

## Immunological aspects of *Giardia* infections

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**Abstract** – Immunodeficiency, particularly antibody deficiency, predisposes to increased intensity and persistence of *Giardia* infections. *Giardia*-infected immunocompetent hosts produce serum and intestinal antibodies against *Giardia* trophozoites. The number of *Giardia muris* trophozoites, in mice with *G. muris* infection, is reduced by intra-duodenal administration of anti-*G. muris* antibody. *Giardia intestinalis* antigens that are recognised by human anti-trophozoite antibodies include variable (variant-specific) and invariant proteins. Nitric oxide (NO) appears to contribute to host clearance of *Giardia* trophozoites. Arginine is a precursor of NO and is metabolised by *Giardia* trophozoites, possibly reducing its availability for generation of NO by the host. Work with mice suggests that T lymphocytes and interleukin-6 (IL-6) contribute to clearance of *Giardia* infection via mechanisms independent of antibodies.

**Key words:** *Giardia intestinalis*, Giardiasis, NO.

**Résumé** – Aspects immunologiques des infections à *Giardia*. L'immunodéficience, particulièrement la déficience en anticorps, prédispose à une augmentation de l'intensité et de la durée des infections à *Giardia*. Les hôtes immunocompétents infectés par *Giardia* produisent des anticorps sériques et intestinaux contre les trophozoïtes de *Giardia*. Le nombre de trophozoïtes de *Giardia muris*, chez la souris infectée par *G. muris*, est réduit par l'administration intra-duodénale d'anticorps anti *G. muris*. Les antigènes de *Giardia intestinalis* qui sont reconnus par des anticorps humains anti-trophozoïtes comprennent des protéines variables (spécifiques aux variants) et des protéines invariantes. L'oxyde nitrique (NO) semble contribuer à débarrasser l'hôte des trophozoïtes de *Giardia*. L'arginine est un précurseur de NO et est métabolisé par les trophozoïtes de *Giardia*, ce qui réduit peut-être sa disponibilité pour la production de NO par l'hôte. Les travaux sur la souris suggèrent que les lymphocytes T et l'interleukine-6 (IL-6) contribuent à éliminer *Giardia* par des mécanismes indépendants des anticorps.

*Giardia intestinalis* (synonyms: *G. duodenalis*, *G. lamblia*) is a protozoan parasite that colonises the small intestinal lumen of vertebrate hosts. In human subjects, *G. intestinalis* infections range in clinical severity from asymptomatic colonisation to a debilitating syndrome that includes chronic diarrhoea and malabsorption. The two-stage life cycle of *Giardia* species comprises the motile trophozoite, with eight flagella and a ventral adhesive disc by which it adheres to the luminal surface of intestinal epithelial cells (and thereby resists peristaltic expulsion from the host's intestine), and the thick-walled cyst, which is excreted from the host. Previously uninfected hosts become infected by oral ingestion of *Giardia* cysts.

*Giardia* infections are increased in intensity and/or duration in human or non-human mammalian hosts with various forms of immunodeficiency, in comparison with their immuno-

competent counterparts [9, 14, 20]. This situation indicates that host immunological responses limit the intensity and/or duration of these infections. The extant literature suggests that impaired production of anti-*Giardia* antibodies is the main reason why immunodeficiency states predispose to severe/prolonged *Giardia* infections [4]. From the 1980s onwards, it has been known that *Giardia*-infected human and non-human hosts generate serum and intestinal antibody responses against *Giardia* trophozoites [10, 28]. Anti-*Giardia* IgA is present in the intestinal lumen of *Giardia*-infected hosts and has also been detected in human milk [10, 32]. Colostrum from cows with *G. intestinalis*-infected calves contains anti-*Giardia* IgG [19]. Intraperitoneal or intraduodenal administration of anti-*G. muris* antibody leads to reduction in the number of intestinal *G. muris* trophozoites, in mice infected with this parasite [1, 3]. This result is consistent with a role for antibodies in clearing *G. muris* from the mouse intestinal lumen.

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*Giardia* trophozoite antigens that are recognised by antibodies of *Giardia*-infected hosts include heterogeneous “variant-specific surface proteins” (VSPs), and non-variable (structurally conserved) proteins [21]. Of a repertoire of approximately 150 (or more) VSPs in *Giardia intestinalis*, only one VSP appears to be expressed on an individual *Giardia* trophozoite at any one time, other than during antigenic “switching” [17, 18]. It has been speculated that antigenic switching by *Giardia* trophozoites, whereby expression of one VSP changes to that of a different VSP, might be an immune evasion strategy (an adaptation by the parasite to the presence of host antibodies directed against whichever VSP is initially expressed by a population of trophozoites in the intestinal lumen) [17]. The observation that *G. intestinalis* trophozoites switch from the expression of one VSP to another in the absence of antibodies, during *in vitro* culture [18], does not rule out the possibility that antibodies might select against the persistence of initially expressed VSP(s) in the host. The biological role, if any, of VSPs appears to be unknown, although it has been postulated that expression of a particular VSP might influence the relative ability of *Giardia* trophozoites to colonise a particular species of host [26]. *Giardia* trophozoites genetically engineered to express “numerous” VSPs simultaneously can act as a vaccine (whether given as live organisms, or as an inanimate mixture of antigens) to generate protective anti-*Giardia* immunity in a gerbil host [24]. The implications of this finding for understanding the “normal” mechanism(s) of host protective immunity against *Giardia* infection(s) are, however, unclear.

