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REVIEW

Giardia duodenalis: Biology and Pathogenesis

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SUMMARY *Giardia duodenalis* captured the attention of Leeuwenhoek in 1681 while he was examining his own diarrheal stool, but, ironically, it did not really gain attention as a human pathogen until the 1960s, when outbreaks were reported. Key technological advances, including *in vitro* cultivation, genomic and proteomic databases, and advances in microscopic and molecular approaches, have led to an understanding that this is a eukaryotic organism with a reduced genome rather than a truly premitochondriate eukaryote. This has included the discovery of mitosomes (vestiges of mitochondria), a transport system with many of the features of the Golgi apparatus, and even evidence for a sexual or

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parasexual cycle. Cell biology approaches have led to a better understanding of how *Giardia* survives with two nuclei and how it goes through its life cycle as a noninvasive organism in the hostile environment of the lumen of the host intestine. Studies of its immunology and pathogenesis have moved past the general understanding of the importance of the antibody response in controlling infection to determining the key role of the Th17 response. This work has led to understanding of the requirement for a balanced host immune response that avoids the extremes of an excessive response with collateral damage or one that is unable to clear the organism. This understanding is especially important in view of the remarkable ranges of early manifestations, which range from asymptomatic to persistent diarrhea and weight loss, and longer-term sequelae that include growth stunting in children who had no obvious symptoms and a high frequency of postinfectious irritable bowel syndrome (IBS).

KEYWORDS *Giardia*, *Giardia lamblia*, giardiasis

INTRODUCTION

Giardia duodenalis (synonyms *Giardia lamblia* and *Giardia intestinalis*) has caught the interest of scientists and clinicians since its initial description by van Leeuwenhoek in 1681 as he described his own diarrheal stools and identified motile organisms that fit the description of the organism that was ultimately given the name of *Giardia* (1). *G. duodenalis* is the most prevalent protozoan human intestinal pathogen and is found worldwide, causing infections that ranges from asymptomatic to chronic diarrhea and malabsorption.

The life cycle of *G. duodenalis* consists of two stages, the trophozoite and cyst. The trophozoite is the vegetative form and replicates in the small intestine of the host. The eight flagella provide motility, and the ventral disk mediates attachment to the intestinal wall, where it gains its nutrients. More distally, in the small intestine and even extending to the large intestine, the trophozoite encysts into a cyst that is environmentally stable and can be transmitted to the next host through the fecal-oral route. The majority of outbreaks of human infection are associated with contaminated water, but food and direct human-to-human transmission via the fecal-oral route are also important.

CLASSIFICATION OF *GIARDIA*

Classification of *Giardia* as a Eukaryotic Organism

Giardia species have traditionally been categorized with other flagellated protozoa that are pathogenic to humans, but most of these other flagellates are not closely related to *Giardia*. Of the other human pathogens, *Trichomonas vaginalis* is, along with *Giardia*, part of the superclass Fornicata (2). *Giardia* species are the only human pathogens that are classified as diplomonads, while *Trichomonas* species are parabasalids. Diplomonad means two bodies, and the diplomonads typically have two symmetrically placed nuclei. The other diplomonads include *Spironucleus* and *Hexamita* species, which may be free-living or parasites of nonhuman animals (2).

Giardia species have an anaerobic metabolism and lack mitochondria, the Golgi apparatus, and other canonical eukaryotic organelles, and were thought at one time to be one of the earliest divergences of the eukaryotic organisms (3). As such, these species were of particular interest to evolutionary biologists. However, genome sequencing demonstrated that *G. duodenalis* has genes for many of the "missing" organelles and favored the alternative view that *Giardia* has a secondarily reduced genome (4). Although it is probably a eukaryotic organism that diverged early, it diverged after the acquisition of these "missing" organelles. Perhaps the most notable finding has been the identification of mitochondrial remnants called mitosomes (5). The identification of functional equivalents for other organelles has added to the current view that the organism has a minimized genome and cell biology. This is expanded on here in the sections on genomics and cell biology.

TABLE 1 *Giardia* species

Species name	Hosts	Morphology determined by:		Reference(s) for species description	Genotype(s) (reference[s])
		Light microscopy	Electron microscopy		
G. duodenalis	Humans, numerous mammals	Pear-shaped trophozoite; claw-shaped median body		7, 318	AI (4) AII (17) B (16, 17) C and D (19)
G. muris	Rodents	Short and rounded; small, rounded median body		7; EM (319)	
G. agilis	Amphibians	Long and slender; teardrop-shaped median body		7; EM (320)	
G. psittaci	Psittacine birds	Same as *G. duodenalis*	Incomplete ventrolateral flange, no marginal groove	321	
G. microti	Rodents	Same as *G. duodenalis*	Cysts contain two trophozoites with mature ventral disks	322	
G. ardeae	Herons	Same as *G. duodenalis*	Ventral disk and caudal flagellum similar to *G. muris*	323	
G. peramelis	Quenda (small marsupial)	Same as *G. duodenalis*		324	
G. cricetidarum	Hamsters	Short and rounded; closer to *G. muris* than other species		325	

Classification of *Giardia* Species and Genotypes

Giardia species are flagellated anaerobic protozoan (or protist) organisms characterized by their dyadic symmetry and presence of two symmetrical nuclei in the trophozoites. They are intestinal parasites of animals that range phylogenetically from amphibians to mammals, depending on the *Giardia* species. The initial classification of *Giardia* species was done on the basis of host of origin and was supported by early observations that there was relatively little cross-transmission of *Giardia* species to different hosts (6). Subsequently, there was a move to the opposite extreme, with a designation of just three species that were based on differences that were readily observable during light microscopic evaluation of trophozoites (7). Filice (7) described the trophozoites of *Giardia agilis*, which is long and narrow and found primarily in amphibians, *Giardia muris*, which is short and wide and found in rodents, and *G. duodenalis*, which he obtained from rabbits but which had the same pear-shaped morphology as the human parasite.

The organism he called *G. duodenalis* on the basis of light microscopy is found in humans and in a broad range of other mammals. However, subsequent investigation using techniques with greater discriminatory power, primarily electron microscopy and molecular characterization, have defined differences within *G. duodenalis* that are frequently associated with host specificity. The first round of subdividing *G. duodenalis* into subgroups consisted of the formal designation of new species on the basis of morphologic differences seen by electronic microscopy (EM) (Table 1) and included *Giardia psittaci*, *Giardia microti*, and *Giardia ardeae*. The distinctness of these new species has subsequently been supported by DNA sequence comparisons.

Even after excluding the organisms with morphological differences at an EM level, there is substantial variability at a DNA sequence level, and for many of them, there is a difference in host specificity (Table 2). The first recognition of differences among *G. duodenalis* isolates from humans was in the 1980s and consisted of different isoenzyme patterns in five axenized isolates (cell-free *in vitro* culture) (8, 9). In 1985, surface antigen (10) and restriction fragment length polymorphism (RFLP) patterns (11) demonstrated three groups, one of which was different enough from the other two to support the proposal that these represented different species. Subsequent refinement has been done on the basis of sequence differences in housekeeping genes (12–15). These

TABLE 2 *G. duodenalis* genotypes

Genotype[a]	Hosts	Proposed species name[b]	Reference(s)[c]
AI	Primarily animals, but also in humans	*Giardia duodenalis*	4, 11
AII	Humans, numerous other mammals	*Giardia duodenalis*	11, 17
B	Humans, numerous other mammals	*Giardia enterica*	11, 16, 17
C	Dogs	*Giardia canis*	326, 327
D	Dogs	*Giardia canis*	326, 327
E	Cows, sheep, alpacas, goats, pigs	*Giardia bovis*	18, 328
F	Cats	*Giardia cati*	327
G	Rats and mice	*Giardia simondi*	327
H	Seals (marine vertebrates)	NA	329

[a]Other proposed subgenotypes of A are less well documented than AI and AII.
[b]From reference 23. NA, not applicable.
[c]Initial description and genome sequence where available.

different groups have subsequently been divided into assemblages or genotypes that now extend from A to H. Whole-genome sequences have been reported for a number of human isolates, which are found in genotypes (assemblages) A and B. The first genome was reported for the WB isolate (genotype AI), followed by those for the GS isolate (genotype B) (16, 17) and the DH isolate (genotype AII) (17). In addition, the livestock genotype (genotype E) from a pig (18) and genotypes C and D (both dog isolates) (19) have also been sequenced. Genotypes C and D were sequenced by amplifying DNA from single cysts. Phylogenetically, genotypes C and D fall into a single clade but are about as distinct from each other as genotypes A and B are from each other.

All of the eight *G. duodenalis* genotypes are found in mammals (Table 2). Two of these, A and B, are routinely found in humans and occasionally in other mammals. The genotype A isolates have been divided into two groups (AI and AII) on the basis of sequence and biological differences. Genotype AI is a highly homogeneous group in which sequence differences among isolates are rare (20) and the level of allelic heterozygosity is very low (4). Recent data suggest that AI is found primarily in animals and should be considered zoonotic, while AII is seen primarily in humans (20–22). Some of the other genotypes are occasionally found in humans, but not at a frequency to implicate them as disease-causing organisms in humans. Currently, these are usually referred to as part of the same species. The name "*Giardia lamblia*" has typically been used in medical writing, while "*Giardia intestinalis*" and, later, "*G. duodenalis*" have been commonly used in the scientific literature. It is possible that many or all of the genotypes will ultimately be given separate species names. Interestingly, there is a greater phylogenetic difference between the human genotypes, A and B, than between these human genotypes and some of the other genotypes (C to H). Thus, it has been proposed that these two genotypes should be designated separate species (11), and subsequently, species names have been proposed; *G. duodenalis* for genotype A and *Giardia enterica* for genotype B (23). However, the argument has been made that a clonal or near-clade approach should be used for organisms such as *Giardia* and that assigning separate species names is premature (24). There would also be the challenge on the clinical side that molecular typing, which is not currently available, would be required for identifying these at a species level. For the purposes of this review, the term "*G. duodenalis*" is used for all of these genotypes, with the recognition that there remains a lack of consensus on the preferred name.

THE *GIARDIA* LIFE CYCLE

The Cyst

The *G. duodenalis* cyst is the environmentally stable stage of the parasite life cycle that facilitates the transmission of cysts passed in the feces of one host into the environment to be ingested by the subsequent host. The cyst is 5 μm by 7 to 10 μm in size with a two-layered cyst wall. The outer filamentous layer is covered by filaments 7 to

20 nm in length (25) and with *N*-acetylgalactosamine as the major sugar (26). The cyst has four nuclei, rather than the two found in trophozoites. The metabolic rate is only 10% to 20% that of the trophozoite (27), allowing prolonged survival in the environment, especially in cool, moist settings, perhaps explaining the higher frequency of giardiasis in the northern part of the United States compared to that in the southern areas (28).

