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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 (excyzoites) 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 multigene 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 exits 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 mitosomal 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 mitosomal outer membrane protein 35 (MOM35) and 13 additional mitosomal 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 CO_2. 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].

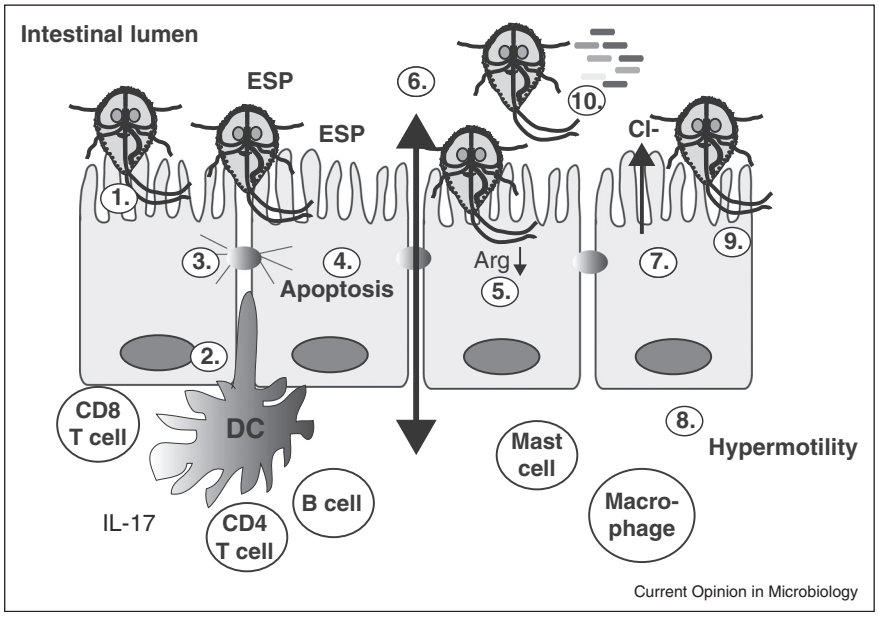
Treatment of giardiasis

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).