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Pathogenesis of Intestinal Amebiasis: From Molecules to Disease

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INTRODUCTION

Amebiasis is the infection of the human gastrointestinal tract by *Entamoeba histolytica*, a protozoan parasite that is capable of invading the intestinal mucosa and may spread to other organs, mainly the liver. *Entamoeba dispar*, an ameba morphologically similar to *E. histolytica* that also colonizes the human gut, has been recognized recently as a separate species with no invasive potential (8, 35, 41, 90). The acceptance of *E. dispar* as a distinct but closely related protozoan species has had profound implications for the epidemiology of amebiasis, since most asymptomatic infections found worldwide are now attributed to this noninvasive ameba.

Currently, there is no low-cost laboratory test available for the differentiation of *E. histolytica* from *E. dispar* infections. The development of this valuable diagnostic tool for use in clinical laboratories and large-scale epidemiological studies has been made a priority (8) and is the subject of intense research (2, 20, 57, 60, 63, 96, 129, 161, 170). Preliminary data obtained from the application of these methods confirm the presence of *E. dispar* in most asymptomatic amebic infections, although *E. histolytica* asymptomatic colonization is not uncommon (19, 58, 59, 130, 171). Of note, the prevalence rates of both species in different geographical areas is still difficult to estimate due to the small number of samples analyzed.

Invasive amebiasis due to *E. histolytica* is more common in developing countries. In areas of endemic infection, a variety of conditions including ignorance, poverty, overcrowding, inadequate and contaminated water supplies, and poor sanitation favor direct fecal-oral transmission of amebas from one person to another. Being responsible for approximately 70 thousand deaths annually, amebiasis is the fourth leading cause of death due to a protozoan infection after malaria, Chagas' disease, and leishmaniasis and the third cause of morbidity in this organism group after malaria and trichomoniasis, according to recent World Health Organization estimates (177).

The motile form of *E. histolytica*, the trophozoite, lives in the lumen of the large intestine, where it multiplies and differentiates into the cyst, the resistant form responsible for the transmission of the infection. Cysts are excreted in stools and may be ingested by a new host via contaminated food or water. The parasite excysts in the terminal ileum, with each emerging quadrinucleate trophozoite giving rise to eight uninucleated trophozoites. Trophozoites may invade the colonic mucosa and cause dysentery and, through spreading via the bloodstream, may give rise to extraintestinal lesions, mainly liver abscesses.

Depending on the affected organ, the clinical manifestations of amebiasis are intestinal or extraintestinal. There are four clinical forms of invasive intestinal amebiasis, all of which are generally acute: dysentery or bloody diarrhea, fulminating colitis, amebic appendicitis, and ameboma of the colon. Dysenteric and diarrheic syndromes account for 90% of cases of invasive intestinal amebiasis. Patients with dysentery have an average of three to five mucosanguineous evacuations per day, with moderate colic pain preceding discharge, and they have rectal tenesmus. In patients with bloody diarrhea, evacuations are also few but the stools are composed of liquid fecal material stained with blood. While there is moderate colic pain, there is no rectal tenesmus. Fever and systemic manifestations are generally absent. These syndromes constitute the classic ambulatory dysentery and can easily be distinguished from that of bacterial origin, where the patient frequently complains of

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systemic signs and symptoms such as fever, chills, headache, malaise, anorexia, nausea, vomiting, cramping abdominal pain, and tenesmus (reviewed in reference 89).

Although *E. histolytica* can infect almost every organ of the body, the most frequent form of extraintestinal amebiasis is the amebic liver abscess. This condition, which results from the migration of trophozoites from the colon to the liver through the portal circulation, is 10 times more common in adults than in children and 3 times more frequent in males than in females (144). In general, the onset is abrupt, with pain in the right hypochondrium radiating toward the right shoulder and scapular area. The pain usually increases with deep breathing, with coughing, and while stepping on the right foot during walking. When the abscess is localized to the right lobe, symptoms include an irritative cough that is sometimes productive and a pleuritic type of chest pain. Abscesses in the upper left lobe can cause epigastric, sometimes dyspneic pain, at times spreading to the base of the neck and to one or both shoulders. Fever between 38 to 40°C is found in 85 to 90% of patients with amebic liver abscess. The patient commonly has chills and profuse sweating in the afternoon and at night. Other symptoms include anorexia, nausea, vomiting, diarrhea (with or without blood), and dysentery. On physical examination, the cardinal sign of amebic liver abscess is painful hepatomegaly. Digital pressure and fist percussion will often produce intense pain in the liver region. On palpation, the liver is soft and smooth, in contrast to the rough, hard, irregular character of the liver in patients with cirrhosis and hepatocarcinoma. Jaundice is present in 8% of the patients who respond well to treatment. When jaundice is severe, multiple abscesses should be suspected. Diarrhea or dysentery is seen in fewer than one-third of patients. Complications of amebic liver abscess include perforation to the pericardial space, pleura, or peritoneal cavity (reviewed in reference 89).

The diagnosis of invasive intestinal amebiasis is still based on the microscopic identification of *E. histolytica* trophozoites in rectal smears or recently evacuated stools and on the results of rectosigmoidoscopy. Trophozoites are most likely to be found in the bloody mucus and in the yellowish exudate covering the mucosal ulcerations obtained during rectosigmoidoscopy. Diagnostic problems arise when only cysts are identified in stools of healthy or diarrheic individuals. A commercially available laboratory test based on the identification of specific *E. histolytica* antigens in stool (60) is able to discriminate *E. histolytica* from *E. dispar* cysts (W. A. Petri, unpublished observations). However, the high cost and lack of knowledge of this test have hindered its use in clinical laboratories, especially in countries where amebiasis is endemic. Until these new diagnostic tests are widely available to clinical laboratories, these samples should be reported as containing *E. histolytica*/*E. dispar* (8).

The diagnosis of amebic liver abscess is sometimes difficult. In areas of endemic infection or when there is a history of travel to such places, amebic abscess should be suspected in patients with spiking fever, weight loss, and abdominal pain in the upper right quadrant or epigastrium and in patients with tenderness in the liver area. The presence of leukocytosis, a high alkaline phosphatase level, and an elevated right diaphragm suggest a hepatic abscess. The diagnosis is confirmed by ultrasonography or by computed tomography (CT) scans. The CT scan is the most precise method for identifying hepatic abscesses, especially when they are small, and following intravenous injection of contrasting agents, it is of great value in the differential diagnosis of other focal lesions of the liver (144). The high cost of this method, however, limits its use to cases when there are doubts about the diagnosis.

