphocytes (IEL) against parasitic infections have been characterized in a number of protozoan and helminth infections (Guk et al., 2003; Moretto et al., 2004; Little et al., 2005). In giardiasis, however, it was recently proposed that increased numbers of IELs in patients with chronic disease in fact were associated with a malabsorption syndrome including the impairment of the glucose/sodium uptake (Troeger et al., 2007).

Reduction in levels of some intestinal disaccharidases, sucrase, lactase, maltase, and trehalase, have been shown to occur in human giardiasis (Levinson and Nastro, 1978) and in experimental infections in mice (Gillon et al., 1982), rats (Cevallos et al., 1995), and gerbils (Buret et al., 1991). In one study of *G. muris* infections in mice carried out by Daniels and Belosevic (1995), disaccharidase deficiency during primary infection was strongly associated with parasite number. In another study with the same model, the decrease in jejunal disaccharidase activities correlated with a diffuse shortening of brush border microvilli (Buret et al., 1990). Scott et al. (2000) showed that brush border damage and diassacharidase deficiency did not develop in nude mice infected with *G. muris*. Adoptive transfer of $CD8^+$, but not $CD4^+$ T cells, from infected wild-type mice into naïve recipients induced both symptoms, indicating that immune responses are key to the brush border defects commonly observed following infection. It still remains unclear how *Giardia* infection induces $CD8^+$ T cell responses and how these cells induce the damage. Interestingly, since $CD8^+$ T cells are not required to control the infection (Singer and Nash, 2000a), it is theoretically possible to induce protective immunity without inducing nutrient malabsorption.

The final mechanism contributing to pathology is intestinal smooth muscle hypercontactility. These muscular contractions contribute to the severe cramps associated with symptomatic disease, but also increase the rate of transit of gut contents through the intestines reducing the time available for nutrient uptake and water reabsorption. Recent work in our lab and others has demonstrated that increased transit rates occur in wild-type mice following *G. duodenalis* and *G. muris* infections, but not in SCID mice even though these mice have higher parasite loads (Li et al., 2006; Andersen et al., 2006). Increased transit requires coordinated contractions and relaxation of smooth muscle. Interestingly, nitric oxide (NO) production from the neuronal isoform of nitric oxide synthase (NOS1) is required for enhanced transit. NO is normally an inhibitor of muscle contractions in the enteric system. In contrast, we have shown that enhanced muscular contractions occur following infection due to release of mast cell granule contents (Li et al., 2007). Activation of muscular contractions could be triggered in organ baths by addition of the hormone cholecystokinin (CCK), and this response was blocked by agents that interfered with mast cell function. Given that *Giardia* parasites consume bile and that a normal physiological function of CCK is to induce contraction of the gall bladder and release of bile into the small intestine, it appears as though the parasite has evolved to co-opt the host's normal physiology in order to provide an essential nutrient for its growth. Moreover, variation in the ability of different parasites to consume bile or in the host to produce

CCK or recruit mast cells following infection could help explain the variation in pathology seen in giardiasis.

Several studies have shown differences in pathology among *Giardia* isolates. There is contradicting evidence showing that a specific genotype of *G. duodenalis* (i.e. assemblage A or B) is associated with symptomatic disease. Singh et al. (2009), for example, showed that most isolates examined from symptomatic patients in Nepal were assemblage B. In contrast, other studies identified assemblage A to be associated with symptomatic disease (Haque et al., 2005). Furthermore, Kohli et al. (2008) found no differences in the ability of assemblage A and B parasites to cause symptomatic infections in children in Brazil. One possibility is that the differences observed (if any) reflect variation within these genotypic classifications, especially the presence or absence of specific virulence factors. Alternately, given that host immune responses are, in part, responsible for pathological effects induced by *G. duodenalis*, it is possible that different isolates may elicit host immune responses in different ways. For example, Williamson et al. (2000) found distinct differences in the infection dynamics, histopathological responses and serum antibody responses in a neonatal mouse model of infection with two different strains of *G. duodenalis* (although the genotypes were not reported). Collection of immune response data in parallel with patient symptoms and parasite genotypes may provide answers to this difficult issue.