Ingestion and Excystation

Infection of the host is initiated when cysts are ingested and pass through the acidic stomach into the duodenum where excystation occurs upon exposure to bile and a more alkaline pH. *In vitro* application of that sequence of pH change and exposure to bile results in a high percentage of successful excystation (29, 30). However, it is also possible to excyst *Giardia* at a neutral pH (31), and humans whose gastric pH is raised naturally or by medical intervention are susceptible to *Giardia* infection (32). In fact, they may be more susceptible to chronic giardiasis, as suggested in a report of two cases of gastric giardiasis in patients with hypochlorhydria as a result of treatment with a proton pump inhibitor, one of which resolved after discontinuing the proton pump inhibitor and without specific antimicrobial treatment (33).

Encystation

The trophozoites replicate in the small intestine, where some of the organisms will differentiate into cysts. Recent animal model data suggest that the trophozoites cluster into foci throughout the small intestine and even into the cecum and that encystation begins shortly after infection and peaks in a week. The encystation occurs in these clusters of increased organism density (34).

The development of *in vitro* encystation was accomplished by three different laboratories (35–37). The initial report of *in vitro* encystation used primary bile salts to induce encystation (37), an approach modified to a two-step process in which the second step utilized an alkaline pH of 7.8 and porcine bile (38). Alternative approaches have included high-bile medium (39) or cholesterol starvation (40). One of early events in encystation is the development of encystation-specific vesicles (ESVs) (41). These ESVs have a number of properties of the Golgi apparatus, including their involvement in protein trafficking that includes cell wall proteins 1 to 3 (CWP1 to CWP3), sensitivity to brefeldin A, and their emergence from active ER sites (42). During encystation, the trophozoites become rounded, and some of the key trophozoite cytoskeletal components are disassembled (43). There are two cycles of chromosome replication and one cycle of nuclear division, resulting in a mature cyst that has four nuclei, each of which is 4n. Then, when viable cysts are exposed to appropriate conditions, an opening at one pole of the cyst allows the emergence of the flagella and the cell body of the excyzoite (44), which undergoes two rounds of division, resulting in four trophozoites that are each 4n (two diploid nuclei).

The *Giardia* genome has 27 cysteine proteases that are part of the CA clan of cysteine proteases (45). Twenty-five of those cysteine proteases are expressed, with GlCP2 (CP2) being the most abundantly expressed. CP2 is a cathepsin B-like protease that is involved in excystation (46) and encystation (45, 47). It is found in the ESVs, and purified CP2 is able to cleave recombinant CWP2 into a 26-kDa fragment, which is the size found in encysting organisms (45). The roles of the other cysteine proteases remain to be confirmed.

The transcriptomics of encystation using serial analysis of gene expression (SAGE) (48) and microarray (49) identified that CWP1 to CWP3, a high-cysteine nonvariant cyst protein (HCNCp), and the transcription factor Myb are highly upregulated in the first 3 h. HCNCp was the first characterized protein from a family of high-cysteine membrane proteins (HCMPs or MCMPs) (50) (see "High-cysteine membrane proteins," below). These and additional genes are upregulated at 7 h, including genes with Myb-binding sequences (49). A subsequent proteomic evaluation of encystation using tandem mass spectrometry found that the variety of variant-specific proteins (VSPs) had decreased 4

h after initiation of encystation in comparison to the baseline level (42). In addition, there were multiple changes in metabolic and cytoskeletal proteins. The mechanisms of initiating encystation are not well understood, but there are data suggesting the inhibition of encystation by nitric oxide (51), histone deacetylase inhibitors (52), or by the presence of lactoferrin (53). In addition, a study of the one Rho family GTPase found in *Giardia* (Rac) showed its localization to the ER and ESVs and demonstrated that its expression increased CWP1 expression (54). CWP1 then accumulated in the extracellular environment and was used by surrounding trophozoites to support encystation. The authors proposed that this mechanism may explain why encystation is frequently found in clusters of organisms.

GENOMICS AND PROTEOMICS OF *GIARDIA*

Genomics

The first *Giardia* genome was published (4) in 2007 and has been refined by optical mapping (55), and recently by extended reads with optical mapping (56) for a nearly complete genome of the WB isolate (genotype AI). There are five chromosomes that range in size from about 1 to 5 Mb (57) with a ploidy of four (two diploid nuclei) (44). The entire genome is about 12 Mb and is compact, with minimal noncoding regions. Introns are rare, with eight *cis*-spliced and five *trans*-spliced introns having been identified (56, 58). The genome consists of 4,863 protein-coding genes, 2,099 of which are hypothetical proteins; the others are annotated (56). In addition, there are 306 pseudogenes. The genomes of the isolates and species that have been sequenced can be found at https://Giardiadb.org/, along with multiple genome- and proteome-related resources. The database is updated frequently, with version 52 released 20 May 2021. Noncoding small RNAs (sRNAs) have also been identified in the genome and include microRNAs (miRNA), which have been associated with control of vsp gene expression (59), as well as endogenous siRNA (endo-siRNA) and tRNA-derived sRNA (60).

Giardia trophozoites are tetraploid with two diploid nuclei (see "Nuclear and chromosomal structure and replication," below) and were assumed to be asexual in their reproduction (also discussed below). In this case, there would be an expectation of increasing allelic heterozygosity over time, as has occurred with certain bdelloid rotifers (61, 62). However, contrary to that expectation, the WB genome (genotype AI) has an extremely low level of allelic heterozygosity and was estimated at $<$0.01% in the initial genome assembly (4), which was subsequently revised to 0.03% (56). In comparison, the GS isolate (genotype B) has a heterozygosity level of about 0.425% (17), while the DH isolate (genotype AII) is intermediate, with a heterozygosity level of 0.037%. The level of allelic sequence heterozygosity for the dog genotypes (C and D) is estimated at 0.52 to 0.58% (19). The reasons for these differences are not known. The additional question to address is whether the heterozygosity is seen at a single-cell level. This question was addressed for genotype B by using clones that were selected by limiting dilution (63) and by sequencing single cells obtained by micromanipulation (64). These studies have confirmed heterozygosity at a single-cell level. Other studies of heterozygosity within genotype B have found that the frequency of silent substitutions in housekeeping genes make it very difficult to subtype these organisms into specific subgenotypes or subassemblages (65, 66).

In sexually reproducing organisms, the level of allelic heterozygosity is limited by the need for chromosome pairing during meiosis, and the levels found in *Giardia* that range from $<$0.01% to 0.5% are within the range expected in sexually reproducing organisms. This has raised the question of whether *Giardia* undergoes sexual reproduction. Support for sexual reproduction could come from examples of recombination among isolates or by documentation from cell biology of meiosis. The initial evidence for recombination among *G. duodenalis* isolates was derived from sequence analysis of single-copy genes of genotype AII field isolates from Peru (67, 68). Reports suggesting recombination between genotypes A and B (69) have not been confirmed by subsequent studies (19). However, recent work has suggested recombination between A

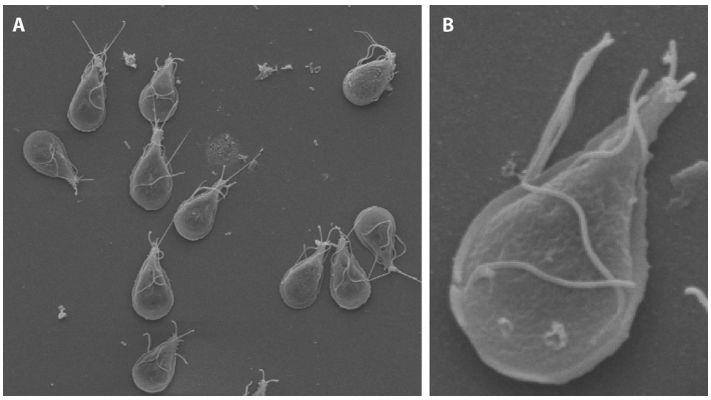

FIG 1 A scanning electron micrograph shows multiple trophozoites adhering to a surface (A) and a magnified view (B). Note the flagella extending from the trophozoite.

and E (A is much closer phylogenetically to E than to B) (22) (see "Chromosomal organization," below).

Proteomics and Transcriptomics

The rarity of introns in the genome, as well as the short 5′ and 3′ untranslated regions and the conserved putative polyadenylation signal, makes it relatively easy to generate the amino acid sequences directly from the genome. In addition, the transcript patterns (transcriptome) are available for a number of conditions for trophozoites or cysts. Despite that, a relatively small number of 3D protein structures have been determined experimentally, and existing sequence-based searches, including BLAST or hidden Markov model (HMM), have poor accuracy when there is a low amino acid identity to the sequences in the database. However, the alternative of structure-based searches with machine learning using the I-TASSER software suite has been used to examine the set of approximately 5,000 *Giardia* proteins (70). The authors generated a library of 1,095 structural models, which included 212 hypothetical proteins, at a high level of confidence and then used this library to explore the redox pathways that could contribute to resistance to nitroimidazoles.

CELL BIOLOGY

Trophozoite Cytoskeleton, Structure, Motility, and Adhesion

G. duodenalis trophozoites are pear-shaped, with a length of 12 to 15 μm and a width of 8 μm. They have four pairs of flagella and a concave ventral side that is surrounded by the lateral crest and a flange (Fig. 1) that allows the organism to attach to the intestinal epithelium. The dorsal surface is convex, and two symmetrically placed nuclei are in the anterior half of the organism (Fig. 2).

The organism is remarkably adapted for survival within the small intestine, even in the absence of tissue invasion, and the cytoskeleton plays a central role in this adaptation. The cytoskeletal components include the ventral disk, the median body, and the eight flagella with their basal bodies. The core composition of the cytoskeleton consists of microtubules that are formed from a family of alpha- and beta-tubulins (71–74). The ventral disk and the median body are unique to *Giardia* species. The ventral disk is composed of a spiral of microtubules and associated sheets that are called microribbons (Fig. 2). There are also hundreds of disk-associated proteins, including ankyrins (initially called alpha-giardins) (75), all of which add rigid structure to the concavity of the disk (76). The disk itself has the ability to contract during attachment and utilizes the lateral crest (Fig. 2) to help generate the initial attachment (77, 78), as well as to remain attached when confronted with shear forces (79).