Serological tests for antiamebic antibodies are positive in approximately 75% of patients with invasive colonic amebiasis and in over 90% of patients with amebic liver abscesses. Of all tests available, the Centers for Disease Control and Prevention has chosen the enzyme immunoassay as its standard serological reference test for amebiasis. However, in areas of endemic infection, the high prevalence of antiamebic antibodies in the general population reduces the usefulness of serological tests for diagnosis (reviewed in reference 89).

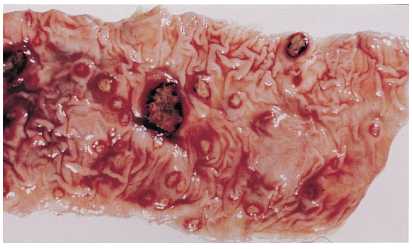

FIG. 1. Intestinal specimen from a patient with acute amebic colitis. Several nodular lesions show characteristic rounded, slightly elevated areas of the mucosa with irregular necrotic centers surrounded by edematous hyperemic tissue. The necrotic centers are filled with a yellowish mucoid material, except in two ulcers, where the center is hemorrhagic. The intervening mucosal folds have a mostly normal appearance, although one segment is congested and edematous.

The powerful lytic activity of *E. histolytica*, for which the parasite was named, has inspired a variety of approaches aimed at understanding the pathogenesis of invasive amebiasis. Most studies have focused on a single factor in an attempt to dissect the multiple mechanisms used by the parasite that ultimately result in tissue destruction. The aim of the present review is to provide an overview of the pathological lesions of human intestinal amebiasis and to discuss recent advances in the study of the molecular mechanisms of amebic pathogenicity.

ACUTE INTESTINAL AMEBIASIS

Intestinal invasive amebiasis may be associated with a variety of anatomical alterations such as acute ulcerative colitis, toxic megacolon, ameboma, or amebic appendicitis. Amebic ulcerative colitis is by far the most frequent and is thus the focus of this discussion.

Macroscopic Lesions

Typical intestinal amebic ulcers are found in the colon (primarily in the cecum), sigmoid colon, and rectum. Two types of ulcers, nodular and irregular, have been described. Nodular lesions appear as small (0.1- to 0.5-cm), rounded, slightly elevated areas of the mucosa with irregular necrotic centers surrounded by a rim of edematous tissue. The necrotic center may appear depressed or hemorrhagic but more often is filled with a yellowish mucoid material (Fig. 1). When there are few ulcers, the intervening mucosal folds may appear normal. However, lesions can also cover most of the entire mucosa of the colon, with the uninvolved segments being congested and edematous. Irregular or serpinginous ulcers 1 to 5 cm in length are usually found in the cecum and ascending colon. These ulcers are characteristically shallow with broad, elevated margins and are filled with fibrin. Congestion and edema of the narrow strips of uninvolved mucosa and edematous thickening of the entire intestinal wall are frequently observed accompanying these large ulcers. Both types of intestinal ulcers are often present in the same patient. In surgical specimens, extensive superficial mucosal denudation is observed (reviewed in reference 113).

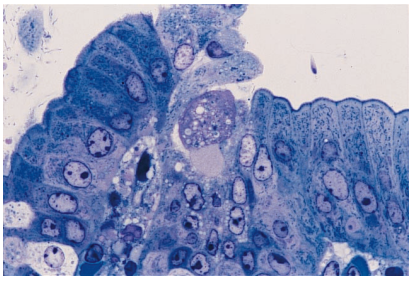

FIG. 2. Experimental intestinal amebiasis in the guinea pig. An invading *E. histolytica* trophozoite proceeds through the interglandular epithelium. A large pseudopod is extended by the parasite during penetration to the lamina propria. A semithin section stained with toluidine blue is shown.

Microscopic Findings

The microscopic changes characteristic of amebic ulcerative colitis have been studied in human rectal biopsy specimens. Little has been added to the now classical description by Prathap and Gilman, published in 1970, who found five types of lesions that seem to correspond to the progression of damage caused by *E. histolytica* trophozoites (120).

In the initial stages, trophozoites produce nonspecific lesions characterized by thickening of the mucosa, due to glandular hyperplasia and stromal edema, with an intact although wavy surface epithelium. Mild to moderate infiltration of neutrophils is observed within and around capillaries, but small collections can also be identified beneath the surface epithelium. Lymphoid aggregates show reactive hyperplasia with numerous histiocytic cells. Amebas are present in small numbers in the surface exudate. As the lesion progresses, there is a mucopenic depression due to loss of mucin from the surface and from glandular epithelial cells, which decrease in height from columnar to cuboidal and flattened dividing cells. A larger neutrophil infiltrate is evident, and plasma cells, eosinophils, macrophages, and lymphocytes can also be identified. Amebas may be present in large numbers on the luminal surface but are particularly abundant adjacent to the sites of epithelial lysis. They are surrounded by a proteinaceous exudate composed mainly of fibrin, mucin, erythrocytes, and occasionally neutrophils and mononuclear cells. Invasion of the colonic and cecal mucosa by *E. histolytica* begins in the interglandular epithelium, forming the early invasive lesions with superficial ulceration (Fig. 2 and 3) (120). Depending on the extent of damage, these can be small interglandular foci of microinvasion, with little tissue necrosis and inflammatory cell response, or larger areas of superficial ulceration where amebic aggregates are separated from surviving tissue by a thin zone of necrosis and neutrophils. Cell infiltration around invading amebas leads to rapid lysis of inflammatory cells and tissue necrosis; thus, acute inflammatory cells are seldom found in biopsy samples or in scrapings of rectal mucosal lesions (120).

The late invasive lesion with deep ulceration corresponds to the flask ulcer described in the classical 1891 monograph of Councilman and Lafleur (37). The mucosal ulcer extends deep into a larger area of the submucosa, which seems to be particularly susceptible to the lytic action of the parasite, and produces abundant microhemorrhages. This explains the finding of hematophagous amebas in stool specimens or in rectal scrapings, still the best indication of the amebic nature of a case of dysentery or bloody diarrhea. A thick exudate containing acellular proteinaceous material, red blood cells, and strands of fibrin is seen on the floor of the ulcer, where groups of amebas are identified, especially in the deeper layers. A deeply eosinophilic zone of fibrinoid necrosis separates the exudate from underlying viable submucosa. The latter is edematous, hyperemic, and heavily infiltrated with plasma cells. Neutrophils are abundant only at the ulcer edge, some lymphocytes and macrophages are present, and eosinophils are rare (120). The presence of viable amebas in the internal muscle layer of the colonic wall in the absence of tissue damage or inflammatory reaction has been reported in nearby tissues (91). Finally, loss of mucosa and submucosa with formation of granulation tissue is characteristic of the granulating ulcer. No undermining of the ulcer edge is observed, and amebas are not found within the tissue, but are occasionally present in the surface exudate (120).

