H Protozoal Diseases

271 Introduction to Protozoal Diseases

Poonum S. Korpe, Jonathan I. Ravdin, and William A. Petri, Jr.

The protozoans known to infect humans are a diverse group, as indicated by phylogeny (Table 271.1), epidemiology (Table 271.2), clinical manifestations (Table 271.3), preferred diagnostic studies (Table 271.4), and chemotherapeutic agents effective in eradicating or arresting infection (see Chapter 42).[1,2] The phylum Protozoa is composed of morphologically simple eukaryotic organisms. Protozoa may be divided, for convenience, into four distinct groups based on method of locomotion: Mastigophora (flagella), Sarcodina (pseudopodia), Apicomplexa (microtubule complex, commonly referred to as sporozoa), and Ciliophora (ciliates) (see Table 271.1). Protozoans such as *Plasmodium* spp., *Entamoeba histolytica,*

TABLE 271.1 Classification of Protozoans That Infect Humans

Phylum I. Sarcomastigophora (flagella, pseudopodia)
- Subphylum I. Mastigophora (flagella)
  - Class 2. Zoomastigophorea
    - Order 2. Kinetoplastida
      - Suborder 2. Trypanosomatina
        - *Leishmania, Trypanosoma*
    - Order 5. Diplomonadida
      - Suborder 2. Diplomonadina
        - *Giardia*
    - Order 7. Trichomonadida
      - *Dientamoeba, Trichomonas*
- Subphylum III. Sarcodina (pseudopodia)
  - Superclass 1. Rhizopoda
    - Class 1. Lobosea
      - Subclass 1. Gymnamoebia
        - Order 1. Amoebida
          - Suborder 1. Tubulina
            - *Entamoeba*
          - Suborder 5. Acanthopodina
            - *Acanthamoeba*
        - Order 2. Schizopyrenida
          - *Naegleria*

Phylum III. Apicomplexa (apical microtubule complex)
- Class 2. Sporozoea
  - Subclass 2. Coccidia
    - Order 1. Piroplasmida
      - *Babesia*
    - Order 3. Eucoccidia
      - Suborder 2. Eimeriina
        - *Cryptosporidium, Cystoisospora, Cyclospora, Sarcocystis, Toxoplasma*
      - Suborder 3. Haemosporina
        - *Plasmodium*
      - Suborder 3. Piroplasmia

Phylum VII. Ciliophora (ciliated)
- Class 1. Kinetofragminophorea
  - Subclass 2. Vestibuliferia
    - Order 1. Trichostomatida
      - Suborder 1. Trichostomatina
        - *Neobalantidium (Balantidium)*

Data from Committee on Systematics and Evolution of the Society of Protozoologists. A newly revised classification of the protozoa. J Protozool. *1980;27:37–58. For an updated phylogeny of Sarcodina, see Pawlowski J, Burki F.J. Untangling the phylogeny of amoeboid protists.* Eukaryot Microbiol. *2009;56:16–25.*

TABLE 271.2 Geographic Distribution and Mechanism of Transmission of Protozoal Infections

ORGANISM	GEOGRAPHIC DISTRIBUTION	MEANS OF TRANSMISSION
Acanthamoeba spp.	Undefined	Water
Babesia spp.	North America, Europe	Tick-borne, blood transfusions
Neobalantidium (Balantidium) coli	Worldwide	Zoonosis (pigs), water,[a] fecal-oral
Blastocystis species *(hominis)*	Unknown	Fecal-oral, water
Cryptosporidium spp.	Worldwide	Water, fecal-oral, zoonosis
Cystoisospora	Worldwide	Fecal-oral, suspected zoonosis
Entamoeba histolytica	Worldwide	Water, fecal-oral, foodborne
Giardia duodenalis (lamblia)	Worldwide	Water, fecal-oral, foodborne
Leishmania spp.[b]		Female sand fly, blood transfusion[16]
L. donovani	India, Pakistan, East Africa, China	
L. tropica	Middle East, Central Asia	
L. major	Middle East, India, Pakistan	
L. aethiopica	Ethiopia, Kenya	
L. mexicana	Central America, Texas	
L. amazonensis	South America	
L. chagasi	Latin America	
L. viannia braziliensis	Latin America	
Naegleria spp.	Worldwide	Fresh water, intranasal exposure
Plasmodium spp.	Africa, Asia, South and Central America, Oceania	Female anopheline mosquito, inoculation of infected blood
Sarcocystis spp.	Unknown	Foodborne (meat)
Toxoplasma gondii	Worldwide	Zoonosis (cats), foodborne (meat), blood or organ transplant, congenital
Trichomonas vaginalis	Worldwide	Venereal, during birth (?); nonvenereal, sexually transmitted
Trypanosoma spp.		
T. cruzi	South and Central America	Reduviid bugs
T. brucei gambiense	West Africa	Tsetse fly
T. brucei rhodesiense	East Africa	Tsetse fly

[a]Ingestion of water contaminated with fecal material.
[b]Other *Leishmania* spp. also infect humans but are less common.

TABLE 271.3 Clinical Syndromes Caused by Protozoan Infection

ORGANISM (DISEASE)	MAJOR CLINICAL SYNDROME
Acanthamoeba spp.	Keratitis, granulomatous amebic encephalitis
Babesia spp. (babesiosis)	Fever, malaise, hepatosplenomegaly, and hemolytic anemia, especially in the asplenic
Neobalantidium (Balantidium) coli (balantidiosis)	Colitis
Blastocystis species *(hominis)* (blastocystosis)	Diarrhea
Cryptosporidium spp. (cryptosporidiosis)	Self-limiting noninflammatory diarrhea, chronic severe diarrhea in children and immunocompromised adults, and cholangitis in AIDS patients[17]
Cystoisospora spp. (cystoisosporiasis)	Diarrhea
Dientamoeba fragilis	Diarrhea
Entamoeba histolytica (amebiasis)	Diarrhea, colitis, liver abscess
Giardia duodenalis (lamblia) (giardiasis)	Noninflammatory diarrhea with malabsorption
Leishmania spp. (cutaneous and visceral leishmaniasis)	Cutaneous or mucosal ulceration, visceral disease with fever, hepatosplenomegaly
Leptomyxida (Balamuthia mandrillaris)	Granulomatous amebic encephalitis
Naegleria spp.	Meningoencephalitis
Plasmodium spp. (malaria)	Paroxysmal fever, chills, headache, hepatosplenomegaly
Sarcocystis spp.	Myositis, fever
Toxoplasma gondii (toxoplasmosis)	Fever, malaise, lymphadenopathy; chorioretinitis; congenital abnormalities; in immunocompromised host, encephalitis, myocarditis, pneumonitis
Trichomonas vaginalis (trichomoniasis)	Vaginitis, urethritis
Trypanosoma spp. (African sleeping sickness and Chagas disease)	Fever, lymphadenopathy, meningoencephalitis, myocarditis; megaesophagus and megacolon; congestive cardiopathy

AIDS, Acquired immunodeficiency syndrome.

TABLE 271.4 Diagnostic Tests for Protozoal Diseases

DISEASE	PREFERRED DIAGNOSTIC TESTS
Amebiasis	
Intestinal	Stool antigen or PCR, serologic tests, colonoscopy
Liver	Ultrasonography or computed tomography, serologic tests, PCR on liver abscess aspiration
Amebic keratitis	Corneal scraping for microscopy and culture
Babesiosis	Giemsa or Wright staining of thin smear, PCR, serology[18,19]
Cryptosporidiosis	Stool antigen or PCR or acid-fast and auramine-rhodamine staining of fecal samples
Giardiasis	Stool antigen or PCR
Granulomatous amebic encephalitis	Brain biopsy
Leishmaniasis	
Cutaneous and mucocutaneous	PCR, antigen detection, biopsy, touch preparation, culture, serologic tests
Visceral	PCR, antigen detection, bone marrow or splenic aspiration, touch preparation, culture, serologic tests, lymph node biopsy
Malaria	Wright or Giemsa stain of thin and thick blood smear, antigen detection or PCR
Primary amebic meningitis	Cerebrospinal fluid examination, wet mount of CSF for amebic trophozoites, CSF PCR,[20] culture for amebas
Toxoplasmosis	Serologic tests, PCR, Wright-Giemsa stain of tissue, antigen detection
Trichomoniasis	Microscopy, culture, PCR, or antigen detection in genital secretions
Trypanosomiasis	
Chagas disease	Fresh blood or Giemsa-stained smear, PCR, xenodiagnosis; serologic tests for chronic disease
African sleeping sickness	Giemsa-stained blood smear, PCR, serologic tests, CSF examination[21]

CSF, Cerebrospinal fluid; *PCR,* polymerase chain reaction.

Trypanosoma spp., and *Leishmania* spp. are major worldwide pathogens and are among the leading causes of morbidity and mortality in areas of Africa, Asia, and Central and South America. *Giardia lamblia* and *Cryptosporidium* are frequent causes of diarrhea in developing areas and established industrialized countries. *Toxoplasma gondii, Cryptosporidium* spp., *Cyclospora, Cystoisospora belli, Trypanosoma cruzi*, and *Leishmania* spp. all have been noted to cause severe diseases in patients with acquired immunodeficiency syndrome.

Important advancements in the past several years include the first vaccine for a parasite to show some degree of effectiveness in humans, the RTS,S circumsporozoite-based vaccine for malaria; radical changes in parasite diagnostic techniques as microscopy is replaced with more accurate and sensitive techniques (e.g., antigen detection and the polymerase chain reaction assay); and improved understanding of pathogenesis as host, parasite, and environmental factors influencing disease are unraveled. Limited therapeutic options have led to a drive for novel drug discovery. Finding new uses for old drugs, that is, drug repurposing, has shown promising results for treating ulcerative leishmaniasis, second-stage trypanosomiasis, primary amebic meningoencephalitis, and enteric protozoa.[3–9]

Advances even in taxonomy of the protozoan parasites have been profound. A fifth species of malaria, *Plasmodium knowlesi,* is now known to infect humans, as well as a fourth member of the *Entamoeba histolytica-dispar* complex, *E. bangladeshi.*[10–12] Cryptosporidia that infect humans are now recognized to include not only *Cryptosporidium parvum* but also *Cryptosporidium hominis* and additional zoonotic species.[13] Within species, differentiation of genotypes is proving to be important for organisms such as *Giardia, Blastocystis,* and *Toxoplasma,* where it is becoming clear that genotypes differ in their pathogenicity for humans.[13–15]

The key to the recognition of protozoal infection is a knowledge of epidemiologic risk factors, such as the parasites' geographic distribution (see Table 271.2), and the most common modes of clinical presentation (see Table 271.3). The clinical diagnosis of protozoal infection presenting outside normal areas of high prevalence is usually dependent on physicians considering this possibility in their differential diagnosis. Given present levels of travel, changing immigration patterns, and the immunosuppressive effects of infection with human immunodeficiency virus, all clinicians need to have a heightened awareness of diseases caused by the protozoans. Diagnosis and therapy often require a specialized expertise with the use of tests (see Table 271.4) or drugs with which most physicians lack experience. Infectious disease consultants will frequently be called on to diagnose and manage protozoal infection; this requires the maintenance of an updated, in-depth database as provided by the chapters in this section.

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272 *Entamoeba* Species, Including Amebic Colitis and Liver Abscess

William A. Petri, Jr., Rashidul Haque, and Shannon N. Moonah

SHORT VIEW SUMMARY

Definition

- Amebiasis is defined as human infection by *Entamoeba* species, including *E. histolytica,* which is the cause of amebic colitis, liver abscess, and, rarely, brain abscess; *E. moshkovskii,* which causes diarrhea; and *E. bangladeshi,* and *E. dispar,* which are nonpathogenic.

Epidemiology

- Amebiasis is spread by fecal-oral transmission from person to person, and is also waterborne and foodborne spread.
- Most infections are in impoverished communities in the developing world.
- Amebic colonization, diarrhea, and colitis are most common in infants and children through the preschool years.
- Amebic liver and brain abscesses occur 90% of the time in young men.
- *E. histolytica* is a common cause of diarrhea in returning international travelers.
- Men who have sex with men (MSM) are at risk of both human immunodeficiency virus (HIV) infection and amebiasis, and one should consider the possibility of HIV in the setting of amebiasis in MSM.

Microbiology

- Cyst form is infectious, environmentally stable, and resistant to chlorination.
- Trophozoite is the tissue-invasive stage.

Diagnosis

- Stool ova and parasite examination should not be used because it is insensitive and nonspecific. Instead, diagnosis is best accomplished in the laboratory through fecal antigen detection or quantitative polymerase chain reaction, in combination with serologic tests for antiamebic antibodies (which can be negative early in illness).
- Colonoscopy and abdominal imaging techniques (ultrasound, computed tomography, and magnetic resonance imaging) are useful adjuncts for diagnosis of intestinal and extraintestinal disease, respectively.

Therapy (See Table 272.2)

- Noninvasive infection is treated with paromomycin: 30 mg/kg/day orally in three divided doses per day for 5 to 10 days.
- Invasive infection is treated with tinidazole (2 g once daily for 5 days) or metronidazole (750 mg three times daily by mouth for 10 days), followed by paromomycin (to prevent relapse from gut lumen parasites).
- Consider percutaneous drainage for liver abscesses of 5 to 10 cm or greater in diameter or if they are in the left lobe.

Prevention

- Prevention is accomplished by sanitation and clean water.
- Vaccine is in preclinical development.

