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PROTOZOA (GIARDIA) (SM SINGER, SECTION EDITOR)

The Immunological Enigma of Human Giardiasis

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Abstract Giardiasis is a major cause of enteritis in humans worldwide. The disease is caused by two very distinct genetic groups (referred to as assemblages A and B) of the species complex *Giardia duodenalis*. The trophozoites stage colonizes the duodenum and jejunum of the small intestine and may cause a broad variety of disease outcomes that reach from asymptomatic carriers to patients with acute or chronic severe gastrointestinal complaints. Studies in immunocompromised patients imply that antibody-mediated acquired immune responses and a minimal T cell availability are of major importance for parasite clearance. These mechanisms likely play in concert with natural resistance mechanisms that are present in the intestinal mucosa. In humans no sterile immunity is acquired after infection. Epidemiological studies further suggest passive protection from symptomatic giardiasis in breastfed children. However, there is a fundamental lack of knowledge about the underlying immunological mechanisms of human giardiasis.

Keywords Giardia duodenalis · Human giardiasis · Immune response

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Introduction

The purpose of the present review is to recapitulate what the outcome of *Giardia* infection in immunodeficient patients teaches us and to review recent advances in our knowledge of immunology of human giardiasis. This knowledge is compared to the evolving concepts in immunology with the eventual aim to identify current research gaps that need attention. For the interested readers, we also would like to refer to more detailed reviews on immunology and pathophysiology of giardiasis by others [1•, 2–4, 5•, 6].

Our knowledge about the immunological and pathophysiological mechanisms triggered during human infections with *Giardia duodenalis* (syn., *G. intestinalis*, *G. lamblia*) remains fragmented. The causative agents of giardiasis are the trophozoites that establish the infection in the duodenum and jejunum after excystation from the infectious dauer form, the cysts. Trophozoites are able to strongly attach to the luminal side of the epithelial cell layer with a highly specialized adhesive disc. Trophozoites possess eight flagella that enable motility. The parasites multiply by simple binary fission and reside exclusively extracellularly in the gut lumen. Their surface is dominated by variant surface protein. When reaching the lower small intestine, trophozoites transform (encystation) again into the infectious cyst stage that is shed with feces. A new infection is established by ingestion of infectious cysts, e.g., with contaminated food or water or by direct transmission from host to host [1•].

We know that manifestation of *G. duodenalis* infections is highly variable. While many of the infected people remain asymptomatic, others show clinical symptoms that are rather unspecific and include serious diarrhea, abdominal pain and cramps, bloating, nausea, and vomiting. In chronically infected patients, symptomatic outcome is correlated with altered intestinal transport and barrier dysfunction [7]. Symptoms start

usually after a prepatency period of about 7–10 days. Acute symptomatic infections mostly resolve spontaneously after 2–3 weeks. Recovery seems not to lead to immunity to reinfection. Moreover, chronic infection and treatment refractory courses of infection are common [8, 9]. Postinfection sequelae, such as irritable bowel syndrome, chronic fatigue, and reactive arthritis [10, 9, 11], have been described to occur after *Giardia* infections, but reasons and mechanisms remain obscure.

We also know that the variability in manifestation of giardiasis is matched by an even greater diversity in the etiologic agent. *G. duodenalis* is considered a species complex that consists of eight morphological identical but genetically distinct groups, designated as assemblages A–H. Assemblages A and B are pathogenic for humans and a wide range of animals and therefore are potentially zoonotic. Due to high sequence diversity, assemblage A and B are predicted to share only 78 % amino acid sequence homology in proteins based on current genome examples, assemblages may have to be considered as two distinct species [12]. Both assemblage A and B parasite populations show significant sequence polymorphisms within themselves, a fact that is commonly used to further sub-divide the species complex. There is evidence from epidemiological studies that clinical manifestations may be related to infections with one or the other assemblage. However, studies yielded conflicting results which may be due to the rather low resolution of current typing methods and/or differences in the patient strata investigated.

