



## Host-parasite interactions and mechanisms of infection in amebiasis

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### ABSTRACT

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Amebiasis is caused by *E. histolytica*, which is the only species pathogenic in humans, while the pathogenicity of *E. dispar* and *E. moshkovskii* is still unclear. The disease is endemic in the developing countries, mainly due to poor sanitation and lack of clean water supplies. Infection occurs by ingestion of *E. histolytica* cysts in fecally contaminated food or water. Excystation in the small intestine releases motile invasive trophozoites which migrate to the large intestine, adhere to the colonic epithelium by means of galactose and an amebic surface antigen, N-acetyl-D-galactosamine-specific lectin. This results in killing of epithelial cells, neutrophils, and lymphocytes by the trophozoites, presumably through secretion of the pore-forming proteins called amebapores and activation of caspase 3. The trophozoite virulence factor, cysteine proteinase, induces an inflammatory response, resulting in neutrophil-mediated damage. Hematogenous spread of trophozoites causes extraintestinal amebiasis, particularly amebic liver abscess (ALA), in the formation of which caspase 3 presumably also plays a role. The trophozoites in the liver induce tissue destruction, cellular necrosis and formation of microabscesses that coalesce into a large solitary abscess in 65-75% of cases. Results from pediatric studies reveal that partial immunity is acquired after infection with *E. histolytica*, the immunity however declining with age.

**Keywords:** Amebiasis, host-parasite interactions, host defenses

### INTRODUCTION

Diarrhea still constitutes a public health problem in many developing countries, being associated with the morbidity rate and the impact on children in the form of retarded growth and sequelae. It has been reported that around 2.5 million deaths occur annually in developing countries as a result of diarrheal diseases.<sup>(1)</sup> The annual prevalence of dysenteric diarrhea caused by the *Shigella* bacterium

worldwide is around 164.7 million, with 163.2 million in the developing countries.<sup>(1)</sup> Another disease besides shigellosis that also induces symptoms of dysentery is amebiasis, which globally is a parasitic infection with a high mortality rate. The World Health Organization (WHO) jointly with the Pan American Health Organization (PAHO) have defined amebiasis as an infection caused by *Entamoeba histolytica*, irrespective of clinical symptoms.<sup>(2)</sup> The parasite has a worldwide distribution, but

it has been reported that prevalences exceeding 10% are frequently encountered in developing countries. In developed countries the parasite infects mainly travelers to developing countries and immigrants. There are reports from Bangladesh that dysenteric diarrhea due to *E. histolytica* kills 1 in 30 children under 5 years of age.<sup>(3)</sup> The presumed diagnosis of *E. histolytica* infection is mainly based on stool examination against the parasite, but this method is not sensitive because *E. histolytica* is morphologically indistinguishable from nonpathogenic *Entamoeba* species.

The genus *Entamoeba* consists of many species, six among them being *E. histolytica*, *E. dispar*, *E. moshkovskii*, *E. polecki*, *E. coli* and *E. hartmanni*, located in the human intestinal lumen. *E. histolytica* is the sole species that is definitely pathogenic in humans. Although several recent studies found *E. dispar* and *E. moshkovskii* in patients with gastrointestinal symptoms, there is no unequivocal evidence of an association between both species and the pathology and symptoms exhibited by the patients.<sup>(4-6)</sup>

The clinical picture of *E. histolytica* amebiasis ranges from asymptomatic colonization to dysenteric diarrhea and invasive extraintestinal amebiasis, commonly in the form of liver abscess. Although effective therapy is available, the morbidity and mortality of amebic infections is still high, a mortality rate of 100,000 per 50 million patients with liver abscess per year having been reported.<sup>(7)</sup> From Hue City, Vietnam,<sup>(8)</sup> it was reported that the annual incidence of amebic liver abscess is around 25 per 100,000 population, whereas in the United States oral-fecal transmission rarely occurs. Amebiasis is more severe in very young and old patients,<sup>(9)</sup> indicating the ineffectiveness of interventions performed for elimination of this disease. Man is the sole host for this parasite, such that there is a need for an effective control program designed for eradication of amebiasis. One of the most important clinical questions arising

is with regard to the existence of protective immunity against amebiasis. There is still a scarcity of data from human studies on protective immunity and most of them are difficult to interpret, because in the majority of cases infection by *E. histolytica* is almost indistinguishable from colonization by *E. dispar*.<sup>(10)</sup>