Entamoeba histolytica is an invasive enteric protozoan parasite that is the cause of amebiasis.[1] *Entamoeba histolytica* is morphologically indistinguishable from three other species of human intestinal amebae, *Entamoeba dispar, Entamoeba moshkovskii,* and the more recently described *Entamoeba bangladeshi. E. dispar* and *E. bangladeshi* are nonpathogenic, while *E. moshkovskii* causes noninvasive diarrhea.[2–6] Because *E. histolytica, E. dispar, E. moshkovskii,* and *E. bangladeshi* cannot be distinguished by a stool ova and parasite (O&P) test, which has been the traditional diagnostic method, it is important clinically to use *E. histolytica*–specific diagnostic tests (stool antigen detection or polymerase chain reaction [PCR]) whenever possible, especially since *E. dispar* and *E. moshkovskii* are often as prevalent as *E. histolytica* in many regions of the world.[7–10]

Entamoeba spp. are taxonomically within the subphylum Sarcodina, class Lobosea, and family Entamoebidae.[11] Revisions of this taxonomy have been proposed.[11a] At least seven additional species of amebae (*Entamoeba coli, Entamoeba hartmanni, Entamoeba polecki, Entamoeba chattoni, Dientamoeba fragilis, Iodamoeba bütschlii,* and *Endolimax nana*) infect the human intestine.[11–19] However, these are generally accepted as commensal organisms, although *E. polecki, D. fragilis,* and *I. bütschlii* have occasionally been implicated as causes of diarrhea.[14–16]

Hippocrates (460–377 BC) wrote that "Dysentery, if it commence with black bile, is mortal," and the Old Testament and Huang Ti's *Classic of Internal Medicine* (140–87 BC) also made reference to dysentery. In 1828, James Annesley may have made the first association of dysentery to liver abscess when he wrote in *Prevalent Diseases of India* that "hepatic disease seems to be induced by the disorder of the bowels, more particularly when this disorder is of a subacute or chronic kind." Approximately 3 decades later, in 1855, Vilém Lambl described amebae in the stool of a child who had diarrhea.[20] In 1875, Fedor Lösch described ameba in the stool of a young farmer with chronic dysentery that resulted in death. He described the amebae as "round, pear shaped or irregular form and which are in a state of almost continuous motion." Autopsy studies revealed ulcerations of the colon, and Robert Koch's postulates were met when the patient's stool inoculated orally and rectally into a dog caused dysentery with amebic ulcers.[21] Stephen Kartulis is credited with first demonstrating amebae in liver and brain abscesses in the 1880s.[22,23]

The first North American case of amebiasis was reported in 1890 by Sir William Osler: "Dr. B., age 29, resident in Panama for nearly six years, where he had had several attacks of dysentery, or more correctly speaking a chronic dysentery, came north in May, 1889." Subsequently in 1890 the patient developed tenderness and hepatosplenomegaly, and amebae were observed in the stool and abscess fluid: "The general character of the amoeba [found in the stool] correspond in every particular with those found in the liver." A year later, Osler's colleagues William Councilman and Henri Lafleur proceeded through a classic investigation of 14 cases of amebic dysentery to distinguish amebiasis from bacterial dysentery, and they coined the terms *amebic dysentery* and *amebic liver* abscess.[23]

Ipecac bark was used in the treatment of dysentery for centuries in Peru. Piso introduced ipecac bark to Europe in 1658. Helvetius used ipecac to successfully treat the dysentery of King Louis XIV and subsequently sold it as a secret remedy to the French government. Not until 1858 was the use of large doses of ipecac for the treatment of dysentery promoted by the surgeon E. S. Docker in Mauritius. He demonstrated that ipecac (60 grains two to three times a day) decreased mortality from as much as 18% to only 2%. However, large doses of ipecac by mouth were complicated by severe nausea and vomiting and necessitated the coadministration of opium, chloral hydrate, or tannic acid. An alternative therapy was discovered by Leonard Rogers in India, who found that emetine, the principal alkaloid in ipecac, killed amebae

in the mucus of stools from patients with dysentery at dilutions as high as 1:100,000. In 1912 he reported successfully treating three patients in Calcutta, who had been unable to tolerate oral ipecac, by injection of emetine.[24]

The life cycle of *E. histolytica* was described by Dobell.[25] The cyst form of *E. histolytica* was implicated as the infective form of the parasite by Walker and Sellards[26] in the Philippines in 1913, and the parasite's life cycle was outlined by Dobell in 1925. Brumpt[27] proposed that *E. histolytica* and *E. dispar* were identical morphologically, but only *E. histolytica* was pathogenic for humans. Axenic culture of *E. histolytica* (free of any associated microorganisms) was accomplished by Diamond[28] at the National Institutes of Health in 1961. In 1978, Sargeaunt and colleagues[2] wrote that *E. histolytica* and *E. dispar* species could be differentiated using zymodeme analysis, and in 1989, Tannich and associates[3] demonstrated that their DNA genomes were distinct.

ORGANISM

Species of *Entamoeba*

Many *Entamoeba* species infect humans, but of greatest significance because of their prevalence are the four morphologically identical amebae *E. histolytica, E. dispar, E. bangladeshi,* and *E. moshkovskii.* Only *E. histolytica* is a cause of invasive amebiasis, whereas *E. moshkovskii* is associated with a noninvasive diarrhea and other two are nonpathogenic.[1,4–6] All of these species are in the quadrinucleated cyst clade of *Entamoeba.* The *E. histolytica* and *E. dispar* genomes share 90% identity in genic regions, and *E. moshkovskii* is also closely genetically related.[29] In most industrialized countries, *E. dispar* is 10 times more common than *E. histolytica,*[1,30–34] and *E. histolytica* and *E. dispar* can be equally prevalent even in a developing country.[18] The presence of ingested erythrocytes was the sole morphologic characteristic of some usefulness in identifying *E. histolytica,* but in one study, this characteristic was present in only 68% of cases of *E. histolytica* but also in 16% of cases of *E. dispar.*[18] *E. moshkovskii* is also prevalent and geographically widely distributed.[5,6,29,35–37] In preschool children from an urban slum, *E. moshkovskii* was present in 21%, *E. histolytica* in 16%, and *E. dispar* in 36%.[6,30] In another study in Tanzania, among approximately 100 human immunodeficiency virus (HIV)–infected individuals with diarrhea, *E. histolytica* was present in 4%, *E. moshkovskii* in 13%, and *E. dispar* in 5%.[36] In Sydney, Australia, 50% of cases of *Entamoeba* identified by stool O&P examination were *E. moshkovskii.*[37] *E. moshkovskii* has recently been shown to be a cause of diarrhea.[5]

E. hartmanni is also in the quadrinucleated cyst clade, but it is smaller than *E. histolytica,* having trophozoites of 3 to 12 µm in diameter and cysts of 10 µm in diameter, whereas *E. histolytica* trophozoites are 12 to 60 µm in diameter, and cysts are 10 to 20 µm in diameter. *E. coli* cysts have up to eight nuclei, with trophozoites of size similar to those of *E. histolytica. Entamoeba gingivalis* does not form a cyst and is not an inhabitant of the intestine; instead, it is observed in the mouth in gingival scrapings. Human infection with the uninucleated cyst amebae *E. polecki, E. chattoni,* and *E. suis* is generally rare. *E. polecki* and *E. suis* infections are linked to contact with pigs, and *E. chattoni* infection is linked to contact with monkeys.[19,38,39] Other nonpathogenic amebae include *I. bütschlii,* which has characteristic glycogen vacuoles in the cysts; *E. nana,* which has a characteristic nuclear structure that lacks peripheral chromatin; and *D. fragilis,* which is more closely related to the flagellates than to the ameba with binucleate trophozoites.[12,13]

Genotypes of *Entamoeba histolytica*

In addition to the genetic differences between the three morphologically identical amebae *E. histolytica, E. dispar,* and *E. moshkovskii,*[4,5,11] genetically distinct strains or genotypes also exist within *E. histolytica.* Genotypes have been distinguished by the use of isoenzymes, polymorphisms in protein-coding DNA, and polymorphisms in short tandem repeat loci linked to transfer RNA genes (reviewed by Ali and colleagues[30]). Important findings from genotyping include the following: *E. histolytica* contains many genotypes[40–46]; patients may be infected with more than one genotype at a time[46]; and certain genotypes are associated with diarrhea, others with colonization, and others with amebic liver abscess formation.[45,46] In contrast to this high level of diversity in repetitive noncoding DNA, individual protein-encoding genes such as the galactose and *N*-acetyl-D-galactosamine (Gal/GalNAc) lectin are conserved between genotypes.[44] An additional nuance to genotypes is that in a study of patients with amebic liver abscess, comparison of the amebae in the intestine and liver demonstrated in every case that each patient had different genotypes in the two locations. This surprising result suggested that there is a genetic bottleneck between the intestine and liver, and only a subset of intestinal isolates is capable of causing extraintestinal disease.[46]

Life Cycle

The life cycle of *E. histolytica* begins with an infectious quadrinucleated cyst and continues with an invasive uninucleated trophozoite. The cyst is ingested from fecally contaminated food or water or through oral-anal sexual practices and, in the intestine, excysts to eight trophozoites (Fig. 272.1). The environmental stability of the cyst and relative resistance to chlorine has resulted in waterborne outbreaks caused by contamination of municipal water supplies.[47] In most laboratory-based studies of the cyst, the reptilian parasite *Entamoeba invadens* has been used

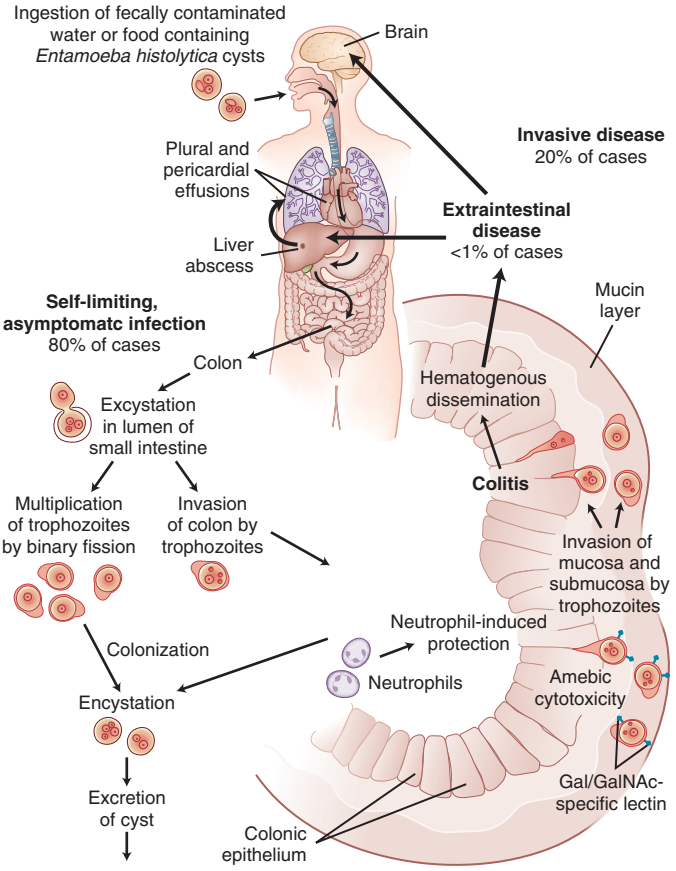

FIG. 272.1 Life cycle of *Entamoeba histolytica.* Infection is normally initiated by the ingestion of fecally contaminated water or food containing *E. histolytica* cysts. The infective cyst form of the parasite survives passage through the stomach and small intestine. Excystation occurs in the bowel lumen, where motile and potentially invasive trophozoites are formed. In most infections, the trophozoites aggregate in the intestinal mucin layer and form new cysts, which results in a self-limited and asymptomatic infection. In some cases, however, adherence to and lysis of the colonic epithelium, mediated by the galactose and *N*-acetyl-D-galactosamine (Gal/GalNAc)–specific lectin, initiates invasion of the colon by trophozoites.[104] Neutrophils responding to the invasion contribute to cellular protection at the site of invasion. Once the intestinal epithelium is invaded, extraintestinal spread to the peritoneum, liver, and other sites may follow. Factors controlling invasion, as opposed to encystation, probably include parasite "quorum sensing" signaled by the Gal/GalNAc-specific lectin, interactions of amebae with the bacterial flora of the intestine, and innate and acquired immune responses of the host. *(From Haque R, Huston CD, Hughes M, Houpt E, et al. Current concepts: amebiasis.* N Engl J Med. *2003;348: 1565–1573.)*

because *E. histolytica* does not encyst in culture. Studies of the process of encystation in *E. invadens* have demonstrated the role of quorum sensing through a surface Gal/GalNAc lectin to initiate encystation[48] after an initial environmental signal such as osmotic shock, low glucose level, or interaction with colonic mucins. Later steps in formation of the cyst require signaling through β-adrenergic receptors and autophagy.[49,50] The cyst wall of *E. invadens* contains a chitin-binding lectin.[51] Transcriptional networks associated with encystation were identified in cultures of clinical isolates of *E. histolytica* that contain encysting organisms.[52,52a] A total of 672 cyst-specific transcripts were identified, including chitin synthetase, some of the transmembrane kinases, and cysteine proteinases, as well as genes involved in transcription, such as those for chromodomain proteins and an *myb*-like protein, EhMyb.[52]

Metabolism

No tricarboxylic acid cycle or oxidative phosphorylation exists for *E. histolytica*.[53] Many metabolic enzymes appear to have been acquired through lateral gene transfer from bacteria.[54] Functional mitochondria, or any other compartmentalized energy generation system, are lacking, although there is a remnant of the mitochondria called the *mitosome*.[55] Glycolysis is the major pathway for adenosine triphosphate generation and occurs in the cytosol.[56] Catabolism of amino acids is a second energy source.[57] Pyruvate ferredoxin oxidoreductase is essential in both glycolysis and amino acid catabolism and also serves to activate metronidazole through its reduction of ferredoxin, which suggests that metronidazole resistance would probably not develop.[53] Energy stores are predominantly in glycogen that occurs in cytoplasmic granules.[53]

Pathways for the biosynthesis of amino acids, with the exception of serine and cysteine, are lacking.[58,59] Cysteine is of special importance because it is the major thiol. Synthetic enzymes for cholesterol fatty acid and phospholipids also have been identified. In contrast, de novo purine synthesis is lacking.[60] More than 100 transporters have been identified in the genome, but their characterization is incomplete at this time.[53]

