Amebiasis

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INTRODUCTION

Entamoeba histolytica is one of six parasitic amebae of the genus Entamoeba that are known to infect humans (24, 38). Entamoeba coli, E. gingivalis, E. moshkovskii, and E. hartmanni are not associated with pathologic sequelae, but E. polecki and E. histolytica are. Lösch in 1875 was the first to describe the trophozoite form of E. histolytica and the pathology associated with the infection in a patient from St. Petersburg, Russia. Although Lösch was able to infect a dog with organisms obtained from the patient, he was not able to mimic the disease produced in humans and failed to recognize the relationship (59). Councilman and LaFleur (26), in a classic monograph, described the clinical and pathological evidence of the association of E. histolytica with dysentery and liver abscesses. Quincke and Roos in 1893 described the cyst form, and Schaudinn in 1903 named E. histolytica and differentiated it from E. coli (59). Early literature used the genus names Endamoeba and Entamoeba to describe E. histolytica. In 1954, the International Commission on Zoological Nomenclature ruled that E. coli was to be the type species and that Entamoeba was to be used in place of Endamoeba to describe E. histolytica.

Walker and Sellards (154) used prisoners in the Philippines to carry out controlled experiments with E. histolytica. They noted that (i) transmission was most likely accomplished by cysts, not trophozoites; (ii) asymptomatic carriers were probably reservoirs and responsible for transmission; (iii) there were differences between individuals’ disease risk; and (iv) there were differences in organism strain virulence. Boeck and Drbohlav (11) were the first to successfully cultivate E. histolytica by using Locke’s egg serum medium. Other media frequently used to culture pathogenic strains include those described by Robinson (123), Balamuth (3), and Jones (54); however, the first axenic cultivation was accomplished by Diamond (32, 33). Culture techniques have greatly aided our understanding of E. histolytica strains and their virulence differences in infected humans.

The unclear pathogenic status of E. histolytica was noted early in the literature with the terminology “small and large race” E. histolytica. Burrows (19, 20) renamed the small race E. hartmanni on the basis of morphological criteria. Although clinical symptoms and pathological sequelae were known to vary considerably in areas where E. histolytica infection was endemic, it was not until reports of infections in homosexuals appeared that the status of nonvirulent and virulent strains took on renewed significance (44).

EPIDEMIOLOGY

E. histolytica infections occur worldwide but are more prevalent in the tropics. It has been estimated that approximately 480 million people, or 12% of the world’s population, are infected and that annual mortality is 40,000 to 110,000 persons (45). The prevalence of the infection differs from one area to another, as does the severity of disease from patient to patient. Differences in prevalence figures often depend on the detection methods used and the number of fecal examinations done. About 10% of those infected every year have clinical symptoms (155). Of the approximately 48 million persons with clinical symptoms, 80 to 98% have symptoms related to the intestinal mucosa (diarrhea or dysentery); however, in only 2 to 20% of the population with clinical symptoms do amebae invade beyond the intestinal mucosa. Evidence of recurrence of invasive colitis or amebic abscess is unusual. DeLeon (27) monitored more than 1,000 patients with amebic liver abscess for 5 years and found a recurrence rate of 0.29%.

Humans are the major reservoir of infection with E. histolytica, although natural infections in macaque monkeys...
and pigs have been reported (50). Ingestion of food and drink contaminated with *E. histolytica* cysts from human feces and direct fecal-oral contact are the most common means of infection. Transmission of *E. histolytica* by water is common in Third World countries, where much of the water supply for drinking is untreated. The use of human feces for fertilizer is also an important source of infection (81). Only the cysts are infective, and most cases originate from asymptomatic human carriers who are cyst passers. The fact that both pathogenic and nonpathogenic isolates can produce cysts may help explain the propensity for family clustering in the transmission of this organism (41). Depending on environmental conditions, cysts may remain viable for as long as 3 months. Viable cysts in water can be destroyed by hyperchlorination or iodination (57, 81).

Recognized high-risk groups include travelers, immigrants, migrant workers, immunocompromised individuals, individuals in mental institutions, and sexually active male homosexuals. *E. histolytica* has been found in as many as 32% of homosexual men in North America (1). Of special interest is the rarity of invasive disease in homosexual men and in patients with AIDS and AIDS-related complex. Almost all the strains of *E. histolytica* isolated from human immunodeficiency virus-positive and homosexual populations have, through isoenzyme analysis, been classified as nonpathogenic (1, 18, 44, 157). Human immunodeficiency virus type 1 antigens, possibly resulting from phagocytosis of infected host cells, have been detected within *E. histolytica*; however, transmission of human immunodeficiency virus type 1 from *E. histolytica* to human cells is not possible (17). The risk factors contributing to predisposition to infection and the factors contributing to increased severity of disease are primarily speculative at present. There are a few publications concerning whether individuals are more readily colonized by nonpathogenic or pathogenic isolates or whether there are virulence differences in pathogenic isolates (34, 58, 95, 97, 98, 124). Evidence supports the view that there are two morphologically identical forms of *E. histolytica*, one nonpathogenic and the other pathogenic. This information will be discussed in the sections on pathogenicity as determined by zymodemes and pathogenesis.

**LIFE CYCLE**

The life cycle of *E. histolytica* was extensively described by Dobell (35) and includes trophozoite, precyst, cyst, metacyst, and metacystic trophozoite stages. Lushbaugh and Miller (75) have reviewed the ultrastructure of *E. histolytica* obtained by scanning and transmission electron microscopy.

**Trophozoite**

Trophozoites vary in size from 12 to 60 μm in diameter, with larger forms found in tissue and smaller forms found in asymptomatic carriers (45). The large tissue forms may result from increased cellular activity, as shown by increases in the DNA and RNA content of the amebae (51, 77). Depending on environmental conditions, motility and pseudopod formation are rapid and unidirectional. However, movement rarely occurs in a straight line. The cytoplasm is clear, and the endoplasm is granular and may contain bacteria and/or erythrocytes in various stages of digestion.

