



An up-date on *Giardia* and giardiasis

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Giardia intestinalis is a non-invasive protozoan parasite infecting the upper small intestine causing acute, watery diarrhea or giardiasis in 280 million people annually. Asymptomatic infections are equally common and recent data have suggested that infections even can be protective against other diarrheal diseases. Most symptomatic infections resolve spontaneously but infections can lead to chronic disease and treatment failures are becoming more common world-wide. *Giardia* infections can also result in irritable bowel syndrome (IBS) and food allergies after resolution. Until recently not much was known about the mechanism of giardiasis or the cause of post-giardiasis syndromes and treatment failures, but here we will describe the recent progress in these areas.

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Introduction

Diarrheal disease is the leading cause of death and illness for children under five years of age in developing countries [1]. The intestinal protozoan parasite *Giardia intestinalis* (synonyms *Giardia lamblia* and *Giardia duodenalis*) is distributed worldwide and estimated to cause 280 million diarrhea infections (giardiasis) annually [2]. The parasite is spread most often *via* contaminated water and many developing countries are considered endemic regions. Giardiasis is part of the WHO's Neglected disease initiative since 2004. Symptoms of infection are variable but typically include watery diarrhea, nausea, epigastric pain and weight loss [2]. The first signs of infection appear after 6–15 days and giardiasis is usually treated with metronidazole or other nitroimidazoles [3]. The parasite is non-invasive and there is relatively limited understanding about the disease-causing mechanisms [4].

The *Giardia* life cycle

Giardia has a simple life cycle with two main stages; the proliferating trophozoite and the infectious cyst. Trophozoites have two nuclei positioned anteriorly and they are both transcriptionally active. The cytoskeleton involves the adhesive disc, a median body and four pairs of flagella that behave differently during motility [2]. *Giardia* infection is initiated by ingestion of infectious cysts, which are stimulated to excyst by the acidic milieu in the stomach and presence of bile and trypsin in the duodenum [2]. The emerging parasites (excystozoites) quickly transform into trophozoites that attach to the intestinal epithelial cells using the adhesive disc. The adhesive disc is essential for attachment and is a major virulence factor of *Giardia* [5]. Several disc-associated proteins have been identified using proteomics [6] and it is clear that the disc is an advanced cytoskeletal structure [7].

Encystation starts as the trophozoite senses a change in the environment as the cell is transported further down in the small intestine. The cell responds by forming a cyst wall that enables the parasite to survive outside the host for several weeks in cold water [2]. The encystation process has been subjected to proteomic and transcriptomic analyses [8*,9,10,11]. These analyses show that encystation is a highly coordinated gene-expression cascade with changes on both the RNA and protein levels. Regulatory factors are encystation-specific transcription factors, chromatin re-modelling enzymes and post-translational modifications [8*]. In the first part of encystation, encystation-specific vesicles (ESVs, vesicles that transport cyst-wall proteins to the parasite surface), develop and mature. At the same time several proteins implicated in metabolic pathways display differential expression (*e.g.* protein folding, cytoskeleton regulatory components and kinases). Several proteins from the cysteine rich multi-gene families variant-specific surface proteins (VSPs) and high cysteine membrane proteins (HCMPs) also change their expression during encystation [8*,9], showing a link between encystation and variation of antigens on the parasite surface. The largest gene expression changes were seen late in encystation [8*]. To date, no proteomic study exists that includes the complete transformation from trophozoite to mature cyst. This, together with analyses of post-translational modifications, would generate a more complete picture of encystation-induced gene expression changes. It will also be important to generate more knowledge about the excystation process.

The mitosome and metabolism

Giardia harbors small mitosomes, highly reduced forms of mitochondria that do not contain any genome and have

lost the capacity to generate energy [2]. They have, however, retained some mitochondrial features such as presence of a double-membrane, synthesis of iron-sulphur (FeS) clusters and requirement of translocation signals for import of mitochondrial proteins [12]. The import machinery found in giardial mitosomes is highly reduced. Known components are porin Tom40 of the outer membrane and inner membrane translocases Pam18 and Pam16 [13]. Recently, the translocase of the inner membrane, a highly divergent Tim44, was elegantly discovered utilizing a compartment-specific biotinylation strategy [14^{*}]. Applying the same technique, a mitochondrial outer membrane protein 35 (MOM35) and 13 additional mitochondrial proteins with unknown function were discovered [14^{*}].