Summary

Immune responses to *Giardia* can be an effective mechanism to control this infection. Development of vaccines that reduce the severity of infection could be possible. Infections with *Giardia* commonly lead to strong immune responses characterized primarily by the production of anti-parasite IgA. Repeated infections appear to produce an immune status in humans where symptoms of disease are reduced, although parasites continue to colonize the host and produce cysts. Recent insights into the molecular epidemiology of this infection, are allowing better assessment of the role of parasite variation in the range of clinical presentations which are commonly observed during giardiasis. These advances are occurring simultaneously with progress in understanding the role of immune response in contributing to pathology as well as protection. Development of vaccines that reduce the severity of infection should therefore be possible, although vaccines which can generate sterile immunity may be more difficult to achieve. Recent insights into the mechanisms involved in initiating immune responses and how these responses both eliminate parasites and contribute to pathology have provided a much better framework for this work to be carried out. Numerous areas of research, however, require more attention. These include how results in animal models relate to human disease, how the different elements of the innate and adaptive immune responses are regulated, and which mechanisms are responsible for parasite prevention of secondary infections as opposed to elimination of the primary infection.

Acknowledgments

SSM is supported by a merit-based scholarship from the Georgetown University Graduate School of Arts and Sciences. Work in the Singer laboratory is supported by a grant from the NIH/NIAID (AI-81033).

References

Abdul-Wahid A, Faubert G. Mucosal delivery of a transmission-blocking DNA vaccine encoding *Giardia lamblia* CWP2 by *Salmonella typhimurium* bactofection vehicle. Vaccine 2007;25:8372–8383. [PubMed: 17996337]

Adam RD. Biology of *Giardia lamblia*. Clinical Microbiology Reviews 2001;14:447–475. [PubMed: 11432808]

Aley SB, Zimmerman M, Hetsko M, Selsted ME, Gillin FD. Killing of *Giardia lamblia* by cryptdins and cationic neutrophil peptides. Infection and Immunity 1994;62:5397–5403. [PubMed: 7960119]

Anderson KA, Brooks AS, Morrison AL, Reid-Smith RJ, Martin SW, Benn DM, Peregrine AS. Impact of *Giardia* vaccination on asymptomatic *Giardia* infections in dogs at a research facility. The Canadian Veterinary Journal 2004;45:924–930.

Andersen YS, Gillin FD, Eckmann L. Adaptive immunity-dependent intestinal hypermotility contributes to host defense against *Giardia spp*. Infection and Immunity 2006;74:2473–2476. [PubMed: 16552082]

Angarano G, Maggi P, Di Bari MA, Larocca AM, Congedo P, Di Bari C, Brandonisio O, Chiodo F. Giardiasis in HIV: a possible role in patients with severe immune deficiency. European Journal of Epidemiology 1997;13:485–487. [PubMed: 9258558]

Ayabe T, Ashida T, Kohgo Y, Kono T. The role of Paneth cells and their antimicrobial peptides in innate host defense. Trends in Microbiology 2004;12:394–398. [PubMed: 15276616]

Bachur TP, Vale JM, Coêlho IC, Queiroz TR, Chaves Cde. S. Enteric parasitic infections in HIV/AIDS patients before and after the highly active antiretroviral therapy. The Brazilian Journal of Infectious Disease 2008;12:115–122.

Bayraktar MR, Mehmet N, Durmaz R. Serum cytokine changes in Turkish children infected with *Giardia lamblia* with and without allergy: Effect of metronidazole treatment. Acta Tropica 2005;95:116–122. [PubMed: 15979046]

Bienz M, Dai WJ, Welle M, Gottstein B, Müller N. Interleukin-6-deficient mice are highly susceptible to *Giardia lamblia* infection but exhibit normal intestinal immunoglobulin A responses against the parasite. Infection and Immunity 2003;71:1569–1573. [PubMed: 12595479]

Buret A, Gall DG, Olson ME. Effects of murine giardiasis on growth, intestinal morphology, and disaccharidase activity. The Journal of Parasitology 1990;76:403–409. [PubMed: 2191103]

Buret A, Gall DG, Olson ME. Growth, activities of enzymes in the small intestine, and ultrastructure of microvillous border in gerbils infected with *Giardia duodenalis*. Parasitology Research 1991;77:109–114. [PubMed: 2027878]

Buret AG, Mitchell K, Muench DG, Scott KG. *Giardia lamblia* disrupts tight junctional ZO-1 and increases permeability in non-transformed human small intestinal epithelial monolayers: effects of epidermal growth factor. Parasitology 2002;125:11–19. [PubMed: 12166516]

Buret AG. Mechanisms of epithelial dysfunction in giardiasis. Gut 2007;56:316–317. [PubMed: 17339241]