On permanent stained slides, the trophozoite nucleus is spherical and one-fifth to one-sixth the size of the trophozoite. The inner surface of the nuclear membrane is evenly lined with a rim of delicate chromatin. A small, compact karyosome is generally located near the center of the nucleus. On permanent stained smears, the organism size may be reduced by 1 to 1.5 μm because of shrinkage by the staining reagents (38) (Table 1).

**Precyst**

In the precyst stage, the trophozoite becomes approximately the same size as the cyst. The cytoplasm is cleared of all food inclusions but usually contains diffusely glycogen deposits and occasional chromatoidal bodies. The chromatoid body is composed of ribosomes; however, its functional role is uncertain (68, 81, 111). The precystic form is unincu late, and the enlarged nucleus contains a karyosome that is more or less eccentric.

**Cyst**

A cyst wall develops around the precystic form, and the single nucleus divides to form the mature quadrinucleate stage. Generally, glycogen and chromatoidal material disappear as the cyst matures. Once immature cysts are cooled to room temperature, they cease to develop, and all the single- and double-nucleated cysts degenerate and die (35). On permanent stained slides, cysts ranges from 8.5 to 19 μm in diameter. Again because of shrinkage caused by the dehydration reagents, cysts may be 1 to 1.5 μm smaller than organisms seen on wet preparations. They are usually spher-
Metacyclic Trophozoite

Outside the cyst, the nuclei within the quadrinucleate ameba begin to separate from the surrounding cytoplasm and undergo division to form eight uninucleate metacytic trophozoites. The resulting trophozoites are always smaller (8 μm) than the trophozoites seen in the bowel of an infected human. The metacytic trophozoites continue to feed and grow, finally achieving the size normally associated with the trophozoite (Table 1).

PATHOGENICITY AS DETERMINED BY ZYMODEMES

In 1925, the pathogenic nature of E. histolytica was questioned by Brumpt, who thought that there were two morphologically similar strains of E. histolytica, one pathogenic and the other nonpathogenic (cited in reference 43). According to epidemiological studies, only 10% of the world's population and a few homosexual males and AIDS patients infected with E. histolytica develop disease (45, 68a, 157). One explanation for this percentage is that most people are infected with nonpathogenic strains (43). Reeves and Bischoff (122) were the first investigators to use enzyme electrophoresis to differentiate "typical" from "atypical" E. histolytica. Typical E. histolytica were those organisms, including those associated with human disease, requiring culture temperatures of 33 to 39°C, whereas atypical E. histolytica organisms could adapt to lower temperatures. This concept was further explored by Sargeaunt and associates in a series of studies designed to differentiate E. histolytica from other intestinal amebae and to distinguish pathogenic strains (36, 134-138).

A zymodeme is defined as a population of amebae in which the electrophoretic mobilities of specific enzymes differ from those in similar populations. The enzymes used to classify E. histolytica include glucosephosphate isomerase (GPI), hexokinase (HK), 1-malate:NAADP+ oxidoreductase, and phosphoglucomutase (PGM) (Fig. 1, Table 2). Zymodeme analysis is performed by observing the migration pattern of isoenzyme bands on a thin-layer starch gel or in polyacylamide gel electrophoresis (63, 82). Only one band is found with 1-malate:NAADP+ oxidoreductase, whereas GPI and PGM show multiple bands that are labeled α through δ. Blanc and Sargeaunt (10) have shown that the concentration of starch in the growth medium can influence the appearance of zymodeme patterns. Expression of γ and δ bands of GPI and PGM was observed more often when higher concentrations of starch were present in the culture media. HK is characterized by two bands, one migrating slowly and the other migrating faster. Pathogenicity is associated with the presence of a fast-migrating HK band, a PGM β band, and no PGM α band. The only known exceptions are zymodeme XIII, which, although considered a pathogenic zymodeme, lacks a fast-migrating HK band, and zymodeme XXI (112). Organisms belonging to zymodeme XXI were recently isolated from a male homosexual. On the basis of clinical history and serological tests, the isolate was considered nonpathogenic. The zymodeme pattern was unusual in that the isolate had a PGM α band and a fast-migrating HK band, a pattern which is often associated with pathogenic zymodemes. Sargeaunt (130, 131) has shown a positive correlation between zymodemes and the clinical histories and serologies.
of more than 6,000 pathogenic and nonpathogenic *Entamoeba histolytica* patient isolates.

In order to perform zymodeme studies, nonaxenic culture techniques must be used to isolate amebae from either feces, exudates, liver aspirates, or other suspect material. The media most frequently used are those described by Robinson (123) and Jones (54). Generally, three to nine subpassages requiring at least 2 days per subpassage are needed before enough organisms are obtained for enzyme electrophoretic analysis. Although the cultures of amebae from patients are xenic, containing multiple bacteria, the bacterial enzyme bands migrate faster than do those of *E. histolytica*. However, it is still necessary to run bacterial and amebic controls when axenic cultures are not obtained. At present, zymodeme analysis is not easily incorporated into routine clinical laboratory work because of the expertise required, technical difficulties, and cost. Although microscopic diagnosis does not address the issue of pathogenicity, it is still the method of choice for detecting amebic infections. The ability to culture organisms from specimens determined by microscopic examination to be positive is limited to approximately 50% (112, 144).

By enzyme electrophoresis, 23 zymodemes of *E. histolytica* have been identified (Fig. 1). Of these, nine have been associated with pathology in the host. These include isolates from stool in which the trophozoites contained ingested erythrocytes and isolates from intestinal ulcers and liver abscess material. Thirteen zymodemes have been obtained from asymptomatic patients passing cysts.