Cell Biology

Vesicular trafficking is of paramount importance to the parasite, inasmuch as endocytosis and phagocytosis serve as mechanisms of nutritional uptake. Exocytosis of the cysteine proteinases and ameba pores implicated in virulence and cyst wall components are also important functions of vesicular trafficking, in addition to the more typical roles in transport to and from the endoplasmic reticulum to the Golgi complex and cell surfaces. This complexity of function is reflected in the presence of 91 *Rab* genes (in comparison with 11 in *Saccharomyces cerevisiae*) involved in vesicle fusion.[61,62] Lysosomal acidification is key in cell amebic trogocytosis and cell killing.[63] N-linked glycosylation of proteins is unusual in that mannose-5 *N*-acetylglucosamine-2 is the most abundant N-linked glycan, whereas in other eukaryotes this would typically be processed by the addition of branching sugars.[64]

Transmembrane kinases are present and extraordinarily diverse (Fig. 272.2).[65–68] These kinases number more than 100 and are part of the family of Gal/GalNAc lectin-related proteins that share the extracellular CXXC and CXC motifs of the lectin intermediate (Igl) subunit. The kinase activity (Ser/Thr vs. Tyr) of the transmembrane kinases, as well as their substrates, ligands, and biologic functions, are all yet to be determined. The immediate downstream effectors of the transmembrane kinases are also yet to be identified, although more than 100 protein phosphatases are known to be present in the genome.[68] An unusual feature of these phosphatases is the presence of leucine-rich repeat domains implicated in protein-protein interactions. There are numerous seven-transmembrane G protein–coupled receptors and trimeric G proteins. A G protein–regulated adenylyl cyclase that functions downstream of an adrenergic ligand receptor has been biochemically identified. Cytosolic proteins involved in signal transduction include Ras, Rac, Rab, Rho, and Arf and their exchange factors: EF-hand motif calcium-binding proteins, phosphatidylinositol 3-kinase, and protein kinase C and mitogen-activated protein kinases.[68] It seems likely that this complex signaling system is required for the adaptation of the parasite to its host.

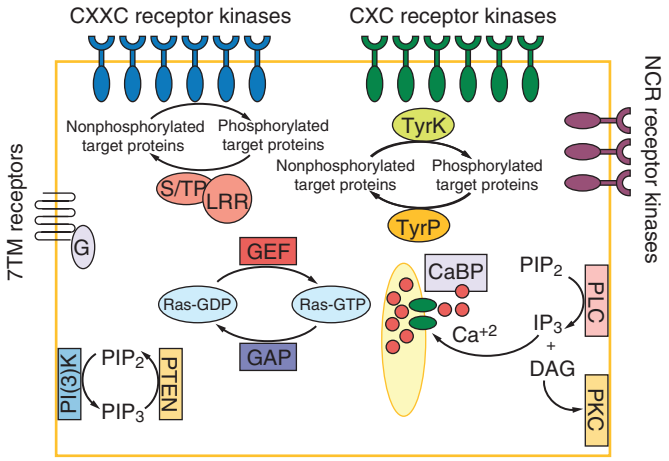

FIG. 272.2 Predicted signal transduction mechanisms of *Entamoeba histolytica*, based on analysis of the genome sequence data. *E. histolytica* possesses three types of receptor serine/threonine kinases: one group has CXXC repeats in the extracellular domain; a second has CXC repeats; and a third has non–cysteine-rich *(NCR)* repeats. *E. histolytica* has cytosolic tyrosine kinases *(TyrK),* but not receptor tyrosine kinases. Some serine/threonine phosphatases *(S/TP)* have an attached leucine-rich repeat *(LRR)* domain. *CaBP,* calcium-binding protein; *DAG,* diacylglycerol; *G,* G protein; *GAP,* guanosine triphosphatase (GTPase)–activating protein; *GEF,* guanine nucleotide exchange factor; *IP_3,* inositol-1,4,5-trisphosphate; *PI(3)K,* phosphatidylinositol 3-kinase; *PIP_2,* phosphatidylinositol-4,5-bisphosphate; *PIP_3,* phosphatidylinositol-3,4,5-trisphosphate; *PKC,* protein kinase C; *PLC,* phospholipase C; *PTEN,* phosphatase and tensin homologue; *Ras-GDP,* Ras protein–guanosine diphosphate; *Ras-GTP,* Ras protein–guanosine triphosphate; *TyrP,* tyrosine phosphatase; *7TM receptors,* seven-transmembrane receptors. *(From Loftus B, Anderson I, Davies R, et al. The genome of the protist parasite* Entamoeba histolytica. Nature. *2005;433:865–868.)*

Some of the unique aspects of the cytoskeleton include the lack of dependence on microtubules for motility, which is instead mediated by actin-myosin motors, and the lack of intermediate filament proteins such as keratins, desmin, and vimentin. Polymerization of actin into polymers of F-actin leads to microfilament assembly, which, through the myosin family of molecular motors, provides vesicular transport and motility by means of pseudopods.[68,69]

Genome Structure

The original genome sequence of *E. histolytica* was from the HM-1:IMSS strain originally isolated from the rectal biopsy sample of a Mexican man with amebic dysentery.[68] Because of a high content of repetitive DNA, the genome has not been completely assembled but instead exists in approximately 1800 fragments, with the average of 12 sequencing reads per fragment. The incomplete nature of the genome ensures that some genes will be missing and that some misassembly will occur. The *E. histolytica* genome is estimated to be 14 chromosomes, 8000 genes, and 24 million base pairs of DNA, a size that is approximately comparable with those of *Plasmodium* and *Trypanosoma* spp. The average gene length of 389 amino acids, however, is approximately half that of *Plasmodium*.[53,68] Approximately 50% of the genome is noncoding DNA, including 20% of the genome that is dedicated to ribosomal RNA genes that are encoded in extrachromosomal circles, and another 10% that encodes transfer RNA genes organized in repetitive linear arrays (probably at chromosome ends). Additional repetitive DNA in the genome includes long interspersed repeated sequence (LINE) and short interspersed repeated sequence (SINE) transposable elements.[68]

The DNA content of *Entamoeba* appears to vary under different growth conditions. The nuclear DNA content of *E. histolytica* was shown to be 10-fold higher in axenic (bacteria-free) than xenic culture. In addition, 40-fold increases in DNA content were observed as trophozoites emerged from *E. invadens* cysts. The most plausible explanation for these observations is that the ploidy of *E. histolytica* varies through a growth-dependent process of DNA replication without nuclear division.[70]

Control of messenger RNA (mRNA) expression in *E. histolytica* shares similarities with later branching eukaryotes: The parasite transcribes mRNA monocistronically by using RNA polymerase II under the control of upstream regulatory elements.[71] Thirty percent of genes are predicted to contain introns, and the pre-mRNA splicing machinery includes conserved U2, U4, and U5 small nuclear RNAs.[72] Most of the protein subunits of RNA polymerase II are also conserved, but not all general transcription factors are identified yet. Other differences from yeast and metazoans include the presence of a third core promoter regulatory element for RNA polymerase II,[73] altered histone code,[74,75] and unique aspects of mRNA silencing through small RNAs.[52,76]

PATHOGENESIS

The pathogenesis of amebiasis centers on the unique tissue-destructive properties for which the organism was named *histolytica*. Tissue invasion involves a contact-dependent process of adherence followed by cell killing that is called "trogocytosis-like" because the host cell is killed by its partial ingestion by the parasite (Fig. 272.3).[77–79]

Adherence

The initial contact of parasite to host is mediated by the parasite's Gal/GalNAc lectin, which binds to carbohydrate determinants on the host.[78–82] Adherence to human colonic mucin glycoproteins,[83] human neutrophils and erythrocytes,[84] certain bacteria,[85] and a variety of cell culture lines[86,87] is inhibited by up to 90% by Gal or GalNAc.[83] Blockade of lectin activity with Gal or GalNAc prevents contact-dependent cytolysis,[78] and glycosylation-deficient mutant cell lines lacking terminal Gal/GalNAc residues on N- and O-linked sugars are nearly totally resistant to amebic adherence and cytolytic activity.[87]

The colonic mucin layer of the large intestine is the first receptor encountered by the trophozoite lectin. Binding of the lectin to colonic mucins inhibits Gal/GalNAc and is of very high affinity (dissociation constant of 8.2×10^{-11} M^{-1}).[83] The mucin layer may protect the host from the parasite's contact-dependent cytolysis by binding to and neutralizing the lectin, while serving as a site of attachment for the parasite to colonize the large bowel. Interaction of trophozoites with colonic mucins appears to be a dynamic process, whereby trophozoites both induce the secretion of colonic mucins and degrade them.[88]

The Gal/GalNAc lectin is composed of a 260-kDa heterodimer of disulfide-linked heavy (170-kDa) and light (35- to 31-kDa) subunits that is noncovalently associated with an intermediate subunit of 150 kDa.[79–82] The 170-kDa subunit contains a carboxyl-terminal cytoplasmic and transmembrane domain adjacent to a cysteine-rich extracellular domain.[89,90] Five distinct genes (termed *hgl1* to *hgl5*) encoding the lectin's heavy subunit have been identified, sequenced, and shown to be expressed in trophozoites.[91] The sequence of the *hgl* genes is nearly completely conserved in isolates of *E. histolytica* from different continents, an important consideration for vaccine design.[44] The carbohydrate recognition domain is located within the cysteine-rich domain of the heavy subunit.[92] The lectin localizes to lipid rafts in the plasma membrane.[93] The lectin can be specifically released from the cell surface through the action of an amebic rhomboid protease, which probably explains earlier observations of both membrane-bound and soluble forms of the lectin.[79,80,94]

The Gal/GalNAc lectin appears to have other biologic functions in addition to adherence. Interference with lectin activity blocks chemotaxis in response to tumor necrosis factor-α (TNF-α).[95] The function of the light subunits may include lateral mobility of the Gal/GalNAc lectin, as evidenced by defects in capping of the lectin in amebae silenced for one of the genes that encodes the light subunits (*lgl* genes).[96] In an animal model, disruption of lectin function by expression of a dominant negative mutant blocked the ability of the parasite to cause liver abscess.[97,98] The lectin may serve as an organizing site for cytoplasmic proteins, with cytoplasmic proteins of the amebae that bind directly or indirectly to the lectin, including a thiol-specific antioxidant, spectrin, actin, myosin, talin, calreticulin, and cysteine proteinase 2.[99–101] The lectin has a role in evasion of serum lytic activity by inhibiting the formation of the complement membrane attack complex through blockade of C5b-9 assembly.[102] As mentioned previously, the lectin also appears to play an initiating role in cyst production.[48]

A recent study has shown that *E. histolytica* invades through the intestinal epithelium via a unique cell biologic process called trogocytosis.[78,103,104]

Cytolysis

Cytolysis occurs after adherence in a process that involves phagocytic ingestion of the host cells in bites, a process called "trogocytosis-like" (see Fig. 272.3). Apposition of amebic and target cell plasma membranes, as can be achieved by centrifugation of target cells and amebae into a pellet, does not lead to cytolysis if the amebic lectin is inhibited with Gal/GalNAc[77] or if the target cell lacks Gal and GalNAc on its surface.[85–87] This is consistent with the fact that lectin not only mediates adherence but also participates in the trogocytosis-like cytolytic event. Anti-lectin monoclonal antibody directed against epitope 1 of the lectin heavy subunit blocked cytotoxicity but not adherence, which implicates the lectin directly in the cytotoxic event. Killing occurs after the lectin engages GalNAc on O-linked target cell surface oligosaccharides: lectin-mediated capping of the O-linked structures (sialic acid-Gal–[sialic acid]–Gal-NAc) could be deduced to mediate killing.