Cevallos A, Carnaby S, James M, Farthing JG. Small intestinal injury in a neonatal rat model of giardiasis is strain dependent. Gastroenterology 1995;109:766–773. [PubMed: 7657104]

Chin AC, Teoh DA, Scott KG, Meddings JB, Macnaughton WK, Buret AG. Strain-dependent induction of enterocyte apoptosis by *Giardia lamblia* disrupts epithelial barrier function in a caspase-3-dependent manner. Infection and Immunity 2002;70:3673–3680. [PubMed: 12065509]

Daniels CW, Belosevic M. Disaccharidase activity in male and female C57BL/6 mice infected with *Giardia muris*. Parasitology Research 1995;81:143–147. [PubMed: 7731922]

Davids BJ, Palm JE, Housley MP, Smith JR, Andersen YS, Martin MG, Hendrickson BA, Johansen FE, Svärd SG, Gillin FD, Eckmann L. Polymeric immunoglobulin receptor in intestinal immune defense against the lumen-dwelling protozoan parasite *Giardia*. The Journal of Immunology 2006;177:6281–6290. [PubMed: 17056558]

Ebert EC. *Giardia* induces proliferation and interferon gamma production by intestinal lymphocytes. Gut 1999;44:342–346. [PubMed: 10026318]

Eckmann L, Laurent F, Langford TD, Hetsko ML, Smith JR, Kagnoff MF, Gillin FD. Nitric oxide production by human intestinal epithelial cells and competition for arginine as potential determinants of host defense against the lumen-dwelling pathogen *Giardia lamblia*. The Journal of Immunology 2000;164:1478–1487. [PubMed: 10640765]

Eckmann L. Mucosal defences against *Giardia*. Parasite Immunology 2003;25:259–270. [PubMed: 12969444]

Erlich JH, Anders RF, Roberts-Thomson IC, Schrader JW, Mitchell GF. An examination of differences in serum antibody specificities and hypersensitivity reactions as contributing factors to chronic

infection with the intestinal protozoan parasite, *Giardia muris*, in mice. The Australian Journal of Experimental Biology and Medical Science 1983;61:599–615. [PubMed: 6689264]

Farthing, MJG. Giardiasis as a disease.. In: Thompson, RCA.; Reynoldson, JA.; Lymbery, AJ., editors. *Giardia*: from molecules to diseases.. CAB International; Oxon: 1994. p. 15-37.

Feitosa G, Bandeira AC, Sampaio DP, Badaró R, Brites C. High prevalence of giardiasis and stronglyloidiasis among HIV-infected patients in Bahia, Brazil. The Brazilian Journal of Infectious Diseases 2001;5:339–344. [PubMed: 12010598]

Geurden T, Levecke B, Cacció SM, Visser A, De Groote G, Casaert S, Vercruysse J, Claerebout E. Multilocus genotyping of *Cryptosporidium* and *Giardia* in non-outbreak related cases of diarrhoea in human patients in Belgium. Parasitology 2009;136:1161–1168. [PubMed: 19631012]

Gillon J, Al Thamery D, Ferguson A. Features of small intestinal pathology (epithelial cell kinetics, intraepithelial lymphocytes, disaccharidases) in a primary *Giardia muris* infection. Gut 1982;23:498–506. [PubMed: 7076025]

Guk SM, Yong TS, Chai JY. Role of murine intestinal intraepithelial lymphocytes and lamina propria lymphocytes against primary and challenge infections with *Cryptosporidium parvum*. The Journal of Parasitology 2003;89:270–275. [PubMed: 12760640]

Haque R, Roy S, Kabir M, Stroup SE, Mondal D, Houpt ER. *Giardia* assemblage A infection and diarrhea in Bangladesh. The Journal of Infectious Diseases 2005;192:2171–2173. [PubMed: 16288384]

Hardin JA, Buret AG, Olson ME, Kimm MH, Gall DG. Mast cell hyperplasia and increased macromolecular uptake in an animal model of giardiasis. The Journal of Parasitology 1997;83:908–912. [PubMed: 9379297]

Heyworth MF, Carlson JR, Ermak TH. Clearance of *Giardia muris* infection requires helper/inducer T lymphocytes. The Journal of Experimental Medicine 1987;165:1743–1748. [PubMed: 2953846]

Homan WL, Mank TG. Human giardiasis: genotype linked differences in clinical symptomatology. International Journal for Parasitology 2001;31:822–826. [PubMed: 11403774]

Istre GR, Dunlop TS, Gaspard GB, Hopkins RS. Waterborne giardiasis at a mountain resort: evidence for acquired immunity. American Journal of Public Health 1984;74:602–604. [PubMed: 6721017]

Isaac-Renton JL, Lewis LF, Ong CS, Nulsen MF. A second community outbreak of waterborne giardiasis in Canada and serological investigation of patients. The Transactions of the Royal Society of Tropical Medicine and Hygiene 1994;88:395–399.