Giardiasis in Immunocompromised Humans

Increased occurrence in patients with acquired or congenital immunodeficiencies have been very informative in other infections to reveal the most critical components of immunity. *G. duodenalis* infections are not particularly rare and we would like to reason that its prevalence of 1–4 % even in high resource countries with very well-developed medical systems is high enough to reveal links to immune disorders if they existed. In the recent past, the AIDS epidemic revealed a number of such links. For example, *Cryptosporidium spp* have been clearly associated with severe enteritis in individuals with reduced $CD4^+$ T cell counts [13]. However, symptoms and incidence of *G. duodenalis* infection in HIV/AIDS patients was not significantly altered. This suggests no direct role of CD4-T cells during giardiasis [14–17]. Although HIV/AIDS patients have a lower anti-*Giardia* Ig response, they are still able to clear infection efficiently [18]. Other patients with cellular immunodeficiencies such as people affected by the DiGeorge syndrome or with severe combined immunodeficiency are also not more prone to symptomatic giardiasis [19, 20]. Only when CD4 counts in HIV/AIDS patients become reduced to ~1 % of normal values was an increased risk of symptomatic *G. duodenalis* infections noted, indicating that only a minimal T cell compartment is necessary to support the control of *Giardia* infection in humans [21, 22, 13].

In contrast to T cell deficient patients, individuals with immune deficiencies that mainly affect B cells such as X-linked agammaglobulinemia are more prone to chronic *Giardia* infection [23, 24, 20, 25]. A similar mutation in the mouse also leads to an elevated duration of *Giardia muris* primary infection [26, 27]. In μMT mice that lack B cells, infections with *G. duodenalis* and *G. muris* have a contradictory outcome. While these mice controlled *G. duodenalis* infection, *G. muris* infection was prolonged [28, 29]. The latter discrepancy between *G. muris* and *G. duodenalis* infection in mice exemplarily highlights the difficulties to use mice infected with *G. duodenalis* as a model for human giardiasis.

From this recapitulation, it follows that symptomatic *G. duodenalis* infections are correlated with and possibly controlled by antibody enhanced effector mechanisms. However, there is also evidence for antibody-independent control mechanisms.

Natural Resistance Mechanisms

Mucosal Fluids Although the parasites colonize the duodenum and jejunum, earlier studies have shown that *Giardia* trophozoites can be killed in vitro by incubation with duodenal fluid from non-infected individuals due to free fatty acids or other products of lipolysis [30]. The toxic effects of these products can be reverted in vitro by addition of bovine serum albumin or human duodenal-jejunal mucus most likely due to trapping of the toxic compounds [30]. Epidemiological studies reveal a correlation between protection from symptomatic giardiasis in newborn children with breastfeeding, and this has been attributed to the occurrence of *G. duodenalis*-specific antibodies (mainly sIgA) in the milk of the mothers [31–34]. But, milk from non-infected mothers was also shown to kill parasites in vitro which was linked to toxic products of lipolysis too [35, 36]. The relative contribution of these toxic products to protection due to breastfeeding is not entirely clear, but they likely act in concert with anti-*G. duodenalis* secretory immunoglobulin A (sIgA). Lactoferrin is another antimicrobial component of the human milk that may interfere with *G. duodenalis* infections in children [37]. Human milk is complex, and in addition to fatty acids, lactoferrin and sIgA, they comprise a multitude of anti-microbial substances. Lysozyme, lactoperoxidases, kappa-casein, haptocorrin, alpha-lactalbumin, complex oligosaccharides, and mucins, and even cytokines and cells of the immune system such as macrophages, granulocytes, and other leucocytes, are present [38–40]. It requires much more research to define to which extend the various components contribute to protection of the newborn from symptomatic giardiasis. It is also an open question how the developing gut of neonates responds to

G. duodenalis infections and how breastfeeding impacts on this system [40].

Mucins G. duodenalis trophozoites require motility for colonization as they need to translocate through the mucus layer in order to adhere to epithelial cells [41, 42]. Apart from motility, little is known how trophozoites are able to pass this layer and how they survive the anti-microbial defense mechanisms associated with the mucin layer in the small intestine. The latter include α-defensins, cathelicidins, lysozymes, angiogenin 4, secreted phospholipase A2, lectins, and collectins [41]. A recent study shows lectin-binding dependent killing of *Giardia* trophozoites by complement, but the role of lectin-binding in the intestinal lumen is unclear [43]. The mucin Muc 2, mainly produced by Goblet cells, is the major gel-forming component of the mucus layer in the small intestine, and it has been suggested that excretory/secretory products of the parasite, likely cysteine proteases and glycosidases, degrade the mucus [44, 45, 42]. On the one hand, mucus may stimulate growth and protect trophozoites from toxic effects of human milk [46–48]; on the other hand, mucus was also shown to inhibit trophozoite attachment to culture dishes [49]. Mechanisms underlying these divergent effects are not understood. As mucus composition and role as anti-parasitic effector can change during infection as recently shown for gastrointestinal nematode infections [50], its role needs to be further investigated in giardiasis.