The flagella have the conventional eukaryotic 9 + 2 microtubule organization and are called the anterior, posterolateral, ventral, and caudal flagella according to the

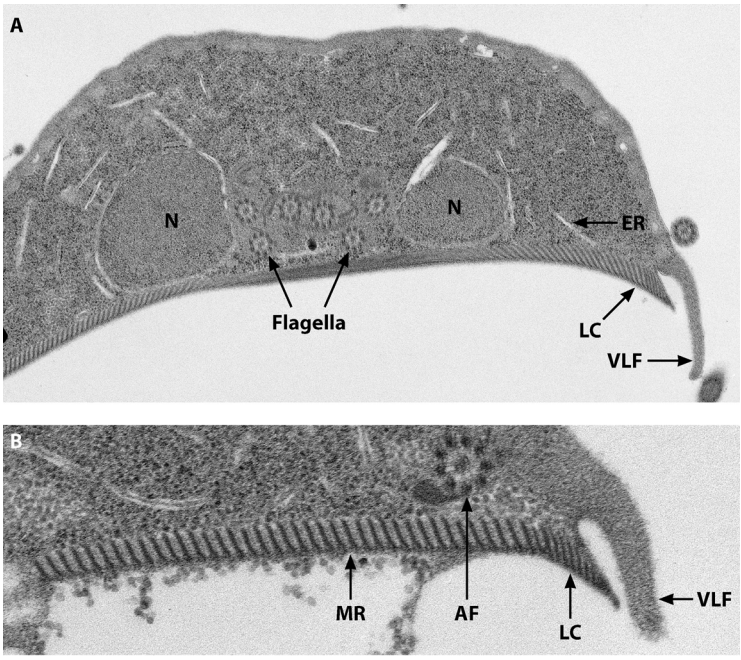

FIG 2 (A) A transverse electron micrograph of a trophozoite through the two nuclei shows the nuclei (N), six flagellar axonemes between the nuclei with the canonical 9+2 arrangement, the ventral disk with the microribbons (MR), and the lateral crest (LC) and ventrolateral flange (VLF). (B) A higher magnification, including the anterior flagellum (AF).

direction of their emergence from the basal bodies located between the nuclei (76) (Fig. 1 to 3). The flagella provide motility for the trophozoites, but their role in attachment continues to be debated. The beating of the ventral flagella occurs in conjunction with attachment to the intestinal epithelium and supports a model in which the ventral flagella would generate a hydrodynamic force resulting in suction by the ventral disk (80). However, more recent data have suggested that the ventral flagella help generate suction through a force that pushes the ventral disk against the intestinal epithelium and helps remove fluid under the disk to allow the initial attachment to occur (81, 82).

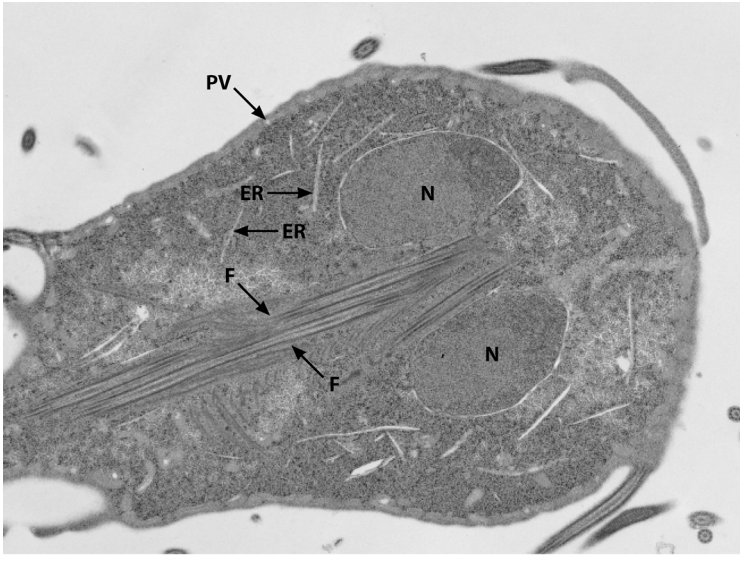

FIG 3 A coronal view through the nuclei (N) also demonstrate the multiple flagellar axonemes (F) traversing through posteriorly. The endoplasmic reticulum (ER) and peripheral vesicles (vacuoles) can also be seen.

TABLE 3 Organelles of *Giardia*

Conventional eukaryotic organelle	Equivalent *Giardia* organelle	Reference(s)
Nucleus	Two nearly identical nuclei	
Nucleolus	rDNA not organized in nucleolar pattern, but genes for nucleolus-localized proteins in genome and candidate nucleolus demonstrated by EM and confocal microscopy	110, 330, 331
Mitochondrion	Mitosome	5
Golgi	Numerous features of Golgi transport done by ER	142
Endosome/lysosome	Peripheral vacuoles or vesicles	145, 332–334
Peroxisome	Peroxisome-like proteins in cytoplasmic vesicles	131
Exosome	Exosome-like vesicles with minimal ESCRT	150

The median body is not only unique to *Giardia* species but is one of the identifying features for the various species. The median body of *G. duodenalis* has been called a "crooked smile" (72, 83). Its function remains unknown, but hypotheses include a role as a reservoir of tubulin subunits during cytokinesis (84) or that it may play a role in detachment (85). A protein called the median body protein (MBP) was initially found in the median body, but a subsequent study found that it is also in the ventral disk and is required for a proper dome shape of the ventral disk and thus for attachment (79).

A single highly divergent actin has been identified in the *Giardia* genome (4), but canonical eukaryotic actin-binding proteins have not been identified. However, recent studies have identified an actin cytoskeleton (86, 87). In addition, the eukaryotic actin-associated protein phosphoserine phosphothreonine 14-3-3 has been shown to interact with actin in *G. duodenalis* (88). However, the role of the actin cytoskeleton has not yet been determined.

Cytokinesis

Cell division is a complex process during which each of the trophozoite's two mastigonts must replicate and segregate to the two daughter trophozoites, all while the organism remains attached to the intestinal wall. Trophozoites replicate by binary fission in a longitudinal plane (89). The ventral disk replicates with the daughter disks being formed at the anterior dorsal side of the trophozoite, followed by division along the longitudinal plane (90). The four pairs of flagella demonstrate semiconservative replication in which each daughter cell receives one parental flagellum in addition to a newly synthesized flagellum. During this process, the newly synthesized flagellum requires three replication cycles to mature, and during this time, the location of the flagella changes from posterolateral and ventral to anterolateral and ultimately to caudal, which is the most mature location and is the organizing center for the ventral disk microtubules (91). Actin appears to play a minimal and as yet not understood role in *Giardia*, and myosin has not been identified in *Giardia*, so the mechanism of cytokinesis must be different from that of canonical eukaryotes, in which cytokinesis depends on actin and myosin. A myosin-independent model has been suggested in which actin positions the microtubule cytoskeleton and works in cooperation with Rab11 to allow the division furrow to progress, with flagellar propulsion finally helping to complete the process (84).

Nuclear and Chromosomal Structure and Replication

Chromosomal organization. Many of the *Giardia* organelles contrast significantly with those of canonical eukaryotes (Table 3), and of course the two transcriptionally active nuclei in dyadic symmetry provide one example. Initial data based on pulsed-field gel electrophoresis revealed the presence of five distinct chromosomes, but with up to four size variants for individual chromosomes (57). The size variants were subsequently shown to result from variation in the size of repeat regions in the subtelomeric regions; some of these repeats consisted of ribosomal DNA (rDNA) (92, 93). The five chromosomes have subsequently been confirmed by optical mapping (55) and by cytogenetic approaches.

Tetraploidy was confirmed by flow cytometry, with the interesting finding that trophozoites spend the majority of time in the G2 phase (after DNA replication) during

in vitro cultivation (44). Thus, some of the earlier quantitative DNA analysis suggesting a ploidy of eight (57) resulted from analyzing organisms that were primarily in the G2 phase. This means that *Giardia* trophozoites in the G1 phase normally have a ploidy of four, with two diploid nuclei that are identical in most ways that have been tested (94). However, a small degree of aneuploidy has been documented by cytogenetic methods (95, 96). An interesting example has been described in the WB isolate and in a cloned line of WB, called WBC6. (WBC6 was cloned from the WB isolate shortly after its initial description in 1982 [97]). The WBC6 line was found to be aneuploid for chromosome 5, with three copies in one nucleus and one in the other (98). Chromosome 5 in the nucleus haploid for that chromosome had a deletion of a subtelomeric region, and five genes from that region were expressed only from one nucleus.

The chromosomes are highly compacted in comparison to many other eukaryotic chromosomes and possess 10-nm fibrils, 30-nm fibrils, and chromomeres (99). During replication, mitosis is semiopen. Prophase chromatin condensation, chromosome alignment, and movement to the spindle poles is similar to that in other eukaryotes, but the nuclear movement to the center of the cell and the telophase spindle structure differ from those of other eukaryotes (89). The centromere can be identified by the presence of a cenH3 histone variant (100). The eukaryotic spindle assembly checkpoint, which monitors attachment of the chromosome to the spindle during metaphase, has not been identified in *Giardia* (101). The telomeres at the end of the chromosomes consist of a 5-nucleotide repeat of TAGGG (92) and, in contrast to the 6-nucleotide human telomere of TTAGGG, the *Giardia* telomere forms a tetramolecular antiparallel structure (102), although the significance of this is unknown.

The above-described findings raise an interesting question regarding the replication of the nuclei of *Giardia*. On one hand, the two nuclei have essentially the same complement of DNA, and both are transcriptionally active (103). On the other hand, there are differences between the nuclei that are maintained through replication, providing evidence for a semiconservative distribution of the nuclei (94, 98). Initial data suggested that the same right-left asymmetry was maintained (94), but subsequent data have supported a model with mirror image replication of the nuclei (89). The semiconservative replication of the nuclei implies that there must be a mechanism of DNA exchange between nuclei at some point, since heterozygosity would continue to accumulate if there were no exchange. Direct evidence for exchange of nuclear material was supported by the EM observation of frequent fused nuclei in cysts, as well as by DNA fluorescent *in situ* hybridization (FISH) of plasmids transfected into the nuclei (104). This led to the conclusion that during encystation, the two nuclear pairs migrate from polar ends, fuse, and separate. Investigators suggested a model in which homologous recombination occurs after nuclear envelope fusion via a parasexual process called diplomixis (104). Indeed, recombination in eukaryotes can occur by canonical meiotic sexual reproduction or by a parasexual process, which is found in the common yeast pathogen, *Candida albicans* (105, 106). Meiotic genes are involved in parasexual processes as well, so the observations of the study support a role for the previously documented meiotic genes in the genome (4, 107).

Nucleolus. The eukaryotic nucleolus is a small structure in the nucleus in which rRNA biogenesis occurs (108). The structure can be seen by electron or light microscopy and contains separate regions called fibrillar centers, dense fibrillar components, and granular components. Nucleoli were long thought to be absent in *Giardia* nuclei, and fibrillarin, which is involved in ribosome assembly, was found diffusely in the nuclei rather than in discrete nucleoli (109). More recently, structures consistent with nucleoli have been identified by light and electron microscopy (110, 111), but there is no evidence of a conventional eukaryotic nucleolar organizing region (112).

DNA repair. *Giardia* has a DNA repair system in which the recombinase DMC1B functions as Rad51 (113), and Rad52 also plays a role in DNA repair (114). The dramatic differences among the genotypes in the level of allelic sequence heterozygosity, especially between genotypes AI and B, raise the question of whether these organisms

differ significantly in the efficiency of their DNA repair systems. In addition, the ability of plasmid DNA to integrate into the genotype B genome but not the AI genome supports the possibility of differences in the repair mechanisms (115).