## EPIDEMIOLOGY

The epidemiology of the *E. histolytica* parasite is still not known with certainty, because of the morphological similarities between the three species of *Entamoeba*, namely *E. histolytica*, *E. dispar* and *E. moshkovskii*. Several reports state that *E. moshkovskii* is frequently found in areas endemic for *E. histolytica*, thus hampering determination of the prevalence of amebiasis.<sup>(11,12)</sup> The worldwide prevalence of *E. histolytica* and *E. dispar* as distinct species has not been investigated extensively and the prevalence of *E. moshkovskii* is practically unknown. On subcontinents such as India, Africa, Asia, and South and Central America, the prevalence is fairly high. In developing countries the prevalence depends on cultural practices, socioeconomic conditions, age, inadequate supply of clean water, population density, and poor sanitation, facilitating fecal-oral transmission of the parasites from one person to another. In developed countries, infections are mostly caused by *E. dispar* and in the majority of cases are limited to certain community groups. Infections by *E. moshkovskii* in humans are reportedly asymptomatic, but the parasite is considered potentially capable of causing disease in humans. Most of the data come from studies using methods that are incapable of differentiating the three aforementioned species. The difficulty of distinguishing the three *Entamoeba* species led the World Health Organization (WHO) to recommend the development and application of improved

methods for specific diagnosis of *E. histolytica* infection.<sup>(2)</sup> Epidemiological surveys on amebiasis should involve the utilization of test instruments capable of differentiating between *E. histolytica*, *E. dispar* and *E. moshkovskii*, individually, simultaneously, and accurately. Single-round PCR assay is reported to be an accurate, rapid, and effective diagnostic method for the detection and discrimination of the three aforementioned *Entamoeba* species as an alternative tool in both routine diagnosis of amebiasis and epidemiological surveys. However, to date no inexpensive laboratory tests are available for use in endemic areas of amebiasis. The identification of *E. histolytica* remains the principal target of the clinical parasitology laboratory and accurate and definite diagnosis constitutes an important step in the management of patients infected with *E. histolytica*.

It has been known for a long time that infection with *E. histolytica* results in pathology in the host, although not in all of those infected. However, recently it became clear that 1 in 4 infections with *E. histolytica* lead to clinical symptoms.<sup>(7,12)</sup> Thus the parasite remains a significant cause of morbidity and mortality for the populations of developing countries. In Bangladesh the annual incidence of amebic dysentery in pre-school children is reported to be 2.2%, in comparison with the annual incidence of 5.3% for *Shigella* dysentery, and 1 in 30 children die of diarrhea or dysentery before the age of 5 years.<sup>(15)</sup>

Acuna-Soto et al.<sup>(16)</sup> in their summary of cases from various reports between 1929 and 1997 found the male-to-female ratio for invasive intestinal amebiasis and asymptomatic carriers to be respectively 3.2:1 and 1:1, where many asymptomatic carriers were due to *E. dispar*.

Some studies reported that the prevalence rate of acute amebic diarrhea was around 1.5% in persons recently returning from travels to Southeast Asia and 3.6% in persons recently returning from Central America, while the overall prevalence rate was 2.7%.<sup>(17)</sup> The

prevalence rate of amebiasis in the United States is around 4%, with asymptomatic *E. dispar* infections being tenfold more frequent than *E. histolytica* infections. In addition, only 10% of the latter are invasive, so that only 1% of individuals with *Entamoeba*-positive stools on microscopic examination exhibit symptoms of amebiasis.