Sera from *G. intestinalis*-infected human subjects contain antibodies directed against trophozoite VSPs [23]. Of possibly greater biological significance, antibodies against *G. intestinalis* trophozoite proteins that are structurally conserved (invariant) have also been identified in sera from *Giardia*-infected individuals. Trophozoite invariant proteins recognised by human serum antibodies include  $\alpha$ -giardins (a group of proteins originally regarded as “internal” in trophozoite adhesive discs, though later identified on trophozoite surface membranes and flagella) [22, 33–35], fructose-1,6-bisphosphate aldolase, and *G. intestinalis* enzymes involved in arginine metabolism (arginine deiminase and ornithine carbamoyl transferase) [21]. Host immunological memory is suggested by the isolation of *Giardia*-reactive CD4+ T lymphocytes from the peripheral blood of human subjects known to have been infected with *G. intestinalis* during an outbreak of giardiasis 5 years previously [7]. One can speculate that these CD4+ T lymphocytes included cells that were able to provide “help” for *Giardia*-specific antibody production by B lymphocytes.

A plausible mechanism for presumed antibody-mediated clearance of *Giardia* infections would involve prevention (by antibodies) of trophozoite attachment to the host intestinal epithelium [8] followed by peristaltic expulsion of these organisms from the intestine. Reportedly, an antibody against  $\delta$ -giardin (a protein in trophozoite adhesive discs) inhibits *G. intestinalis* trophozoite attachment to non-biological surfaces; however, the pertinent antibody may (also) have killed trophozoites, as judged by their morphology after exposure to the antibody [12]. Oral administration of a *Salmonella enterica* strain bioengineered to express *G. intestinalis*  $\alpha$ -1 giardin

induced serum IgG and intestinal IgA antibodies against  $\alpha$ -1 giardin in mice, and conferred some protection against subsequent *G. intestinalis* infection in the animals [11]. Although recombinant  $\alpha$ -1 giardin of *G. intestinalis* binds to human intestinal epithelial cells *in vitro* [34], exposure of *G. intestinalis* trophozoites to antibody directed against  $\alpha$ -1 giardin did not inhibit the ability of these organisms to become attached to a non-biological surface [6]. Further work may be needed to clarify the role, if any, of antibodies against giardin(s) in clearance of/protection against *Giardia* infections.

Experimental work with mice has suggested that T lymphocytes can contribute directly (i.e., in the absence of antibodies) to clearance of infection with a clone of *G. intestinalis* (GS/M-H7) [27]. The mechanism(s) involved in this putative T-cell-mediated clearance of *Giardia* infection does not appear to be known (it may be worth mentioning that a postulated effector role for T cells in the clearance process would not be identical to CD4+ T-cell-mediated help for anti-*Giardia* antibody production) [27].

Studies of *Giardia* infections in rodents have implicated interleukin-6 (IL-6) in anti-*Giardia* immunity. IL-6-deficient mice have a diminished ability to clear infection caused by *G. intestinalis* [2, 36]. The mice studied in the pertinent experiments were able to produce intestinal anti-trophozoite IgA; the findings suggest that IL-6 contributes to clearance of *Giardia* infection in mice (albeit by an unknown mechanism that appears not to involve IgA). Recent work has identified dendritic cells (of bone marrow origin) as a source of IL-6 that promotes clearance of *G. intestinalis* infection in mice [13].

There is evidence that intestinal nitric oxide (NO) contributes to host clearance of *Giardia* trophozoites [5]. In view of the fact that arginine is a substrate for generation of NO, it is interesting that *Giardia* trophozoites appear to compete with the host for arginine [5]. *Giardia* trophozoites are able to metabolise arginine [25]. Uptake and metabolism of arginine by *Giardia* trophozoites has implications for host nutrition (reducing the proportion of dietary arginine available for absorption by the host), as well as for trophozoite survival via reduced availability of arginine for host NO production [29, 30].

Experimental work has implicated mast cells in clearance of *Giardia* infections, at least in mice [16]. There is evidence of increased expression of mast cell protease genes during *Giardia* infections in mice [31], though whether this increased gene expression contributes to a possible role for mast cells in promoting intestinal peristalsis (and consequent expulsion of *Giardia* trophozoites from the intestinal lumen) is unclear [16].

As mentioned above, mice can be partially protected against *Giardia* infection, by oral administration of bacteria that have been genetically engineered to express *Giardia* proteins [11, 15]. There would seem to be little clinical imperative to try to develop a vaccine against human giardiasis, even if doing so were eventually found to be technically feasible.

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