Killing of host cells is not caused by an isolated toxin, inasmuch as parasite extracts have no cytotoxic activity. Cytolysis does require an intact parasite cytoskeleton, as demonstrated by inhibition of Rho,[105] by cytochalasin disruption of the cytoskeleton,[78] and by expression of dominant-negative myosin II.[106] The earliest observed event in a dying cell is a rise in intracellular calcium within seconds of direct contact by an amebic trophozoite; this event is associated with membrane blebbing.[107] Extracellular ethylenediaminetetraacetic acid and treatment of the target cells with the slow sodium-calcium channel blockers verapamil and bepridil[108] significantly reduce amebic killing of target cells in suspension. Isolation of amebic pore-forming proteins similar in function to pore-forming proteins of the immune system has been reported by a number of investigators. A purified 5-kDa amebapore and a synthetic peptide based on the sequence of its third amphipathic α-helix have cytolytic activity for nucleated cells at high concentrations (10–100 μM).[109,110] Silencing of amebapore A blocked the ability of amebae to release monolayer tissue culture cells from a plastic well, although cell death was not specifically measured.[111] The optimal pH of amebapore is 5.3, and amebapore is inactive at a pH of 7, which may be of some significance in view of the inhibition of cytotoxicity with weak base treatment of amebae.[112] Interestingly, no DNA degradation

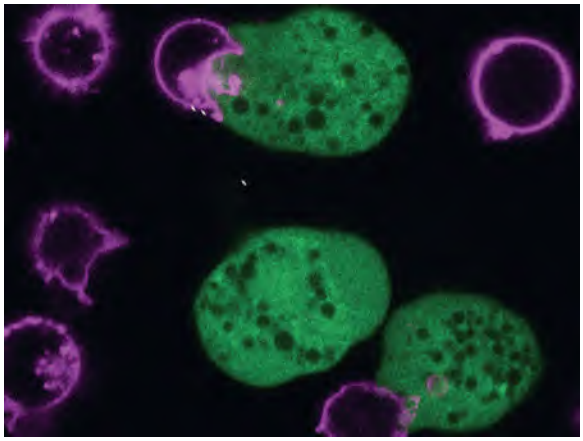

FIG. 272.3 Trogocytosis-like killing of host cells by *Entamoeba histolytica*. Trophozoites of *E. histolytica* (labeled *green* with CMFDA [5-chloromethylfluorescein diacetate]) are shown interacting with Jurkat T cells (labeled *purple* with DiD [1,1′-dioctadecyl-3,3,3′,3′-tetramethylindod icarbocyanine]). The trophozoites at the top and bottom right show partial ingestion of pieces of the Jurkat cells in a trogocytosis-like process by the amebae. Host cell death follows trogocytosis-like partial ingestion. *(From Ralston KS, Solga MD, Mackey-Lawrence NM, et al. Trogocytosis by* Entamoeba histolytica *contributes to cell killing and tissue invasion.* Nature. *2014;508:526–530.)*

was observed in cells lysed in vitro by the purified amebapore, which is suggestive of a different mechanism of cell killing by the purified amebapore than by the intact parasite.[113]

Cells killed by the parasite undergo nuclear chromatin condensation, membrane blebbing, and internucleosomal DNA fragmentation.[113] There is evidence of a nonclassical mechanism of apoptotic killing by *E. histolytica.* Overexpression of the Bcl-2 protein that inhibits apoptosis caused by a variety of cellular stresses (e.g., serum starvation, ultraviolet radiation) did not prevent murine cell DNA fragmentation after exposure to *E. histolytica.*[113] Furthermore, *E. histolytica* caused hepatocyte apoptosis in mice deficient in the Fas/Fas ligand and tumor necrosis factor receptor-1 signaling pathways.[114] Caspase 8–deficient cells, resistant to killing by Fas ligand, were readily killed by *E. histolytica.* Caspase 8–deficient cells treated with a caspase 9 inhibitor (Ac-LEHD-fmk) (at a level sufficient to inhibit apoptosis through etoposide) were readily killed as well. Together, these data suggest that *E. histolytica* initiates host cell apoptosis by directly activating the host cell's distal apoptotic machinery. Caspase 3 was activated within minutes of *E. histolytica* adherence, and the caspase 3 inhibitor Ac-DEVD-CHO at 100 µmol (sufficient to block killing through actinomycin D) blocked *E. histolytica* killing, as measured both by DNA fragmentation and by Cr51 release; this outcome indicates that both apoptotic death phenotype and necrosis were necessary.[115] In conclusion, amebic killing of the host is a result of parasite nibbling (trogocytosis) on the human cell, which results in apoptosis in the host cell at the level of caspase 3 activation.

Phagocytosis

In multicellular organisms, phagocytosis is the final step in the apoptotic pathway and serves to limit inflammation by preventing spillage of toxic intracellular contents of dead cells. Although amebic killing of cells by contrast involves phagocytosis followed by death, phagocytosis could similarly limit the host inflammatory response and enable *E. histolytica* to establish a persistent infection.[67,116,117] Inhibition of endocytosis by galactose and phosphatidylserine is additive, consistent with the Gal/GalNAc lectin and an as-yet unidentified phosphatidylserine receptor acting as coreceptors for ingestion.[118,119] Understanding the molecular mechanisms of engulfment of and subsequent death of the host promises to reveal much about pathogenesis, inasmuch as amebae that are defective in phagocytosis are also defective in virulence.[67,120]

Role of Bacteria

The effect of the gut bacteria on the biologic properties of *E. histolytica* may be profound. As mentioned previously, the genome content of *Entamoeba* is lower when the parasite is grown in the presence of bacteria.[53] In addition, amebae cultured with bacteria are better able to destroy monolayers of tissue culture cells and resist oxidative stress.[121–123] Recently it was shown that the gut microbiome is enriched in *Prevotella copri* in children with amebic diarrhea, and that antibiotic-induced alterations of the gut microbiome act via alterations in neutrophil trafficking to the gut in the mouse model of amebic colitis.[124,124a] Thus ultimate susceptibility to amebic colitis may in part be due to dysbiosis limiting neutrophil migration to the colon.

Cysteine Proteinases

E. histolytica encodes at least 44 genes that are cysteine proteinases, some of which are membrane bound and others predicted to be soluble.[53] These activities are implicated in a number of potentially important activities and include degradation of colonic mucin glycoproteins,[125] digestion of hemoglobin and villin,[126,127] inactivation of interleukin (IL)-18,[128] and digestion of extracellular matrix.[129]

Role of Leptin in Host Resistance

Amebiasis is more common in malnourished children (a physiologic state of leptin deficiency). This suggests that the nutritional hormone leptin could play a protective role in amebiasis. In fact, a mutation in the leptin receptor was discovered that was associated with amebiasis susceptibility in children. The mutation is a nonconservative substitution (Q223R) in the extracellular cytokine receptor homology domain 1. The mutation accounted for the majority of susceptibility to amebiasis in children because it conferred a 3.9-fold greater risk of intestinal amebiasis and was present in almost half the children. The purely nutritional role of leptin did not explain protection because the Q223R mutation was not associated with children's nutritional status. Experiments in mice validated the human study, with leptin-deficient (*ob*/*ob*), leptin receptor–deficient (*db*/*db*), and 223R leptin receptor knock-in mice highly susceptible to intestinal *E. histolytica* infection. The site of leptin action was localized to the gut, because an intestinal epithelial cell–specific deletion of the leptin receptor rendered mice susceptible, whereas lack of the leptin receptor in the central nervous system or bone marrow–derived cells did not. Leptin receptor signal transducer and activator of transcription 3 (STAT3) and Src homology 2 domain–containing phosphatase 2 (SHP2) signaling were required for protection in vivo because susceptibility was conferred by mutation of tyrosine 985 or 1138, which mediate leptin signaling through the SHP2/extracellular signal–regulated kinase and STAT3 pathways, respectively. The importance of leptin signaling in prevention of amebic killing of the host could even be seen in single-cell studies, where transfection of human embryonic kidney cells with the leptin receptor rendered them resistant to amebic killing.[130,131]

Macrophage Migration Inhibitory Factor and Inflammation

Colonic inflammation is a hallmark of *E. histolytica* infection, hence the term *amebic colitis.*[132] *E. histolytica,* like several pathogenic protozoans, secretes a protein homologue of the proinflammatory cytokine macrophage migration inhibitory factor (MIF).[133–138] A recent study found a positive correlation between *E. histolytica* MIF levels and intestinal inflammation in persons with amebic colitis. In the same study, the researchers demonstrated a causal relationship between *E. histolytica* MIF and gut inflammation using cellular and mouse models of amebic colitis.[139] *E. histolytica* MIF-induced mucosal inflammation resulted in increased matrix metalloproteinases (MMPs) production.[139] MMPs are enzymes that break down extracellular matrix proteins and promote cell migration. MMPs were shown recently to promote *E. histolytica* tissue invasion in a human colon explant model.[140] This potential link between *E. histolytica* MIF, inflammation, and tissue invasion might explain why a parasite would encode a proinflammatory cytokine.

IMMUNE RESPONSE AND IMMUNITY

Innate Immunity

Neutrophils and Eosinophils

Neutrophils are the earliest innate cellular immune response for both intestinal and hepatic amebiasis. They occur as a dominant infiltration of polymorphonuclear leukocytes surrounding trophozoites. Lymphocytes, macrophages, and epithelioid cells are recruited to infected tissue by day 3, in association with the formation of granulomas, which contribute to the confinement of invading trophozoites.[141–145] Neutrophils may be recruited by the chemotactic activity of an amebic membrane-bound peptide and chemokines secreted by epithelial cells exposed to *E. histolytica.*[146–149] As the consequence of interaction with trophozoites, neutrophils become activated and release reactive oxygen species and antimicrobial peptides. Many in vitro studies have reported neutrophil amebicidal activity after stimulation by interferon-γ (IFN-γ), TNF-α lipopolysaccharide, or amebic antigens.[84,150] In accordance with a protective role for neutrophils, depletion of neutrophils with anti–Gr-1 neutralizing antibodies resulted in exacerbated amebic hepatic and intestinal disease.[151–153] It is worth noting that the GR-1 antibodies also deplete other granulocytes such as eosinophils, which are also observed as part of the innate immune response and are protective in the murine model of amebic colitis.[154] Neutrophils can also be lysed by virulent amebae.[155,156] This can occur through disruption of the oxidase activities of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH)[157,158]; through protection of the amebae from oxidative damage by amebic peroxiredoxin, a 29-kDa surface protein conferring resistance to host reactive oxygen defenses[99,159,160]; or by inducing neutrophil apoptosis.[158] Neutrophil destruction in turn could result in tissue damage through the release of cytotoxic oxidase and lytic peptidases.[155]

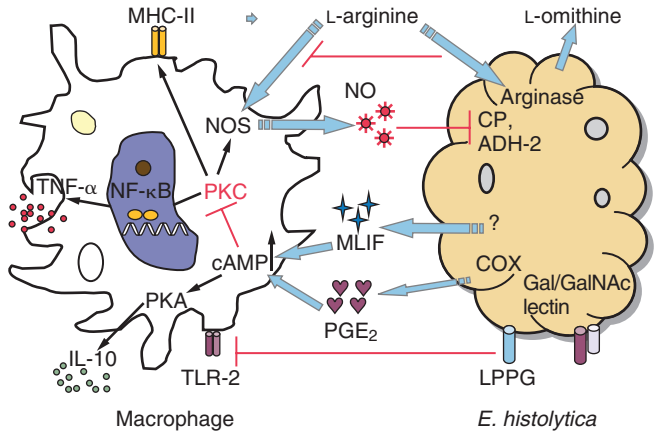

FIG. 272.4 Modulation of macrophage functions by *Entamoeba histolytica*. The killing of *E. histolytica* trophozoites by macrophages is mainly mediated by nitric oxide (NO), derived from L-arginine by nitric oxide synthase (NOS). NO could inhibit amebic cysteine proteinases (CP) and alcohol dehydrogenase 2 (ADH-2), the critical enzymes conferring virulence of *E. histolytica*. The arginase activity was detected in *E. histolytica,* which putatively converts L-arginine into L-ornithine, in turn limiting NO production by macrophage NOS. Prostaglandin E_2 (PGE_2) is an immunoregulatory molecule produced by cyclooxygenase (COX) in amebae or ameba-exposed macrophages. By activating the cyclic adenosine monophosphate (cAMP)–protein kinase A (PKA) pathway, PGE_2 suppresses macrophage effector functions by inhibiting protein kinase C (PKC)–mediated expression of major histocompatibility complex class II (MHC-II) and production of tumor necrosis factor-α (TNF-α), while favoring production of interleukin-10 (IL-10). Monocyte locomotion inhibitory factor (MLIF) produced by amebae may suppress macrophage functions in a manner similar to that of PGE_2. Amebic lipopeptidophosphoglycan (LPPG) might downregulate Toll-like receptor 2 (TLR2) expression on macrophages and thus control the effector mechanisms triggered through TLR2 signaling. *Gal/GalNAc, N*-acetyl-D-galactosamine; *NF-κB,* nuclear factor kappa B. *(From Guo X, Houpt E, Petri WA Jr. Crosstalk at the initial encounter: interplay between host defense and ameba survival strategies.* Curr Opin Immunol. *2007;19:376–384.)*

Macrophages

Macrophages acquire amebicidal activity after in vitro stimulation with IFN-γ, TNF-α, or colony-stimulating factor-1 (Fig. 272.4).[161–163] The Gal/GalNAc lectin of *E. histolytica* upregulates Toll-like receptor (TLR) 2 expression in macrophages, resulting in activation of nuclear factor kappa B and production of proinflammatory cytokines.[164] Macrophages lacking TLR2 and TLR4 showed impaired response to *E. histolytica* lipopeptidophosphoglycan, which suggests that pattern recognition is essential in the macrophage response.[164] Inducible nitric oxide synthase–deficient mice were more susceptible to amebic liver abscess and to *E. histolytica*–induced hepatocytic apoptosis,[165] which suggests that nitric oxide plays a critical role in host defense against amebiasis.

Despite the sensitivity of *E. histolytica* to nitric oxide–mediated cytotoxicity,[165,166] impaired macrophage function has been observed in human and experimental amebiasis, which suggests that amebae have developed strategies to modulate macrophage responses (see Fig. 272.4). Macrophage exposure to *E. histolytica* trophozoites or amebic components suppresses the respiratory burst and nitric oxide production.[167,168] A decrease in TNF-α secretion and IFN-γ–induced expression of major histocompatibility complex class II has also been observed.[169,170] Macrophage suppression may depend at least partially on prostaglandin E_2, an immunoregulator produced by *E. histolytica,* or on macrophages exposed to amebic proteins.[170,171] Prostaglandin E_2 elevates cyclic adenosine monophosphate levels in macrophages, triggering the protein kinase A pathway, which in turn inhibits the expression of major histocompatibility complex class Ia molecules, the release of helper T-cell type 1 cytokines, NADPH-mediated oxidative burst, and nitric oxide synthesis through the protein kinase C pathway (see Fig. 272.4). In addition, monocyte locomotion inhibitory factor, an immunosuppressor synthesized by amebae, also contributes to the modulation of host immune responses. Monocyte locomotion inhibitory factor is a soluble pentapeptide with antiinflammatory properties.[172,173]

Natural Killer Cells and Natural Killer T Cells

Natural killer cells and natural killer target cells have an innate role in host defense by production of IFN-γ and cytolytic peptides. Elevated cytotoxic activity of natural killer cells was found in mice infected with pathogenic amebae, and this may explain gender-dependent differences in the control of amebic liver abscess in C57BL/6 mice.[174,175]

Activated Mast Cells

Activated mast cells produce IL-6 and TNF-α, can recruit phagocytes, and can influence lymphocytic development and functions. Increased mast cell infiltration and upregulated mast cell protease expression have been observed in infected mouse ceca, but whether mast cells contribute to parasite clearance or play a pathologic role in tissue damage remains unanswered.[176]

Complement-Mediated Lysis of *Entamoeba histolytica*

After trophozoites penetrate the epithelial layer, the alternative complement pathway is initiated at least in part through cleavage of C3 and C5 by the amebic cysteine proteinase.[177,178] C3a and C5a act to chemoattract neutrophils to the site of infection. The Gal/GalNAc lectin heavy subunit of *E. histolytica* inhibits the assembly of C8 and C9 into the C5b-9 membrane attack complex, thereby preventing complement-mediated lysis of the parasite.[102]

Intestinal Epithelial Cells

Intestinal epithelial cells serve as the effectors of the mucosal immune system. Coculture of epithelial cell lines with *E. histolytica* trophozoites results in increased production of TNF-α, IL-1α, IL-6, IL-8, growth-regulated peptide-α, and granulocyte-macrophage colony-stimulating factor by the epithelial cells.[179–181] In the murine model of intestinal amebiasis, innate resistance is conferred by nonhematopoietic cells, as expected because leptin signaling is a critical mechanism to protect the epithelium.[130,131] Of note is that hematopoietic IL-10 is required for intestinal epithelial resistance to amebiasis.[182]

Acquired Immunity

Mucosal Immunoglobulin A Response

A mucosal immunoglobulin A (IgA) response directed at the carbohydrate recognition domain of the parasite Gal/GalNAc lectin is linked to protection from both infection and disease (Fig. 272.5).[33,183,184] IgA anti-Gal/GalNAc lectin in breast milk is also associated with passive immunity of infants to amebiasis.[185] These findings suggested that IgA antilectin antibody responses provide both passive and active immunity.