PATHOGENESIS OF INTESTINAL AMEBIASIS

Although the term "pathogenesis," defined as the mechanisms involved in the initiation, evolution, and ultimate outcome of a disease process, relates to both host and parasite factors, this review focuses mainly on the parasitic mechanisms that may be related to invasive intestinal amebiasis.

Nonspecific Lesion

The edematous thickening of the mucosa, the glandular hyperplasia, and the stromal edema described in the nonspecific lesion are probably due to mild irritation of the epithelium produced by soluble amebic products. Trophozoites in axenic cultures are known to secrete a variety of molecules, some of which have been identified as proteases (see below). Irritation stimulates goblet cells to release mucus, at the same time that it increases its production, thus explaining the glandular hyperplasia. Recent experiments carried out with mouse colonic explants show that spent axenic culture medium is capable of inducing glandular hyperplasia, suggesting the presence of still unidentified toxic molecules released by the parasite (M. Espinosa-Cantellano and A. Martínez-Palomo, unpublished observations). Similarly, amebic toxins may produce edema of the underlying epithelium. The waviness of the epithelial surface outline could be the result of focal variations in epithelial mucin content, spasm of the muscularis mucosae, or regional variations in the rate of growth of the glandular epithelium.

Mucopenic Depression

In mucopenic lesions, *E. histolytica* produces small superficial depressions associated with loss of mucin from the surface and glandular goblet cells. The mechanism involved remains obscure, since human colonic mucin effectively prevents the binding of *E. histolytica* to target cells in vitro by inhibiting the galactose and *N*-acetyl D-galactosamine (Gal-GalNAc) adherence lectin of the parasite (33, 54).

The first barrier preventing mucosal injury is the intestinal mucus blanket. In the human colon it is 110 to 160 mm thick and is flanked by two additional barrier structures, a lipid monolayer that forms on the luminal surface of the mucus blanket and the surface coat of the apical plasma membrane of epithelial cells. These two components contribute significantly to the selective permeability of the mucus blanket. The lipids, together with the saccharide chains of mucins, also serve as scavengers against free-radical attack. The main components of colonic mucus are water and mucin glycoproteins. Mucus inhibits bacterial and toxin access to the epithelium and facilitates their removal by the luminal stream. Mucin also allows colonization by members of the native flora that compete with pathogenic organisms and, through short-lived low-affinity bonds between mucin and secretory antibodies, aids the anti-

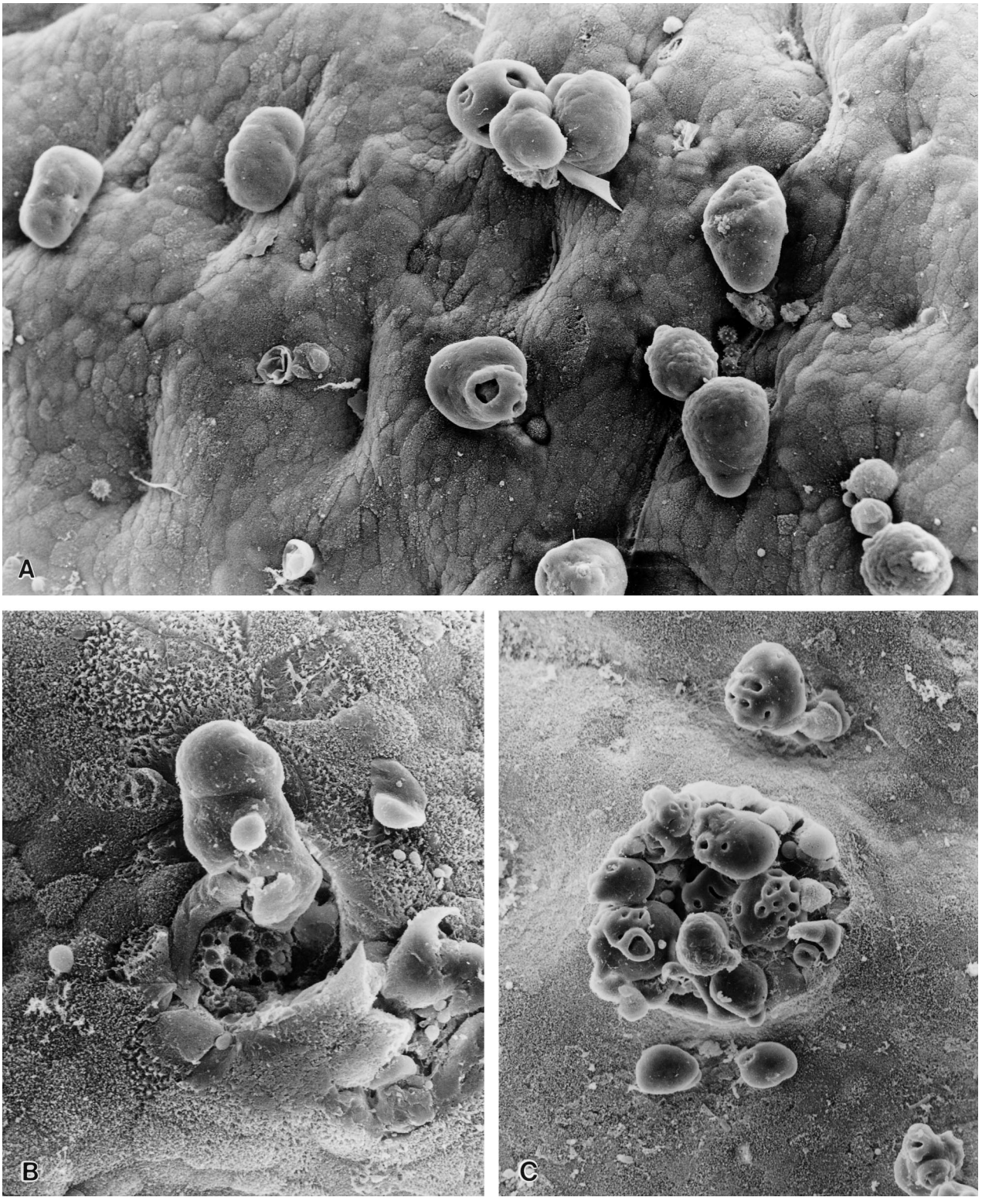

FIG. 3. Scanning electron micrographs of experimental intestinal amebiasis in the guinea pig. (A) Trophozoites of *E. histolytica* adhere preferentially to the elevated interglandular epithelium. (B) A small interglandular region of microinvasion is observed in the early invasive lesion with superficial ulceration. (C) In a more advanced stage of invasion, numerous trophozoites are seen penetrating a colonic ulcer.

bodies in trapping microbial pathogens and antigens. In performing these functions, mucus is secreted continuously and then either shed and discarded or digested and recycled. Mucus secretion and shedding rates can increase dramatically to eliminate toxic substances (36).