Giardia has a minimalistic metabolic capacity as do many other microaerophilic parasites. The parasite lacks pathways for *de novo* biosynthesis of pyrimidines and purines and depends on the host for nucleotide salvage [15,16]. Most metabolic enzymes are soluble and present in the cytosol, thus they are not sub-compartmentalized. Trophozoites mainly use glycolysis and arginine dihydrolase pathways for energy production. The preferred sugar glucose is converted into pyruvate and end products of glucose catabolism are acetate, ethanol, alanine and CO₂. However, small changes in oxygen concentration can affect the metabolism of trophozoites and influence

the end product formation [16]. Despite anaerobic metabolism, oxygen radicals are generated and a detoxification mechanism is necessary. Moreover, the intestinal environment fluctuates in oxygen levels and reactive oxygen species (ROS) are generated by the host [17]. The oxygen-sensitive parasite lacks conventional ROS scavenging pathways such as catalase and superoxide dismutase systems. Instead *Giardia* depends on thioredoxin-like proteins, NADH oxidase, flavodiiron protein, flavohemoglobin, peroxiredoxin and superoxide reductase for detoxification [18,19].

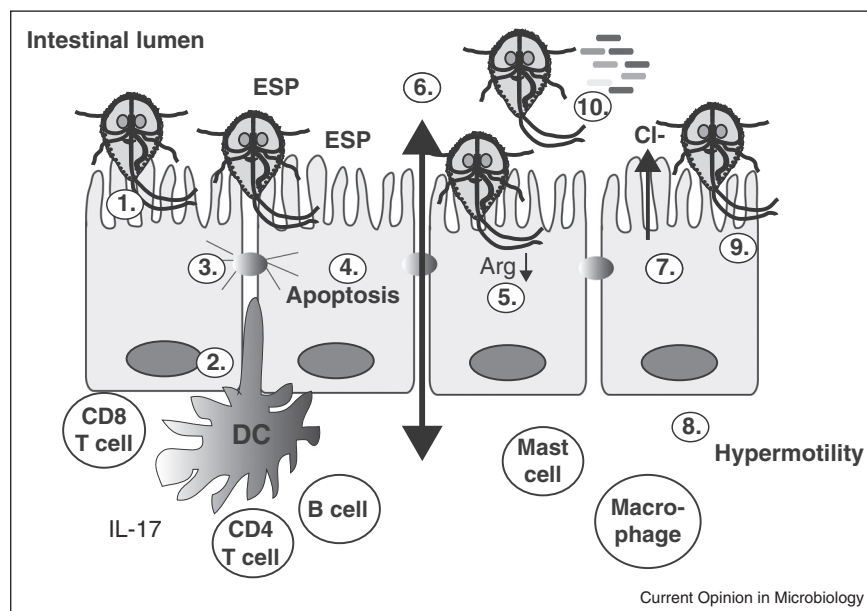
Treatment of giardiasis

There are several anti-giardial drugs, of which the most used are 5-nitroimidazole compounds such as metronidazole. Metronidazole is a pro-drug that needs to be reduced to become toxic, upon which DNA and protein damage is induced in microaerophilic and anaerobic organisms [3,20]. An additional target for metronidazole is the redox enzyme, thioredoxin reductase, whose inactivation causes severe oxidative stress [21]. There are, however, known cases of treatment failure and drug resistance [22] in giardiasis and the search for additional drug targets has been intensified in the last years [23,24,25].

Giardia pathogenesis

Giardiasis is multifactorial disease (Figure 1), reflecting the complex interplay between the host and parasite as

Figure 1



Giardiasis is a multi-factorial diarrheal disease and several different parasite-induced mechanisms are involved in disease induction. (1) Diffuse shortening of microvilli and inhibition of brush border enzymes. This involves attachment of the parasites and CD8⁺ T cells. (2) Induction of chemokines in IECs, resulting in attraction of immune cells like mast cells and dendritic cells (DC). (3) Disruption of tight junctions. (4) Induction of apoptosis. (5) Starvation for arginine in IECs, resulting in less NO, cell cycle arrest and apoptosis. (6) Increased intestinal permeability induced by mechanisms three to five. (7) Increased chloride ion secretion. (8) Intestinal hypermotility. (9) Crypt hyperplasia and increased mucus secretion. (10) Changed composition of bacterial normal flora. The parasite and host cells secrete specific proteins during the interaction (ESPs).