Invasive amebiasis, including amebic liver abscess, is more frequently found in males than in females, while in prepubertal children amebic liver abscess is equally distributed in both genders. However, amebic liver abscess is tenfold more frequent in adults than in children. It is suggested that the higher proportion of amebic liver abscess in males is due to their susceptibility to invasive amebiasis.<sup>(18)</sup>

## **PATHOGENESIS OF AMEBIASIS**

The asymptomatic passage or entry of cysts is the most common manifestation of *E. histolytica*, but this conclusion comes from studies using microscopic examination of fecal samples.<sup>(6)</sup> In the stool, cysts are commonly seen but trophozoites are rarely encountered. Individuals with asymptomatic *E. histolytica* infection may form antibodies, even though there are no invasive abnormalities. However, untreated asymptomatic colonization with *E. histolytica* may lead to amebic dysentery and various invasive disorders.<sup>(9)</sup> Haque et al.<sup>(18)</sup> reported that after a one-year follow-up, 4-10% of individuals with asymptomatic *E. histolytica* colonization had colitis or extraintestinal disorders.

### **Intestinal amebiasis**

Ingestion of quadrinucleate cysts of *E. histolytica* in food or water contaminated with fecal matter initiates infection. This event is a daily occurrence for inhabitants in developing countries and constitutes a threat to inhabitants of developed countries.<sup>(18,19)</sup> The infective form of the cyst survives in the stomach and