Cell-Mediated Response

Cell-mediated production of IFN-γ would be expected to provide protection from amebiasis through its ability to activate neutrophils and macrophages to kill the parasite. In a prospective study of a cohort of preschool children in Dhaka, Bangladesh, amebic antigen–stimulated IFN-γ production by peripheral blood mononuclear cells was associated with protection from future diarrhea caused by *E. histolytica* (Fig. 272.6).[186] An experimental amebic colitis model suggested that $CD4^+$ T cells that produced helper T-cell type 2 cytokines mediated pathogenesis[176]; therefore the pattern of $CD4^+$ T-cell response may be critical. Vaccine-mediated immunity to amebiasis was transferrable by CD4 or CD8 T cells producing IFN-γ and IL-17.[187] In summary, host protection from amebiasis includes innate defenses at the intestinal epithelium mediated by leptin and acquired immunity from anti-Gal/GalNAc lectin IgA and T-cell production of IFN-γ and IL-17.

EPIDEMIOLOGY

The best estimate is that *E. histolytica* infection results in 34 to 50 million symptomatic cases worldwide each year and as many as 100,000 deaths. The bulk of the morbidity and mortality from amebiasis occurs in Central and South America, Africa, and the Indian subcontinent.[188] Local prevalence rates in these regions can be astounding. A carefully conducted national serologic survey in Mexico demonstrated antibody to *E. histolytica* in 8.4% of the population.[189] In the urban slums of Fortaleza, Brazil, 25% of the population tested carried antibody to *E.*

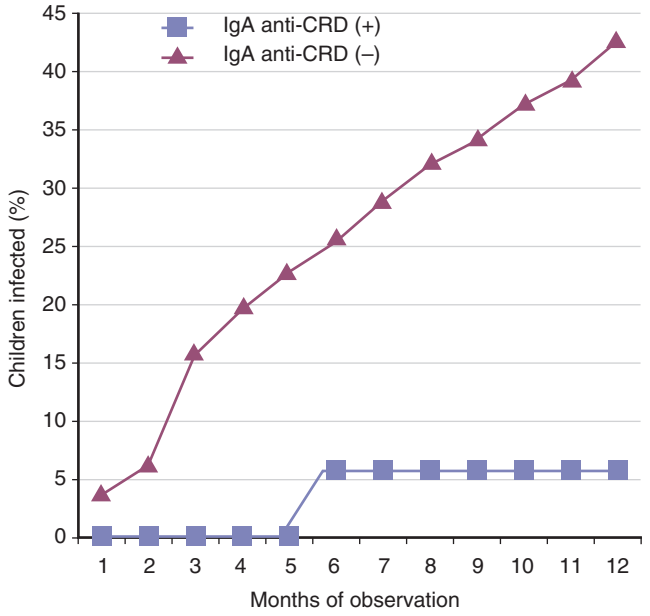

FIG. 272.5 Immunoglobulin A (IgA) anti–carbohydrate recognition domain (CRD) is associated with immunity to *Entamoeba histolytica* infection. Children with fecal IgA antibodies against the *N*-acetyl-D-galactosamine (Gal/GalNAc) lectin CRD (IgA anti-CRD$^+$; $n = 81$) had a lower incidence of new intestinal *E. histolytica* infection than did children who lacked this response (IgA anti-CRD$^-$; $n = 149$). The two groups were statistically significantly different ($P \leq .04$) at every time point. The average duration of protection was 437 days (95% confidence interval, 346–528 days). *(From Haque R, Mondal D, Duggal P, et al. Entamoeba histolytica infection in children and protection from subsequent amebiasis.* Infect Immun. *2006;74:904–909.)*

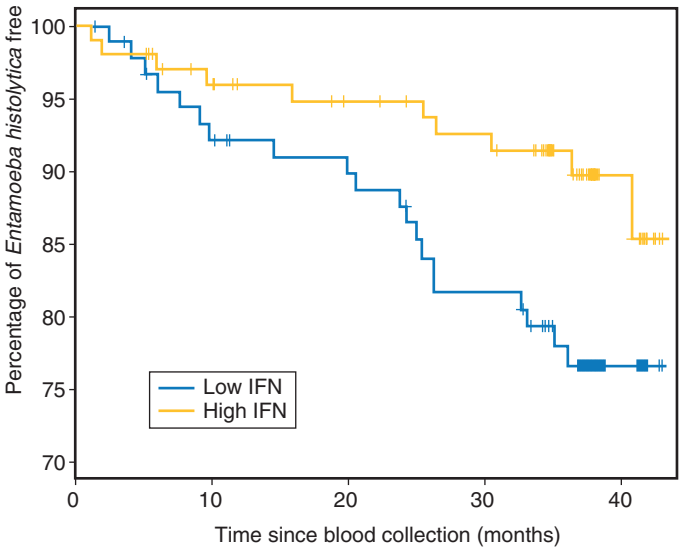

FIG. 272.6 High levels of interferon (IFN)-γ are predictive of increased survival free of *Entamoeba histolytica* diarrhea. Peripheral blood mononuclear cells were stimulated with soluble amebic extract, and children were grouped by IFN-γ production in response to stimulation with soluble amebic antigen. Children were then monitored for 44 months, and the incidence of *E. histolytica* diarrhea was measured. The upper line and lower line indicate percentages of children with and without IFN-γ response, respectively, above the median for all children (580 pg/mL). The two lines are significantly different: logrank test $P = .03$; $n = 92$ for low IFN-γ, and $n = 103$ for high IFN-γ. *(From Haque R, Mondal D, Shu J, et al. Correlation of interferon-gamma production by peripheral blood mononuclear cells with childhood malnutrition and susceptibility to amebiasis.* Am J Trop Med Hyg. *2007;76:340–344.)*

histolytica, and the prevalence in children 6 to 14 years of age was 40%.[190] In Dhaka, Bangladesh, where diarrheal diseases are the leading cause of childhood death, the annual incidence of infection in cohorts of infants and preschool children was greater than 40%.[33,185] The annual incidence of amebic liver abscess was reported to be 21 cases per 100,000 inhabitants in Hue City, Vietnam.[191] Country-specific prevalence data for amebiasis have been reviewed.[30]

Reported prevalences of *E. histolytica* can vary widely, depending on the method of diagnosis used, the patient population, and the area studied. Recent data from the large case-control Global Enteric Multicenter Study identified *E. histolytica* to be one of the top seven pathogens causing dysentery in young children (under the age of 5 years) living in developing countries throughout Africa and Asia.[192]

In the United States, amebiasis is the third most common parasitic infection, after giardiasis and cryptosporidiosis (1.2 cases per 100,000 US population). Travelers to and immigrants from endemic regions and institutionalized individuals are at increased risk of acquiring amebiasis.[193] In returning travelers, diarrhea is the predominant reason for a patient to seek medical care, and amebiasis is the second most common cause of diarrhea in returning travelers (Fig. 272.7).[193] Travel of intimate or household contacts to an endemic area may also be a risk for acquisition of infection.[194] Previously reported high rates of *E. histolytica* infection in homosexual men in the United States actually reflect a high prevalence of *E. dispar* infection in this population.[1] In contrast, in Asia, amebiasis is more frequently a presenting symptom of HIV infection and acquired immunodeficiency syndrome,[195–198] and recent studies from Taiwan and Japan have shown high rates of invasive *E. histolytica* infection in HIV-infected men who have sex with men.[199,200] Several groups are at increased risk of severe invasive amebiasis, including those with extremes of age, malnutrition, pregnancy, and receipt of corticosteroids. Fulminant amebic colitis, the most life threatening complication of amebic infection is associated with high mortality and morbidity; case fatality rates upwards of 40% have been reported.[201]

The National Institute of Allergy and Infectious Diseases also considers *E. histolytica* a Biodefense Research category B pathogen because of its low infectious dose (<100 organisms), chlorine resistance, and

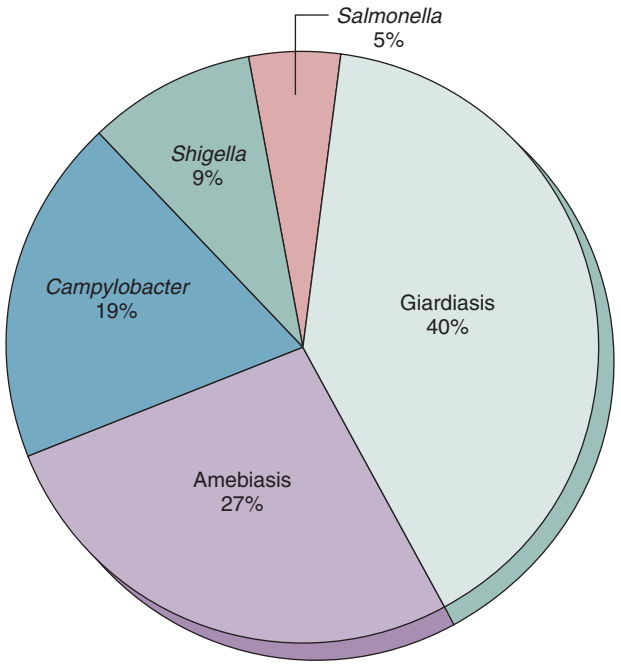

FIG. 272.7 Amebiasis as a cause of diarrhea in returning travelers. Amebiasis is the second most common cause of diarrhea in returning travelers, according to the GeoSentinel Surveillance Network of 30 travel or tropical medicine clinics on six continents. *(Data from Freedman DO, Weld LH, Kozarsky PE, et al. Spectrum of disease and relation to place of exposure among ill returned travelers.* N Engl J Med. *2006;354:119–130.)*

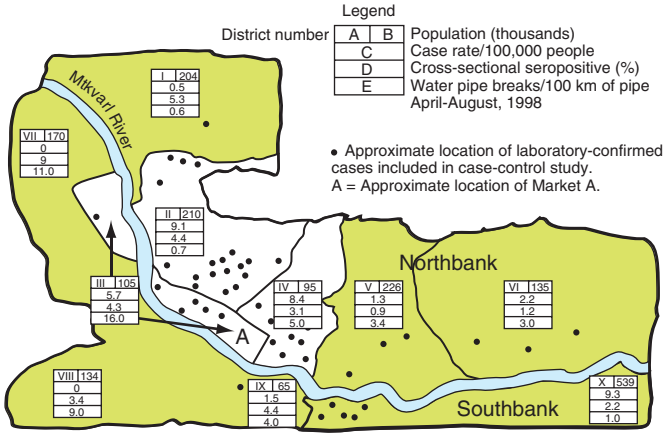

FIG. 272.8 Location of cases of amebic liver abscess in Tbilisi, Republic of Georgia. Cases were mapped by area of residence during the 1998 outbreak. Interruptions in water supply, decreases in water pressure, and increased water consumption were all significantly associated with infection. *(From Barwick R, Uzicanin A, Lareau S, et al. Outbreak of amebiasis in Tbilisi, Republic of Georgia, 1998.* Am J Trop Med Hyg. *2002;67: 623–631.)*

environmental stability. All these properties make it a threat to food and water supplies, as the municipal water outbreak of amebic liver abscess in Tbilisi, Republic of Georgia, demonstrated (Fig. 272.8).[47]

CLINICAL MANIFESTATIONS

Asymptomatic Intraluminal Amebiasis

The most common type of amebic infection is an asymptomatic cyst-passing carrier state. All *E. moshkovskii* and *E. dispar* infections and up to 80% of *E. histolytica* infections are asymptomatic. Asymptomatically infected individuals represent a risk to the community because they are a source of new infections. Asymptomatic infection with *E. histolytica* also carries a small but definite risk to the carrier for the subsequent development of invasive amebiasis. In a study in Bangladesh of children 2 to 5 years old who were colonized with *E. histolytica,* there was a small risk of developing invasive amebiasis with *E. histolytica* colonization: 2 of 17 colonized children developed dysentery during a 1-year follow-up period.[202] A subsequent study in Bangladesh revealed that 4% (25) of 651 asymptomatically infected children went on to develop amebic diarrhea or dysentery.[203] A study in South Africa revealed that of individuals colonized with *E. histolytica,* 10% developed invasive disease within 1 year.[31]

The median duration of asymptomatic colonization in children in Bangladesh was 2 months[33]; in adults in Vietnam, colonization was observed for more than 1 year.[32] In both studies, the same approach to DNA fingerprinting of *E. histolytica* was performed to distinguish continued infection with the same isolate from a second new infection; thus the differences in duration of colonization appear to be real, although currently unexplained.