The zymodeme analysis data include information on isolates obtained from various areas of the world (47, 96, 130, 132). Even 9 years after the organisms were first isolated, Sargeaunt and co-workers have not detected changes in isoenzyme patterns with continued and repeated testing of isolates. Interestingly, mixtures of nonpathogenic zymodemes but no mixtures of pathogenic zymodemes have been found in the same specimen from the same patient. However, two pathogenic zymodemes have been detected from two specimen sources (stool and liver aspirate) from the same patient (132). When specimens are cultured for *E. histolytica*, pathogenic strains always outgrow nonpathogenic strains and all other amebae (112).

Clinically, patients who harbor pathogenic zymodemes generally have high antibody titers, whereas individuals with nonpathogenic zymodemes are either seronegative or have low antibody titers (40, 135). Asymptomatic carriers of pathogenic zymodemes and carriers of nonpathogenic zymodemes have been found in many instances to self-cure within 1 to 9 months (40, 44, 93). On the basis of this evidence, it has been proposed that patients who have *E. histolytica* in their stool specimens but no clinical signs of infection and no antibody titer need not be treated (40, 44, 66, 68a, 112).

Jackson and Gathiram (52) conducted a seroepidemiological study in which they found asymptomatic individuals who were serologically positive but harbored nonpathogenic zymodemes. Whether these individuals were previously treated for invasive amebiasis was not addressed.

It has not been established whether the zymodeme patterns reflect genotypic or phenotypic traits. Sargeaunt (131) proposed that pathogenic and nonpathogenic *E. histolytica* isolates represent distinct subspecies, whereas Mirelman and co-workers and others (12, 14, 86, 87, 90, 97) have presented evidence that zymodeme patterns may be a phenotypic trait and that these patterns could change under environmental influences. If the latter were true, treatment of only symptomatic patients or those who have a serological response could pose potential public health problems if asymptomatic cyst passers who harbor pathogenic zymodemes are not treated; they could serve as an important reservoir for transmission.

The reliability of differentiating between pathogenic and nonpathogenic strains of *E. histolytica* on the basis of phenotypic expression of isoenzyme patterns and the stability of the isoenzymes has been questioned (14, 86, 87, 97). Many authors have published information concerning the influence of bacteria on the virulence of amebae (7, 14, 94, 97, 107, 159, 160). During the process of axenization, Andrews et al. (2) and Mirelman et al. (90) converted a nonpathogenic zymodeme to a pathogenic one in culture. This change was manifested not only by changes in the mobilities of the HK and PGM bands but also by changes in vitro and in vivo pathogenicity. The isolate was capable of destroying tissue culture monolayers and inducing liver abscesses in hamsters. The ameba-bacterium association and the influence of bacteria on the virulence of *E. histolytica* have been reviewed by Mirelman (88). The ability to change from a nonpathogen to a pathogen or vice versa does not appear to be a universal trait among *E. histolytica* isolates (87). Some isolates do not undergo pathogenic conversion. Mirelman suggested that the pathogenic state of *E. histolytica* is interchangeable and that virulence is expressed only under unique growth conditions. However, the mechanisms or conditions that induce these changes are unknown. Through the use of polymerase chain reaction, Mirelman et al. (89) have shown that nonpathogenic isolates contain extrachromosomal circular DNA that hybridizes with DNA probe P-145, which has been used to characterize pathogenic isolates. They suggested that the extrachromosomal circular DNA characteristic of pathogenic isolates was induced to amplify when changes occurred in bacterial

### Table 2. Differential characteristics of zymodeme patterns

<table>
<thead>
<tr>
<th>Isoenzyme band or group</th>
<th>Pathogensic isolates</th>
<th>Non-pathogenic isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>HK</td>
<td>Fast migration</td>
<td>Slow migration</td>
</tr>
<tr>
<td>PGM</td>
<td>Beta</td>
<td>Alpha</td>
</tr>
<tr>
<td>L-Malate:NADP*</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Oxidoreductase G6P</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Zymodeme groups</td>
<td>II, II gamma, VI, VII, XI, XII, XIII, XIV, XIX, and XX</td>
<td>I, III, III alpha, IV, V, VIII, IX, X, XV, XVI, XVII, XVIII, and XX</td>
</tr>
</tbody>
</table>

* Zymodeme XIII is pathogenic but lacks the fast-migrating HK band.

* Zymodeme XXI is nonpathogenic but has a fast-migrating HK band.
flora in the growth medium. If the pathogenic status of an isolate can readily change because of environmental influences, then all E. histolytica isolates would have to be considered potential pathogens.

Sargeant and co-workers (129, 133) and Blanc et al. (9) have shown that genetic transfer between pathogenic strains can occur both in vivo and in vitro, with new zymodeme patterns different from those of the parental strains being detected. The possibility that mutation is responsible for the zymodeme pattern changes was believed to be remote because the frequency of mutation was postulated to be extremely low (9).

Recent work strongly supports the conclusion that there are two species of E. histolytica, one nonpathogenic and one pathogenic (24, 25, 102, 120, 144, 145, 147, 149–151). Genetic studies do not support the genotype interconversion theory but rather provide considerable evidence that two stable genotypes exist (7, 124). Nevertheless, issues of mutation, changes in the cell surface that affect virulence, and whether pathogenic forms have a nonpathogenic stage in the intestinal tract need to be addressed.

