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# *Giardia duodenalis*: The double-edged sword of immune responses in giardiasis

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ABSTRACT

Giardiasis is one of the most common intestinal protozoan infections worldwide. The etiological agent, *Giardia duodenalis* (syn. *Giardia lamblia*, *Giardia 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.



## 1. Epidemiology

Recent studies of *Giardia* have identified eight distinct genotypes within *Giardia 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). Thus, while some studies have shown some protection against experimental infections (Olson et al., 1996, 1997, 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 three 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, Geurden 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

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to the fact that assigning parasites to specific genotypes usually reflects alleles at loci such as glutamate dehydrogenase, 18S RNA and triosephosphate 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.

## 2. 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 5 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 does not prevent infection, but does reduce the pathology which can occur (Kohli et al., 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 *Giardia 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., 2001). 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 et al., 1994). However, the effects of defensins on intestinal flora is bidirectional, and changes in flora can also effect production of defensins (Ayabe et al., 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 agammaglobulinemia (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 poly Ig 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, 8 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