Intestinal Proteases Intestinal proteases are also able to induce *G. duodenalis*' death in vitro, which is partly dependent on the type of variant surface protein expressed on the surface of the trophozoites [51]. The surface of the parasite is dominated by a single member of this vast family of proteins to which it devotes a significant portion of its coding genome. While most of the trophozoites will be dying after treatment with intestinal proteases, some parasites survive presumably because they express a protease resistant variable surface protein (VSP) member on their surface. Biochemical analysis indicated that certain VSPs are resistant to proteolytic cleavage [51, 52]. Studies in SCID mice that lack an adaptive immune system show selective growth of mouse-adapted *G. duodenalis* clones with specific VSPs. This indicates that VSP-dependent selection can occur independently from anti-VSP immunoglobulins [53, 54]. VSP switching is thus thought of as an escape mechanism, but it should be highlighted that assemblages can show very different VSP switching behaviors, e.g., *G. duodenalis* strain WB6 (assemblage AI) switches approximately every 12 generations while strain GS (assemblage B) approximately every 6 generations [55]. This difference may greatly influence a parasite's ability to establish infection and evade control.

Defensins These anti-microbial peptides are mainly produced by epithelial cells and neutrophils. In the gut, high concentrations of specific α-defensins are found in the crypts of the intestinal layer, where they are produced by Paneth cells. In vitro, data suggested that *G. duodenalis* trophozoites are selectively susceptible to different human and mouse defensins [56]. The real impact of intestinal defensins on *G. duodenalis* infection is unclear and understudied, but data from mouse models suggest that they play a role in protection [3, 57•].

Nitric Oxide Inducible nitric oxide (iNOS) is an important effector molecule against many pathogens and is for example produced by proinflammatory macrophages. In mice iNOS (NOS2), depletion alone showed no alteration in parasite load [58]. In vitro studies however confirmed profound giardiastatic effect of NO [59]. Beside iNOS, two other enzyme isoforms in neurons (NOS1) and endothelial cells (NOS3) exist that produce NO as a neurotransmitter. In enteric neurons, NO is involved in the modulation of enteric muscle contraction and bowel movement. It has been shown that NO is one mediator for increased intestinal propulsion that eliminates *G. duodenalis* infection in mice [60, 58]. Studies suggest that NO plays in concert with another neurotransmitter, cholecystokinin (CCK), to increase intestinal compulsion and thus intestinal transit rates [60, 5•]. To date, the validation of findings in murine models with respect to iNOS has met great difficulties in attempts to translate this to the human situation due to the very different nature of triggers of iNOS expression in human cells. Thus, a role for iNOS or, more generally, NO in humans with giardiasis remains speculative.

All these data imply that *G. duodenalis* infections are controlled to a variable degree by natural resistance mechanisms that may play in concert with innate and adaptive immune factors. However, in humans, this subject is greatly understudied, and the role of these natural resistance mechanisms as a selective force during colonization with various assemblages (species) or sub-assemblages of the parasite is not clear and greatly understudied. Their effect on isolates of different genetic composition should be advocated as a focus of future investigations. To date, most studies have been performed using either one of the laboratory strains WB (assemblage A) or GS (Assemblage B), but we lack a systematic analysis of a broader variety of isolates.

Innate Immunity

The key immunological concept governing the initiation of an immune response is the recognition of foreign entities by cells using pattern recognition receptors (PRR) located at external or internal membranes or in the cytoplasm [61]. Many of these receptors are expressed not only by cells of the immune

system but also by mucosal epithelial cells. Arguably, two main cell types first come into contact with *G. duodenalis* trophozoites and may thus mediate the underlying innate and adaptive immune responses; epithelial cells such as enterocytes, Goblet cells, and Paneth cells that derive from the intestinal stem cell lineage lining the intestinal layer and mucosal innate immune cells including intestinal macrophages, intestinal and plasmacytoid dendritic cells (DCs), eosinophils, mast cells, invariant natural killer T cells (iNKT), mucosal-associated invariant T cells (MAIT), and the recently identified innate lymphoid cells (ILC1, ILC2, ILC3) [62]. In giardiasis, responses of only two of these cell types have been analyzed in some detail: enterocytes and mononuclear phagocytic cells (mainly blood monocyte-derived DCs).