Recently, Strachan et al. (144) have used immunofluorescence techniques with monoclonal antibodies to the HK isoenzyme to differentiate pathogenic from nonpathogenic amebae. Others have shown that monoclonal antibodies directed against membrane protein or the galactose-specific adherence lectin can be used as a rapid method of distinguishing pathogenic from nonpathogenic isolates after the organisms have been cultured (101, 144, 150). DNA probes that selectively hybridized to DNA from either pathogenic or nonpathogenic E. histolytica have been developed (39, 149). Use of monoclonal antibodies and DNA probes was as effective as isoenzyme electrophoresis for determining the pathogenicity of the isolate. However, neither technique currently allows one to determine the zymodeme pattern, which may be useful for epidemiological purposes, and the question still unanswered is whether E. histolytica can change its pathogenic status as a result of environmental changes. All of these studies were performed with organisms from culture.

**PATHOGENESIS AND PATHOLOGY**

E. histolytica is unique among the intestinal amebae parasitizing humans because it is able to invade tissue. After the host ingests cysts, metacytic trophozoites escape from the metacyst in the gastrointestinal tract and colonize the cecum. At present, the mechanisms of colonization and pathogenicity are not well understood (43). Bacterial flora are always thought to have a direct role in the virulence of E. histolytica and its ability to colonize the host (14, 86, 106). Clinical syndromes associated with amebiasis vary with the host and the organism. Syndromes may range from an asymptomatic infection to a disseminated fatal disease. When the infection disseminates to extraintestinal sites, it is found most frequently in the right lobe of the liver (113).

Research on the mechanisms of pathogenicity has evolved along two pathways: one considers pathogenesis to be dependent on export of soluble toxins, and the other considers it to be dependent on cellular contact (31). It has been postulated that amebae can export pore-forming proteins (amebaporens) that form aqueous pores in target cell surface membranes and also produce cytotoxic products that are released from the organism and cause the initial cytotoxic effect on the host cell (62, 74, 76, 115, 161). This pore-forming peptide was recently purified by Leippe et al. (69). Recent studies have focused on contact-dependent killing by the trophozoite, including adherence, extracellular cytology, and phagocytosis. Host cell leukocytes also play a major role in pathogenesis (45, 152, 158).

A number of E. histolytica adherence receptors have been identified, but the galactose-specific lectin receptor has been the most thoroughly studied and is thought to be responsible for mediating adherence to both mucosal and colonic epithelial cells (71, 78, 79, 83, 101, 104, 116). Human colonic epithelial cells and mucus contain large numbers of galactose or N-acetylgalactosamine residues (23). Incubation of E. histolytica with colonic mucin prevents trophozoites from attaching to and killing target cells such as those in the intestinal epithelium (23). E. histolytica also enhances mucus secretion, which correlates with its pathogenicity (22). More virulent strains stimulated mucus secretion and depleted goblet cells of mucin, thereby making epithelial surfaces more vulnerable to invasion.

The binding to mucin was reversed by the addition of galactose and was completely inhibited by monoclonal antibodies directed against the amebic lectin receptor (23, 103). Monoclonal antibodies directed against the galactose-specific receptor have also been found to enhance adherence (102, 105). Monoclonal antibodies to the galactose-specific lectin receptor have been used to differentiate pathogenic and nonpathogenic strains, and the primary structure of this lectin has been sequenced (80, 102, 148). It has been noted that a galactose-specific lectin receptor is present on nonpathogenic zymodemes; however, it is antigenically altered (102). The galactose-specific lectin receptor on pathogenic strains is a potential target for therapy or vaccine production. Petri and Ravdin (103) were able to immunize animals with galactose-specific adherence lectin and protect them from liver abscesses.

Leitch et al. (70) postulated that amebae may invade the intestinal epithelium in areas where the mucous blanket is thin and there are fewer galactose or N-acetylgalactosamine residues to protect the host. Penetration of the mucous blanket was thought to be due primarily to mechanical ameboid movement and not to require enzymatic digestion. Talamas-Rohana and Meza (146) have shown that E. histolytica binds to a receptor on fibronectin, a glycoprotein, of the target cell. Whether this receptor contains galactose or N-acetylgalactosamine residues was not discussed. Once the organism was bound to the fibronectin, there appeared to be changes in its cytoskeleton, and the organism rapidly degraded and internalized the fibronectin receptor.

Pore-forming proteins that help destabilize the host cell and bring about cytolysis have been studied by a number of investigators, but no conclusive role for them in cytolysis by E. histolytica has been provided (62, 69, 76, 125, 161). The pore-forming amebapore protein is transferred from the trophozoite to the target cell, causing a disruption of the transmembrane gradient and contributing to the cell’s death by the colloid-osmosis lysis mechanism. Tachibana et al. (145) postulated that the monoclonal antibody they used was directed against the pore-forming protein found only in pathogenic isolates.

Luaces and Barrett (73) and Muller et al. (92) have reported finding extracellular proteinases and hydrolases in E. histolytica. No direct role in host cell lysis has been found for these enzymes, but they might be involved in amebic invasiveness at intercellular junctions, thereby allowing amebae to invade host tissues. Cysteine proteinases are the major proteolytic enzymes detected in E. histolytica (30, 60, 61, 121). Cysteine proteinase can degrade cellular attach-
ment and matrix proteins such as collagen, laminin, and fibronectin. There is a direct correlation between the amount of proteinase activity and the pathogenicity of *E. histolytica* (30, 60, 100, 121, 150, 153). Pathogenic isolates secrete larger amounts of proteinase than do nonpathogenic isolates, and the expression of mRNA is 10- to 100-fold greater in pathogenic than in nonpathogenic zymodemes (60, 121, 150).

The effects of calcium and phorbol esters on lipases and proteinase activity in the cystolytic event have been studied by Long-Krug et al. (72), Ravdin et al. (118, 119), and Weikel et al. (156). Release of intracellular calcium increased the activity of phospholipases, and phospholipase increased the hemolytic activity of *E. histolytica* through the release of free fatty acids (126). Phorbol esters were noted to increase the cystolytic activity of *E. histolytica*, whereas sphingosine inhibited cystolytic events. These two compounds are known to stimulate and inhibit the activity of protein kinase C, which is thought to have a major role in the cystolytic event (29, 156). The definitive chronology of cystolytic events awaits further studies.