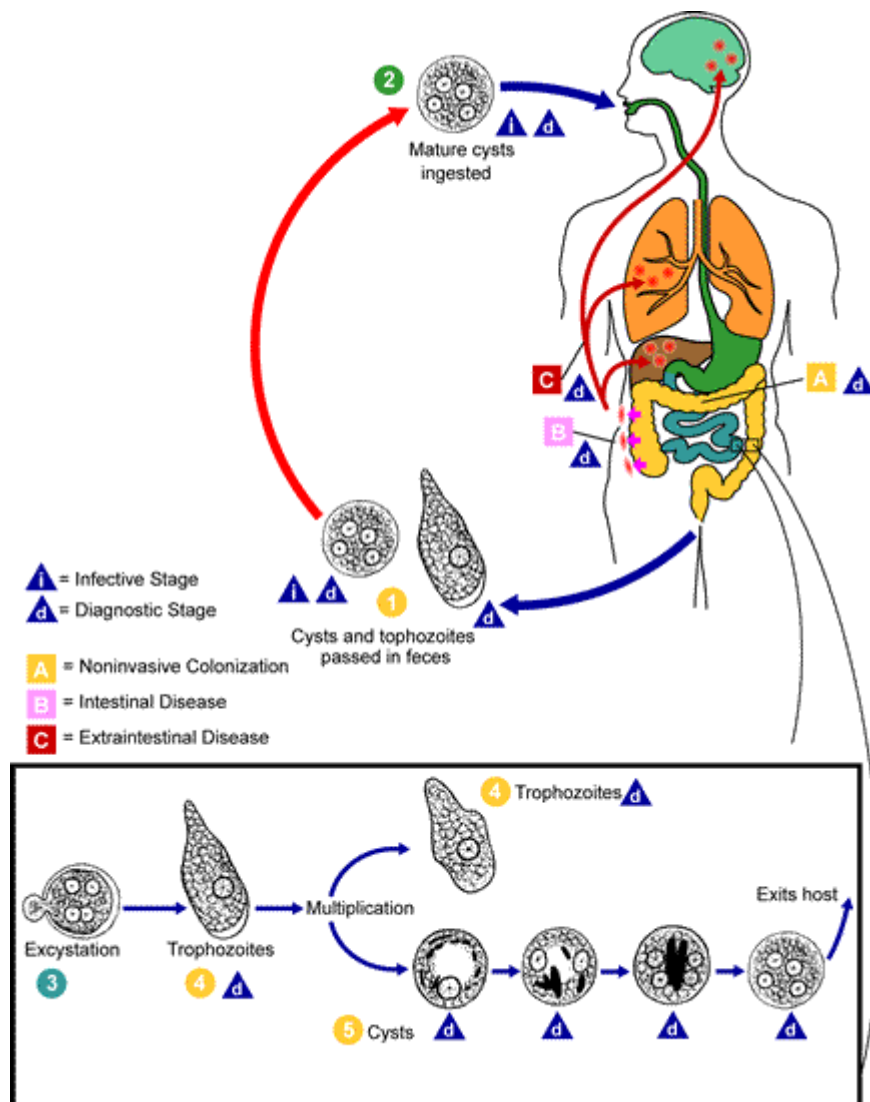


Figure 1. Life cycle of *Entamoeba histolytica*<sup>(22)</sup>

intestines. Excystation takes place within the lumen of the small intestine, whereafter motile invasive trophozoites are released to migrate into the lumen of the large intestine. Trophozoite adherence occurs by means of galactose and N-acetyl-D-galactosamine (Gal/GalNAc)-specific lectin, which is a surface molecule of the ameba. The trophozoite adheres to the colonic mucin layer, thereby colonizing the large intestine.<sup>(20,21)</sup> The reproduction of the trophozoites lacks a sexual cycle and the overall population of *E. histolytica* is presumably clonal. In most infections, the trophozoites aggregate in the

mucin layer and form new cysts by binary fission, thus resulting in a self-limited and asymptomatic infection. The cysts that are excreted in the stool maintain the life cycle by further fecal-oral spread. Being protected by their thick walls the cysts are capable of surviving for days to weeks in the external environment and play a role in the transmission of the infection. In contrast, the trophozoites that are excreted in the stool and come to be outside the human body are rapidly incapacitated and die. Ingested trophozoites do not survive within the human stomach. Commonly the trophozoites remain within the

intestinal lumen as a noninvasive infection in asymptomatic individuals, who thus become carriers releasing new cysts. Cysts are typically found in formed stools whereas trophozoites are found in liquid stools.

In a number of cases, adherence of trophozoites to colonic epithelium results in epithelial lysis, initiating invasion of the colon by trophozoites or hematogenous spread of trophozoites to extraintestinal locations, with their various pathological manifestations. In view of the fact that purified lectin has no cytotoxic effect even in high concentrations, it is assumed that cytolysis is effected by adhesins through stimulation of actin polymerization. Neutrophils react to the invasion and cause cellular injury locally. Invasion of intestinal epithelium is followed by extraintestinal spread to the peritoneum, liver, and other locations.<sup>(9,18)</sup>

Invasive intestinal disease may occur days to years after initial infection and is characterized classically by abdominal pain and bloody diarrhea. Watery or mucus-containing diarrhea, constipation, and tenesmus may also occur. This clinical picture corresponds histologically with trophozoites invading and laterally undermining the intestinal surface to form the so-called flask-shaped ulcers (Figure 2).<sup>(19)</sup>

Colitis occurs when the trophozoites penetrate the intestinal mucosa, which functions as a barrier to invasion by preventing adhesion of the amebae to the epithelium and by decreasing trophozoite motility.<sup>(9,18)</sup> Trophozoite invasion is mediated by the killing of epithelial cells, neutrophils, and lymphocytes by the trophozoites. These events do not occur until lectins of the parasite bind to host N-acetyl-D-galactosamine groups on O-