discussed below. The parasite is non-invasive and replicates attached to the intestinal epithelium. Symptoms of disease typically manifest after approximately 7–12 days. The most common clinical signs of infection are diarrhea (with or without malabsorption syndrome), nausea, vomiting and weight loss [4]. Some patients experience mild illness that resolves spontaneously. Others suffer from long-lasting severe disease that does not respond to the normal treatment. Most patients are usually found between these extremes [4]. Chronic *Giardia* infections are known to occur and can lead to complications such as irritable bowel syndrome (IBS), food allergies, arthritis or chronic fatigue syndrome [4]. Patients infected during a *Giardia* outbreak in a non-endemic area were reported to have long-term symptoms after the parasite was cleared [26*].

As diarrheal disease is a major cause of death in young children in developing countries, many studies have been conducted to try to resolve the role of *Giardia* in this regard. Reports of both acute or persistent disease as well as protection against diarrhea exist [2,4,27]. An extensive meta-analysis has been performed to try and resolve these conflicting findings [28]. There is no statistical evidence of association of acute diarrhea and *Giardia* infections in children older than 5 years in developing countries but an association was found in children up to 1 year of age. Instead a strong association was seen to persistent diarrhea (>14 days) among children in developing countries. Evidence was found that residents of industrialized countries that encounter the parasite in a waterborne outbreak or during travels to endemic areas are prone to develop acute diarrheal disease [28]. Differences in nutrition status, immune status, co-infections and intestinal normal flora could contribute to the differences in disease outcome observed among individuals from developing versus developed countries. It is possible that the observed protective effect of *Giardia* against other types of diarrhea could be due to the recently shown anti-inflammatory activity of *Giardia* [29,30*,31], since many diarrhea-causing organisms cause high levels of inflammation.

The roles of the host's intestinal normal flora and co-infections during *Giardia* infections are still largely unknown. A study in mice showed that adult mice with differences in their normal flora varied in their susceptibility to *Giardia* infections [32]. Co-infections with *Helicobacter pylori* and *Giardia* have been observed in asymptomatic children (age group 1–5) in Uganda [33]. *C. elegans* was recently used as a model system to study effects of *Giardia* on the normal flora. Commensal bacteria from humans were exposed to *Giardia* and thereafter fed to *C. elegans*, resulting in killing of the worms. This was not seen when commensal bacteria was not exposed to *Giardia*, suggesting that the parasite has the ability to affect the host's microbiota and alter the interactions between host and normal flora [34*]. *Lactobacillus* sp.

can be used as a probiotic and administration to mice before or during *Giardia* infections reduced severity and duration of the infection [35].

Observed differences in infection outcome could also be due the parasite itself. *G. intestinalis* consists of eight (A–H) assemblages or genotypes. Human infections are caused by assemblages A and B but few studies exist that links symptoms and assemblages together in a consistent manner [36]. Human infections of assemblage B (~58% of the cases) are more common worldwide compared to assemblage A (~37%). Interestingly, this proportion is not altered when comparing data from developing and developed countries but the prevalence of mixed infections is higher in the developing countries [36]. A large number of publications dealing with *Giardia* genotyping are generated every year but still the link between different genotypes and disease outcome is scarce [36]. It is of course possible that genetic differences between parasites have small or no effects on the infection outcome. Recently, genomes of new clinical isolates belonging to different subtypes have been sequenced, and compared with those of the classical laboratory strains [37,38], revealing a large genetic variability within genotypes. Future genomic studies combined with good patient data can reveal how and if the genotype affects the disease outcome during giardiasis.

Giardia-host cell interactions

The interaction between intestinal epithelial cells (IECs) and *Giardia* trophozoites has mainly been studied *in vitro* using axenic parasite isolates and intestinal cell lines [39,40,41]. Mice, and to some extent gerbils, are used as *in vivo* models of the human infections [42]. Studies in the mouse model sometimes use the rodent parasite *Giardia muris* [43*]. Transcriptomes of parasites during interaction with IECs are available and revealed regulation of hundreds of genes including surface proteins, cysteine proteases, oxygen defense proteins and attachment-associated proteins [39]. The metabolic enzymes arginine deiminase (ADI), enolase and ornithine carbamoyl transferase (OCT) are rapidly secreted upon interaction [44]. ADI and OCT are part of the arginine dihydrolase pathway, which is the primary source of energy for *Giardia* [45,46]. Scavenging of arginine reduces the enterocytes possibility to produce nitric oxide (NO), which is cytotoxic to the parasite [44,45]. Cysteine proteases are also secreted upon interaction [30*,39] and they can cleave IL-8, reducing infiltration of neutrophils (Figure 1). The host cells respond to the parasite by secreting proteins that affect the attachment of the parasites [47*]. Further studies of the secretomes (ESP, excretory secretory products, Figure 1) during host-parasite interactions will reveal additional released proteins and this will be important in order to understand giardiasis.