Enterocytes Gene expression analysis of human intestinal epithelial cells, Caco-2 cells, co-cultured with *G. duodenalis* (WB6) trophozoites, revealed a very early increase of various chemokines and chemokine receptors including CCL20, CCL2, and CXCR4 [63]. Upregulation of several transcription factors imply a complex stimulation cascade in enterocytes upon contact with trophozoites that also involve Nf-kB and mitogen-activated phospho-kinases (MAPK) signaling [63, 64]. This response would be consistent with recognition of the pathogen by a PRR, but the respective receptors have not been identified. However, the response is also compatible with the reaction of epithelial cells to nutritional stress as sensed by the mTOR pathway and linking to an ER stress response pattern [65]. In mice, ER stress response engineered to occur in the mucosal epithelial cells induces inflammation [66].

The release of chemoattractants such as CCL20 by enterocytes has not been further studied, but they may lead to recruitment of cells of the immune system to the gut. Histologically, this would be recorded as inflammation. However, the occurrence of local inflammation in the *G. duodenalis*-infected tissue has been controversial. Data of some studies are interpreted as evidence that inflammation is only rarely associated with *giardiasis* in humans [67, 68, 5•]. The largest study analyzed intestinal biopsies of approximately 19,000 patients, consecutive cases with "unspecific gastrointestinal complaints." In 567 of these specimens, *G. duodenalis* trophozoites were identified, but no significant increase in cell infiltration compared to control specimens was observed [67]. However, this study design did not allow to investigate asymptomatic versus symptomatic cases of *G. duodenalis* infection. In fact, the frequency of infections observed was 0.3 % which is even below the expected prevalence of infection of cross sectional studies. We suggest that this large data set reflects sampling of asymptomatic infections. In contrast, when the study design was focused on chronically infected symptomatic giardiasis patients, a significant increase in CD3-positive intraepithelial lymphocytes (IEL) in the infected tissues was found and was accompanied by epithelial barrier dysfunction [7]. Moreover, clinically manifested disease has been positively correlated to parasite density.

Mononuclear Phagocytic Cells Both mononuclear phagocytic cell types in the gut, intestinal macrophages, and DCs are considered to play a major role for antigen uptake and presentation. In comparison to other macrophage populations in the body, intestinal macrophages express high levels of MHC class II on the surface and there are difficulties to differentiate functionally intestinal macrophages and DC in the gut [62]. There are no *G. duodenalis* studies available on intestinal mononuclear phagocytic cells from humans. To study the response of DC upon stimulation with *G. duodenalis* products, monocyte-derived DCs (MoDC) have been generated from human blood. In congruence with the lack of inflammation in the gut, *G. duodenalis* trophozoites or trophozoites products activate cytokine/chemokine response or maturation of human (and mouse) dendritic cells only weakly if at all [69, 70]. However, *G. duodenalis* extracts alter the response of human MoDCs co-stimulated with known PRR-ligands such as some recognized by Toll like receptors (TLRs). TLR4 (lipopolysaccharide (LPS))-stimulated MoDCs in the presence of *G. duodenalis* extracts secreted lower levels of IL-12 and IL-23 and higher levels of IL-10 and reduced surface expression of CD25, CD83, CD86, and HLA-DR, while TLR2 ligand (Pam)-stimulated MoDCs increased all cytokine levels (IL-12, IL-23, IL-10) and surface markers indicating a modulatory effect on stimulated DCs [70]. A recent study in mice identified intestinal macrophages expressing arginase 1 and nitric oxide synthase 2 in the small intestine during *G. duodenalis* infection [71]. Arginase 1 is one marker of alternatively activated macrophages typically found in a Th2 environment. Whether or not alternatively activated macrophages occur during human giardiasis is unknown.

It should be noted that intestinal mononuclear phagocytic cells are central for antigen presentation to T- and B cells, but it is believed that they respond in concert with a variety of other possible responsive cells such as basophils, mast cells, iNKT, MAIT, innate lymphoid cells (ILCs), stromal cells, and epithelial cells [72, 73, 62]. It is unknown which role these cells play during human giardiasis, and it would be necessary to study the cell-cell network between all these cell types in the human context. It would be of particular interest how *G. duodenalis* infections affect the newly identified ILCs [74] and how these cells may contribute to the immune reaction to giardiasis in humans.

Data, from murine models, suggest a role for mast cells. Mast cell deficient mice (c-kit$^{w/wv}$) are not able to eliminate the *G. duodenalis* infection likely due to a subsequently diminished IgA response and/or reduced bowel movement [75, 60]. Besides DC, mast cells may be important for IL-6

production, which has been shown to be an important factor for parasite clearance in the mouse [76–78].