The host has a bearing on whether infection is asymptomatic. In addition to the aforementioned importance of the leptin receptor mutation in susceptibility,[130,131] children heterozygous for the human leukocyte antigen class II DQB1*0601/DRB1*1501 haplotype were protected from symptomatic infection with amebiasis.[204] The genotype of *E. histolytica* also may determine whether infection is asymptomatic; certain genotypes appear to be associated with the propensity for colonization, as opposed to invasion.[46,47]

Amebic Diarrhea

Amebic diarrhea without dysentery is the most common disease manifestation of infection with *E. histolytica.* Amebic diarrhea is defined as diarrhea in an *E. histolytica*–infected individual; for the diagnosis of amebic diarrhea, mucus need not be visible and microscopic blood need not be present in the stool. In one community-based study of a cohort of preschool children in Bangladesh, the annual incidences of amebic infection, diarrhea, and dysentery were 45%, 9%, and 3%, respectively.[33] In a case-control study of diarrhea severe enough to prompt a patient to go to the hospital, approximately 2% of all such cases were caused by *E. histolytica.* This was true for all age groups. The mean duration of amebic diarrhea was 3 days.[33]

Amebic Dysentery or Colitis

Amebic dysentery, or amebic colitis, is defined as diarrhea with mucus or visible or microscopic blood in a patient with *E. histolytica* infection (Fig. 272.9). Approximately 15% to 33% of cases of *E. histolytica* diarrhea are accompanied by amebic dysentery.[33,205] Of patients with either condition, 70% have a gradual onset of symptoms over the 3 or 4 weeks after infection; increasingly severe diarrhea is the primary complaint, accompanied by general abdominal tenderness. On occasion, the onset may be acute or delayed for several months after infestation. This is different from bacterial causes of dysentery, in which patients usually have symptoms for only 1 to 2 days. The diarrhea is associated with abdominal pain and may be of such severity that an acute abdomen is suspected.[206,207] Surprisingly, fever is present in only the minority of patients with amebic colitis.[206] Abdominal distention or dehydration is unusual. In young children, intussusception, perforation, and peritonitis or necrotizing colitis may develop rapidly.[206–208] Amebic colitis may mimic inflammatory bowel disease.[201] Unusual manifestations of amebic colitis include toxic megacolon, perforation, or peritonitis (accounts for only 0.5% of cases, but is associated with a high mortality and usually necessitates surgical intervention) and ameboma (granulation tissue in colonic lumen whose appearance mimics that of colonic cancer). *Entamoeba histolytica* can also cause acute appendicitis, and was noted in up to 15% of HIV-infected patients presenting with acute appendicitis and undergoing appendectomy in a report from Japan. The only distinguishing feature was higher median white blood cell counts in those with amebic infection, compared to those without.[209] Corticosteroid use has been described as a risk factor for the development of fulminant forms of amebic colitis.[201]

Amebic Liver Abscess

Amebic liver abscess is 10 times more common in men than in women and is uncommon in children (Fig. 272.10); the reason for these differences in gender and age are not well understood. Approximately 80% of patients with amebic liver abscess have symptoms that develop relatively quickly (typically within 2–4 weeks), including fever, cough, and a constant, dull, aching abdominal pain in the right upper quadrant or epigastrium. Involvement of the diaphragmatic surface of the liver may lead to right-sided pleural pain or referred shoulder pain. Associated gastrointestinal symptoms, which occur in 10% to 35% of patients, include nausea, vomiting, abdominal cramping, abdominal distention, diarrhea, and constipation. Hepatomegaly with point tenderness over the liver, below the ribs, or in the intercostal spaces is a typical finding.[210,211] Hepatic amebiasis can develop even 20 or more years after the last visit to an endemic area.[212] The typical patient with an amebic liver abscess in the United States is an immigrant, usually a Hispanic man 20 to 40 years old, who has fever, right upper quadrant pain, leukocytosis, abnormal serum levels of transaminases and alkaline phosphatase, and a defect on hepatic imaging study. The abscess is usually single and is in the right lobe of the liver in 80% of cases.[208,210,211,213–215] Most frequently, patients present with liver abscess but without concurrent colitis, although a history of dysentery within the previous year is often present. Amebae are infrequently seen in the stool at the time of diagnosis of liver abscess. Liver abscess can manifest acutely (with fever and right upper abdominal tenderness and pain) or subacutely (with prominent weight loss and less frequent fever and abdominal pain). The peripheral white blood cell count is elevated, as is the alkaline phosphatase level, in many patients. Early evaluation of the hepatobiliary system with ultrasonography or computed tomography is essential for demonstrating the abscess in the liver.

The differential diagnosis of the lesion in the liver includes pyogenic abscess (less likely if the gallbladder and ducts appear normal), hepatoma, and echinococcal cyst. Aspiration of the abscess is occasionally necessary in order to diagnose amebiasis (although amebae are visualized in the pus in only the minority of cases; if the abscess is pyogenic, the responsible bacteria is seen or cultured, or both). If a space-filling defect in the

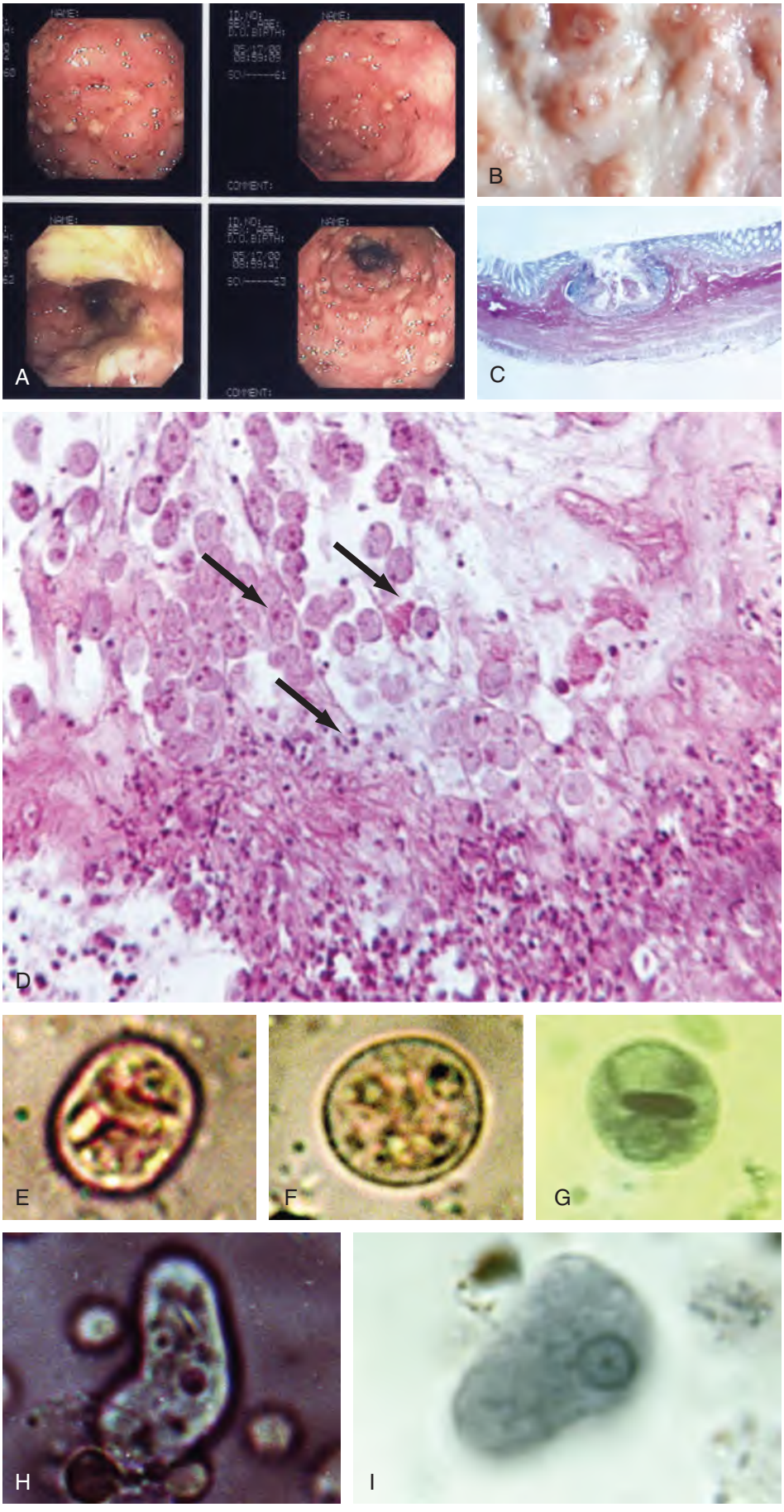

FIG. 272.9 Endoscopic and pathologic features of intestinal amebiasis. (A) Colonoscopic appearance of intestinal amebiasis. (B) Colonic ulcers averaging 1 to 2 mm in diameter on gross pathologic examination. (C) Cross section of a flask-shaped colonic ulcer (hematoxylin and eosin stain; magnification ×20). (D) Inflammatory response to intestinal invasion by *Entamoeba histolytica* (hematoxylin and eosin stain; magnification ×100). Arrows indicate *E. histolytica* trophozoites. (E and F) *E. histolytica* cysts in a saline preparation (magnification ×1000). (G) Iodine-stained cyst from stool (magnification ×1000). (H) *E. histolytica* trophozoite with an ingested erythrocyte, in a saline preparation from stool (magnification ×1000). (I) Trophozoite from stool stained with trichrome (magnification ×1000). *(B, C, and D courtesy the late Dr. Harrison Juniper. From Haque R, Huston CD, Hughes M, et al. Current concepts: amebiasis.* N Engl J Med. *2003;348:1565–1573.)*

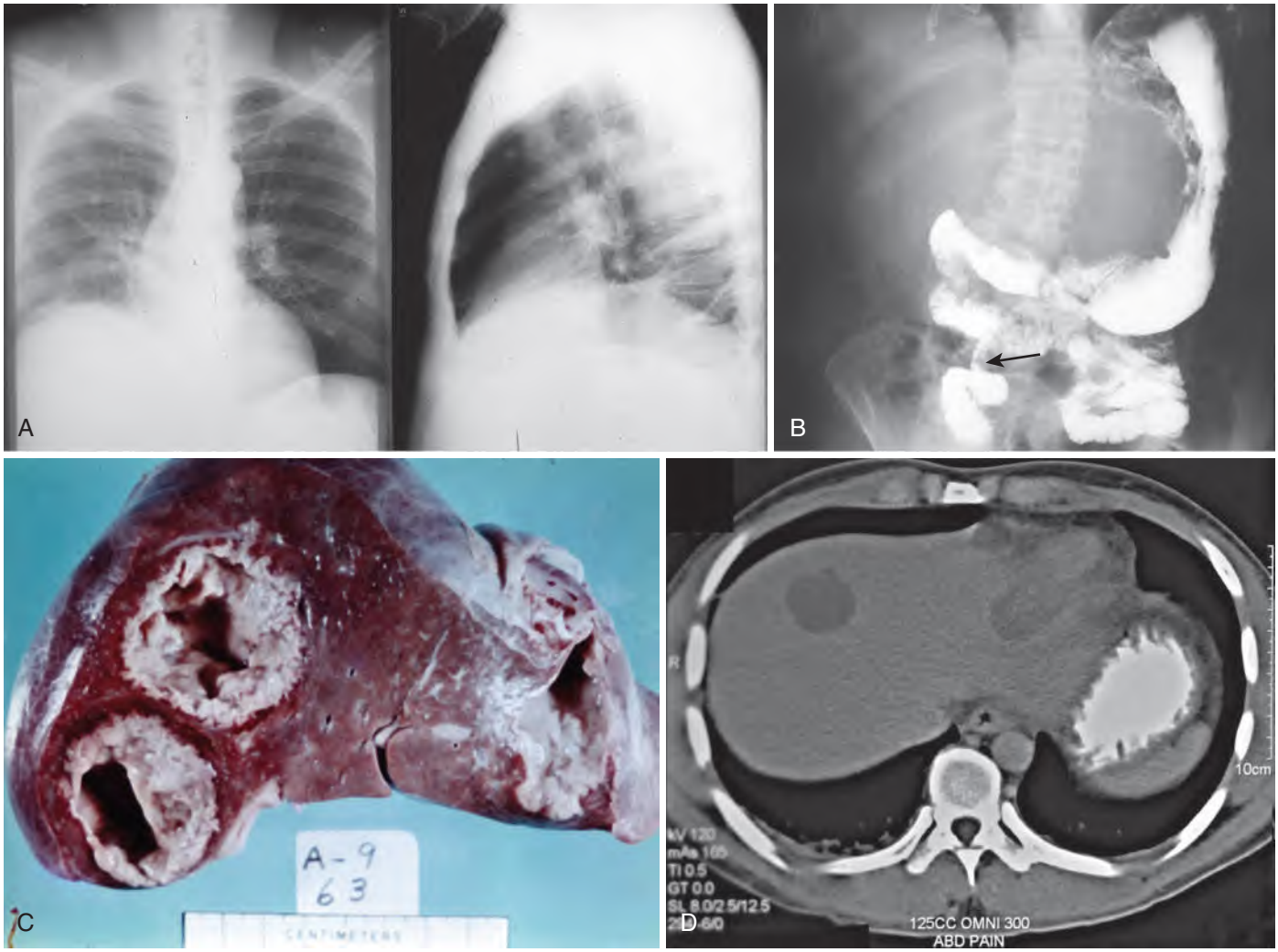

FIG. 272.10 Radiographic and pathologic features of extraintestinal amebiasis. (A) Left posteroanterior and right lateral chest radiographs in a patient with amebic liver abscess. The findings include elevated right hemidiaphragm and evidence of atelectasis. (B) Luminal narrowing *(arrow)* revealed by a barium-enema examination in a patient with ameboma. (C) Two abscesses in the right lobe and one abscess in the left lobe of a patient with amebic liver abscess. (D) One abscess in the right lobe and one abscess in the left lobe in a patient with amebic liver abscess, shown on abdominal computed tomography. *(From Haque R, Huston CD, Hughes M, et al. Current concepts: amebiasis.* N Engl J Med. *2003;348:1565–1573.)*

liver is observed, the differential diagnosis includes (1) amebiasis (most common in men with a history of travel or residence in a developing country; (2) pyogenic or bacterial abscess (suspected in women, patients with cholecystitis, elderly patients, individuals with diabetes, and patients with jaundice); (3) echinococcal abscess (an incidental finding, inasmuch as echinococcal abscess should not cause pain or fever); and (4) cancer. Most patients with amebic liver abscess have detectable circulating antigen in serum, as well as serum antiamebic antibodies.