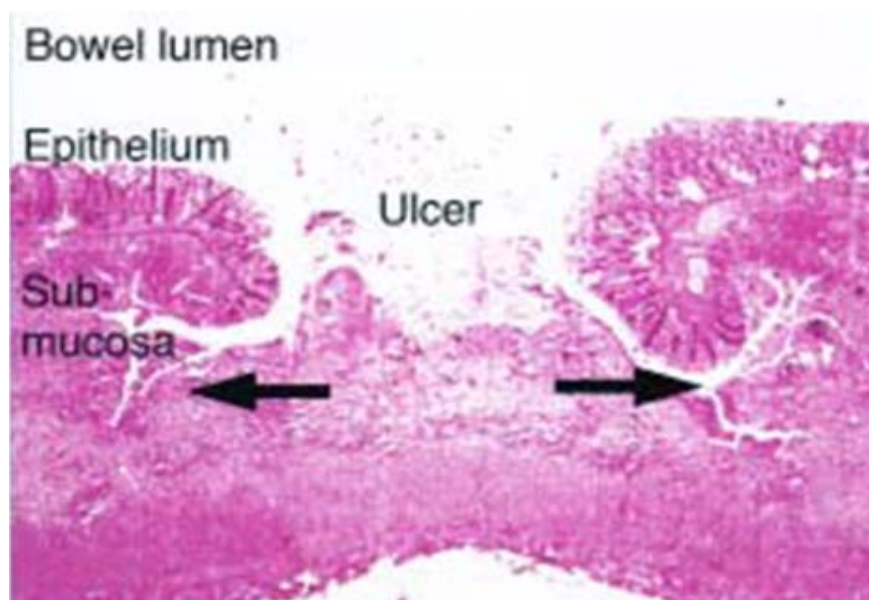


Figure 2. “Flask-shaped” ulcer of invasive intestinal amebiasis (hematoxylin-eosin, original magnification  $\times 50$ ). Note that the apex of the ulcer at the bowel lumen is narrower than the base, accounting for the flask shape. This is formed as trophozoites invade through the mucosa and move laterally into the submucosa (direction of ulcer expansion is marked by arrows). Microscopically, trophozoites are localized to the advancing edges of the submucosal ulcer. Image courtesy of John Williams, CBiol, MIBiol, London School of Hygiene and Tropical Medicine<sup>(19)</sup>

linked cell surface oligosaccharides. The lectin-glycoconjugate interaction is stereospecific and multivalent. The identity of the high-affinity receptors on intestinal epithelial cells is as yet unclear. The secretion by the amebae of amebapores, which are 5-kD proteins capable of forming pores in lipid bilayers, presumably plays a role in aforementioned killing. Activation of caspase 3, a distal effector molecule in the apoptotic pathway, occurs soon after contact with the amebae, and caspases are required for killing of cells in vitro and for the formation of amebic liver abscess in vivo.<sup>(24)</sup>

Patients with amebic colitis typically show symptoms of several weeks duration, such as abdominal cramping, weight loss, and watery or bloody diarrhea. The insidious onset and variability of the symptoms and signs make the diagnosis difficult to establish. The occurrence of diarrhea with bloody stools should lead to consideration of bacterial infections with *Shigella*, *Salmonella*, *Campylobacter* and enteroinvasive and enterohemorrhagic *Escherichia coli*. Uncommon manifestations of amebic colitis may also be found, such as acute necrotizing colitis, toxic megacolon, ameboma and perianal ulceration, the latter potentially leading to fistula formation. Although extremely rare, these manifestations should be properly managed.

In developing countries, intestinal amebiasis may frequently be diagnosed by finding and identifying *Entamoeba* cysts or motile trophozoites on microscopic preparations. A drawback of this method is its poor sensitivity, as it is incapable of differentiating *E. histolytica* infection from *E. dispar* and *E. Moshkovskii* infection.<sup>(25)</sup> For definitive diagnosis, specific tests for fecal *E. histolytica* antigens, serum antiamebic antibodies, or amebic DNA, should be utilized. An important aid to fecal antigen tests is detection of serum antibodies against amebae, which may be found in more than 70% of

patients with symptomatic amebiasis.<sup>(16)</sup> However, these serologic test results remain positive for years after amebic infection, thus making it difficult to differentiate between recent and past infections.