Transcription and translation. The genes are present for eukaryotic RNA polymerases I, II, and III. For RNA polymerase II, the subunits rpb4 and rpb9 have not been identified, and in contrast to RNA polymerase II from other eukaryotes, transcription is resistant to amanitin (116). Although RNA splicing is relatively rare in *Giardia*, a spliceosome, along with core spliceosome proteins and small nuclear RNA candidates, has been identified (117). An earlier report suggested the absence of a 5-methyl-guanosine cap on mature mRNA (118), but subsequent studies confirmed conventional capping (119, 120). In view of the very short 5′ untranslated regions (UTRs), the first AUG is the one that is utilized for translation (119). Eukaryotic translation initiation normally involves the eIF4F trimer complex and the preinitiation complex. The eIF4F complex comprises eIF4A and eIF4E held together by eIF4G. However, the latter has not been identified in the genome, and there is evidence that the two remaining components can interact directly with the preinitiation complex (121), providing yet another example of the minimalization of the genome.

Mitosomes

In aerobic eukaryotic organisms, ATP is generated by oxidative phosphorylation in double-membraned organelles called mitochondria. In certain anaerobic protozoa, such as *Tritrichomonas foetus* (122) and *Tritrichomonas vaginalis* (123), there is an organelle called a hydrogenosome in which ATP is generated by substrate-level phosphorylation with the production of hydrogen. In contrast, *Giardia* trophozoites have an even more rudimentary organelle called a mitosome (5) or mitochondrion-like organelle (MLO) (124, 125). The mitosome is a double-membraned organelle that contains iron-sulfur (Fe-S) clusters, which play an essential role in assembling proteins with Fe-S, but there is no ATP production. The *Giardia* mitosome has the Fe-S cluster proteins and proteins related to biogenesis of the mitosome, but no other known functions (126–128). Unlike conventional mitochondria, the replication of the mitosomes is linked to mitosis (129).

Peroxisomes

Peroxisomes are named after their ability to reduce O_2 to H_2O_2 and then to reduce the H_2O_2. They are nearly universal in eukaryotes but differ greatly in function, with the most common functions being detoxification and fatty acid synthesis (130). *Giardia* has long been believed to have no peroxisomes, but recent evidence suggests that a rudimentary form of this organelle may be present. Candidates for the peroxisome proteins, acyl-coenzyme A (CoA) synthetase long-chain family member 4 (ACSL-4) and peroxin-4 (PEX-4), are present in the *Giardia* proteome (131). Acosta-Virgen et al. used antibodies against these proteins, as well as cytochemical approaches, to localize their localization to vesicles that were distinct from encystation-specific vesicles.

Biosynthesis and Energy Metabolism

In keeping with its status as an obligate parasitic organism and its genomic minimalization, *G. duodenalis* also has very limited biosynthetic and energy production pathways (132). The energy metabolism is anaerobic, lacking cytochrome-mediated oxidative phosphorylation. Glucose is the major carbohydrate source of energy and is metabolized into various concentrations of acetate, ethanol, alanine, and CO_2. The relative concentrations depend on the oxygen concentration, with alanine as the major product under strict anaerobic conditions. Figures 1 to 3 in my prior review (1) give the detailed pathways. At oxygen concentrations greater than 0.25 μM, the production of alanine is inhibited and ethanol is stimulated, but both are produced. At an oxygen concentration of $>$46 μM, both are replaced by acetate and CO_2 (133). This range of oxygen concentration likely represents the range of oxygen exposure seen by the trophozoites in the small intestinal environment.

Giardia trophozoites have very little endogenous amino acid synthesis (reviewed in reference 1) but do use amino acids acquired from the host as an important source of energy. The most studied pathway is the arginine deiminase pathway, in which arginine is metabolized to produce ornithine with the generation of ATP from ADP (1, 134). This pathway is present in other diplomonads, as well in the parabasalids to which *T. vaginalis* belongs (135). In addition to being an important source of energy (134), it may play a role in pathogenesis (see "Proteases and Secreted Substances," below). Aspartate may be an additional energy source through conversion to pyruvate through a malate intermediate.

Many pathogenic protozoa depend on purine salvage, as does *Giardia*. In addition, *Giardia* also depends on pyrimidine salvage, as do the trichomonads (1).

Fatty acids play key roles in the life cycle and metabolism of *Giardia*, including in encystation. Cholesterol and bovine bile from growth medium are able to induce encystation, and genes linked to cholesterol biosynthesis are upregulated during encystation (136). In addition, there is evidence for synthesis of phosphatidylglycerol and phosphatidylethanolamine in vegetative and encysting trophozoites (137), which led to a proposed model of lipid metabolic pathways in *Giardia* (see Fig. 4 in the review of Yichoy et al. [138]).

The Endomembrane Transport System

The eukaryotic Golgi apparatus is a distinct-appearing membrane-bound organelle consisting of membrane stacks and vesicles. It accepts molecules from the ER, modifies them as required, and delivers them to their required location on the basis of appropriate signal sequences. Thus, it has always been surprising that Golgi apparatus are not found in vegetatively growing *Giardia* trophozoites. For many years, it was assumed that *Giardia* had no Golgi apparatus and perhaps had emerged from the eukaryotic lineage before the development of the Golgi apparatus (139, 140). However, the discovery of structures consistent with the Golgi apparatus in encysting trophozoites (41) and the identification of protein transport that was inhibited by brefeldin A, which is used as a tool for studying Golgi transport (141), gave an indication that at least some of the functions of Golgi transport were present. Subsequent studies have suggested that the endoplasmic reticulum (ER) fills many of the Golgi functions in *Giardia* (142). In a canonical eukaryotic transport system, a COPII coat assembly mediates the transfer of its cargo from the ER to the Golgi apparatus, and transfer from the Golgi apparatus to the ER is mediated by COPI. In *Giardia*, members of the COPII complex are located at specific sites in the ER where cyst wall protein 1 (CWP1) is transported and transferred to ESVs (143). This occurs in vegetative and encysting trophozoites. Members of the COPII complex interact physically with CWPI at these sites (142). The eukaryotic KDEL receptors function in protein transport at the Golgi apparatus-ER junction, and the single *Giardia* KDEL receptor is also found at the same specific ER site. These findings all suggest that the ER has taken over the functions of the Golgi apparatus in *Giardia* (140).

Endocytosis is performed by the peripheral vesicles (PV), which are acidified for importing materials required by the trophozoite (144) and function as endosome-lysosomes (145). Invaginations of the dorsal side of the trophozoite that are associated with the presence of PVs on the inside suggest that import of these materials occurs by means of membrane fusion (146). The PVs also interact with the *Giardia* clathrin heavy chain, which is involved in eukaryotic endocytosis, and with associated proteins (146).

Exocytosis involves the VSPs in particular, which are secreted in high quantities (147) and form a protein coat over each trophozoite (148, 149). The secretion system is not well characterized, but a recent study identified exosome-like vesicles in *in vitro* cultures and demonstrated the role of ESCRT-associated proteins and ceramide in forming intraluminal vesicles inside the PVs (150). This supports the idea that the secretory process of *Giardia* also depends on the ER and the PVs.

Membrane and Surface Proteins

The VSPs and *vsp* genes. The variant-specific surface proteins (VSPs) were initially called cysteine-rich protein (CRP) (151), trophozoite surface antigen (TSA) (152), and

trophozoite surface protein (TSP) (153). They are currently called VSPs (154) because of their ability to undergo antigenic variation at a rate of up to 10^{-3} per cell division (155). These proteins are cysteine rich (about 12% cysteine), with frequent CXXC motifs (151). The *vsp* genes that were initially described all encoded a C-terminal CRGKA (154). They are heterogeneous in size, ranging from about 2 to 6 kb, corresponding to about 60 to 180 kDa for the translated protein. The size of the *vsp* gene repertoire for WB has been estimated at 270 to 300, making up 4% of the genome. It includes 30 with tandem repeats (as does *vspA6*, the first *vsp* described), and 14 in pairs with head-to-head or tail-to-tail arrangements (156). How many of these are functional *vsp* genes cannot be determined by a genomic approach. Thus, a study to determine the sequence motifs that are required to result in expression on the surface of the organism determined that the CRGKA motif was not required but that two other motifs (called motifs 1 and 2) are required for localization to the cell membrane (157). Motif 1 consists of 45 AA with CXXC flanking 12 to 15 AA near the C terminus, and motif 2 consists of the 38 hydrophobic AA at the C terminus. Li et al. identified 73 genes meeting these criteria and proposed them as true *vsp* genes. However, five were missing the CRGKA, and the CRGKA was modified in another three. Since all experimentally described *vsp* genes include the CRGKA, it may still be that this motif plays another essential role. This also opens the question of whether the additional 200 putative *vsp* genes are expressed and, if so, what their function is.

The VSPs appear to play a role in immune evasion (see "Immune response," below). Some VSPs protect the trophozoites from intestinal proteases (158) and may play other roles in adaptation to different hosts (159, 160). The biological role of the VSP is not known, but based on the amount of the genome dedicated to the encoding genes, it most likely plays an important role.

A number of studies on the mechanism of antigenic variation of the *vsp* genes have been performed, but the mechanism of change is only partially understood. The rapid change in expression, estimated at every 6 to 13 generations (155) suggests an epigenetic mechanism. There is no current evidence that DNA sequence alteration or recombination plays a role in antigenic variation. This is in contrast to the antigenic variation that occurs in African trypanosomes, in which sequence alterations and rearrangements are commonly associated with antigenic variation (161).

A study of antigenic variation of the *vspA6* (CRP170) gene indicates the loss of the expressed version in variants, which likely means the loss of the expressed allele (162). It is possible that the loss of the expressed version represents loss of the chromosome containing the expressed *vspA6* (see evidence for aneuploidy in a paper by Tůmová et al. [98]).

Control of antigenic variation was studied for the *vspH7* gene from the GS isolate (genotype B) by using stable transfection with homologous integration of a hemagglutinin (HA)-tagged *vspH7* gene into the *vspH7* genome location (163). The authors showed that expression of the native *vspH7* and the HA-tagged *vspH7* were independent of each other, implying that expression was allele specific. In addition, lysine acetylation occurred immediately upstream of the integration in association with expression, suggesting a potential epigenetic mechanism for allele-specific expression.

There is also evidence for the role of RNA interference (RNAi) in the control of *vsp* expression. Organisms expressing a single *vsp* have one stable transcript, but other *vsp* genes are transcribed but do not result in stable transcripts. These silenced genes correlate with small RNAs consistent with an RNAi process preventing their expression (164). The *Giardia* genome encodes an RNA-dependent RNA polymerase (RdRp), Argonaute, and Dicer as components of an RNAi system (164, 165). When the RNAi pathway was disrupted by constitutively expressing antisense transcripts for RdRp, Argonaute, and Dicer, multiple *vsp* genes were expressed simultaneously (164). *Giardia* has 25 small nucleolar RNAs (snoRNAs) (166), and two snoRNAs were identified that were precursors of the miRNAs miR6 and miR10, which are processed by Dicer into a mature miRNA duplex (166). Forty-four of the *vsp* genes have potential binding sites for miR6 in the 3′ UTR, and 159 have potential binding sites for miR10 at the 3′ end of the open reading frame (ORF); 33 have binding sites for both. Gerbils infected with

G. duodenalis could be protected from subsequent infection by exposure to RNAi-disrupted organisms expressing multiple VSPs, but not by those expressing a single VSP, providing evidence that antigenic variation is important for survival of the organism in the host intestine (167).