Taken together, the reaction and role during human giardiasis of mucosal innate immune cells is almost completely unknown. However, based on the currently available data, we would like to suggest that *G. duodenalis* is not registered by the immune system for want of ligands of PRRs. Instead, sensing the infection requires high parasite densities able to cause nutritional stress which provides the required signal to the host cells. Keeping colonization density below a not yet known threshold by natural resistance mechanisms as discussed above will enable *G. duodenalis* to remain below the limit of the immune systems' surveillance capacity. Once crossing the threshold, an inflammatory reaction triggered initially by nutritional stress could cause inflammation and symptomatic disease but also adaptive immunity which together with IgA may eventually amplify the effectiveness of the natural resistance mechanisms to control pathogen densities.

Adaptive Immune Responses

Data from mice suggest that innate immune responses triggered by nutritional stress or ER stress appear not to instruct adaptive immune responses that are highly skewed to e.g., type 1 or type 2 responses [66]. In human giardiasis, the few studies available reveal that *G. duodenalis* infection does not induce a highly biased cytokine response during the course of infection, i.e., no clear T cell polarization is associated with human giardiasis. In two studies using the same samples, IL-5, IL-6, IL-13, TNF-α, and INF-γ levels were analyzed before and 2 and 8 weeks after treatment and an increase of all cytokines at all time points compared to healthy controls was found [79, 80]. In the same studies, NO and IgE were analyzed and also revealed a higher serum level compared to the control sera. In another study, only a significant increase in IL-2 was detectable but there was no significant increase of IL-4 or IL-10 in giardiasis patients [81, 82], but measuring the latter is notoriously difficult in human cells. A study in children showed only an increase in certain cytokines (IL-1, IL-6, IL-8) or C-reactive protein (CRP) and NO when giardiasis was in addition associated with allergy [82]. A study in Venezuela analyzed IL-13, IFN-γ, IL6, and IL-10 levels in *G. duodenalis*-infected children and found also only a moderate increase of all cytokines in the serum compared to uninfected controls. These levels were profoundly increased by *Ascaris lumbricoides* co-infection [83]. The same group also found that giardiasis may enhance the outcome of atopic dermatitis allergic reactions in school children [84]. But *G. duodenalis* infection was also found to reduce granulocyte infiltration in an in vivo model of bacterial toxin-induced colitis and attenuates inflammation in human intestinal tissue suggesting modulating immune responses during giardiasis [85].

Analysis of local responses by lamina propria lymphocytes from naïve human individuals revealed an increased proliferation and elevated IFN-γ response after stimulation with *Giardia* antigen and has been interpreted as a "mitogenic" effect by the parasite [86]. A study on fecal cytokine excretion has found that elevated levels of MCP-1, IFN-γ, IL-4, and IL-5 are associated with a longer *G. duodenalis* infection outcome. In contrast, elevated IL-8 levels were associated with shorter infection times in the studied child population [87]. A recent study analyzing the cellular proliferation of blood lymphocytes in patients who had suffered from giardiasis 5 years earlier ("Bergen outbreak") revealed a significant elevation of *Giardia*-specific proliferation indicating T cell memory. Activation markers were also analyzed but were inconclusive [88•].

Experimental *G. duodenalis* infections in humans and epidemiological studies reveal that no sterile immunity is mounted. Rather, protection against symptomatic infections is acquired through primary infections [89–91]. It is not known whether and if yes to what extent infection with one assemblage protects from symptomatic reinfection with another assemblage. Recent publications suggested *G. duodenalis* genotype specific differences in pathogenesis in animal models [92•, 93, 94]. However, and as mentioned before, epidemiological studies in humans that try to link infection with different assemblages to clinical outcome are inconclusive [95–97, 31, 98, 99].