### Extraintestinal amebiasis

The most common extraintestinal manifestation of amebiasis is amebic liver abscess (ALA). ALA is a progressive disorder caused by hematogenous spread of invasive trophozoites from the colon through the portal vein into the liver. The abscess is four times more common in the right lobe, as this part receives most of its blood supply from the cecum and ascending colon. Although amebic liver abscess may affect all ages and both genders, it occurs with greater frequency in males between 20 and 40 years of age. In certain individuals ALA is encountered concomitantly with amebic colitis, but frequently without symptoms, while microscopic examination of the stool for trophozoites and cysts of *E. histolytica* yields negative results. The possibility of amebic liver abscess should be suspected in individuals from endemic areas with fever, pain in the right upper quadrant and tenderness in the hepatic region, but rarely with jaundice. The most serious complications of amebic liver abscess are rupture of the abscess and bacterial superinfection.

The trophozoites may have infected the patient's liver for months or years before an abscess develops. In the intervening period hopefully an immune response is induced in the patient's body, resulting in the formation of circulating immunoglobulins. As mentioned above, the dominant surface antigen of the parasite is the Gal/GalNAc-specific lectin. Around 80% patients with ALA have circulating IgG against the antigens of the parasite, but this does not result in recovery from infection caused by the parasite, thus suggesting the presence of other defense mechanisms.<sup>(9,18,25)</sup>

During liver invasion by *E. histolytica*, the host will both sequentially and simultaneously deploy a number of mechanisms to kill the parasite. The first line of tissue defense is composed of innate immune system cells that recognize pathogen-associated molecular patterns (PAMPs) and trigger an inflammatory response. It was previously reported that female and male mice differ in their early responses to *E. histolytica* liver invasion.<sup>(26)</sup> In in vitro models, the effects of gamma interferon (IFN  $\gamma$ ) can be bypassed by the recognition of PPGs of *E. histolytica* by Toll-like receptor 2 (TLR2) and TLR4, which results in direct tumor necrosis factor (TNF), interleukin-12p40 (IL-12p40), and IL-8 production.<sup>(27)</sup> This proinflammatory cytokine profile underlines the importance of the early recognition of PAMPs (such as proteophosphoglycans/PPGs) and appropriate inflammatory cell recruitment and activation during ALA onset.<sup>(28)</sup>

Although the body of the host mounts a massive inflammatory response against *E. histolytica*, the parasites are capable of survival within their niche. Virulence of the parasites in their invasion of the host confers survival and invasive potential. The clonal origin of the trophozoite population cannot explain adequately why some of the trophozoites are able to survive within the host body and cause infection, whereas other trophozoites are unable to survive. This suggests the presence of local and individual adaptation mechanisms of the parasites, leading to the hypothesis of specialization of subpopulations, in which the trophozoites are capable of defense against the complement system of the host. These trophozoite subpopulations subsequently invade the host tissues, causing cell death through parasite cytotoxicity.<sup>(23)</sup>

Hepatic invasion by amebic trophozoites induce tissue destruction, cellular necrosis and formation of microabscesses that gradually coalesce. In 65-75% of cases a solitary abscess is found, although multiple abscesses may also be formed.<sup>(29)</sup> The abscesses consist of soft,

necrotic, acellular yellow-brown debris, called "anchovy paste".<sup>(30)</sup> The occurrence of severe jaundice indicates the possibility of multiple abscesses. The parasites themselves are rarely identifiable, as they are located in the peripheral parts of the lesions. In general, the symptoms of amebic liver abscess are pain in the right hypochondrium radiating to the right shoulder and scapular region. The pain increases on deep inspiration, on coughing, and on stamping the right foot when walking.