**Asymptomatic Infection**

Most human infections are asymptomatic, with the parasite acting as a harmless commensal organism in as many as 90% of those infected (31, 45). Individuals harboring the organisms may have either a negative or a weak antibody titer and show no occult blood in stool samples, and cysts can usually be detected in the stool when examinations for ova and parasites are done. If trophozoites are detected, they do not contain any phagocytized erythrocytes. Isoenzyme analyses of organisms isolated from asymptomatic individuals have usually shown that the isolates belong to nonpathogenic zymodemes (130).

Many asymptomatic individuals never become symptomatic and usually excrete cysts in the stool for only a short time, as shown by repeat examinations for ova and parasites or by culture. This pattern is found in persons infected with pathogenic and nonpathogenic strains (1, 4, 16, 40, 44, 93).

**Symptomatic Infection**

About 10% of individuals infected with *E. histolytica* have clinical symptoms, with intestinal and/or extraintestinal pathology. Although this proportion of infected individuals is small, the disease represents a major public health problem in terms of morbidity and mortality in Third World countries. Patients with noninvasive infection may exhibit non-specific gastrointestinal symptoms, including abdominal pain and increased frequency of bowel movements, that may be intermittent or chronic. When amebae invade host tissues, only trophozoites are seen and the organism loses its ability to encyst. Amebic invasion of the intestinal mucosa occurs most frequently (in descending order) in the cecum, ascending colon, sigmoid colon, appendix, descending colon, and transverse colon (56). The symptoms associated with invasive intestinal amebiasis are usually nonspecific. The onset of symptoms is usually gradual, with abdominal pain, diarrhea, dysentery, or weight loss occurring, depending on the severity of disease. Almost all patients have occult blood in the stools, and nearly one-third exhibit fevers. Histological examination of tissue may be required to rule out malignancy and inflammatory bowel disease.

When amebic colitis is detected, it is usually (70% of cases) associated with segmentary ulceration of the colon (21). If ulceration with perforation occurs, it occurs mainly in the cecum (53). Dissemination of amebic infection to extraintestinal sites most frequently involves the liver, lungs, pericardium, brain, and skin.

Colonic mucosal invasion begins in the interganglular epithelium, where the overlying mucous layer is noticeably thin (110). With initial microulceration, there is an outflow of tissue fluids, erythrocytes, neutrophils, lymphocytes, eosinophils, plasma cells, epithelial cells, and Charcot-Leyden crystals (108). The inflammatory response to amebic invasion is minimal and may be due to the lysis of tissue cells by *E. histolytica*. One or several ulcerations may be present, but if there are several ulceration sites, they are usually separated. At first, the ulceration is superficial, and cellular infiltration and tissue necrosis are limited to the invasion site. Proctoscopic examination reveals an edematous and friable mucosa. The ulcer has a pinhead center that is composed of eosinophilic granular debris, some inflammatory cells, and *E. histolytica* trophozoites. One can see normal mucosal surface between ulcers (109). As the ulcer deepens and progresses, it forms the classic flask-shaped ulcer of amebic colitis, which extends from the mucosa and muscularis mucosa into the submucosa. When ulcers coalesce, the mucosa undergoes necrosis and sloughs. Amebae are found on the advancing edges of the ulcer and usually not in the necrotic areas. Many times, the ulcers become secondarily infected with bacteria. "Toxic megacolon" is the result of total confluence of ulcerations and necrosis of the colon. This form of amebiasis has a very high mortality rate.

Ameboma, the result of chronic ulceration, is predominately found in the cecum, sigmoid colon, and rectum (15). These tumorlike lesions are often mistaken for malignancies and are sometimes present as palpable masses. Histologically, the ameboma is nonfibrotic and contains granulation tissue with lymphocytes and giant cells (15).

Perforation of the colon by an amebic ulcer occurs in as many as 21% of patients with acute amebic colitis (15) and may result in peritonitis and liver abscess. Perforations occur most commonly in the cecum (58). Peritonitis is frequently the result of liver abscesses rather than intestinal perforation, but the latter has a high mortality rate (91).

Amebic infection of extraintestinal sites is the result of previous intestinal infection. Liver abscess can occur concurrently with colitis; however, there is frequently no clinical history of recent *E. histolytica* intestinal infection or evidence of parasites in stool specimens. Onset may be acute, with abdominal pain and fever, or subacute, with weight loss; less than 50% of patients have fever or abdominal pain. Patients may have leukocytosis without eosinophilia, elevated alkaline phosphatase and transaminase levels, and a high erythrocyte sedimentation rate. *E. histolytica* trophozoites infecting the muscularis mucosa frequently digest the walls of mesenteric venules, enter the circulation, and are carried to the liver via the portal venules (15). The trophozoites are detected more frequently in the right lobe rather than the left lobe of the liver, perhaps because of increased perfusion of the larger liver mass (15). Liver abscess is diagnosed more frequently in adults (ages 20 to 60 years) than in children and more frequently in males than in females (142).

The liver abscess has a thin capsular wall with a necrotic center composed of a thick fluid, an intermediate zone of coarse stroma, and an outer zone of nearly normal tissue that is being invaded by amebae (15). The contents of the abscess have been described as resembling response to trauma because of the light brown color. Generally, the abscess is bacteriologically sterile. Secondary bacterial invasion of an amebic
abscess occurs infrequently. Microscopic examination of the abscess fluid reveals granular eosinophilic debris with no or few cells (67).