The highly glycosylated mucins could be the targets of glycosidic cleavage by the parasite. Glycosidase activities detected in *E. histolytica* include glucosidase (99, 150, 160), galactosidase (150, 160), mannosidase (160), fucosidase (150, 160), xylosidase (150, 160), glucuronidase (160), *N*-acetyl-D-glucosaminidase (14, 99, 150, 160, 176), *N*-acetyl-D-galactosaminidase (14, 150), *N*-acetylneuraminate lyase (160), neuraminidase (160, 165), amylase (99, 160), and hyaluronidase (160). Glycosidases are generally more active at acidic pH, suggesting their lysosomal origin. The highest activities are found for the glycogen-degrading enzymes α-amylase and α-glucosidase, consistent with the use of glycogen as the main carbohydrate source, as well as for the β-*N*-acetyl-D-glucosaminidase. However, the other enzymes show low activity, casting doubts on their participation in mucin degradation. The contribution of β-*N*-acetyl-D-glucosaminidase to the pathogenicity of the parasite through the digestion of mucin carbohydrate side chains is questionable, since both *E. histolytica* and the noninvading *E. dispar* produce the enzyme and since the same enzyme of the host is present extracellularly without pathologic effect (160). In addition, rat mucin internalized by whole trophozoites is exocytosed without undergoing proteolytic degradation (34). Since glycosidases are not secreted by the trophozoite (150), their activity on intestinal mucus could be due mostly to the liberation of enzymes from lysed trophozoites.

If mucin is not degraded exclusively through proteolytic cleavage by the parasite, how can mucus depletion be explained? It may be suggested that the continuous irritation could cause exhaustion of mucus-producing cells, which are responsible for the large amounts of mucus that constitute one of the pathognomonic signs of invasive amebic intestinal infections, i.e., diarrhea with mucus and blood. It has been reported that *E. histolytica* trophozoites in contact with the mucus layer of human adenocarcinoma cell lines stimulate the release of mucus glycoproteins by 300%, which suggests the presence of mucin secretagogues in the parasite (15, 66). However, more studies are needed to clarify this crucial step that allows access of the parasite to the underlying epithelium.

Early Invasive Lesions with Superficial Ulceration

Three main consecutive events occur at this stage: a focal superficial erosion of the mucosa, small glandular foci of microinvasion, and mild to moderate infiltration of the lamina propria.

Superficial epithelial erosion. Once the mucus barrier has been broken down, *E. histolytica* reaches the luminal surface of enterocytes and initially produces a contact-dependent focal and superficial epithelial erosion (Fig. 3). In vitro, time-lapse microcinematography has shown that the aggressive mechanism of *E. histolytica* is a complex multifactorial phenomenon that includes adhesion, a contact-dependent "hit-and-run" damage to the plasma membrane of effector cells, phagocytosis, and intracellular degradation of ingested cells (86). The adhesion and cytolytic events have been related to three types of molecules: lectins, amebapores, and proteases.

(i) Gal-GalNAc lectin. Adhesion of the parasite occurs mainly through a surface Gal-GalNAc lectin which binds to exposed terminal Gal-GalNAc residues of target cell glycoproteins (116). Other molecules include a 220-kDa lectin (131), a 112-kDa adhesin (10), and a surface lipophosphoglycan (151). It has been suggested that the Gal-GalNAc lectin could be translocated to the basolateral surface of enterocytes before the parasite exerts its cytolytic effect (77), but the benefits of this action for the parasite remain unclear.

The Gal-GalNAc adhesin is a multifunctional protein composed of an heterodimer of heavy (170-kDa) and light (35/31-kDa) subunits (116). Evidence for the participation of this molecule in the adhesion event of the parasite has been demonstrated by reduced amebic adherence to human erythrocytes, neutrophils, colonic mucins, and epithelia and to certain bacteria when the lectin is inhibited by galactose (17, 29, 33, 124). Complete inhibition of *E. histolytica* adherence to target cells or colonic mucins is not observed, even when the lectin is blocked with high (100 to 500 mM) concentrations of galactose or GalNAc monomers (42, 77, 115). It has been suggested that the spacing of multiple GalNAc residues on the surface of target cells is important for optimal lectin binding (1). In addition, other molecules (mentioned above) could participate in the adhesion event. In addition to its role in amebic adherence, the adhesin may participate in the cytolytic event, since contact-dependent target cell lysis is reduced in the presence of galactose and a monoclonal antibody against the heavy subunit is capable of partially inhibiting cytolysis without blocking adherence (133). Considering that the purified lectin has no cytotoxic effect even at high concentrations, it has been proposed that the adhesin is involved in the signaling of cytolysis, probably through the stimulation of actin polymerization (84, 133). Furthermore, the adhesin binds to purified C8 and C9 components of complement and blocks the assembly of the complement membrane attack complex on the amebic plasma membrane, suggesting a role in mediating amebic resistance to complement lysis through components C5b through C9 (18).

The heavy subunit of the Gal-GalNAc adhesin is encoded by five genes in *E. histolytica* strain HM1:IMSS (83, 121, 155). The derived amino acid sequences suggest that the heavy subunit is an integral membrane protein with a small cytoplasmic tail and a large extracellular N-terminal domain containing a cysteine-rich region. The cytoplasmic tail has sequence identity with β_2-integrin cytoplasmic tails and seems to be involved in the inside-out signaling that controls the extracellular adhesive activity of the amebic lectin (169). On the other hand, the cysteine-rich region seems to be essential for several activities described for the lectin. With the aid of monoclonal antibodies, several antigenic determinants that affect adherence and complement resistance have been mapped to this region (reviewed in reference 92). Moreover, the carbohydrate recognition domain is contained within the cysteine-rich region of the lectin (43). The light subunit shows two 35- and 31-kDa isoforms, with the latter having a glycosylphosphatidylinositol anchor. This subunit is also encoded by a gene family that have 79 to 85% nucleotide sequence identity (123). Its function is not yet clear but seems to involve the modulation of parasite cytopathic activity (7, 109).

Comparison between *E. histolytica* and *E. dispar* reveals very similar Gal-GalNAc adhesins that efficiently bind to target cells and colonic mucins (29, 42, 119). Derived amino acid sequences encoded by homologous genes show that *E. dispar* heavy- and light-subunit clones are 86 and 79% identical in their primary structures, respectively, to their *E. histolytica* counterparts (42, 119). In vitro, *E. dispar* exhibits adherence and cytotoxicity to target cells that is mediated by the Gal-GalNAc lectin (42), although to a lesser extent than *E. histolytica* (42, 49). These observations are in accordance with the ability of *E. dispar* to produce superficial erosions in the colonic mucosa of experimental animals (28) but leaves unan-

swered the question why this ameba does not invade any further.