### Host immunity and inflammatory response

The interaction of the parasites with the intestinal epithelium results in an inflammatory response marked by activation of nuclear factor  $\kappa$ B and secretion of lymphokines. The epithelial response depends on the trophozoite virulence factor cysteine proteinase, causing various intestinal abnormalities through neutrophil-mediated damage.<sup>(25)</sup> However, the neutrophils may also be protective, such as in the activation of neutrophils or macrophages by tumor necrosis factor  $\alpha$  (TNF  $\alpha$ ) or interferon  $\gamma$ , which in vitro are capable of killing amebae and of limiting the size of the liver abscess. In contrast with the severe inflammatory response that is typical of early invasive amebiasis, the inflammation occurring after the formation of colonic ulcers and liver abscess is of minimal intensity only.

In the case of the infection becoming chronic, *E. histolytica* maintains itself against the host immune response in several ways. The Gal/GalNAc-specific lectin exhibits sequence homology and antigenic cross-reaction with CD59, a leukocyte antigen that prevents the formation of the C5b-C9 membrane attack complex. Amebic cysteine proteinase rapidly degrades complement anaphylatoxins C3a and C5a, and also degrades secretory IgA and serum IgG, presumably protecting the amebas from opsonization.<sup>(18)</sup>

The immunity against *E. histolytica* infection is associated with the mucosal IgA response to the Gal/GalNAc-specific lectin.

After a period of around one year, children with the above response experience less reinfection than do children without the response. The cell-mediated response is reportedly found in patients with amebic liver abscess, characterized by lymphocyte proliferation and lymphokine secretion that is amebicidal in vitro. It should be realized that the pandemic of acquired immunodeficiency syndrome (AIDS) has not shown increased amebic invasion, although asymptomatic intestinal colonization is frequently encountered. However, this statement is still controversial, because there are reports stating that both in endemic and non-endemic areas amebic liver abscess is an infectious disease that threatens the individual with HIV-AIDS. Among 31 hospitalized patients with amebic liver abscess at the Seoul National University Hospital in 1990-2005, ten of them (32%) were HIV-AIDS patients.<sup>(31)</sup>


Recently a pediatric cohort study conducted by Haque et al.<sup>(12)</sup> included determination of fecal IgA antilectin antibodies and found that children with IgA antibodies against amebic adherence lectins showed resistance against reinfection with *E. histolytica*. However, the immunity was short-lived, and around 20% of the children in the cohort study suffered from a second episode of *E. histolytica* infection. These findings demonstrate that immunity to amebiasis may occur in a number of children after a prior infection, but that the immunity is of temporary character.

At the time of invasion of the liver by *E. histolytica*, a number of responses take place in the host, in the form of defense mechanisms for killing the parasites, consisting of immune cells that trigger an inflammatory response. Experimentally, female mice have been reported to mount a distinct response against invasion of the liver by *E. histolytica*. These animals rapidly clear the parasites from the liver, release natural killer T cells (NKTC) in larger numbers into the infected areas and

produce higher levels of gamma interferon (IFN- $\gamma$ ).<sup>(32)</sup> Deficiencies of NKTC as well as of IFN- $\gamma$  result in a higher survival rate of the parasites. IFN- $\gamma$  is an important regulator in the initial inflammatory process, because it activates the synthesis of tumor necrosis factor (TNF), which increases NO synthesis by neutrophils and macrophages.

The role of monocytes in hepatic *E. histolytica* infection is not known in detail, but it is certain that the parasite strongly activates peritoneal macrophages and circulating monocytes, leading to synthesis of cytokines that subsequently cause the destruction of the parasite.

## CONCLUSIONS

*Entamoeba histolytica* is the causative organism of the globally distributed human amebiasis. Most infections are asymptomatic, but invasion of the tissues by the parasite may result in amebic colitis and liver abscess, which is the most common extraintestinal amebic infection. The occurrence of invasive amebiasis in some individuals undergoing colonization by *E. histolytica* and of secondary infection indicates that acquired immunity against the parasite is only partial. However, it is clear that amoebic factors potentially activate peritoneal macrophages and circulating monocytes, leading to cytokine and reactive oxygen species (ROS) production and then parasite and tissue destruction. 

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