In animal models, amebae are not directly responsible for the damage to the liver cells. The tissue destruction apparently results from lysis of leukocytes and macrophages by the amebae (152). Neutrophils are attracted to amebae and are rapidly killed on contact with *E. histolytica*. Although the neutrophils have little or no cytolytic effect on the virulent organisms (46), the release of toxic intracellular products from these destroyed host cells promotes lysis and necrosis of the hepatic cells.

If the amebic lesions heal, they invariably heal without forming scar tissue (15). Because of the cytolytic action of the amebae, leukocytes may be prevented from accumulating in the abscess area, thereby inhibiting the initiation of a cellular immune response. *E. histolytica* trophozoites inhibit the mobility of monocytes but not leukocytes (42). Monocytes (macrophages) play a crucial role in any wound-healing process by stimulating collagen deposition (37, 64, 65).

**DIAGNOSIS**

*E. histolytica* infections can be diagnosed in the laboratory by the detection of organisms in stool preparations, sigmoidoscopy smears, tissue biopsy samples, or hepatic aspirate samples or by serological tests (Table 3). Organism detection depends on proper specimen collection, processing, and examination by trained personnel. Examination for ova and parasites in a minimum of three stool specimens, using concentration and permanent stain techniques, is the recommended standard for the detection and identification of the organism (38). Reliance on inadequately trained personnel for laboratory diagnosis may lead to missed infections or, more commonly, false-positive results because of misidentification of leukocytes as *E. histolytica*. Although wet mounts of fresh specimens can be screened for motile trophozoites containing erythrocytes, most patients do not harbor trophozoites with erythrocytes. If fresh stool samples are examined, they should be examined within 30 min after passage of the specimen, not 30 min after arrival in the laboratory. If the specimen cannot be examined within 30 min, it should be preserved in a suitable fixative such as polyvinyl alcohol (PVA). Although some textbooks advocate the use of direct wet mounts to identify *E. histolytica*, the identification should always be confirmed by using a permanent-stained slide. The importance of the stained slide cannot be overemphasized, particularly when organisms are few in number in the stool specimen.

Even if sigmoidoscopy is performed, a minimum of three stool specimens should also be collected and examined. No fewer than six areas of the mucosa should be sampled, with examination slides made at the bedside or in the examination room. Material scraped or aspirated from the mucosal surface can be examined by three methods. If there is no delay, a wet mount in 0.85% NaCl can be made and examined for ameboid movement. It may be several minutes after the NaCl preparation has been made before the amebae exhibit any motility. Microscopic examination may be difficult because of the need for low light intensity and difficulties in differentiating the unstained amebae from cellular material. In wet preparations, other protozoa such as *E. coli* may resemble *E. histolytica* (Table 3). Only careful examination will distinguish amebae from other host cells, such as polymorphonuclear leukocytes, which mimic *E. histolytica* cysts, and macrophages, which may be mistaken for *E. histolytica* trophozoites (Table 4). The mucosal material may also be smeared on a slide and immediately immersed in Schaudinn’s fixative. An alternative method of preparing a permanent fixed-slide specimen is to add 2 to 3 drops of PVA directly to the mucosal material directly on the slide, mix it, and allow the slide to air dry. These two methods provide a permanent mount that can be stained with trichrome stain (38).

Histologic examination of tissue biopsy sections can be done with periodic acid-Schiff and hematoxylin-eosin stains (Table 3). In histologic material, all the nuclear detail may not be seen in one organism, and sequential sections may have to be examined to identify the organism. The amebae must be differentiated from normal host cells such as histiocytes. Hepatic aspirates can be examined for the organism, but this examination is rarely performed and rarely done correctly. Aspirated material should be collected in a number of different containers as it is obtained from the abscess. The amebae are sparse in necrotic material from the center of the abscess, but they are more abundant on the marginal walls. Therefore, they are more commonly found in the last portions of aspirated material. Enzyme liquefaction has been used to facilitate the search for organisms by freeing the organisms from aspirate material (5). Demonstration of the organisms is often very difficult because they may be trapped in viscous pus or debris and will not exhibit typical motility.

In extraintestinal amebiasis, organisms may or may not be found in the stool; therefore, one of the many types of available serological tests may be necessary for diagnosis (48, 99). Serology should not be used solely for the diagnosis of intestinal amebiasis, because antibody titers may be low.

### TABLE 3. Laboratory diagnosis of amebiasis: specimen type

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fixative</th>
<th>Examination</th>
<th>Stain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stool</td>
<td>PVA, 10% Formalin, Schaudinn’s fixative, SAF</td>
<td>Concentrate, permanently stained slide</td>
<td>Gomori trichrome, iron hematoxylin</td>
</tr>
<tr>
<td>Sigmoid colon Aspirate Direct</td>
<td>PVA, Schaudinn’s fixative</td>
<td>Permanently stained slide</td>
<td>Gomori trichrome, iron hematoxylin</td>
</tr>
<tr>
<td>Fixed Biopsy Blood</td>
<td>None</td>
<td>Wet mount with or without enzyme digest</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>PVA, Schaudinn’s fixative</td>
<td>Permanently stained slide</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Formalin</td>
<td>Routine histology</td>
<td>None</td>
</tr>
</tbody>
</table>

* Fixative examination: PVA, PVA with either HgCl₂ or CuSO₄; SAF, sodium acetate-acetic acid-Formalin; PAS, periodic acid-Schiff (organism stains intensely pink and has a distinct outline, but cytoplasmic and nuclear details are obscured); H&E, hematoxylin and eosin stain (cytoplasm is pink and nucleus is blue; in sections, the nucleus may not always be present).

b Standard serological tests include indirect hemagglutination, counterimmunoelectrophoresis, indirect fluorescent antibody, and enzyme immunoassay.
and/or impossible to interpret, particularly with patients from an area of endemicity where a high prevalence of seropositivity already exists. Asymptomatic cyst passers usually have negative serological test results, but there is good correlation between infection with pathogenic zymodemes and positive serological tests (130). Positive indirect hemagglutination, indirect fluorescent-antibody, and immunodiffusion tests give results that correlate well with the presence or absence of an amebic liver abscess. An important major problem common to most serological tests is the persistence of antibody for prolonged periods (up to 2 years) after diagnosis and therapy. These tests should therefore be interpreted with caution. However, indirect fluorescent-antibody titers decrease within 6 to 12 months of successful therapy. Serological tests can also be used to rule out amebiasis in patients with inflammatory bowel disease (49).