(ii) Amebapores. Once *E. histolytica* establishes contact with mammalian cells in vitro, a rapid cytolytic event takes place that results in swelling, surface blebbing, and lysis of the inadvertent target cell, including lymphocytes, polymorphonuclear leukocytes, and macrophages, leaving the parasite unharmed. The similarity of this event to the perforin-mediated lysis of target cells by cytotoxic T lymphocytes (162) initially suggested the participation of a channel-forming protein called the amebapore, whose activity had been identified in *E. histolytica* lysates (82, 132, 178).

The amebapore of *E. histolytica* is a channel-forming peptide of 77 amino acid residues, which has now been purified; the protein has been sequenced, and the respective genes have been cloned (68, 69). Three isoforms, amebapores A, B, and C, are present at a ratio of 35:10:1, respectively, with the genes showing 35 to 57% deduced amino acid sequence identity. The molecules share six cysteine residues at identical positions and a histidine residue near the C terminus. Structural modeling suggests a compact tertiary structure composed of four α-helical structures stabilized by three disulfide bonds (72). Thus, amebapores are different from the much larger (65- to 70-kDa) perforins that contain three amphipathic segments, two α-helices and one β-sheet (162). However, similarities at the structural and functional levels have been found between amebapores and NK-lysin, a polypeptide present in natural killer (NK) cells and cytotoxic T lymphocytes of pigs (reviewed in references 73 and 75).

Like other pore-forming peptides, amebapores are readily soluble but are capable of rapidly changing into a membrane-inserted stage (75). Early observations suggested that the molecule forms multistate channels with similar properties to those found in the barrel-stave aggregates of toxins such as alamethacin (65). With the elucidation of the primary and secondary structures, it is now believed that amebapores aggregate through the arrangement of their amphipathic α-helices. In a model proposed by Andrä and Leippe (5), amebapores bind to negatively charged phospholipids via protonated lysine residues; this is followed by the insertion of the peptide into the lipid bilayer driven by the negative membrane potential of the target membrane. Oligomerization of the peptide occurs with the participation of a key histidine residue (His^{75}), that may either interact with another monomer through the formation of hydrogen bonds or stabilize the predicted fourth α-helix. The oligomer forms a channel through the plasma membrane, allowing the passage of water, ions, and other small molecules and thus lysing the target cell.

In vitro, amebapores exert cytolytic activity against several human cultured cell lines. Amebapore C seems to be the most effective, while amebapore A is not efficient in lysing erythrocytes. In addition, the peptides show potent antibacterial activity against gram-positive bacteria by damaging their surface membranes. Damage to the outer membrane-shielded gram-negative bacteria requires high concentrations of amebapore or removal of the wall with lysozyme (6, 71, 72). With the use of synthetic peptides, of the four α-helices present in the molecule, helix 3 was found to be responsible for the membrane penetration, displaying the highest antibacterial activity. Interestingly, helix 3 is also the most highly conserved domain in the three isoforms (71). The peptide derived from isoform C was the most active of several synthetic peptides studied, reaching a magnitude of activity in the range of the whole molecule (6).

Amebapores are localized in cytoplasmic vesicles, as evidenced by positive immunofluorescence staining and by the presence of typical signal peptides of intracellular transport in its primary translation products. The peptides show maximum activity at acidic pH, which is consistent with previous observations that lysis of target cells by *E. histolytica* required a pH of 5.0 within amebic vesicles (69).

A peptide homologous to *E. histolytica* amebapore A has been identified in *E. dispar* (70). The molecules have common structural and functional properties, such as insertion into negatively charged liposomes, highest activity at low pH, localization in cytoplasmic vesicles, 95% identity of primary structures, and a high degree of similarity of secondary-structure predictions. In spite of these similarities, the specific activity of the *E. dispar* amebapore is 60% lower than that of the one in *E. histolytica*. This may be related to a shortened amphipathic helix in the former (70).

Surprisingly, in spite of all the advances in the biochemistry and molecular biology of amebapores, their participation in the cytolytic event produced by *E. histolytica* has not yet been demonstrated. Amebapores are not spontaneously secreted from viable trophozoites (74). Whether the molecule is able to insert into target cell membranes upon adherence in vivo remains to be established.

The presence of pore-forming activity in the noninvasive *E. dispar* suggests that the primary function of amebapores is to destroy phagocytosed bacteria, the main intestinal source of nutrients of amebas; thus, they have a similar function to defensins found in mammalian phagocytes that kill bacteria and fungi to prevent intracellular microbial growth within digestive vacuoles (reviewed in references 75 and 158). The anaerobic environment found in the colon may favor oxygen-independent mechanisms as a means of destroying bacteria, rather than using oxygen metabolites and nitric oxide. Amebapores and other proteins, like the recently characterized ameba lysozyme that colocalizes to the same cytoplasmic granules of amebapores (61, 105), could synergistically enhance the antibacterial activity.

Interglandular foci of microinvasion. Focal superficial epithelial erosions produced by *E. histolytica* in humans are followed by small interglandular foci of microinvasion. Electron microscopy studies of experimentally infected rodents have confirmed the invasion of trophozoites through the interglandular epithelium (Fig. 3), where pronounced shedding of desquamating epithelial cells takes place. The superficial desquamation seems to render this area particularly susceptible to invasion, which occurs through an active displacement of amebas with large pseudopodia extending toward the basal epithelial layers (88, 149). During their passage to deeper layers of the intestine, trophozoites must lyse surrounding cells and degrade the extracellular matrix (ECM) components of the colonic mucosa. Thus, this stage of lesion is characterized by continuing lysis of cells, penetration through locomotion, and proteolytic degradation of ECM (Fig. 2). The way in which the parasite exerts its cytolytic action is described above. The last two events, locomotion and ECM degradation, seem to be closely related and possibly occur through cycles of anchorage to ECM components, forward movement, and degradation of ECM.

(i) Invasion through locomotion. In mammals, cell locomotion involves at least three processes: (i) extension of a leading edge, (ii) attachment to the substrate surface through adhesion plaques, and (iii) pulling forward of the remainder of the cell. The motile force underlying cytoplasmic streaming is a Ca^{2+}-activated interaction between actin and myosin, similar to the one described for skeletal muscle contraction. In *E. histolytica*, actin polymerization occurs during pseudopod extension (47). No reports are available on fluctuations in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) during locomotion, but the presence of

myosin II in leading lamellae (47) suggests that the mechanism for leading-edge extension in amebas is similar to that in mammalian motile cells.