Jiménez JC, Fontaine J, Grzych JM, Dei-Cas E, Capron M. Systemic and mucosal responses to oral administration of excretory and secretory antigens from *Giardia intestinalis*. Clinical and diagnostic laboratory immunology 2004;11:152–160. [PubMed: 14715563]

Jung HC, Eckmann L, Yang SK, Panja A, Fierer J, Morzycka-Wroblewska E, Kagnoff MF. A distinct array of proinflammatory cytokines is expressed in human colon epithelial cells in response to bacterial invasion. The Journal of Clinical Investigation 1995;95:55–65. [PubMed: 7814646]

Kamda JD, Singer SM. Phosphoinositide 3-kinase-dependent inhibition of dendritic cell interleukin-12 production by *Giardia lamblia*. Infection and Immunity 2009;77:685–693. [PubMed: 19047410]

Kohli A, Bushen OY, Pinkerton RC, Houpt E, Newman RD, Sears CL, Lima AA, Guerrant RL. *Giardia duodenalis* assemblage, clinical presentation and markers of intestinal inflammation in Brazilian children. Transactions of the Royal Society of Tropical Medicine and Hygiene 2008;102:718–725. [PubMed: 18485429]

Larocque R, Nakagaki K, Lee P, Abdul-Wahid A, Faubert GM. Oral immunization of BALB/c mice with *Giardia duodenalis* recombinant cyst wall protein inhibits shedding of cysts. Infection and Immunity 2003;71:5662–5669. [PubMed: 14500486]

Lee P, Faubert G. Expression of the *Giardia lamblia* cyst wall protein 2 in *Lactococcus lactis*. Microbiology 2006a;152:1981–1990. [PubMed: 16804173]

Lee P, Faubert GM. Oral immunization of BALB/c mice by intragastric delivery of *Streptococcus gordonii*-expressing *Giardia* cyst wall protein 2 decreases cyst shedding in challenged mice. FEMS Microbiology Letters 2006b;265:225–236. [PubMed: 17081198]

Levinson JD, Nastro LJ. Giardiasis with total villous atrophy. Gastroenterology 1978;74:271–275. [PubMed: 620900]

Li E, Zhou P, Petrin Z, Singer SM. Mast cell-dependent control of *Giardia lamblia* infections in mice. Infection and Immunity 2004;72:6642–6649. [PubMed: 15501797]

Li E, Zhou P, Singer SM. Neuronal nitric oxide synthase is necessary for elimination of *Giardia lamblia* infections in mice. The Journal of Immunology 2006;176:516–521. [PubMed: 16365445]

Li E, Zhao A, Shea-Donohue T, Singer SM. Mast cell-mediated changes in smooth muscle contractility during mouse giardiasis. Infection and Immunity 2007;75:4514–4518. [PubMed: 17620354]

Little MC, Bell LV, Cliffe LJ, Else KJ. The characterization of intraepithelial lymphocytes, lamina propria leukocytes, and isolated lymphoid follicles in the large intestine of mice infected with the intestinal nematode parasite *Trichuris muris*. The Journal of Immunology 2005;175:6713–6722. [PubMed: 16272327]

Matowicka-Karna J, Dymicka-Piekarska V, Kemona H. IFN-gamma, IL-5, IL-6 and IgE in patients infected with *Giardia intestinalis*. Folia Histochemica et Cytobiologica 2009;47:93–97. [PubMed: 19419945]

Merluzzi S, Frossi B, Gri G, Parusso S, Tripodo C, Pucillo C. Mast cells enhance proliferation of B lymphocytes and drive their differentiation toward IgA-secreting plasma cells. Blood 2010;115:2810–2817. [PubMed: 20101023]

Moretto M, Weiss LM, Khan IA. Induction of a rapid and strong antigen-specific intraepithelial lymphocyte response during oral *Encephalitozoon cuniculi* infection. The Journal of Immunology 2004;172:4402–4409. [PubMed: 15034055]

Morrison HG, McArthur AG, Gillin FD, et al. Genomic minimalism in the early diverging intestinal parasite *Giardia lamblia*. Science 2007;317:1921–1926. [PubMed: 17901334]

Nash TE. Antigenic variation in *Giardia lambli*a and the host's immune response. Philosophical Transactions of The Royal Society of London. Series B. Biological Science 1997;352:1369–1375.