Although numerous methods for the serological detection of amebiasis have been reported in the literature, the use of these methods in clinical laboratories is severely limited by the unavailability of commercial reagents or test kits (38). Newer diagnostic methods being proposed include the use of purified antigens and assays for immunoglobulin M and A antibodies (6, 28, 55, 102, 117, 139). The Centers for Disease Control offer amebic serological tests. Submission regulations vary from state to state; therefore, laboratories should check their county or state public health department for guidelines.

Recently, culture techniques with Robinson’s medium, Diamond’s medium, Jones’s medium, and Locke’s egg yolk serum medium in conjunction with isoenzyme electrophoresis have been used to differentiate infections caused by pathogenic and nonpathogenic *E. histolytica* strains (85–87, 136). At present, this technique is not practical for routine clinical laboratories. Results usually are not available for 4 days or more. Even though a patient may be diagnosed by clinical history, serological tests, or zymodeme analysis as harboring a nonpathogenic strain, the question of whether or not the strain will revert to pathogenicity in vivo has been raised (42, 85–87, 89, 130, 131).

To provide clinically relevant information to physicians

### TABLE 4. Differential characteristics of host cells and *E. coli*, commonly mistaken for *E. histolytica*, in wet preparations

<table>
<thead>
<tr>
<th>Cell</th>
<th>Diam or length (µm)</th>
<th>Motility</th>
<th>Nucleus No.</th>
<th>Cytoplasmic appearance (stained)</th>
<th>Inclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. histolytica</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trophozoites</td>
<td>12–60; usual range, 15–22</td>
<td>Progressive, with hyaline fingerlike pseudopodia; may be rapid</td>
<td>1</td>
<td>Clear differentiation of ectoplasm and endoplasm; vacuoles usually small, if present; looks like ground glass</td>
<td>Presence of erythrocytes diagnostic</td>
</tr>
<tr>
<td>Cysts</td>
<td>10–20; usual range, 12–15</td>
<td>None</td>
<td>1–4</td>
<td>1:2–1:3</td>
<td>Clear</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trophozoites</td>
<td>15–50; usual range, 20–25</td>
<td>Sluggish, nondirectional; blunt, granular pseudopodia</td>
<td>1</td>
<td>Granular, little differentiation into ectoplasm and endoplasm; usually vaculated</td>
<td>Bacteria, yeast cells, other debris</td>
</tr>
<tr>
<td>Cysts</td>
<td>10–35; usual range, 15–25</td>
<td>None</td>
<td>1–8</td>
<td>≥16 nuclei seen occasionally</td>
<td>Clear</td>
</tr>
<tr>
<td>Host cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polymorphonuclear</td>
<td>Avg, 16</td>
<td>None</td>
<td>1</td>
<td>2–4 segments; if lobed nucleus fragments may mimic the 4 nuclei found in <em>E. histolytica</em> cyst (1:1)</td>
<td>Granular</td>
</tr>
<tr>
<td>leukocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>None</td>
</tr>
<tr>
<td>Macrophages</td>
<td>20–60; may be 5–10</td>
<td>Sluggish</td>
<td>1</td>
<td>Large, may be irregular in shape (like monocyte); may mimic <em>E. histolytica</em> trophozoite; can also ingest erythrocytes</td>
<td>Coarse; may be highly vacuolated</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Usually contain ingested debris, polymorphonuclear leukocytes, and erythrocytes</td>
</tr>
</tbody>
</table>
for treatment of patients infected with pathogenic strains of *E. histolytica*, methods using monoclonal antibodies, purified antigens, or DNA probes may be required (8, 13, 39, 102, 127, 143, 144, 147, 151). Although there have been numerous reports of using techniques such as enzyme immunoassay to detect antigens in specimens, none of these methods are routinely used in clinical laboratories (84). If zymodeme patterns are found to remain stable, these techniques could be incorporated into a routine diagnostic laboratory, provided that they can be used directly on clinical specimens rather than after the isolation of organisms by culture. Zymodeme patterns could be very useful for epidemiological purposes in tracking organism transmission and potential differences in organism invasiveness, virulence, and therapeutic susceptibility. The use of purified antigens and RNA or DNA probes for routine diagnostic testing would offer significant improvements over current serological or direct detection methods. The serological techniques commonly used for diagnostic purposes are not sensitive or specific enough to be solely relied on clinically. Reliable probes would help to eliminate false-positive test results due to misidentification of *E. coli* or host cells such as polymorphonuclear leukocytes or macrophages. Commercial kits that make use of purified antigens or DNA probes are not available at this time. For a more comprehensive review of laboratory diagnostic techniques, see Garcia and Bruckner (38).

**THERAPY**

Two classes of drugs are used in the treatment of amebic infections. Luminal amebicides, such as iodoquinol and diloxanide furoate, act on organisms in the intestinal lumen and are not effective against organisms in tissue. Tissue amebicides, such as metronidazole, chloroquine, and dehydroemetine, are effective in the treatment of invasive amebiasis but less effective or ineffective in the treatment of organisms in the bowel lumen.