In summary, there is evidence supporting at least the following two mechanisms for control of *vsp* expression: (i) lysine acetylation, which can limit expression to even one allele of four, and (ii) an RNAi system that limits expression to one *vsp* at a post-transcriptional level. However, the actual mechanisms for switching from expression of one *vsp* gene to another remain to be determined.

High-cysteine membrane proteins. In addition to the *vsp* genes, there is a family of cysteine-rich HCMPs that do not undergo antigenic variation, but some of these are upregulated during encystation (50). The estimated number of HCMP genes is now 116 on the basis of a more complete genome assembly of the WB isolate (56). The HCMPs localize to the nuclei of trophozoites. During encystation, they are transported by the encystation-specific vesicles (ESV) and can be found in the cell body as well as in the wall of mature cysts. The role of these proteins remains to be determined.

Other membrane-associated proteins have also been described but are not yet characterized (168).

Giardiavirus (*Giardia lamblia* Virus)

Giardiavirus (*Giardia lamblia* virus [GLV]) (169) is a double-stranded RNA virus found in *Giardia* trophozoites from genotypes A and B. It is a member of the *Totiviridae* family, which also has members that infect fungi, *Trichomonas*, and *Leishmania* (170). After internalization by what may be receptor-mediated endocytosis, it is concentrated in the peripheral vacuoles (171). Although many of the isolates are not infected, the majority are susceptible to infection when exposed to the virus. The RNA is 6.2 kb in length and encodes a virus composed of a 100-kDa viral capsid protein and an RNA-dependent RNA polymerase (RdRp). Giardiavirus-infected trophozoites do not appear to differ from uninfected organisms in their fitness or virulence (172, 173). An miRNA encoded by the RdRp gene has been identified that may limit the viral copy number (174).

EPIDEMIOLOGY

G. duodenalis is distributed worldwide, but there is important variation in geographic distribution and epidemiology. Cysts survive in the environment longer in cool, moist areas. Thus, in temperate climates, which tend to be low-prevalence areas, giardiasis is often seen in the form of symptomatic waterborne outbreaks (28, 175). In fact, it was in the 1960s and 1970s with reports of outbreaks among Finnish travelers (from a low-prevalence region) to St. Petersburg (Leningrad), which had inadequately treated water at that time, that *Giardia* became generally accepted as a human pathogen. Some of the reported series had attack rates of over 50% (176–178). Similarly, important waterborne outbreaks have been documented in northern regions, such as Bergen, Norway, in 2004 (179, 180) and British Columbia (181). In the United States, it was found that in outbreaks of giardiasis at ski resorts, visitors were at higher risk than local residents, suggesting partial acquired immunity in the residents (182). The partial protection of the local residents suggests that the outbreak-associated pattern of high attack rates is due to infection in hosts without prior immunity. In contrast, giardiasis regions where it is highly endemic, particularly in low-income areas in tropical and subtropical areas (e.g., shantytowns of Lima, Peru) (183), is typically subclinical.

Within the United States, giardiasis may be outbreak related and is usually associated with drinking water or recreational water exposure. Food-borne outbreaks also occur but are less frequently documented. For sporadic cases, the risk factors are numerous and include direct and indirect fecal contact, as well as host factors (175, 184). Major risk factors found in a study of reported cases from Colorado and Minnesota, the major risk factors are shown in Table 2 of Reses et al. (184).

Some risk factors, such as male-male sexual contact and international travel, have very high odds ratios, but on a population basis, additional risk factors with lower odds

ratios are still important because of their high prevalence; these include daycare exposure, swimming in or drinking from natural water bodies, and even chronic gastrointestinal conditions or the use of antibiotics. Animal exposure has not been a risk factor in recent studies (175, 184), and the role of animals remains controversial. There are human cases with reasonably well-documented animal sources, but quantitatively, animals play a small role (see for example, references 67 and 185). In fact, data from Brazil suggest that transmission from humans to animals is more common than that from animals to humans (186). Food-borne outbreaks are occasionally reported but are likely underrecognized, and sporadic cases due to food are very difficult to identify (187), so the true importance of foodborne transmission remains unknown.

Numerous studies of the relative importance of genotypes A (usually AII) and B have been reported, but the results of these studies do not clearly identify a difference in epidemiology. In contrast, there is accumulating evidence that genotype AI is primarily a zoonotic infection (21, 22).

PATHOGENESIS

G. duodenalis is a noninvasive pathogen of the small intestine and produces a wide range of clinical presentations, including chronic diarrhea with weight loss, postinfectious complications of irritable bowel and chronic fatigue, growth stunting, and asymptomatic infections. These varied manifestations may result from host, parasite, or microbiota differences and make it particularly difficult to elucidate the mechanisms resulting in this range of illness. Nevertheless, there has been substantial progress over the past 2 decades in elucidating some of the mechanisms. Recent in-depth reviews of these advances are also available (188–191).

The trophozoites adhere tightly to the small intestinal mucosa, leaving a detectable imprint when they detach from the intestinal epithelium (192), so the possibility of direct pathogenesis from the mechanical attachment has been raised. However, there is currently no evidence to support this possibility. Rather, current data suggest that a combination of secreted proteases and other *Giardia* factors, the host immune response, and the interaction of these factors with the intestinal microbiota contribute to the various manifestations (191, 193). Through the entire immune response, it is actually remarkable that in patients who have biopsies for symptomatic giardiasis, the findings consist of flattening of the villi but no obvious inflammatory changes. However, an inflammatory picture can be seen and may actually be separate from the location of the trophozoites. A series of cases was reported in which trophozoites were seen in ileal biopsy specimens from ileocolonoscopy of symptomatic patients but inflammatory changes were found in the duodenal biopsy specimens of these patients (194). All patients had ileal blunting or atrophy, 6 of 11 had neutrophilic infiltration, and one had findings consistent with celiac disease. The finding of trophozoites in the ileum is consistent with the animal model in which trophozoites were concentrated in the proximal small intestine but were sometimes found in the ileum or even the cecum (34). These findings raise interesting new questions about the pathogenesis of giardiasis that are being pursued by ongoing studies.

Proteases and Secreted Substances

The role of excretory secretory products (ESPs), most notably the cysteine proteases, has received substantial attention over the last decade (193). Although serine proteases are also present in the ESP, the major portion of the protease activity is from the cysteine proteases (195). It may be that the primary role of these proteases is to control encystation and excystation, but they also play roles in the interaction between the trophozoite and the host intestinal epithelium.

Trophozoites interfere with the intestinal tight junction by number of mechanisms that include the cysteine proteases (193, 196, 197). A study of the effect of CP2 (called giardipain 1 in this study) on an intestinal epithelial cell line (IEC-6) monolayer demonstrated damage to the cell junctions that was prevented by the protease inhibitor E-64 (198). In addition, CP2 induces apoptosis and damages the villi by its interaction with villin (199). The intestinal mucus layer is a key component of innate defense against

pathogens and, as such, it is notable that the cysteine proteases have a complex effect on the mucus. MUC2 (human mucus protein) is degraded *in vitro*, and MUC2 gene expression in goblet-like cells is increased, leading to the hypothesis that the *Giardia* cysteine proteases may deplete the normal intestinal mucus reserve (200). In the same study, the mucus was thinned in infected wild-type mice, and Muc2-deficient mice developed weight loss when infected. Bacterial translocation was also increased in the wild-type mice (200).

It is likely that many additional ESPs play a role in pathogenesis, so a systematic approach was used to identify the secretome of *Giardia* and intestinal epithelial cells (IECs) differentiated from Caco-2 cells using two different isolates (WB from genotype AI and GS from genotype B) (201). Proteins related to metabolism were among the dominant *Giardia* ESPs and included those related to glycolysis, arginine metabolism, phospholipid remodeling, and nucleotide salvage. In addition, proteins involved in encystation, VSPs, HCMPs, alpha-giardins (annexins), and tenascins were increased. When the GS and WB isolates were compared, the classes of proteins were similar, although the number of proteins differed between the isolates. In addition to the effect of IECs on *Giardia*, the response of IECs to *Giardia* was also studied. The response included chemokine production for recruitment of immune cells, impact on glucose and lipid metabolism, and induction of apoptosis.

A transcriptomic study of the interaction of WB (genotype AI) trophozoites with IECs was done to explore which genes were upregulated (202). Overall, the HCMPs showed the greatest increase in expression, but were varied in their responses. Another study of the secretome of these two *Giardia* isolates, focused particularly on the tenascins, found similar results (203). The mammalian tenascins are extracellular matrix proteins, but tenascins are not well characterized in unicellular eukaryotes. The *Giardia* tenascins have been proposed as virulence factors, but specific studies have not yet been reported.

Arginine consumption by *Giardia* reduces nitric oxide production (51, 204) and proliferation of IECs (205). The *Giardia* arginine deiminase is a cytoplasmic enzyme that is secreted upon interaction with IECs in abundant quantities (206) and is responsible for arginine depletion. Arginine depletion also impacts the immune response to *Giardia* by increasing tumor necrosis factor alpha (TNF-α) and decreasing interleukin 10 (IL-10) in dendritic cells (207) and by reducing T-cell proliferation in intestinal epithelial cells (204). Thus, arginine deiminase can be considered a virulence factor, in addition to its role in energy production for trophozoites.

Immune Response

Early assumptions and even dogma about the central role of the antibody response for immunity to *Giardia* infections was supported by observations of prolonged giardiasis with total immunoglobulin or IgA deficiency. Those observations were never well documented, and the story has subsequently become much more complicated and interesting. There is evidence for a significant role of the IgA response in view of the observation of a brisk IgA response in infected humans that is directed particularly against semiconserved portions of the VSP (208).

Since the VSPs undergo antigenic variation, this raises the question of whether the antigenic variation is to avoid the host immune system. Earlier data in gerbils (160) showed that after the change in VSP expression that occurred within the first 7 days, there were no further changes by 28 days, suggesting that antigenic variation might be unrelated to avoiding the host immune response. However, in human volunteers, the data on the change in VSP expression were more consistent with a role for antigenic variation in survival in the host (159). Subsequent data have accumulated that strongly support a role for antigenic variation in surviving the host immune response. A vaccination study was done using a WB isolate that had been transfected to silence Dicer and the RNA-dependent RNA polymerase, with the effect of suppressing RNAi and allowing multiple VSPs to be expressed simultaneously. This organism was used to infect gerbils and induced effective immunity against reinfection (167). Gerbils were

also protected by immunization with a vaccine composed of the multiple VSPs. The early immune response was primarily Th1, including gamma interferon (IFN-γ), TNF-α, and IL-17, and it was followed by a Th2 response. A similar vaccine was given to domestic animals and provided protection from infection (209). The ability to develop a protective immune response after exposure to multiple VSPs suggests their important role in evoking an immune response, as well as the role that antigenic variation plays in surviving the host immune response.