In children, abdominal pain is reported infrequently with amebic liver abscess.[208,213,214] More commonly, high fever, abdominal distention, irritability, and tachypnea are noted. Some of these children are admitted to the hospital with a fever of unknown origin. Hepatomegaly occurs frequently, but elicitation of hepatic tenderness is not well documented. In one report, four of five children younger than 5 years of age died with amebic liver abscesses because the diagnosis was not suspected. Unusual extraintestinal manifestations of amebiasis include direct extension of the liver abscess to pleura or pericardium and brain abscess. Death usually results from rupture of the liver abscess into the peritoneum, thorax, or pericardium but may follow extensive hepatic damage and liver failure.[208,213–215]

Metastatic Amebiasis

Extraabdominal amebiasis presumably follows direct extension from liver abscesses rather than direct dissemination from the intestine.[1,211,216] Thoracic amebiasis is the most common type of extraabdominal amebiasis and occurs in about 10% of patients with amebic liver abscess.[208,211] Symptoms depend on the type of involvement. Empyema, bronchohepatic fistulas, or extension of a pleuropulmonary abscess into the pericardium may occur. Pericardial involvement is the next most common form of extraintestinal amebiasis and may result from rupture of a liver abscess in the left lobe of the liver into the pericardium or through extension of the right-sided pleural amebiasis.[208,216–218] It is estimated to occur in 3% of patients with hepatic abscesses. It manifests as acute pericarditis with tamponade and, on occasion, as pneumopericardium.[217,218] Amebic liver abscess in the left lobe also may rupture directly into the left side of the chest. Cerebral amebic abscesses were found in 0.66% to 4.7% of patients with amebic liver abscess.[219] In 18 patients with proven cerebral amebiasis, initial neurologic examination yielded normal findings in 13, and only 1 later developed seizures. Other foci of infection are rare, but amebic rectovesical fistula formation and involvement of pharynx, heart, aorta, and scapula have been reported. Cutaneous extension after the adherence of perforated, inflamed bowel to the skin is an extremely painful but rare complication.[220,221] This situation also may arise after invasion of the skin by trophozoites emerging from the rectum.

DIAGNOSIS

Diagnosis of amebiasis is best accomplished by the combination of serology and identification of the parasite in feces through antigen

TABLE 272.1 Sensitivity of Tests for Diagnosis of Amebiasis

TEST	COLITIS[a]	LIVER ABSCESS[a]	COMMENTS
Microscopy			
Microscopy: stool	25%–60%	10%–40%	Requires specialized expertise to perform, and even the most experienced observer cannot differentiate the cysts of *E. histolytica* in the stool from less pathogenic/nonpathogenic species (*E. dispar, E. moshkovskii,* and *E. bangladeshi*)
Microscopy: abscess fluid	N/A	~40%	
Antigen Detection			
Stool antigen detection	90%	~40%	Offers improved sensitivity and specificity over stool microscopy. Relatively easy to perform and rapid.
Serum antigen detection	65%	>95%	
Molecular Detection			
Real-time polymerase chain reaction (PCR)	>95%	>95%	Best performance of all diagnostic tests. Increasing availability through incorporation on multiplex PCR panels. Can be performed on stool and abscess fluid.
Serologic Testing (Indirect Hemagglutination)			
Acute	70%	70%–80%	Antibodies detectable within 5–7 days of infection but persist for years, and so does not readily distinguish between acute or past infection in endemic areas.
Convalescent	>90%	>90%	Negative result has a strong negative predictive value.

[a]Diagnostic test sensitivity reported is for before the initiation of therapy.
N/A, Not applicable.
Modified from Haque R, Huston CD, Hughes M, et al. Current concepts: amebiasis. N Engl J Med. *2003;348:1565–1573.*

detection or by molecular analysis, or at extraintestinal sites of invasion (such as liver abscess pus). The traditionally utilized microscopic examination of stool is of limited value and should not be utilized if other modalities are available for diagnosis. This section reviews the use of the O&P examination, culture, antigen detection, PCR, serologic study, colonoscopy and biopsy, and imaging for the diagnosis of amebiasis (Table 272.1).

Stool Ova and Parasite Examination

Inadequacies of the stool O&P examination have been appreciated since at least 1978, when Krogstad and colleagues[222] showed that its insensitivity and lack of specificity led to frequent misdiagnosis of amebiasis, with at times fatal results. Surprisingly, 30 years later the stool O&P examination remained the most common test ordered by US physicians when intestinal amebiasis was suspected.[223] In this survey of 2800 physicians from five US states, 97% of respondents believed that a routine O&P examination tested for *E. histolytica.*[223] The glaring problems with stool O&P examination were highlighted in a prospective study of 112 patients presenting at three Canadian centers with amebiasis symptoms (at least three loose bowel movements per day, abdominal pain, bloody stool, or weight loss) or risk factors (travel to or immigration from the tropics in the previous 2 years; men who have sex with men) or both. The specificity of stool O&P examination in community laboratories was an astoundingly low 10%.[34] The problems with stool O&P examination were magnified by the fact that only 5% (3) of the 65 positive results for *E. histolytica–E. dispar* complex were in fact *E. histolytica.*[34] This is because it is not possible with a stool O&P examination to distinguish morphologically the three closely related and common amebae: pathogenic *E. histolytica* and commensal *E. dispar* and *E. moshkovskii.*

In most industrialized countries, *E. dispar* is 10 times more common than *E. histolytica,*[1,30,34] and even in a developing country, *E. histolytica* and *E. dispar* can be equally prevalent.[18] The presence of ingested erythrocytes was the sole morphologic characteristic that was of some use in identifying *E. histolytica;* however, in one study, it was present in only 68% of cases of *E. histolytica* but also present in 16% of cases of *E. dispar.*[18] A third species of *Entamoeba* that is morphologically identical to *E. histolytica* is *E. moshkovskii.*[5,6,35–37] In a study of preschool children from an urban slum, *E. moshkovskii* was present in 21%, *E. histolytica* in 16%, and *E. dispar* in 36%[6]; in another study from Tanzania of approximately 100 HIV-infected individuals with diarrhea, *E. moshkovskii* was present in 13%, *E. histolytica* in 4%, and *E. dispar* in 5%.[36] In Sydney, Australia, 50% of *Entamoeba* organisms identified by stool O&P examination were *E. moshkovskii.*[37] Anecdotal experience confirms continued difficulties in misdiagnosis in the United States.[208,224] We conclude that the stool O&P examination suffers from insensitivity and the inability to distinguish *E. histolytica* from *E. dispar* and *E. moshkovskii.*

Culture

Culture of *E. histolytica* from stool samples is available in only a few research laboratories worldwide.[225] Culture is, in general, more sensitive than stool O&P examination but significantly less sensitive than antigen detection or PCR.[7] It is also not specific for *E. histolytica,* and thus an *E. histolytica*–specific antigen detection or PCR test must be used on the cultured material.

Antigen Testing for Amebiasis

The only fecal antigen test that distinguishes *E. histolytica* from *E. dispar* and *E. moshkovskii* is the TechLab (Blacksburg, VA) *E. HISTOLYTICA II* enzyme-linked immunosorbent assay (ELISA). This microwell ELISA, which detects the Gal/GalNAc adherence lectin of *E. histolytica,* is more sensitive than stool O&P examination or culture, and it is rapid (<2 hours).[7] A limitation is the need for fresh or frozen stool samples for the analysis by antigen detection. Other antigen detection tests are the RIDASCREEN *E. histolytica* immunoglobulin G (IgG) enzyme immunoassay for serum antibody (R-Biopharm, Darmstadt, Germany) and the Triage Micro Parasite Panel (Biosite Diagnostics, San Diego).[226] Neither of these tests can distinguish *E. histolytica* from *E. dispar,* and so they would at best serve as screening tools, with additional specific testing for *E. histolytica* required for all positive results. The TechLab *E. HISTOLYTICA II* ELISA was also found to be more sensitive than the RIDASCREEN enzyme immunoassay.[227] The Triage Micro Parasite Panel has the advantage of coupling the testing of *E. histolytica–E. dispar* with that of *Giardia lamblia* and *Cryptosporidium parvum,* covering the three most common parasites that cause diarrhea in the United States; its disadvantage is that it requires a tedious multistep process of fecal processing and filtration. The TechLab *E. HISTOLYTICA QUIK CHECK,* a rapid test for detection of *E. histolytica* in stool, has proved sensitive and specific in studies from Bangladesh,[7] Canada,[34] The Netherlands,[228] the United Kingdom,[229] and India.[230] In a single study, researchers observed a discrepancy between the results of PCR and antigen detection,[231] and it is not clear if this discrepancy was attributable to false-positive PCR results, which is a recognized issue with some PCR formats for *E. histolytica.*[232] Antigen detection is also useful in the diagnosis of amebic liver abscess. In one study, the TechLab *E. HISTOLYTICA II* assay detected Gal/GalNAc lectin in the sera of 22 of 23 patients (96%) with amebic liver abscess before they underwent treatment with the antiamebic drug metronidazole.[7] For liver abscess pus, it was 41% to 74% sensitive[7,233] for detection of the parasite. Furthermore, for stool specimens collected at the time of diagnosis of amebic liver abscess (and before metronidazole treatment), it detected the parasite in 43% (3 of 7).[7] We conclude that the TechLab *E. HISTOLYTICA II* ELISA antigen detection test has sensitivity and specificity superior to those of stool O&P examination, and its sensitivity is inferior but its specificity

is comparable to those of PCR, but it is technically simpler to perform. Recently, clinical evaluation of the *E. HISTOLYTICA QUIK CHEK* stool antigen detection test showed that the sensitivity and specificity are 98% and 100%, respectively, compared with the *E. HISTOLYTICA II* ELISA.[234] False-negative results should be rare. In one case of amebic dysentery reported from Spain, the enzyme immunoassay was initially negative, but became positive with subsequent testing. Interference of antigen detection by pH or amebic lytic activity was postulated by the authors. Repeat testing of the stool should be considered if high clinical suspicion remains, and serology is also a useful adjunct in such cases.[235]

Polymerase Chain Reaction Testing for Amebiasis

Real-time PCR is superior in sensitivity to stool antigen detection, and is considered the gold standard for diagnosis.[233] PCR tests are becoming more widely available through US Food and Drug Administration–cleared gastrointestinal panels such as the xTAG Gastrointestinal Pathogen Panel (Luminex, Austin, TX) and the BioFire FilmArrray GI Panel (BioFire Diagnostics, Salt Lake City, UT), though *E. histolytica* is not a target of the VERIGENE Enteric Pathogens Test (Luminex).[236,237] There are several real-time PCR assay formats for *E. histolytica,*[8,9,233,238] many of which are reviewed by Qvarnstrom and colleagues[232] and Fotedar and associates.[239] These tests are more sensitive than conventional PCR. Real-time PCR is also a sensitive test for detection of *E. histolytica* in liver abscess pus.[240] In one study, real-time PCR yielded positive results in 20 of 23 liver abscess pus specimens; the 3 specimens with negative findings had been collected from patients who had already received antiamebic therapy (8 days for one patient and 30 days for two patients).[233] Detection of *E. histolytica* DNA in saliva and urine could also be used as a diagnostic tool for amebic liver abscess.[241]

Serologic Tests for Amebiasis

Serologic tests are a cornerstone of the diagnosis of amebic liver abscess and are an important adjunct in the diagnosis of intestinal amebiasis. However, it is important to realize that false-negative results may be obtained early in the course of intestinal amebiasis and amebic liver abscess.[7,206,210,242] The serologic tests are therefore best used in conjunction with antigen detection or PCR for *E. histolytica.* In general, different assays for amebic antibodies behave similarly, but commercially the enzyme immunoassay is more widely available than the indirect hemagglutination assay.[243,244] In one study, the RIDASCREEN enzyme immunoassay was reported to have sensitivity of 97%.[245] The Microtiter ELISA (LMD Laboratories, Carlsbad, CA), the ImmunoTab assay (Institut Virion, Wurzburg, Germany), and an indirect hemagglutinin assay (Behring Diagnostics, Marburg, Germany) were compared in one study of amebic liver abscess patients from Kuwait.[244] All three tests had equal sensitivities of 98% to 99%. The ImmunoTab and Microtiter ELISA specificities were 95%, less than the 99.8% calculated specificity of the indirect hemagglutinin assay. The lower specificity was attributed to the high (5%) background titer of *E. histolytica* infection in individuals from endemic countries. The specificity of antibody tests for amebiasis is limited by the 5% to 10% baseline seropositivity of populations from endemic countries, because patients will remain seropositive for years after infection.