As the trophozoites colonize the host, induction of apoptosis in the IECs has been detected [48]. Additional evidence from studies of biopsies from chronically infected patients exists [49]. Transcriptome studies of host cells during infection under *in vitro* settings highlighted induction of various chemokines and apoptosis. In addition, decreased expression of genes responsible for cell proliferation was found [41]. The cell cycle progression can be inhibited by arginine starvation caused by the parasite's consumption of arginine. This decreased rate of IEC proliferation will affect the cell turnover in the intestine, which might help the parasite to prolong its colonization since it can stay attached to the IECs longer [45].

Tight junctions between the enterocytes create an important selective barrier between the intestinal lumen and underlying tissue. *Giardia* increases the permeability by altering the proteins important for maintaining tight junctions (e.g. F-actin and zonula occludens-1 (ZO-1)) [50] (Figure 1). Also during chronic human infections, an increase of intestinal permeability was observed and these patients had decreased mucosal surface area [49]. CD8⁺ T cells have been reported to be responsible for diffuse microvilli shortening [51]. Reduction of the microvilli length decreases the absorptive area of the intestine, resulting in malabsorption of water, Na⁺ and disaccharides, combined with hypersecretion of Cl⁻ [49] (Figure 1). *Giardia* infections elicit both innate and adaptive immune responses in humans and animals but the exact mechanisms controlling infection are not fully understood. Early in infection, operating defence mechanisms are B-cell-independent and include NO, reactive oxygen species (ROS), defensins, lactoferrins, immune cells such as phagocytes, mast cells and dendritic cells, and chemokines (Figure 1). IL-17 has been shown to be important during *Giardia* infections in mice and in humans [43,52,53]. Later, an antibody-dependent phase comes into play and is responsible for parasite clearance.

Conclusions

Giardia infection induces pathophysiological effects in the host intestines through cellular attachment and the excretion of parasite products, ESPs. This causes structural damages that compromises the integrity of intestinal epithelia as well as the function of brush border enzymes, and induces apoptosis in cells. These changes affect the electrolyte balance and cause increased intestinal permeability, leading to the production of diarrhea. The pathological outcomes, however, are dependent on isolate virulence and thus, might not be seen in all *Giardia* infections. The next step in the understanding of *Giardia* infections is to identify and characterize specific virulence factors and this will be the focus of *Giardia* research in the coming years.