Oberhuber G, Kastner N, Stolte M. Giardiasis: a histologic analysis of 567 cases. Scandinavian Journal of Gastroenterology 1997;32:48–51. [PubMed: 9018766]

Olson ME, Morck DW, Ceri H. The efficacy of a *Giardia lamblia* vaccine in kittens. Canadian Journal of Veterinary Research 1996;60:249–256. [PubMed: 8904660]

Olson ME, Morck DW, Ceri H. Preliminary data on the efficacy of a *Giardia* vaccine in puppies. The Canadian Veterinary Journal 1997;38:777–779.

Olson ME, Ceri H, Morck DW. *Giardia* Vaccination. Parasitology Today 2000;16:213–217. [PubMed: 10782082]

Olson ME, Hannigan CJ, Gaviller PF, Fulton LA. The use of a *Giardia* vaccine as an immunotherapeutic agent in dogs. The Canadian Veterinary Journal 2001;42:865–868.

Pérez PF, Minnaard J, Rouvet M, Knabenhans C, Brassart D, De Antoni GL, Schiffrin EJ. Inhibition of *Giardia intestinalis* by extracellular factors from Lactobacilli: an in vitro study. Applied and Environmental Microbiology 2001;67:5037–5042. [PubMed: 11679323]

Ringqvist E, Palm JE, Skarin H, Hehl AB, Weiland M, Davids BJ, Reiner DS, Griffiths WJ, Eckmann L, Gillin FD, Svärd SG. Release of metabolic enzymes by *Giardia* in response to interaction with intestinal epithelial cells. Molecular and Biochemical Parasitology 2008;159:85–91. [PubMed: 18359106]

Roberts-Thomson IC, Mitchell GF. Giardiasis in mice. I. Prolonged infections in certain mouse strains and hypothymic (nude) mice. Gastroenterology 1978;75:42–46. [PubMed: 401094]

Roskens H, Erlandsen SL. Inhibition of *in vitro* attachment of *Giardia* trophozoites by mucin. The Journal of Parasitology 2002;88:869–873. [PubMed: 12435122]

Roxström-Lindquist K, Ringqvist E, Palm D, Svärd S. *Giardia lamblia*-induced changes in gene expression in differentiated Caco-2 human intestinal epithelial cells. Infection and Immunity 2005;73:8204–8208. [PubMed: 16299316]

Roxström-Lindquist K, Palm D, Reiner D, Ringqvist E, Svärd SG. *Giardia* immunity--an update. Trends in Parasitology 2006;22:26–31. [PubMed: 16303332]

Salzman NH, Underwood MA, Bevins CL. Paneth cells, defensins, and the commensal microbiota: a hypothesis on intimate interplay at the intestinal mucosa. Seminars in Immunology 2007;19:70–83. [PubMed: 17485224]

Scott KG, Logan MR, Klammer GM, Teoh DA, Buret AG. Jejunal brush border microvillous alterations in *Giardia muris*-infected mice: role of T lymphocytes and interleukin-6. Infection and Immunity 2000;68:3412–3418. [PubMed: 10816492]

Shant J, Ghosh S, Bhattacharyya S, Ganguly NK, Majumdar S. Mode of action of a potentially important excretory-secretory product from *Giardia lamblia* in mice enterocytes. Parasitology 2005;131:57–69. [PubMed: 16038397]

Shant J, Ghosh S, Bhattacharyya S, Ganguly NK, Majumdar S. The alteration in signal transduction parameters induced by the excretory-secretory product from *Giardia lamblia*. Parasitology 2004;129:421–430. [PubMed: 15521630]

Shant J, Bhattacharyya S, Ghosh S, Ganguly NK, Majumdar S. A potentially important excretory-secretory product of *Giardia lamblia*. Experimental Parasitology 2002;102:178–186. [PubMed: 12856314]

Singer SM, Nash TE. T-cell-dependent control of acute *Giardia lamblia* infections in mice. Infection and Immunity 2000a;68:170–175. [PubMed: 10603384]

Singer SM, Nash TE. The role of normal flora in *Giardia lamblia* infections in mice. The Journal of Infectious Diseases 2000b;181:1510–1512. [PubMed: 10751141]

Singh A, Janaki L, Petri WA Jr. Houpt ER. *Giardia intestinalis* assemblages A and B infections in Nepal. American Journal of Tropical Medicine and Hygiene 2009;81:538–539. [PubMed: 19706929]

Smith PD. Pathophysiology and immunology of giardiasis. Annual Review of Medicine 1985;36:295–307.