TH17 cells produce IL-17A as well as other cytokines and, in addition to protective immunity, play a role in autoimmune diseases, including inflammatory bowel disease, meaning that precise regulation is required (210, 211). IL-17A has been recognized as a key component of the immune response to *Giardia* infection and has been shown in mice infected with *G. duodenalis* (212) and with *Giardia muris* (213) and facilitates the IgA response (214). Studies have also shown that the IL-17 receptor A (IL-17RA) is a regulator of the immune response to *Giardia* infection and that upregulation of the receptor is required for a local IgA response (214, 215). When IL-17RA knockout mice were infected with *G. muris*, expression of many of the genes that are normally upregulated by *Giardia* infection was inhibited (215). These animal model studies suggest a key role of IL-17 A and IL-17RA in controlling *Giardia* infection. A Th1 (CD4) response with IL-17A-producing CD4 cells has also been documented in infected human travelers (216), as well as a trend toward increased Il-17 levels in children in Brazil (217), showing that the mouse model correlates with human infections. A study of the Th17 response to *G. muris* was done in BALB/c mice compared to C57BL/6 mice. The BABL/c mice had an impaired Th17 response that correlated with impaired clearance of *Giardia*, leading to the conclusion that a correctly balanced Th17 response appears to be required for effective *G. muris* infection in mice (218). An ineffective response results in prolonged infection, while an excessively exuberant response may result in a host inflammatory response that is damaging to the host. Perhaps inadequately regulated immunity can explain the great variability in symptoms, as well as that in clearing the organism.

Mast cells are recruited to the intestine following infection, play a role in controlling infection (189), and may also contribute to the abdominal cramping that is common in symptomatic giardiasis (219). A recent study has suggested that mast cells may be activated by arginine deiminase or by its product, citrulline (220). There is also an accumulation of macrophages that produce arginase 1 and nitric oxide synthase 2 in mouse lamina propria following *G. duodenalis* infection (221), but macrophage depletion does not impair clearance of the trophozoites (222), so the significance of the macrophages is not yet clear.

In addition to the inflammatory response induced by *Giardia* infection, there are also examples where *Giardia* actually attenuates the inflammatory response. *G. duodenalis* extracts increase IL-10 production by dendritic cells (223, 224) and decrease IL-12 (223) through the phosphoinositide-3-kinase (PI3K) pathway (223). The importance of this attenuation is emphasized by a study in which *G. muris* infection of IL-10-deficient mice caused colitis (225). The colitis was Myd88 dependent and was facilitated by the intestinal microbiota. *G. muris* also reduces the severity of intestinal disease in mice caused by the attaching and effacing pathogen *Citrobacter rodentium* (226). This was dependent on the NLRP3 inflammasome and was associated with increased antimicrobial peptide production by the host (149). *G. duodenalis* also reduced neutrophil infiltration in biopsy specimens from humans with active Crohn's disease (227). *G. duodenalis* cathepsin B cysteine proteases degraded the neutrophil chemoattractant IL-8 in biopsy specimens infected with nontyphoidal *Salmonella* (228).

Malnutrition and Long-Term Sequelae

Over the last decade, there has been an increasing appreciation of the pathological and physiological roles of the intestinal microbiota. In fact, for about 50 years, there has been a recognition that *Clostridioides difficile* (formerly *Clostridium difficile*) colitis is facilitated by the predominance of bacterial flora that allows the colitis to develop, confirmed by evidence that repopulating the colonic flora using bacteria from healthy

hosts substantially reduces the risk of *C. difficile* colitis (229). More recently, the use of next-generation sequencing has allowed systematic evaluation of the microbial makeup of the intestine and its association with health and disease. A landmark study on the role of the microbiota in malnutrition was done in twins in Malawi who were discordant for severe malnutrition (230). Colonic flora from the malnourished children, but not that from their healthy twins, reproduced malnutrition in a mouse model. Subsequently, alterations in intestinal flora have been associated with disorders that range from inflammatory gastroenterological diseases (210) to metabolic diseases, including diabetes (231).

Early data specific to *Giardia* regarding the importance of bacterial flora came from evidence that germfree animals would not develop disease from *Giardia* despite being susceptible to infection (232, 233). Mice also differ in susceptibility to *Giardia* infection with differences in microbial flora (234). These observations emphasize the importance of understanding the interplay between *Giardia*, the intestinal microbiota, and the host immune response (191). The impact of *Giardia* infection was examined in a mouse model of *G. duodenalis* infection using the GS isolate (genotype B), which establishes infection more easily than genotype A organisms and requires less use of antibiotics to establish infection (235). The investigators infected animals with *Giardia* (with and without antibiotic supplementation) and found that the number of *Giardia* organisms in the proximal small intestine was much greater in the animals who received antibiotics. The alpha diversity, a measure of diversity within the microbiota, was not changed. However, there was a shift in the microbiota (dysbiosis) induced by *Giardia* in animals, whether those animals were treated with antibiotics or not, particularly a shift to *Proteobacteria* and away from *Firmicutes*. This provides an interesting contrast with a study in which a human microbiota was exposed to *Giardia* trophozoites (genotype not stated) and then infused into germfree mice, resulting in a microbiota in these mice that was enriched in *Clostridiales* species from the *Firmicutes* group. These effects on microbiota substantiate the complex interaction between the bacterial flora and *Giardia* during *Giardia* infection. Other investigators using the GS isolate in a mouse model found that *Giardia* infection resulted in decreased sucrase activity, an effect that was attenuated by an antibiotic combination with a broad spectrum of Gram-positive and Gram-negative activity, but without activity against *Giardia* (236). They also found that pathological T-cell CD8 activation was prevented by antibiotics, suggesting a role of the microbiota in altering the immune response to giardiasis. Others have used an *ex vivo* approach to study the interaction of *Giardia* trophozoites with colonic biopsy material with a microbial biofilm. They found that *Giardia*, by its excretory secretory proteases, altered the biofilm. The microbiota enhanced lymphocyte production of proinflammatory cytokines and, in epithelial cells, decreased tight junction ZO-1 protein expression and increased proinflammatory CXCL-8 production and Toll-like receptor 4 (TLR4) expression. This led to their hypothesis that the pathogenesis of giardiasis is mediated through a TLR4 response and could explain its association with irritable bowel syndrome (IBS) (237). The complex interaction between *Giardia* and the intestinal bacteria was also demonstrated by the disruption of the tight junction after a resolved *Giardia* infection in a mouse model using GS (genotype B) *Giardia*. The disruption of the tight junction persisted after resolution of the *Giardia* and led to an influx of bacteria along with a neutrophilic inflammatory reaction (238). These findings from IECs contrast with a mouse model in which TLR2-deficient mice displayed enhanced clearance of *Giardia* along with increased proinflammatory cytokine production (239). In the latter study, there was no modification of the microbiota.

Several observational studies of humans have now been reported. A microbiota study of fecal samples of 20 people from the Ivory Coast, some with abdominal pain and some with no obvious symptoms, found that the 10 infected with *Giardia* had a dysbiotic pattern, as measured by a decreased *Faecalibacterium prausnitzii*:*Escherichia coli* ratio (240). A study from Colombia evaluated 23 fecal samples from 290 initial samples, each containing only one species of parasite, using 16S sequence analysis. Nine

had *Giardia* with or without other parasites. They found that children with giardiasis had a distinct microbiota type that was characterized by an increased prevalence of *Prevotella* spp. (241). A study of 37 asymptomatic children in Argentina using whole-genome sequencing found that the 13 with >1 fg/μl of *Giardia* DNA in stool had decreased alpha diversity, along with increased abundance of *Prevotella* spp. (242). A study was reported using 1,004 participants from the Global Enteric Multicenter Study (GEMS) who had microbiome data, 215 of whom had *Giardia* identified. The GEMS study included children from low-resource countries who had moderate to severe diarrhea (243). In addition, there were about 913 fecal samples from Peruvian children under 2 years of age who were enrolled in the Malnutrition and the Consequences for Child Health (MAL-ED) study (244) and had 16S sequencing done. Those from the GEMS study (with acute symptoms) had increased abundance of *Prevotella* spp. and decreased abundance of *Gammaproteobacteria*, and those from the MAL-ED study (without acute symptoms) also had increased *Prevotella* and decreased *E. coli* (the most abundant member of the *Gammaproteobacteria* family) abundance (245). These observational studies, including one with a large sample size, showed consistent findings of increased *Prevotella* and decreased *Gammaproteobacteria* abundance in association with *Giardia* infection and represent the beginning of research that is likely to move from investigating associations to determining causality.

A neonatal rat model of postgiardiasis irritable bowel syndrome (IBS) has been developed, recreating some of the features of human IBS, including visceral hypersensitivity (246). These rats had persistent visceral hypersensitivity after *Giardia* infection, in addition to an impaired tight junction and increased translocation of commensal intestinal bacteria. C-fos has been a marker for the painful stimulus of IBS, and its expression was increased in this model, supporting the validity of the model.

A study done to understand the mechanism of malnutrition caused by *Giardia* used a weaned mouse model infected with *Giardia* cysts to explore the impact of *Giardia* on malnutrition (247). Mice were given normal or protein-deficient diets and infected with cysts from the GS isolate. *Giardia* caused epithelial hyperplasia and villous blunting through a Th2 immune response, along with worsened malnutrition, primarily in the protein-deficient mice. In addition, the malnutrition prevented an effective immune response. Thus, there was a synergistic effect of malnutrition and giardiasis in this model. These investigators also demonstrated a synergistic pathogenesis between *Giardia* and enteroaggregative *E. coli*, in which *Giardia* prevented a microbiota-dependent clearance of the *E. coli* bacteria (248). These animal model studies provide an important insight into potential mechanisms of *Giardia* persistence, as well as associated malnutrition.

CLINICAL MANIFESTATIONS

Giardia infection is best known for a subacute onset of diarrhea beginning 1 to 2 weeks after exposure, which consists of loose, greasy stools and flatulence and is frequently accompanied by weight loss (Table 4). Fever and other systemic symptoms are uncommon, and patients typically present for medical evaluation days to weeks after the onset of symptoms. However, other clinical manifestations frequently receive less attention, and the majority of infections are subclinical, especially in regions where giardiasis is endemic.