Conflict of interests

The authors declare no competing interests.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, Wu Y, Sow SO, Sur D, Breiman RF *et al.*: **Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study.** *Lancet* 2013, **382**:209-222.
2. Ankarklev J, Jerlström-Hultqvist J, Ringqvist E, Troell K, Svärd SG: **Behind the smile: cell biology and disease mechanisms of *Giardia* species.** *Nat Rev Microbiol* 2010, **8**:413-422.
3. Watkins RR, Eckmann L: **Treatment of giardiasis: current status and future directions.** *Curr Infect Dis Rep* 2014, **16**:396.
4. Bartelt LA, Sartor RB: **Advances in understanding *Giardia*: determinants and mechanisms of chronic sequelae.** *F1000Prime Rep* 2015, **7**:62.
5. Dawson SC: **An insider's guide to the microtubule cytoskeleton of *Giardia*.** *Cell Microbiol* 2010, **12**:588-598.
6. Hagen KD, Hirakawa MP, House SA, Schwartz CL, Pham JK, Cipriano MJ, De La Torre MJ, Sek AC, Du G, Forsythe BM *et al.*: **Novel structural components of the ventral disc and lateral crest in *Giardia intestinalis*.** *PLoS Negl Trop Dis* 2011, **5**:e1442.
7. Brown JR, Schwartz CL, Heumann JM, Dawson SC, Hoenger A: **A detailed look at the cytoskeletal architecture of the *Giardia lamblia* ventral disc.** *J Struct Biol* 2016, **194**:38-48.
8. Einarsson E, Troell K, Hoepfner MP, Grabherr M, Ribacke U, Svärd SG: **Coordinated changes in gene expression throughout encystation of *Giardia intestinalis*.** *PLoS Negl Trop Dis* 2016, **10**:e0004571.
9. Emery SJ, Pascovi D, Lacey E, Haynes PA: **The generation gap: Proteome changes and strain variation during encystation in *Giardia duodenalis*.** *Mol Biochem Parasitol* 2015, **201**:47-56.
10. Wampfler PB, Tosevski V, Nanni P, Spycher C, Hehl AB: **Proteomics of secretory and endocytic organelles in *Giardia lamblia*.** *PLoS One* 2014, **9**:e94089.
11. Liao JY, Guo YH, Zheng LL, Li Y, Xu WL, Zhang YC, Zhou H, Lun ZR, Ayala FJ, Qu LH: **Both endo-siRNAs and tRNA-derived small RNAs are involved in the differentiation of primitive eukaryote *Giardia lamblia*.** *Proc Natl Acad Sci U S A* 2014, **111**:14159-14164.
12. Nyindodo-Ogari L, Schwartzbach SD, Estrano CE: ***Giardia* mitochondrial protein import machinery differentially recognizes mitochondrial targeting signals.** *Infect Disord Drug Targets* 2014, **14**:23-29.
13. Jedelsky PL, Dolezal P, Rada P, Pyrih J, Smid O, Hrdy I, Sedinova M, Marcincikova M, Voleman L, Perry AJ *et al.*: **The minimal proteome in the reduced mitochondrion of the parasitic protist *Giardia intestinalis*.** *PLoS One* 2011, **6**:e17285.
14. Martincova E, Voleman L, Pyrih J, Zarsky V, Vondrackova P, Kolisko M, Tachezy J, Dolezal P: **Probing the biology of *Giardia intestinalis* mitochondria using in vivo enzymatic tagging.** *Mol Cell Biol* 2015, **35**:2864-2874.
15. Morrison HG, McArthur AG, Gillin FD, Aley SB, Adam RD, Olsen GJ, Best AA, Cande WZ, Chen F, Cipriano MJ *et al.*: **Genomic minimalism in the early diverging intestinal parasite *Giardia lamblia*.** *Science* 2007, **317**:1921-1926.

A study that identified new mitochondrial proteins using a biotinylation *in vivo* tagging.