Giardia Factors Known to Interact with the Immune System

Only a few virulence factors and immune-reactive entities are described for *G. duodenalis*, most of which refer to structural features like the motility through flagella and the ventral adhesive disc (e.g., giardins/annexins) [1•]. The highly antigenic VSP coat on the parasite surface is thought to enable immune evasion by antigen-switching to escape protective antibody responses and is thought to mediate protection to natural resistance mechanisms as described above [55]. However, how much this VSP surface repertoire is contributing to pathogenicity and virulence in humans is still understudied in particular with respect of the broad variety of genetic differences in human isolates. Besides this, only very few candidate virulence factors are described. Candidate proteins may be immunogenic and therefore recognized by *G. duodenalis*-specific serum antibodies. This idea has led to identification of a number of antigens, including heat shock proteins, tubulins and several other not further analyzed proteins [100–107]. A prominent candidate is the arginine deiminase (ADI) that has been identified as one of 16 immuno-dominant, non-variant

G. duodenalis proteins (examples of additional antigens are α-giardins, ornithine carbamoyl transferase, and fructose-1,6-bisphosphate aldolase) in a screening assay with human sera from individuals with acute giardiasis [108]. As other metabolic enzymes, ADI is released by an unknown mechanism upon contact with epithelial cells. ADI-dependent hydrolysis of arginine has been linked to inhibited production of the immune effector NO, to reduced proliferation of epithelial cells and of T lymphocytes [59, 109–111]. Arginine depletion by ADI also modulates the phenotype and cytokine release from LPS-activated MoDC which in part involves mTOR signaling, one of the sensing pathways of nutritional stress [112].

The mechanisms by which *G. duodenalis* trophozoites modulates the immune response of enterocytes and DCs and possibly other cell types remains unknown, and we strongly favor nutritional stress as one component of it. However, a recently identified "*G. duodenalis* binding immunoglobulin protein" suggests also a direct pathway to trigger—in this case—murine DC maturation via TLR4 and MAPK pathways, [113] but this awaits confirmation using human cells. A pattern recognition-independent stimulation of immune responses by *G. duodenalis* is reminiscent of Th2-type responses induced during helminth's infections, which also in many cases do not prime via PRRs [73]. Th2 responses have been associated with a broad variety of initiating mechanisms including protease activity, metabolic stresses induced by the pathogen or by pathogen tissue damage [73]. In this respect, it is noteworthy that *G. duodenalis* parasites secrete a broad variety of cysteine proteases [85, 45, 114, 115] that may also play a part in a PRR-independent immune response. Such proteins were shown to stimulate a mixed Th1/Th2-like in mice [116]. Cysteine proteases may also be involved in mediating apoptosis in enterocytes and immune evasion, e.g., by cleavage of IL-8 released from enterocytes [45, 115, 85]. Together with secretion of metabolic enzymes such as ADI that may deplete arginine locally which may result in a nutritional stress environment, cysteine proteases may influence the outcome of the immune response in particular in newborn humans as it has been shown for bacterial infections [117]. Studying the impact of these enzymes during human *G. duodenalis* infection deserves more attention and needs to be integrated with the elucidation of *G. duodenalis* assemblage specific differences in these factors that could explain genotype-clinical phenotype correlations [92•, 93, 99].

Conclusion

Functional studies on human giardiasis are scarce. Available data point towards a mixed type immune response with an antibody-driven component important to control *G. duodenalis* infections. It is unknown which *G. duodenalis* factors shape the immune response and whether they are polymorphic between assemblages A and B and within these genotypic units. Our interpretation of current knowledge as presented above is certainly an oversimplification but is intended to identify major knowledge gaps (Box 1) and to provide a framework to formulate testable hypotheses.

Box 1: Important open research questions in immunology of human giardiasis.

Q1: What are the adaptive immune response dependent and independent protective components of human milk? Do they shape the parasite population present in breast fed infants?

Q2: What is the particular role of gut associated natural resistance mechanisms such as mucins, defensins and proteases also as selecting factors for the variety of G. duodenalis assemblages and sub-assemblages infecting the human gut?

Q3: What is the response of the various stem cell derived epithelial cells, e.g., Paneth cells, goblet cells and primary enterocytes, in the small intestine to G. duodenalis infection?

Q4: What is the mechanistic trigger of the response of mucosal innate immune cells such as intestinal macrophages, intestinal and plasmacytoid DCs, eosinophils, mast cells, invariant natural killer T cells (iNKT), mucosal-associated invariant T cells (MAIT), and the recently identified innate lymphoid cells (ILC1, ILC2, ILC3) in giardiasis? How do these cells communicate to each other and to other cell types in the gut like epithelial cells and lymphocytes?

Q5: What is the effector mechanism underlaying immunoglobulin-dependent control of Giardia infections in humans?

Q6: What are the main G. duodenalis virulence factors and are they functionally polymorphic in different assemblages or sub-assemblages?

Compliance with Ethics Guidelines

Conflict of Interest The authors declare that they have no competing interests.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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