**Asymptomatic Patients**

Asymptomatic patients who are cyst passers may be treated with diloxanide furoate, iodoquinol, or paromomycin (38, 81, 112, 128, 140, 141). Like iodoquinol, diloxanide furoate is a luminal amebicide. Diloxanide furoate is available only from the Centers for Disease Control on special request. Although known as a luminal amebicide, diloxanide furoate is readily hydrolyzed in the intestinal tract and absorbed into the bloodstream. For asymptomatic patients who are passing both cysts and trophozoites in the stool, the recommended treatment is metronidazole plus iodoquinol (38, 81, 128, 140, 141). From zymodeme analysis, it has been determined that many asymptomatic patients harbor non-pathogenic zymodemes, and the use of drug therapy in these individuals is controversial. As discussed above, methods to differentiate pathogenic from nonpathogenic *E. histolytica* strains are not easily incorporated into routine clinical laboratory use. Therefore, from a public health viewpoint, it is best to treat all infected patients.

**Symptomatic Patients**

The treatment of symptomatic patients varies with the clinical stage of the infection. Different therapeutic approaches are employed for intestinal and extraintestinal amebiasis (38, 81, 114, 128, 140, 141).

Mild intestinal disease due to amebic dysentery should be treated with metronidazole plus iodoquinol or with metronidazole plus diloxanide furoate. Patients should be warned to refrain from ingesting alcohol when taking metronidazole, for they may have a reaction similar to that to disulfiram. Symptoms may be abrupt and severe and include abdominal pain, nausea, vomiting, and headache. The safety of iodoquinol taken during pregnancy has not been determined. In cases of dehydration, fluid and electrolyte replacement may be necessary.

**Extraintestinal Disease**

A number of combination therapies have been used for treating extraintestinal amebiasis (38, 81, 114, 128, 140, 141). These include metronidazole and dehydroemetine with chloroquine. Chloroquine is useful for treating amebic liver abscess because it is concentrated in the liver; however, chloroquine is not useful for intestinal amebiasis. Dehydroemetine can be toxic to the heart, so patients should be monitored throughout therapy. Although surgical drainage of larger abscesses may be necessary, aspiration of small and moderate-sized abscesses can usually be avoided. Smaller abscesses resorb, and their disappearance can be monitored by scanning procedures (computerized tomography, liver scan, or ultrasound). Extraintestinal therapy should be followed with a luminal amebicide such as iodoquinol or diloxanide furoate.

**Follow-Up**

All treated patients should be followed up clinically and with laboratory tests. Patients who had positive stool sample results should have follow-up stool examinations at 2 to 4 weeks posttherapy. The stool examination must include a permanent stained slide. Patients with extraintestinal amebiasis should show a clinical response after therapy and can be monitored with the scanning procedures mentioned above. Although antibody titers in serum may remain elevated for prolonged periods and may be of little use in follow-up, indirect fluorescent-antibody titers have been shown to decrease within 6 to 12 months of successful therapy.

**SUMMARY AND FUTURE DIRECTIONS**

Although approximately 12% of the world’s population is infected with *E. histolytica*, only 10% of those infected will develop clinical symptoms, and of this group, only 2 to 20% will have invasive disease. It has been postulated that the large number of asymptomatic people are infected with a nonpathogenic strain that cannot be distinguished morphologically from pathogenic strains. It has been shown that pathogenic and nonpathogenic strains can be differentiated by the use of isoenzyme (zymodeme) analysis, monoclonal antibodies, and molecular probes, and the results correlate with clinical symptoms and serological tests. Whether the isoenzyme patterns are a phenotypic or genotypic trait will have to be answered by future research. It is possible to transfer genetic material between organisms, thus transforming one pathogenic zymodeme into a completely different pathogenic zymodeme, and the presence or absence of specific bacterial flora has been associated with the interconversion of nonpathogenic and pathogenic strains in culture. Mirelman (87) found that nonpathogenic *E. histolytica* isolates contain extrachromosomal DNA that hybridizes with
pathogen-specific DNA probes. However, through the use of recombinant DNA technology, two genetically distinct forms of *E. histolytica* have been found to exist. Genetic analysis has shown substantial differences between the non-pathogenic and pathogenic forms. Until our understanding of the pathogens is elucidated, there will continue to be questions concerning the influence of bacterial flora and genetic exchange between isolates. Because of the uncertainty of isoenzyme stability, the expression or repression of pathogenic genes, or transfer of genetic material between organisms, the designation of an organism as non-pathogenic or pathogenic should not be solely relied on for therapeutic purposes.

Although recent research has helped to elucidate the molecular mechanisms of organism attachment, cytology, and phagocytosis of host cells, pathogenesis is still not well understood, even though *E. histolytica* has been studied for over 100 years. Most recent work cited has dealt with the galactose-specific lectin receptor of *E. histolytica*. Prevention or alteration of attachment may eliminate the pathogenicity of this organism. The sequencing of this receptor and others involved in attachment may lead in the near future to a vaccine or a specific therapeutic agent that will prevent or reduce the pathological sequelae associated with pathogenic zymodemes.

Diagnosis of *E. histolytica* infections depends primarily on examinations for ova and parasites and on serological tests. There are no commercially available or practical and reliable methods for differentiating pathogenic from nonpathogenic strains for routine use in clinical laboratories. Currently, there are no antigen tests for routine clinical laboratory use, and monoclonal antibodies and DNA probe tests have been used only with culture isolates to detect pathogenic zymodemes.

With the current interest in developing genomic libraries (51) and in using amplification methods to selectively look at specific functions of the organism, our knowledge of *E. histolytica* will be considerably enhanced. Amplification methods and serological tests that are sensitive and specific enough to directly detect very small numbers of organisms are currently needed. In the near future, clinical laboratories will be evaluating a number of new test products for diagnosing infections caused by pathogenic *E. histolytica*.

REFERENCES