16. Lloyd D, Williams CF: **Comparative biochemistry of *Giardia*, *Hexamita* and *Spironucleus*: Enigmatic diplomonads.** *Mol Biochem Parasitol* 2014, **197**:43-49.
 17. Ma'ayeh SY, Knörr L, Svärd SG: **Transcriptional profiling of *Giardia intestinalis* in response to oxidative stress.** *Int J Parasitol* 2015, **45**:925-938.
 18. Mastronicola D, Falabella M, Forte E, Testa F, Sarti P, Giuffrè A: **Antioxidant defence systems in the protozoan pathogen *Giardia intestinalis*.** *Mol Biochem Parasitol* 2016, **206**:56-66.
 19. Mastronicola D, Falabella M, Testa F, Pucillo LP, Teixeira M, Sarti P, Saraiva LM, Giuffrè A: **Functional characterization of peroxiredoxins from the human protozoan parasite *Giardia intestinalis*.** *PLoS Negl Trop Dis* 2014, **8**:e2631.
 20. Muller J, Rout S, Leitsch D, Vaithilingam J, Hehl A, Muller N: **Comparative characterisation of two nitroreductases from *Giardia lamblia* as potential activators of nitro compounds.** *Int J Parasitol Drugs Drug Resist* 2015, **5**:37-43.
 21. Leitsch D, Schlosser S, Burgess A, Duchene M: **Nitroimidazole drugs vary in their mode of action in the human parasite *Giardia lamblia*.** *Int J Parasitol Drugs Drug Resist* 2012, **2**:166-170.
 22. Ansell BR, McConville MJ, Ma'ayeh SY, Dagley MJ, Gasser RB, Svärd SG, Jex AR: **Drug resistance in *Giardia duodenalis*.** *Biotechnol Adv* 2015, **33**:888-901.
 23. Kulakova L, Galkin A, Chen CZ, Southall N, Marugan JJ, Zheng W, Herzberg O: **Discovery of novel anti-giardiasis drug candidates.** *Antimicrob Agents Chemother* 2014, **58**:7303-7311.
 24. Ranade RM, Zhang Z, Gillespie JR, Shibata S, Verlinde CL, Hol WG, Fan E, Buckner FS: **Inhibitors of methionyl-tRNA synthetase have potent activity against *Giardia intestinalis* trophozoites.** *Antimicrob Agents Chemother* 2015, **59**: 7128-7131.
 25. Garcia-Torres I, de la Mora-de la Mora I, Marcial-Quino J, Gomez-Manzo S, Vanoye-Carlo A, Navarrete-Vazquez G, Colin-Lozano B, Gutierrez-Castrellon P, Sierra-Palacios E, Lopez-Velazquez G *et al.*: **Proton pump inhibitors drastically modify triosephosphate isomerase from *Giardia lamblia* at functional and structural levels, providing molecular leads in the design of new anti-giardiasis drugs.** *Biochim Biophys Acta* 2016, **1860**:97-107.
 26. Litleskare S, Wensaas KA, Eide GE, Hanevik K, Kahrs GE, Langeland N, Rortveit G: **Perceived food intolerance and irritable bowel syndrome in a population 3 years after a giardiasis-outbreak: a historical cohort study.** *BMC Gastroenterol* 2015, **15**:164.
- Paper dealing with patients with food intolerance and IBS after a water-borne giardiasis outbreak, showing that *Giardia* can give long-term problems after clearance of the parasite.
27. Muhsen K, Cohen D, Levine MM: **Can *Giardia lamblia* infection lower the risk of acute diarrhea among preschool children?** *J Trop Pediatr* 2014, **60**:99-103.
 28. Muhsen K, Levine MM: **A systematic review and meta-analysis of the association between *Giardia lamblia* and endemic pediatric diarrhea in developing countries.** *Clin Infect Dis* 2012, **55**(Suppl 4):S271-S293.
 29. Cotton JA, Amat CB, Buret AG: **Disruptions of host immunity and inflammation by *Giardia Duodenalis*: potential consequences for co-infections in the gastro-intestinal tract.** *Pathogens* 2015, **4**:764-792.
 30. Cotton JA, Bhargava A, Ferraz JG, Yates RM, Beck PL, Buret AG: ***Giardia duodenalis* cathepsin B proteases degrade intestinal epithelial interleukin-8 and attenuate interleukin-8-induced neutrophil chemotaxis.** *Infect Immun* 2014, **82**:2772-2787.
- Paper showing that *Giardia* has cysteine protease activities that can break down IL-8, partly explaining the low levels of inflammation during giardiasis.
31. Cotton JA, Motta JP, Schenck LP, Hirota SA, Beck PL, Buret AG: ***Giardia duodenalis* infection reduces granulocyte infiltration in an in vivo model of bacterial toxin-induced colitis and attenuates inflammation in human intestinal tissue.** *PLoS One* 2014, **9**:e109087.
 32. Singer SM, Nash TE: **The role of normal flora in *Giardia lamblia* infections in mice.** *J Infect Dis* 2000, **181**:1510-1512.
 33. Ankarklev J, Hestvik E, Lebbad M, Lindh J, Kaddu-Mulindwa DH, Andersson JO, Tylleskar T, Tumwine JK, Svärd SG: **Common coinfections of *Giardia intestinalis* and *Helicobacter pylori* in non-symptomatic Ugandan children.** *PLoS Negl Trop Dis* 2012, **6**:e1780.