Interaction of *E. histolytica* trophozoites with fibronectin (FN) and other extracellular matrix substrates induces the formation of adhesion plaques. Ligand recognition and binding seem to involve a 37-kDa FN-binding protein (152) and a 140-kDa integrin-like receptor (153, 154). Isolated amebic adhesion plaques are composed mainly of actin filaments and at least four actin binding proteins: vinculin, α-actinin, tropomyosin, and myosin I (166). Vinculin may serve as a link between actin and an integral component during anchoring of the cytoskeleton to the plasma membrane in higher eukaryotes. Its identification in amebic adhesion plaques suggests a similar function in this parasite. The presence of α-actinin in *E. histolytica* had already been reported (13). However, the identification of this gel-forming protein in adhesion plaques suggests a role in the stabilization of actin filaments. In mammalian cells, tropomyosin is not present in adhesion plaques but is found in stress fibers, which are absent in amebas. In muscle cells, this cofilamentous protein sterically blocks the interaction of actin and myosin but shifts its position when the $[Ca^{2+}]_i$ is raised, thus allowing contraction to take place. As discussed below, adhesion of *E. histolytica* trophozoites to FN induces a sustained rise of $[Ca^{2+}]_i$. The unexpected finding in amebic adhesion plaques of myosin I, a molecule known to participate in the intracellular transport of vesicles in higher eukaryotes, awaits further explanation.

The mechanism of the third process involved in cell motility, pulling forward of the remainder of the cell, is still obscure in *E. histolytica*. During locomotion, the cell body of amebas move toward the pseudopod, leaving a trailing uroid. Actin and myosin II have been identified in the uroid (9), but no regulatory proteins have been reported.

The most mysterious aspect of locomotion, however, is related to its control. Only recently have signaling pathways in *E. histolytica* started to be explored. As stated above, interaction of *E. histolytica* with FN induces a marked reorganization of the actin cytoskeleton, with the formation of adhesion plaques. FN binding transduces information into the trophozoites, activating a protein kinase C (PKC) pathway with the production of inositol triphosphate and the phosphorylation of several proteins, probably including PKC itself (137). Activation of PKC through binding of FN or direct stimulation with phorbol myristate acetate shows a direct relationship to actin polymerization and assembly of various actin binding proteins in the adhesion plaques (reviewed in reference 94). Although the targets of PKC have not been identified, its activation sheds some light on one signaling pathway in the parasite.

A second signaling pathway seems to occur via a focal adhesion kinase, $pp125^{FAK}$, identified in immunoblots of isolated amebic adhesion plaques induced by FN (166). Moreover, adhesion of *E. histolytica* trophozoites to collagen induces the phosphorylation of several proteins, one of which has been identified as $pp125^{FAK}$, further suggesting its participation in signal transduction (111). It has been proposed that activation of $pp125^{FAK}$ might initiate a signal transduction pathway that activates the mitogen-activated protein kinase cascade, allowing the flow of information from the ECM to the cell interior. This is supported by the identification of $p42^{MAPK}$ in tyrosine-phosphorylated polypeptides induced by collagen-stimulated trophozoites (111).

Incubation of trophozoites with FN produces a sustained rise in $[Ca^{2+}]_i$, which promotes the stabilization of adhesion plaques and focal contacts by polymerizing soluble actin. External Ca^{2+} influx is responsible for the increased $[Ca^{2+}]_i$, since cytoplasmic Ca^{2+} stores are rapidly depleted (30). In the absence of Ca^{2+} following the addition of chelating agents, poor adhesion of trophozoites is observed. Since tropomyosin has been identified in isolated adhesion plaques, it is possible that this protein regulates actin-myosin interactions, as occurs in muscle cells, where increased Ca^{2+} levels induce a conformational change in troponin that shifts the position of tropomyosin, thus allowing the interaction between actin and myosin.

Recent studies indicate that actin organization might have different levels of regulation, namely, changes in actin gene expression and balance between the monomeric G-actin and polymerized F-actin configurations. Treatment of *E. histolytica* trophozoites with drugs known to increase cytoplasmic cyclic AMP levels (forskolin and dibutyryl cyclic AMP) or to activate PKC (phorbol myristate acetate) produces a shift in the actin equilibrium to the F-actin form, which in turn increases actin mRNA levels (85). This suggests an actin feedback-regulatory mechanism, previously described in other cells, where the gene product and the configuration of the microfilaments could directly regulate actin gene expression (85). Evidence for another regulatory mechanism in actin organization is provided by the recent isolation and cDNA cloning of the *E. histolytica* profilin basic isoform (16). Profilin acts as a buffer in actin organization by either inhibiting or promoting actin filament formation.

(ii) Degradation of ECM components by cysteine proteases. Once regarded as a passive support element for cells and tissues, the ECM is now considered a highly active tissue that participates in cellular migration, proliferation, differentiation, and immune system signaling. In the human colon, the basement lamina underlying the epithelium consists mainly of type IV collagens, laminins, and proteoglycans and contains a number of basement lamina-associated molecules such as fibronectin, tenascin C, and entactin (114). In addition, fibronectin, laminin, and collagens type I, III, IV, and VI have been identified in the lamina propria and muscularis mucosae (3).

The amebic adhesion plaques described above seem to be important not only in the adhesion and locomotion of the parasite but also in the degradation of ECM components. This is supported by the detection of several protease activities in isolated adhesion plaques (166).

Cysteine proteases are the most abundant proteases in the parasite. Potent cysteine protease activities ranging from 16 to 116 kDa are found in amebic extracts electrophoresed on substrate gels (11, 38, 108, 112, 138, 147). Cell fractionation studies indicate that although present in the cytosol, they are enriched in the plasma and internal membranes. In addition, secreted protease activities of 16, 26, and 56 kDa have been reported (64, 80, 81). A total of six distinct genes (*EhCP1* through *EhCP6*) encoding prepro forms of cysteine proteinases have been identified. The enzymes encoded by three of these genes have been purified and characterized, namely, EhCP1 (ACP3; previously known as amebapain) (138, 156), EhCP2 (ACP2; earlier reported as histolysin) (80, 157), and EhCP5, a membrane-bound protease (62). These three enzymes, all with a molecular mass of ~30 kDa, account for ~90% of cysteine protease transcripts and for virtually all cysteine protease activity found in *E. histolytica* lysates (24). It remains to be established whether the different molecular masses reported for other protease activities represent different enzymes or the expression of different states of one of the six genes identified (97).