Serologic tests for *E. histolytica* intestinal infection are generally less sensitive than those for amebic liver abscess, and seropositivity is rarely detected in asymptomatic carriers.[7,31,246] In a study in Egypt, IgG antibodies to the Gal/GalNAc lectin were found in the sera of 89% of patients with amebic colitis,[247] and in a small study from the Netherlands, 86% (six) of seven patients with intestinal amebiasis were seropositive. Serologic study can be particularly helpful when *E. histolytica*–specific stool diagnostic techniques (antigen detection or PCR) are not available because infection with *E. histolytica,* and not *E. dispar* or *E. moshkovskii,* results in seroconversion.[1,242] Recently, a sensitive bead assay has been used to detect IgG responses against *E. histolytica* lectin adhesion antigen.[248] In conclusion, serologic study is an important part of the diagnosis of intestinal and extraintestinal amebiasis. A limitation of current tests is the lack of a point-of-care test for single use at the patient's bedside. False negatives have occasionally been reported.[249]

Colonoscopy and Biopsy

Colonoscopy and biopsy can be helpful in the diagnosis of intestinal amebiasis.[250–252] Amebae can be difficult to visualize in the biopsy samples, and periodic acid–Schiff stains or, ideally, immunoperoxidase with anti–*E. histolytica* antibodies, can help to identify the parasites (see Fig. 272.9).[253] A limitation of colonoscopy is that it is an invasive procedure and not widely available in developing nations.

Imaging

Ultrasound, computed tomography, and magnetic resonance imaging can all be useful imaging modalities in the diagnosis of amebic liver abscess (see Fig. 272.10). By ultrasound, a solitary cystic intrahepatic hypoechoic lesion is often seen, most typically involving the right lobe. With computed tomography scanning, an abscess with a nonenhancing center surrounded by a rim of inflammation can be seen following contrast administration.[254]

THERAPY

It is recommended that all infections with *E. histolytica* be treated; however, therapy for invasive infection differs from therapy for noninvasive infection (Table 272.2).

TABLE 272.2 Drug Therapy for Treatment of Amebiasis

DRUG	ADULT DOSAGE	SIDE EFFECTS
Amebic Liver Abscess[a]		
Metronidazole[b]	750 mg PO tid × 10 d	Primarily GI side effects: anorexia, nausea, vomiting, diarrhea, abdominal discomfort, or unpleasant metallic taste; disulfiram-like intolerance reaction to alcoholic beverages; neurotoxicity, including seizures, peripheral neuropathy, dizziness, confusion, irritability
or		
Tinidazole	2 g PO once daily × 5 d	Primarily GI side effects and disulfiram-like intolerance reaction to alcoholic beverages for 5 days
Followed by a luminal agent		
Paromomycin	30 mg/kg/d PO in three divided doses per day × 5–10 d	Primarily GI side effects: diarrhea, GI upset
or		
Diloxanide furoate	500 mg PO tid × 10 d	Primarily GI side effects: flatulence, nausea, vomiting Pruritus, urticaria
Amebic Colitis[c]		
Metronidazole	750 mg PO tid × 10 d	Same as for amebic liver abscess
Tinidazole	2 g PO once daily × 5 d	Same as for amebic liver abscess
Plus a luminal agent (same as for amebic liver abscess)		
Asymptomatic Intestinal Colonization		
Treatment with luminal agent as for amebic liver abscess		

[a]Amebic liver abscess may necessitate antiparasitic treatment plus percutaneous or surgical drainage. Nitazoxanide may be effective therapy as well, but clinical experience is limited.
[b]Drug of choice for treatment of amebic liver abscess.
[c]Amebic colitis may necessitate antiparasitic treatment plus surgical treatment if complicated by toxic megacolon, perforation, peritonitis, or other intraabdominal complication.
GI, Gastrointestinal; *PO,* by mouth; *tid,* three times daily.
Modified from Haque R, Huston CD, Hughes M, et al. Current concepts: amebiasis. N Engl J Med. *2003;348:1565–1573.*

Noninvasive Infections

Noninvasive infections, characterized by asymptomatic detection of infection with *E. histolytica,* may be treated with a luminal agent, among which the nonabsorbable aminoglycoside paromomycin is recommended. Diiodohydroxyquin and diloxanide furoate can also eliminate intestinal cysts, but safety concerns limit the use of iodoquinol, which has been associated with optic atrophy and peripheral neuropathy, in particular with long courses or high doses.

Invasive Infections

The nitroimidazoles, particularly tinidazole and metronidazole,[255] are the mainstay of therapy for invasive amebiasis, including amebic colitis and amebic liver abscess (see Table 272.2). Nitroimidazoles with longer half-lives (namely, tinidazole, secnidazole, and ornidazole) are better tolerated and allow shorter periods of treatment. Metronidazole is as effective as tinidazole at clearing parasites.[256,257] Tinidazole and metronidazole are both available in the United States. Approximately 90% of patients with mild-to-moderate amebic dysentery have a response to nitroimidazole therapy. Nitazoxanide is a relatively newer alternative therapy. In a small, single-center trial performed in Egypt, over 90% of patients treated for hepatic or intestinal amebiasis with nitazoxanide demonstrated both clinical and microbiologic response.[258] Of concern was the high response rate in the placebo group, with 40% to 50% of those receiving placebo showing clinical and microbiologic resolution, so until more is known, a nitroimidazole should be considered the preferred treatment over nitazoxanide, when available.

In the rare case of fulminant amebic colitis, it is prudent to add broad-spectrum antibiotics to treat intestinal bacteria that may spill into the peritoneum. Metronidazole is often administered intravenously in these patients, if they are unable to tolerate enteral administration, though there is limited experience with intravenous metrondiazole.[259] Surgical intervention is indicated for acute abdomen, gastrointestinal bleeding, or toxic megacolon.

Parasites persist in the intestine in up to 40% to 60% of patients who receive nitroimidazole. Therefore, tinidazole or metronidazole treatment should be followed with the nonabsorbable aminoglycoside paromomycin or the second-line agent diloxanide furoate to cure luminal infection. A nitroimidazole and paromomycin should not be given at the same time because diarrhea, a common side effect of paromomycin, may make it difficult to assess the patient's response to therapy.[260–262]

Therapeutic aspiration of an amebic liver abscess is occasionally required as an adjunct to antiparasitic therapy. Drainage of the abscess should be considered in patients who have no clinical response to drug therapy within 5 to 7 days or those with a high risk of abscess rupture, as defined by a cavity with a diameter of greater than 5 to 10 cm or by the presence of lesions in the left lobe.[254] Bacterial coinfection of amebic liver abscess has occasionally been observed (both before and as a complication of drainage), and it is reasonable to add antibiotics, drainage, or both to the treatment regimen in the absence of a prompt response to nitroimidazole therapy. Imaging-guided percutaneous treatment (needle aspiration or catheter drainage) has replaced surgical intervention as the procedure of choice for reducing the size of an abscess.[254]

PREVENTION

Prevention of fecal contamination of food and water has dramatically reduced transmission of amebiasis in industrialized nations. Transmission can be further reduced by the use of safe sexual practices in men who have sex with men and by proper maintenance of municipal water supplies to prevent access of chlorine-resistant amebic cysts to the treated water supply. In developing nations, however, more than 1 billion people still have no access to safe food and water.[263]

Travelers to endemic areas should be given advice on the avoidance of risk factors for acquisition of infection. Infection with *E. histolytica* should be considered prior to the diagnosis of inflammatory bowel disease in anyone with a relevant travel history, given the overlapping clinical presentation. Similarly, infection with *E. histolytica* should be excluded with appropriate diagnostic screening prior to the administration of corticosteroids in anyone with a relevant travel history to prevent the development of fulminant colitis in the setting of unrecognized amebiasis.[201]

An effective vaccine is a desirable and feasible goal. The high incidence of amebiasis in community-based studies suggests that an effective vaccine would improve child health in developing countries. Because humans naturally acquire partial immunity against intestinal infection, an effective acquired immune response should be achieved. Aiding vaccine design is the demonstration that several recombinant antigens, including the Gal/GalNAc-specific lectin, provide protection in animal models of amebiasis and that human immunity is linked to intestinal and breast milk IgA against the lectin.[185,264–269] The clonal-population structure of *E. histolytica* and, specifically, the high degree of sequence conservation of the Gal/GalNAc-specific lectin suggest that a vaccine could be broadly protective. Finally, the absence of epidemiologically significant animal reservoirs suggests that herd immunity could interrupt fecal-oral transmission in humans. The challenges will be to design vaccines capable of eliciting durable mucosal immunity, to understand the correlates of acquired immunity, and, most important, to enlist the continued support of industrialized nations to combat diarrheal diseases of children in developing countries.

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The complete reference list is available online at Expert Consult.

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273 Free-Living Amebae

Anita A. Koshy, Brian G. Blackburn, and Upinder Singh[a]

SHORT VIEW SUMMARY

Microbiology and Epidemiology

- Free-living amebae are protists that are common in the environment worldwide but rarely cause recognized human infections. The trophozoite stages of these organisms feed on bacteria and debris in the environment.
  - *Naegleria fowleri* is widely distributed globally and has been isolated from fresh water, most commonly in warm environments.
  - *Acanthamoeba* spp. are ubiquitous members of the environment and are found worldwide in soil and fresh water.
  - *Balamuthia mandrillaris* is also likely widely distributed and has been isolated from soil.

Clinical Manifestations and Diagnosis

- *N. fowleri* typically causes a fulminant meningoencephalitis in healthy, immunocompetent young patients, usually in association with immersion in warm fresh water. The disease is nearly always fatal, with few survivors reported to date.
  - The cerebrospinal fluid (CSF) profile of patients with N. fowleri primary amebic meningoencephalitis (PAM) is similar to that of bacterial meningitis (high white cell count and neutrophil predominance, low glucose, high protein) but with a negative Gram stain and culture. Motile trophozoites can sometimes be seen on wet mount of the CSF. Polymerase chain reaction (PCR) on CSF is the preferred diagnostic test.
  - Neuroimaging studies in patients with PAM are usually nonspecific.
- *Acanthamoeba* spp. and *B. mandrillaris* cause the subacute onset of focal neurologic deficits and mental status changes (granulomatous amebic encephalitis [GAE]) related to central nervous system mass lesions. *Acanthamoeba* is mostly seen in immunocompromised and debilitated individuals. *Balamuthia* occurs in both immunocompromised and immunocompetent patients and has been associated with both acute and subacute presentations. The case-fatality rate for these infections is also high.
  - In the United States, *Balamuthia* GAE may be more common in male Hispanic patients than in other groups.
  - CSF studies of patients with GAE are usually nonspecific, and it is rare to isolate organisms from the CSF.
  - Neuroimaging studies generally reveal multiple space-occupying lesions in the brain, with or without contrast enhancement. Hemorrhage within the lesions may suggest *Balamuthia*.
  - Biopsy of involved tissues (skin, brain, etc.) can be diagnostic, usually via histopathologic examination/immunohistochemical staining or PCR. Next-generation sequencing technology that does not require an a priori suspicion for *Acanthamoeba* or *Balamuthia* is often diagnostic in the right clinical circumstance; some institutions are now making this approach readily available for clinical samples (see http://nextgendiagnostics.ucsf.edu/our-diagnostic-lab/ and http://nextgendiagnostics.ucsf.edu/providers/).
- *Acanthamoeba* spp. and *B. mandrillaris* can involve other sites (lungs, sinuses, adrenal glands, kidneys, and skin).
- *Acanthamoeba* spp. also cause sight-threatening keratitis in otherwise healthy individuals in association with contact lens use. Diagnosis depends on a high clinical suspicion, in conjunction with in vivo confocal microscopy or demonstration of *Acanthamoeba* in corneal scrapings or biopsy specimens by histopathologic examination, culture, or PCR.

Therapy

- Therapeutic regimens for free-living amebic infections of humans are not well defined.
- Treatment for *N. fowleri* PAM should include high-dose intravenous amphotericin deoxycholate; intrathecal amphotericin may also provide some benefit, and the addition of azoles, rifampin, azithromycin, miltefosine, other antimicrobials, and dexamethasone should be strongly considered, along with aggressive management of increased intracranial pressure.
- *Acanthamoeba* keratitis should be treated with topical chlorhexidine or polyhexamethylene biguanide; adjunctive surgical therapy may also be necessary.
- *Acanthamoeba* GAE should be treated with combination antimicrobial regimens, possibly including pentamidine, an azole, a sulfonamide, miltefosine, and flucytosine. However, the most efficacious regimen is not currently known.
- *B. mandrillaris* GAE should be treated with combination antimicrobial regimens, possibly including pentamidine, flucytosine, a sulfonamide, albendazole, an azole, a macrolide, amphotericin, and/or miltefosine. However, the most efficacious regimen is not currently known.
- Surgical débridement may play an adjunctive role in the management of both forms of GAE.

Free-living amebae (FLAs) are aerobic, eukaryotic protists that comprise several genera. Clinically apparent infection of humans with FLAs is an infrequent but often fatal occurrence in both normal and immunocompromised individuals. Central nervous system (CNS) invasion by *Naegleria fowleri, Acanthamoeba* spp., and *Balamuthia mandrillaris* has been reported in hundreds of patients worldwide, with thousands of *Acanthamoeba* keratitis cases described.[1–4] Other FLA species have each been reported as the cause of disease in single human cases and are discussed briefly below.

Distinct from other pathogenic protozoa by nature of their free-living existence, these organisms have no known insect vectors, no human carrier states of epidemiologic importance, and little relationship between poor sanitation and their transmission. Four distinct clinical syndromes are caused by the FLAs that infect humans: (1) primary amebic meningoencephalitis (PAM), (2) granulomatous amebic encephalitis (GAE), (3) disseminated granulomatous amebic disease (e.g., skin, pulmonary, and sinus infection), and (4) amebic keratitis.

PAM is caused almost exclusively by *N. fowleri* and occurs most commonly in healthy children and young adults with recent recreational freshwater exposure, usually warm lakes, rivers, or streams. Increasingly, however, it is being recognized that *N. fowleri* can be found in household water systems, with some infections occurring through tap

[a]Dr. Blackburn and Dr. Singh are co-senior authors and contributed equally to the writing of this text.