 34. Gerbaba TK, Gupta P, Rioux K, Hansen D, Buret AG: ***Giardia duodenalis*-induced alterations of commensal bacteria kill *Caenorhabditis elegans*: a new model to study microbial-microbial interactions in the gut.** *Am J Physiol Gastrointest Liver Physiol* 2015, **308**:G550-G561.
- Paper presenting a new model system for *Giardia* infections, *C. elegans*. *Giardia* can affect the bacterial normal flora in the worm and that has harmful effects on the worm.
35. Goyal N, Shukla G: **Probiotic *Lactobacillus rhamnosus* GG modulates the mucosal immune response in *Giardia intestinalis*-infected BALB/c mice.** *Dig Dis Sci* 2013, **58**: 1218-1225.
 36. Ryan U, Caccio SM: **Zoonotic potential of *Giardia*.** *Int J Parasitol* 2013, **43**:943-956.
 37. Ankarklev J, Franzen O, Peirasmaki D, Jerlström-Hultqvist J, Lebbad M, Andersson J, Andersson B, Svärd SG: **Comparative genomic analyses of freshly isolated *Giardia intestinalis* assemblage A isolates.** *BMC Genomics* 2015, **16**:697.
 38. Hanevik K, Bakken R, Brattbakk HR, Saghaug CS, Langeland N: **Whole genome sequencing of clinical isolates of *Giardia lamblia*.** *Clin Microbiol Infect* 2015, **21**:e191-193.
 39. Ma'ayeh SY, Brook-Carter PT: **Representational difference analysis identifies specific genes in the interaction of *Giardia duodenalis* with the murine intestinal epithelial cell line, IEC-6.** *Int J Parasitol* 2012, **42**:501-509.
 40. Fisher BS, Estrano CE, Cole JA: **Modeling long-term host cell-*Giardia lamblia* interactions in an in vitro co-culture system.** *PLoS One* 2013, **8**:e81104.
 41. Roxström-Lindquist K, Ringqvist E, Palm D, Svärd S: ***Giardia lamblia*-induced changes in gene expression in differentiated Caco-2 human intestinal epithelial cells.** *Infect Immun* 2005, **73**:8204-8208.
 42. Tako EA, Hassimi MF, Li E, Singer SM: **Transcriptomic analysis of the host response to *Giardia duodenalis* infection reveals redundant mechanisms for parasite control.** *MBio* 2013, **4**:e00660-00613.
 43. Dreesen L, De Bosscher K, Grit G, Staels B, Lubberts E, Bauge E, Geldhof P: ***Giardia muris* infection in mice is associated with a protective interleukin 17A response and induction of peroxisome proliferator-activated receptor alpha.** *Infect Immun* 2014, **82**:3333-3340.
- First paper showing the importance of IL-17 during *Giardia* infections.
44. Ringqvist E, Palm JE, Skarin H, Hehl AB, Weiland M, Davids BJ, Reiner DS, Griffiths WJ, Eckmann L, Gillin FD *et al.*: **Release of metabolic enzymes by *Giardia* in response to interaction with intestinal epithelial cells.** *Mol Biochem Parasitol* 2008, **159**:85-91.
 45. Stadelmann B, Merino MC, Persson L, Svärd SG: **Arginine consumption by the intestinal parasite *Giardia intestinalis* reduces proliferation of intestinal epithelial cells.** *PLoS One* 2012, **7**:e45325.
 46. Stadelmann B, Hanevik K, Andersson MK, Bruserud O, Svärd SG: **The role of arginine and arginine-metabolizing enzymes during *Giardia* - host cell interactions in vitro.** *BMC Microbiol* 2013, **13**:256.
 47. Emery SJ, Mirzaei M, Vuong D, Pascovici D, Chick JM, Lacey E, Haynes PA: **Induction of virulence factors in *Giardia duodenalis* independent of host attachment.** *Sci Rep* 2016, **6**:20765.
- First paper showing that secreted host factors have a role in *Giardia* pathogenesis.
48. Koh WH, Geurden T, Paget T, O'Handley R, Steuart RF, Thompson RC, Buret AG: ***Giardia duodenalis* assemblage-specific induction of apoptosis and tight junction disruption in**

- human intestinal epithelial cells: effects of mixed infections. *J Parasitol* 2013, **99**:353-358.
49. Troeger H, Eppe 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.
50. Di Genova BM, Tonelli RR: **Infection strategies of intestinal parasite pathogens and host cell responses.** *Front Microbiol* 2016, **7**:256.
51. 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.** *Infect Immun* 2000, **68**:3412-3418.
52. Dann SM, Manthey CF, Le C, Miyamoto Y, Gima L, Abraham A, Cao AT, Hanson EM, Kolls JK, Raz E *et al.*: **IL-17A promotes protective IgA responses and expression of other potential effectors against the lumen-dwelling enteric parasite *Giardia*.** *Exp Parasitol* 2015, **156**:68-78.
53. Saghaug CS, Sornes S, Peirasmaki D, Svärd S, Langeland N, Hanevik K: **Human memory CD4+ T cell immune responses against *Giardia lamblia*.** *Clin Vaccine Immunol* 2016, **23**: 11-18.