Amebic cysteine proteases are active against a variety of substrates and increased activity has been reported in clones of high virulence (103). Of the ECM components that *E. histo-*

TABLE 1. Cysteine proteases of *E. histolytica*

Enzyme	Size	pH	Substrates	Other features	Reference(s)
Amebapain (EhCP1 or ACP3)	27 ± 2 kDa,[a] 24 kDa[b]	5.0–8.5	Human laminin and FN, bovine collagen type I, and human collagens types IV (70-kDa component) and V (α_1-chain)	Multicopy gene, high levels of expression	24, 139–141, 156, 157
Histolysin (EhCP2 or ACP2)	26–29 kDa,[a] 23.6 kDa[b]	5.5–9.5	Human collagen type IV, bovine nasal cartilage; not active against rabbit skin collagen type I or elastin	Single-copy gene, high levels of expression	24, 80, 157
EhCP3 or ACP1	NA[c]	NA	NA	Only primary structure available; single copy-gene, low levels of expression	24, 25, 95, 125
EhCP4	33,726 Da[b]	NA	NA	Only primary structure available; multicopy gene, very low levels of expression	24
Membrane bound protease (EhCP5)	30 kDa,[a] 24 kDa[b]	6.0–8.0	FN, immunoglobulin G, C3 and C9 components of complement	Single-copy gene, high levels of expression; high content of hydrophobic regions can explain membrane association	12, 24, 62
EhCP6	35,324 Da[b]	NA	NA	Only primary structure available; single-copy gene, very low levels of expression	24
Major neutral protease	56 kDa[a]	6.0–7.0	Rat-derived laminin, fibronectin, collagen type I, immunoglobulin G, C3 (α-chain) and C5 components of complement, and anaphylatoxins C3a and C5a	Secreted enzyme	64, 126–128, 159
Hemoglobinase	82 kDa[a]	7.0	Human, bovine, and porcine hemoglobin	Two additional minor bands of 116 and 21 kDa with hemoglobinase activity are described	147

[a] Reported molecular mass calculated after gel electrophoresis run with known molecular mass standards.
[b] Reported molecular mass deduced from the amino acid sequence of the enzyme.
[c] NA, not available.

lytica encounters during colonic invasion, laminin, collagen types I and IV, and FN are good targets for amebapain, histolysin, EhCP5 membrane-bound protease, and the neutral 56-kDa protease (Table 1).

E. dispar seems to possess four homologous cysteine protease genes, although none of the enzymes (EdCP2, EdCP3, EdCP4, and EdCP6) have been purified. In addition, the presence of the neutral 56-kDa protease in *E. dispar* has not been reported yet. If *E. dispar* indeed lacks several of the most potent *E. histolytica* cysteine proteases (EdCP1, EdCP5, and the neutral protease), it is possible that this difference could partially explain its noninvasive nature.

In addition, *E. histolytica* possesses a membrane-bound metallocollagenase that degrades collagen types I and III and is more active against the former (100). The degree of collagenolytic activity has been linked to the virulence of different isolates (53, 101, 145, 164). Activation of the enzyme apparently results in translocation of the enzyme from internal membranes to the plasma membrane (87).

In vitro, incubation of trophozoites on collagen-coated wells induces the formation and release of electron-dense granules (EDGs) through a cytoskeleton-driven, Ca^{2+}-calmodulin-dependent mechanism (87, 102, 146). In addition to a collagenolytic activity, EDGs seem to contain at least 25 polypeptides with acidic pIs (6.06 to 6.59), 9 gelatinase activities, actin, small molecules including inorganic phosphate (P_i) and pyrophosphate (PP_i), and several ions. Six of these polypeptides (108, 106, 104, 97, 68, and 59 kDa) and two protease activities of 40 and 85 kDa have been claimed to be detected in EDGs but not in total-trophozoite extracts (76). Whether some of the above-mentioned components represent contamination with cytoplasmic molecules not found in EDGs remains to be established.

In summary, *E. histolytica* possesses the necessary machinery to degrade the ECM components it encounters during invasion. However, the participation of the different proteases during in vivo infections has yet to be demonstrated.

Neutrophil infiltration of the lamina propria. The last observation of the early invasive lesion with superficial ulceration is a mild to moderate infiltration of the lamina propria. At this stage, cell infiltration around invading amebas leads to rapid lysis of inflammatory cells and tissue necrosis. These observa-

tions have been confirmed in rodent models of intestinal amebiasis (88, 149).

Neutrophils, representing over 90% of the circulating granulocytes, respond to a variety of cytokines and soluble factors during the inflammatory response. C5a, the cleavage product of complement component C5, is one of the most potent chemotactic and activation molecules for phagocytes. In amebic intestinal lesions, however, the participation of C5a in the neutrophil infiltration of the lamina propria is uncertain, since *E. histolytica* trophozoites release a neutral cysteine protease that degrades this molecule (128).

In recent years it has become evident that gut inflammation is not only the result of the immune system response to an intestinal insult but also the result of a complex interplay of immune and nonimmune cell interactions that involve epithelial, mesenchymal, endothelial, and nerve cells, as well as components of the ECM (51). Studies on mucosal immunity have provided growing evidence that intestinal epithelial cells, for instance, constitutively express or can be induced to express a number of immunologically active cytokines and soluble factors, including interleukin-8 (IL-8), monocyte chemoattractant protein 1, granulocyte-macrophage colony-stimulating factor, and tumor necrosis factor alpha (TNF-α) (93). Thus, in addition to their fundamental absorptive and secretory functions, intestinal epithelial cells are integral and essential components of the host's innate and acquired immune system.

Neutrophils are rapidly recruited and activated in response to the proinflammatory cytokine IL-8. In human colon epithelial cell lines, *E. histolytica* trophozoites increase the secretion of IL-8 and TNF-α (45, 179). At least three possible mechanisms have been described for the upregulation of IL-8. Some colon epithelial cell lines increase the secretion of IL-8 in response to the preformed IL-1α released from the cells lysed by *E. histolytica*. In other cell lines lacking preformed IL-1α, increased IL-8 production seems to be mediated by the adherence of trophozoites through the Gal-GalNAc lectin by a mechanism that involves, at least partially, an increase in $[Ca^{2+}]_i$ (45). In a third mechanism, *E. histolytica* secreted products could induce the secretion of IL-8 in the absence of trophozoite contact (179). Whether any of these mechanisms take place during natural intestinal infection remains to be established, but further support for the importance of intestinal epithelial cells as producers of inflammatory cytokines comes from studies using a severe combined immunodeficient (SCID) mouse-human intestinal xenograft model (148). In this model, increased IL-1α and IL-8 production and extensive neutrophilic infiltration followed infection of the xenograft with *E. histolytica*. Moreover, both cytokines were of epithelial origin from the human intestinal xenograft (148).

Finally, a chemoattractant activity for neutrophils has been found in *E. histolytica* (32). Although the molecule responsible for this activity has not been purified, enzymatic treatment suggests that it corresponds to a membrane-bound peptide.