In these settings where giardiasis is highly endemic, children are infected early in life and usually do not have diarrhea. A study in a high-prevalence area of Peru reported in 1988 (183) that within 6 months after treatment with a nitroimidazole, 98% again had *Giardia* detected in stool specimens. However, these children had no symptoms and no excess fat excretion in association with their giardiasis. A study from the same area in Peru showed a lack of correlation of *Giardia* infection with diarrhea or growth stunting (249). Subsequent studies in other areas where *Giardia* is highly endemic have confirmed the lack of acute symptoms (250), with some even finding that *Giardia* was associated with reduced diarrhea (183, 251), resulting in the suggestion that *Giardia* might be protective. Despite the general absence of diarrhea, some

TABLE 4 Signs and symptoms of giardiasis

Symptom	% Reported in reference:							Median %
	Brodsky et al. (335) (*N* = 324)	Moore et al. (336) (*N* = 56)	Walzer et al. (176) (*N* = 32)	Kent et al. (337) (*N* = 240)	Shaw et al. (338) (*N* = 183)	Osterholm et al. (339) (*N* = 29)	Steen and Damsgaard (340) (*N* = 200)	
Diarrhea or loose stools	96	93	72	98	92	100	95	95
Malaise or weakness	72	80	88	86	20	97		83
Foul-smelling stools	57	75				79		75
Abdominal cramps	61	77	59	85	70	83	53	70
Wt loss	62	73		69	13	59	6	60
Nausea	60	59	59	74	58		59	59
Decreased appetite		60	56		2			56
Greasy stools		55	52					54
Bloating or distension	42	62	34	75	9	79	34	42
Flatulence	35		30	89	6			32
Vomiting	29		34	36	23	17		29
Belching	26		30					28
Fever	17		17	15	28	21		17
Constipation		9			1			5

studies of giardiasis in children have found evidence of malnutrition and/or growth stunting (252–255), while others have not (249, 256). The multicenter MAL-ED study prospectively followed 2,089 children to determine the impact of giardiasis (257), and in this study, only one site in Pakistan showed a negative association of giardiasis and diarrhea. In that one site, the apparent association mostly resolved when controlling for the use of metronidazole shortly before the diagnosis of giardiasis. In contrast, there was evidence across the sites for malnutrition, including growth stunting and an increased lactulose:mannose fecal excretion ratio suggestive of malabsorption. Higher zinc and vitamin A levels were associated with reduced incidence of subsequent giardiasis, indicating that reduced levels of these micronutrients were a risk factor for subsequent giardiasis. Whether the levels were also reduced as a result of giardiasis was not answered. In the same study, *Giardia* infection was not associated with elevated fecal inflammatory markers, in contrast to increased levels with other pathogens, including *Campylobacter* and enteroaggregative *E. coli* (258). Quantitative PCR was done in 1,469 of the children during a 2-year follow-up period, and *Giardia*, along with *Shigella*, *E. coli*, and *Campylobacter*, was associated with a decreased growth rate (259). *Giardia* infection was specifically associated with malnutrition in a cohort of children between 6 months and 2 years of age from Bangladesh who were selected based on impaired growth (260). In summary, the cumulative evidence suggests that *Giardia* infection is associated with growth retardation and other measures of malnutrition, even in children with no overt symptoms.

Postinfectious IBS is common after infection with a variety of gastrointestinal pathogens (261), but until recently, little attention was given to IBS following giardiasis. The occurrence of a large outbreak of symptomatic giardiasis in Bergen, Norway, provided an opportunity to prospectively follow these patients to determine long-term sequelae. In the fall and winter of 2004, an outbreak of giardiasis included over 1,500 patients with laboratory-diagnosed giardiasis (179, 180) and a total of 2,500 who were treated for giardiasis. These patients were followed long term for sequelae, including IBS and chronic fatigue syndrome (CFS). A case-control study of 817 of these patients found that 3 years later, 46% had IBS by Rome III criteria compared to 14% of controls (262), and 46% had CFS compared to 12% of controls. Even 6 years after the exposure, 39% still had IBS and 31% had CFS. In comparison, a U.S. study based on insurance information found an IBS rate of 3.8% after giardiasis compared to 0.4% for controls for an adjusted hazard ratio of 3.9 (263), and a study of U.S. military personnel showed a 2.1-fold increased risk (264). Thus, symptomatic giardiasis connotes a risk for IBS and CFS as sequelae, which may be higher than that reported for other gastrointestinal pathogens (265).

A risk for postinfectious joint pain has also been suggested on the basis of U.S. insurance data (odds ratio, 1.5) (266). Other sequelae, such as urticaria (267), have been reported

TABLE 5 Commercially available tests for fecal diagnosis of giardiasis[a]

Manufacturer	Test name	Antigen detected	Method
Meridian	ImmunoCard Stat *Giardia*/*Cryptosporidium*	Not stated	Immunochromatographic assay
Meridian	Merifluor *Giardia*/*Cryptosporidium*	Not stated	Direct fluorescent antibody
TechLab	Pt5012-*Giardia* II	CWP1	ELISA[b]
TechLab	Tri-Combo parasite screen		Enzyme immunoassay
Biosite	Triage parasite panel for *Giardia*, *Entamoeba histolytica*, and *Cryptosporidium*		Enzyme immunoassay
Becton Dickinson	ColorPAC *Giardia*/Crypto	CWP1	Immunochromatographic assay
Remel	X/pect *Giardia* or *Giardia*/Crypto	Not stated	Rapid immunoassay
Luminex	xTAG GPP		Multiplex PCR
Becton Dickinson	BD Max		Multiplex PCR
bioMérieux BioFire	FilmArray gastroenteritis panel		Multiplex PCR
Savyon	NanoCHIP GIP		Multiplex PCR
Genetic Signatures	EasyScreen parasite detection		Multiplex PCR
Roche	LightMix modular assay gastro parasites		Multiplex PCR

[a]Reproduced from reference 347 with permission of Elsevier. Numerous antigen-based and nucleic acid-based tests are on the market. The list in this table is a partial list. The major focus in nucleic acid testing has been on multiplex tests, some of which are limited to parasites and others of which include parasites, bacteria, and viruses (341–344).

[b]ELISA, enzyme-limited immunosorbent assay.

rarely, primarily at a case report level or on the basis of finding an increased frequency of *Giardia* organisms in the stool, so causality and level of risk are more difficult to determine.

DIAGNOSIS

The diagnosis of giardiasis should be considered in a patient who presents with subacute or chronic diarrhea, especially when accompanied by the typical symptoms (Table 4). The "gold standard" for clinical diagnosis consists of microscopy of a fecal sample. Ideally, this should consist of a wet preparation of a fresh specimen for detection of trophozoites and a fixed specimen for detection of cysts or trophozoites. However, one limitation of microscopic identification is the dependence on expertise of the microscopist, since there may be artifacts in fecal specimens that can be confused with *Giardia*. Immunologic detection of stool antigen has a sensitivity similar to that of microscopy, as well as good specificity, and it is less labor-intensive, so in some settings, it is a good substitute for microscopy. There are also numerous nucleic acid detection tests that are now on the market, including many that are multiplex (Table 5). Some of the multiplex tests are specific to parasitic agents, while others cover the entire range of fecal pathogens (268–270). These tests are highly specific and are more sensitive than microscopy, but they are expensive, and availability is limited. In addition, the greater sensitivity of the test may result in diagnosing giardiasis in a greater number of asymptomatic people; whether to treat asymptomatic patients is not always clear, especially in settings where giardiasis is highly endemic. In these patients, submitting more than one stool for ova and parasite microscopy is discouraged (270). However, there are some patients with giardiasis who present with prolonged diarrhea and malabsorption for whom microscopy or other tests are negative. For these patients, the traditional gold standard has been performance of microscopy on three fecal specimens collected on different days, since cysts are intermittently detected in fecal specimens (271). However, the data suggest that PCR on a single fecal specimen is more sensitive than microscopy performed on three consecutive specimens, so in settings where PCR is available, it should be considered for these patients (272).

Endoscopic biopsy and examination of duodenal contents can be considered with prompt microscopic examination of the intestinal contents for trophozoites. An advantage of this approach is the potential of identifying other conditions leading to diarrhea and malabsorption, such as gluten enteropathy. An alternative method is the Entero-Test (string test), in which the patient swallows a capsule on the end of a string (273, 274). The string is left in place for 4 h to overnight and is then removed for microscopic examination for trophozoites or other parasitic organisms.

Serum antibody testing has been used in research settings to study the epidemiology of giardiasis, but has no role in clinical diagnosis because of limited sensitivity and

specificity. Finally, *G. duodenalis* can be cultured *in vitro*, but the process of establishing an *in vitro* culture is laborious and unpredictable and is not used in the clinical laboratory setting. The reader is referred to an extensive review of the diagnosis of gastrointestinal parasites for greater detail on these tests (270).

TREATMENT

In view of the range of clinical manifestations of *Giardia* infection from asymptomatic to prolonged diarrhea with malnutrition, the clinical challenge is to determine who should be treated for giardiasis. In settings where it is highly endemic, there is probably no value in treating giardiasis in people with no symptoms. Even though *Giardia* infection is associated with growth stunting, reinfection rates may be so high that the effectiveness of treatment in eradicating the infection is minimal (183). In addition, the long-term effect of nitroimidazoles and other antibiotics on antimicrobial resistance and alteration of the microbiota of the person being treated are not known well enough to advise treatment in this group. A stronger case for treatment may be made for asymptomatic people in a low-prevalence setting, in view of the potential of reducing transmission to others, but no data exist for this situation. In contrast, symptomatic giardiasis should normally be treated, since the duration is frequently long in the absence of treatment. In fact, a duration of diarrhea of at least 5 or 7 days is sometimes used as a cutoff for epidemiological studies of giardiasis.

The first drug that came into common use for treatment of giardiasis was quinacrine, which was considered the drug of choice for many years. It has a long half-life (5 to 14 days) and is well absorbed from the gastrointestinal tract. Clinical studies have resulted in cure rates of 77% to 100%, and it may even decrease cyst viability (27). However, there are some important disadvantages of the drug as well. It leaves a bitter taste and frequently causes vomiting and can cause toxic psychosis. In addition, it must be administered 3× daily for five to 7 days, making it difficult for some patients to complete a treatment course. Its potential role is primarily as a single agent or as part of combination therapy for treatment-resistant giardiasis, but it is no longer available in the United States.

Metronidazole was the first 5-nitroimidazole to be developed and came into widespread use in the 1960s for giardiasis and a number of other infections caused by anaerobic protozoa or bacteria (275). It is reduced to toxic intermediates in the anerobic cell and has a variety of effects, including DNA reactivity. Pyruvate-ferredoxin oxidoreductase (PFOR) (276) and thioredoxin reductase (TrxR) (277–279) have been proposed as contributing to its activity, but there are likely to be other contributing pathways as well (275). Because of the DNA reactivity, metronidazole has been considered to possibly having carcinogenic activity. However, clinical studies have found little or no correlation (280–283). The drug is not teratogenic (284) and has been used widely in pregnancy after the first trimester. Metronidazole, is now, along with other nitroimidazoles, the drug of choice for giardiasis, although, ironically, it has never been given FDA approval for that indication. Metronidazole is 100% absorbed orally and is even absorbed fairly well from mucosal surfaces, including oral, vaginal, and rectal mucosa. A small study demonstrated the efficacy of rectally administered metronidazole for treating giardiasis in children (285). Because of its efficient absorption, levels in the intestinal lumen are relatively lower than those for drugs that are less efficiently absorbed, but whether that affects clinical activity is not known.