Snel SJ, Baker MG, Kamalesh V, French N, Learmonth J. A tale of two parasites: the comparative epidemiology of cryptosporidiosis and giardiasis. Epidemiology and infection 2009;137:1641–1650. [PubMed: 19393124]

Snider DP, Skea D, Underdown BJ. Chronic giardiasis in B-cell-deficient mice expressing the xid gene. Infection and Immunity 1988;56:2838–2842. [PubMed: 3262587]

Solaymani-Mohammadi S, Genkinger JM, Loffredo CA, Singer SM. A meta-analysis of the effectiveness of albendazole compared with metronidazole as treatments for infections with *Giardia duodenalis*. PLoS Neglected Tropical Diseases 2010;4:e682. [PubMed: 20485492]

Stäger S, Gottstein B, Müller N. Systemic and local antibody response in mice induced by a recombinant peptide fragment from *Giardia lamblia* variant surface protein (VSP) H7 produced by a *Salmonella typhimurium* vaccine strain. International Journal for Parasitology 1997;27:965–971. [PubMed: 9292314]

Stark D, Barratt JL, van Hal S, Marriott D, Harkness J, Ellis JT. Clinical significance of enteric protozoa in the immunosuppressed human population. Clinical Microbiology Reviews 2009;22:634–650. [PubMed: 19822892]

Stein JE, Radecki SV, Lappin MR. Efficacy of *Giardia* vaccination in the treatment of giardiasis in cats. Journal of American Veterinary Medicine Association 2003;222:1548–1551.

Stevens DP, Frank DM, Mahmoud AA. Thymus dependency of host resistance to *Giardia muris* infection: studies in nude mice. The Journal of Immunology 1978;120:680–682. [PubMed: 621403]

Teoh DA, Kamieniecki D, Pang G, Buret AG. *Giardia lamblia* rearranges F-actin and alpha-actinin in human colonic and duodenal monolayers and reduces transepithelial electrical resistance. The Journal of Parasitology 2000;86:800–806. [PubMed: 10958459]

Thompson RC, Reynoldson JA, Mendis AH. *Giardia* and giardiasis. Advances in Parasitology 1993;32:71–160. [PubMed: 8237618]

Thompson RC. *Echinococcus, Giardia* and *Cryptosporidium:* observational studies challenging accepted dogma. Parasitology 2009;136:1529–1535. [PubMed: 19366481]

Troeger H, Epple HJ, Schneider T, Wahnschaffe U, Ullrich R, Burchard GD, Jelinek T, Zeitz M, Fromm M, Schulzke JD. Effect of chronic Giardia lamblia infection on epithelial transport and barrier function in human duodenum. Gut 2007;56:328–335. [PubMed: 16935925]

Uehlinger FD, O'Handley RM, Greenwood SJ, Guselle NJ, Gabor LJ, Van Velsen CM, Steuart RF, Barkema HW. Efficacy of vaccination in preventing giardiasis in calves. Veterinary Parasitology 2007;146:182–188. [PubMed: 17350765]

von Allmen N, Christen S, Forster U, Gottstein B, Welle M, Müller N. Acute trichinellosis increases susceptibility to *Giardia lamblia* infection in the mouse model. Parasitology 2006;133:139–149. [PubMed: 16677442]

Williamson AL, O'Donoghue PJ, Upcroft JA, Upcroft P. Immune and pathophysiological responses to different strains of *Giardia duodenalis* in neonatal mice. International Journal for Parasitology 2000;30:129–136. [PubMed: 10704595]

Zhou P, Li E, Zhu N, Robertson J, Nash T, Singer SM. Role of interleukin-6 in the control of acute and chronic *Giardia lamblia* infections in mice. Infection and Immunity 2003;71:1566–1568. [PubMed: 12595478]

Zhou P, Li E, Shea-Donohue T, Singer SM. Tumour necrosis factor alpha contributes to protection against *Giardia lamblia* infection in mice. Parasite Immunology 2007;29:367–374. [PubMed: 17576366]