Metronidazole is given for a period of 5 to 10 days, and numerous studies have demonstrated high efficacy for treatment of giardiasis (see File S1 in the supplemental material) (286, 287). However, the required duration of treatment sometimes limits compliance and tolerability, so single-dose treatment has also been evaluated, but with poor results. Newer 5-nitroimidazoles have been developed, including tinidazole, which is a derivative of metronidazole and has been used in Europe since 1969, but it was not approved by the FDA until 2004 (288). Secnidazole was approved in 2017, but not for treatment of giardiasis, and ornidazole has not yet been approved. These drugs

have longer half-lives than metronidazole and have all been evaluated for single dose use (File S1) (286). Tinidazole has been studied the most extensively, and a review of the literature that included 60 randomized controlled trials (RCTs) concluded that tinidazole is more effective than metronidazole or albendazole (286), so it may be considered the drug of choice.

The benzimidazoles are broad-spectrum antihelminthics that bind to beta-tubulin and cause irreversible damage to the parasite (289). Mebendazole has been studied, but with generally disappointing results. However, albendazole has demonstrated excellent efficacy when given as a 5-day course. Oral absorption is 1% to 5%, and it is metabolized to the active drug albendazole sulfoxide (290). Some studies, including a review with meta-analyses (291), have suggested that it is better tolerated than the nitroimidazoles (Table 6). However, albendazole is known to be embryotoxic in animals and must be avoided during pregnancy.

Nitazoxanide is a nitroheterocyclic compound that is notable as the only effective drug for treatment of *Cryptosporidium* spp., and it is also effective for giardiasis (292). After absorption from the gastrointestinal tract, it is rapidly converted to the active metabolite tizoxanide meaning that nitazoxanide is not detected in the serum. It has been proposed to work as a noncompetitive of PFOR (293, 294). When given as a 3-day course, it is well tolerated, but the average efficacy of 71% is significantly lower than those for the nitroimidazoles or albendazole (286).

Paromomycin is a nonabsorbed aminoglycoside that is moderately effective and is sometimes used in the first trimester of pregnancy because of lack of adequate data for the safety of nitroimidazoles in the first trimester (295). However, paromomycin is less effective, and in someone with malnutrition/weight loss, the more effective treatment should be given even during the first trimester. The antimalarial chloroquine has also been studied in children, and its efficacy is comparable to that of the nitroimidazoles (296, 297).

Many of these drugs have been compared in various combinations in children and/or adults and in symptomatic or asymptomatic individuals (File S1). A number of meta-analyses or other reviews have included many of these studies (286, 287, 298–300).

Clinical treatment failure accompanied by microscopic evidence for persistence of *Giardia* is occasionally seen following treatment of symptomatic giardiasis (301). These observations have raised the question of drug resistance, particularly as it related to the nitroimidazoles. Indeed, a number of studies have demonstrated trophozoites with *in vitro* resistance to metronidazole (302). However, documentation of microbiologically resistant isolates requires isolation from the host with a clinically resistant infection, followed by demonstration of *in vitro* resistance, which is difficult to do because of the challenges of adapting the organisms to *in vitro* culture. In addition, no genetic markers of resistance have been identified. Thus, it is more accurate to refer to treatment-refractory cases than to resistance. However, there are a few clinical correlation studies to suggest that true resistance is found for the nitroimidazoles (298). The increase in treatment failure from 15% in 2008 to 40% in 2013 for travelers returning to England suggests a true development of resistance (303), as does the higher frequency in travelers to Asia than to other areas (298). It is not yet known how many of these treatment failures are due to true resistance. Combination therapy with a nitroimidazole plus quinacrine (304) or a nitroimidazole plus albendazole (305) results in a high success rate in patients with treatment failure. In addition, quinacrine alone may be effective (298, 306).

There are also other potential reasons for the persistence or recurrence of clinical symptoms in the absence of resistance. This may be due to postinfectious IBS or to the existence of a second diagnosis, especially when the symptoms are not typical of giardiasis. Thus, when a patient has suspected treatment failure, it is critical that the presence of parasites be documented. If parasites are absent, these other possibilities must be considered. Retreatment with antimicrobial therapy is not indicated when organisms are not found on the follow-up fecal examinations (307).

TABLE 6 Drugs for treatment of giardiasis[a]

Class	Drug	Oral bioavailability (% if known)	Half life[c]	Dose (adult and max pediatric dose) and duration	Availability in United States[b]	FDA approved for giardiasis[b]	Common side effects	Concerns
Nitroimidazole	Metronidazole	100	6–10 h	5 mg/kg t.i.d. (250 mg), 5–10 days	Y	N	Metallic taste, nausea, anorexia	DNA mutagenesis and possibility of carcinogenesis
	Tinidazole	100	12 h	50 mg/kg (2 g), single dose	Y	Y	Metallic taste, nausea, anorexia	DNA mutagenesis and possibility of carcinogenesis
	Ornidazole	100	11–14 h	40 mg/kg (2 g), single dose	N	N	Metallic taste, nausea, anorexia	DNA mutagenesis and possibility of carcinogenesis
	Secnidazole	100	14–20 h	30 mg/kg (1.5 g), single dose	Y	N	Metallic taste, nausea, anorexia	DNA mutagenesis and possibility of carcinogenesis
Benzimidazole	Albendazole	1–5	8–12 h	15 mg/kg q.i.d. (400 mg), 5 days	Y	N		Embryotoxicity
Aminoglycoside	Paromomycin	0	NA	10 mg/kg t.i.d. (500 mg), 5–10 days	Y	N		
Nitrofuran	Furazolidone		10 min	2 mg/kg q.i.d. (100 mg), 7–10 days	N[d]	Y		Adverse effect on reproduction, mutagenesis
Others	Nitazoxanide	Parent drug not detected	1.3–1.8 h	500 mg b.i.d. (adult), 100 mg b.i.d. (ages 1–3), 200 mg b.i.d. (ages 4–11), 3 days	Y	Y		
	Quinacrine	Good	5–14 days	2 mg/kg t.i.d. (100 mg), 5–7 days	N[d]	Y	Nausea and vomiting	Toxic psychosis, common nausea and vomiting

[a]Reproduced from reference 345.
[b]Y, yes; N, no.
[c]NA (not applicable).
[d]No longer generally available in the United States.
[e]t.i.d., three times a day; q.i.d., four times a day; b.i.d., twice a day.

TABLE 7 Axenic growth medium for *G. duodenalis*[a]

Component	Quantity per liter	Final concn
K_2HPO_4-H_2O	1 g	4.4 mM
KH_2PO_4	600 mg	4.4 mM
Trypticase (casein peptone)	20 g	
Yeast extract	10 g	
D-Glucose	10 g	56 mM
NaCl	2 g	34 mM
Cysteine-HCl	2 g	16.5 mM
Ascorbic acid	200 mg	1.1 mM
Ferric NH_4 citrate (Sigma F-5879)	22.8 mg	
Bovine bile (Sigma B-8381)-NaOH	40 ml of 6.5% solution to bring pH to 7.0–7.2 (1.65 ml of 10 M solution)	
Serum (bovine or fetal calf)	100 ml	10%

[a]Reproduced from reference 1. Ingredients in the formula of TYI-S-33 (309), the most commonly used medium for growth of *G. duodenalis* trophozoites. The ferric NH_4 citrate and bovine bile are generally kept as solutions at 4°C. All ingredients except the serum are dissolved in water, brought to 200 ml, sterilized with a 0.22- or 0.45-μm filter, and added to 700 ml sterilized water. Heat-inactivated (56°C for 20 min) serum is then added. During *in vitro* growth, the organisms are grown in sealed glass containers filled nearly full with medium. When this is impossible, such as during cloning by limiting dilution in 96-well plates (346), a sealed bag containing an anaerobic generator is used. At 4°C, the shelf life is limited to 5 to 7 days, primarily because of the degradation of the cysteine.

TOOLS FOR STUDYING *GIARDIA*

A number of tools are now available for studying *Giardia*, many of which are summarized by Jex et al. (308). The advance that has made the single biggest impact on the study of *G. duodenalis* has been the development of an axenic (without organisms other than *Giardia*) *in vitro* culture system (309) (Table 7). The medium described in that report is still used today, frequently with minor modifications, such as the use of fetal calf serum in place of bovine serum if the study requires minimalization of potentially confounding antibodies.

The development of *in vitro* excystation (30, 310) and encystation (35–37) have made it possible to study the life cycle of *Giardia*.

The development of genomics and proteomics databases began with the initial *Giardia* genome project of the WB isolate (genotype A), reported in 2007 (4), and was followed by reports of the GS isolate (genotype B) (16, 17). These are found at the *Giardia*DB web site (https://Giardiadb.org/) and have provided the foundation for proteome analyses and databases as well (70, 311, 312).

A variety of tools for alteration of gene expression have been developed, beginning with transient transfection using a luciferase reporter (313). This was followed by stable transfection of a plasmid containing a gene for puromycin acetyltransferase (115) and using a bacterial neomycin phosphotransferase gene (*neo*) gene (314). Interestingly, the plasmids were maintained episomally in the WB isolate (genotype A), but in the GS isolate (genotype B), linear or circular DNA was integrated by recombination. Subsequent studies have used a variety of modifications to this initial approach. One of the limitations of transfection systems is that knockouts cannot easily be obtained, since the trophozoites are tetraploid. Thus, RNAi has also been used to study antigenic variation (164) and has been used for gene silencing to search for new drug targets (315). CRISPR interference (CRISPRi) (316) and CRISPR/Cas9 (317) have also been developed for stable repression of gene expression.

Advances in microscopic techniques that range from electron microscopy to fluorescence/confocal microscopy have included functional components, as well as improved preparation techniques, and are contributing greatly to improved understanding of the cytogenetic and cytoskeletal aspects of the organism. Numerous animal models have been developed, primarily with mice or other rodents. Some have used *G. duodenalis*, while others have used *G. muris*. Many of these are discussed in the sections on immune response and malnutrition and long-term sequelae.

These new tools for investigation of the biology of *Giardia* and pathogenesis of infections caused by this organism will contribute to ever accelerating advances in understanding of this organism and its impact on its host.

CONCLUSIONS

G. duodenalis as an organism caught immense interest when it was considered to be one of the earliest-diverging eukaryotes. With the finding that it actually has a minimalized genome with minimization of many canonical eukaryote functions, the emphasis has switched to understanding how that adaptation works for an organism that is an obligate intestinal parasite. As such, the organism has the ability to persist in the host intestine in the absence of tissue invasion and despite the mounting of a host immune response. This is especially remarkable in view of the requirement for trophozoites to replicate while their ventral disk adheres to the intestinal wall. The spectrum of illness caused during human infection with *G. duodenalis* ranges from asymptomatic to prolonged diarrhea and weight loss. Ongoing studies of pathogenesis and the immune response are beginning to shed light on the causes of these differences and will ultimately translate into more accurate guidance regarding appropriate therapy in various settings.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

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