Role of neutrophils. Cell infiltration around invading amebas leads to rapid lysis of inflammatory cells followed by tissue necrosis. The reasons for the inability of neutrophils to destroy *E. histolytica* are unknown. In other parasitic infections, neutrophils kill invading microorganisms by both O_2-dependent and -independent mechanisms; they produce an intense oxidative burst, and their secretory granules contain highly cytotoxic molecules. *E. histolytica* could avoid the oxidative burst through an iron-containing superoxide dismutase that can be induced by superoxide anions to produce H_2O_2 (22). In addition, a bifunctional NADPH:flavin oxidoreductase containing NADPH-dependent disulfide reductase and H_2O_2-forming NADPH oxidase activities could aid in the detoxification of hydroperoxides produced during an oxidative stress (23, 27). Although the ameba does not contain catalase, a cysteine-rich 29-kDa protein in amebas has been shown to eliminate H_2O_2 (21, 26, 52). There is no evidence, however, on how *E. histolytica* might escape the action of the cytotoxic molecules released by the neutrophils. The parasite must possess still unexplored mechanisms of protection against its own lytic peptidases, but whether it uses them to avoid attack by external cytotoxic molecules is unknown.

Alternatively, it could be speculated that neutrophils present in human *E. histolytica* lesions are not properly activated. As stated above, humans are the only natural host for *E. histolytica*. Unfortunately, no experimental model has been developed to date that reproduces the invasive intestinal amebic lesions seen in human intestinal amebiasis. Although the results are highly reproducible with the best model available, the gerbil, they can only mimic the initial stages of infection but resolve in 96 h (149). This has been interpreted as an innate resistance to *E. histolytica*. Recent studies using the model of induction of amebic liver abscess have started to explore the cellular basis of this innate resistance in mice (167). Following intrahepatic inoculation of trophozoites, neutropenic mice developed larger liver lesions than did normal mice, suggesting a role for neutrophils, albeit partial, in the mechanism of resistance in these rodents (167). Also, in the highly susceptible hamster liver model, in contrast to what occurs with *E. histolytica*, neutrophils effectively eliminate *E. dispar* within 24 h (48). Comparison of the responses induced by the two species of amebas in experimental liver and intestinal infection might shed some light on their strikingly different pathogenic behaviors.

Neutrophils not only fail to resist *E. histolytica* but may in fact contribute to host tissue damage through their destruction and release of cytotoxic granules (88, 163). In vitro, human neutrophils are killed even by low-virulence strains of *E. histolytica* in a 400:1 ratio of neutrophils to amebas, although many of these parasites succumb in the process. Highly virulent strains, however, emerge victorious after incubations with 3,000 neutrophils per ameba (55).

In addition to the contribution of neutrophils, amebas play a direct role in tissue necrosis. Evidence for this is available only from the model of induction of amebic liver abscess, where the parasite is able to produce liver damage in the absence of an inflammatory reaction in neutropenic mice (167). In the same report (167), images suggesting apoptosis of hepatocytes close to the inflammatory infiltrate or in areas of the liver parenchyma distant from amebas or inflammatory cells are shown.

Late Invasive Lesion with Deep Ulceration

As invasion progresses, the mucosal ulcer extends deep into a larger area of the submucosa. Once the interglandular epithelium has surrendered to amebic penetration, the underlying tissue offers little resistance, allowing extension of the ulcer into the typical flask form. While continuing the invasion process through degradation of ECM and locomotion, the parasite might use its hemoglobinases to digest phagocytosed erythrocytes, thus obtaining the necessary iron for its survival (147). As a result of lysed cells and necrosis, the observed thick exudate containing acellular proteinaceous material, red blood cells, and strands of fibrin is explained, as is the deeply eosinophilic zone of fibrinoid necrosis separating the exudate from the underlying viable submucosa.

At this stage, infiltration by abundant neutrophils and some lymphocytes and macrophages is observed, while eosinophils

are rare. The paucity of macrophages is noticeable and probably results from the release of a monocyte locomotion-inhibitory factor, a small thermolabile amebic product capable of inhibiting chemotaxis, chemokinesis, and random mobility of human mononuclear phagocytes but not of polymorphonuclear leukocytes (67). In addition, the degradation of anaphylatoxins C3a and C5a by the secreted neutral cysteine protease (128) could inactivate these important proinflammatory cytokines.

Role of macrophages. The role of macrophages in acute intestinal amebiasis remains obscure. In vitro, human polymorphonuclear leukocytes, peripheral blood mononuclear cells, and monocytes are rapidly killed by *E. histolytica* trophozoites (134). An amebicidal effect has been reported only after activation of human monocytes and murine spleen, peritoneal, and bone marrow-derived macrophages with high doses of gamma interferon (IFN-γ) (10^2 to 10^3 U/ml), TNF-α (10^4 to 10^5 U/ml), or colony-stimulating factor 1 (10^4 U/ml), with a macrophage-to-ameba ratio of 200:1 (40, 78, 134). However, a high proportion of activated macrophages are still killed during this interaction (40, 134). The amebicidal effect exerted by activated macrophages is contact dependent and involves both oxygen-dependent (NO, H_2O_2, O_2^-) and -independent (cytolytic protease) pathways (40, 78, 79, 134, 143).

In contrast to these in vitro observations with activated macrophages, a deficiency in parasite-specific cell-mediated immunity occurs in human amebiasis, which gradually recovers following anti-amebic therapy (107, 135). Experimental models confirm that *E. histolytica* infections are associated with suppression of cell-mediated immunity and particularly with impaired macrophage effector functions. Thus, in the rodent model of induction of amebic liver abscess, macrophages isolated from amebic liver granulomas are refractory to IFN-γ and lipopolysaccharide activation, defective for the production of H_2O_2, IL-1, and TNF-α, and ineffective in amebic killing (39, 173, 174). During the course of cecal amebiasis in gerbils, peritoneal exudate cells are significantly more cytotoxic to trophozoites than are macrophages isolated from mesenteric lymph nodes (31). This higher activity has also been reported for spleen and peritoneal macrophages isolated from intrahepatically infected animals (39), suggesting a local suppression of macrophage functions while distant macrophage populations remain unaffected. In vitro, exposure of naive macrophages to amebic proteins inhibits the production of TNF-α, IL-1, H_2O_2, and NO_2^- as well as the IFN-γ-induced macrophage surface Ia antigen synthesis and IAβ-mRNA expression (39, 173–175). The possible mechanisms for this amebic-specific transient anergy state have only started to be explored.

Macrophage activation is a complex phenomenon whose effector functions depend on environmental and maturational effects and on the lymphokines and inflammatory stimuli to which they are exposed. To become activated, macrophages respond to external signals through signal transduction pathways like the PKC and PKA cascades. PKC has been linked to macrophage activation, while PKA is associated with decreased TNF-α mRNA accumulation in macrophages. The c-*fos* gene is among the early genes expressed during macrophage activation, with expression being rapid and transient in PKC stimulation and stable for hours in PKA stimulation. In vitro, naive bone marrow-derived macrophages from BALB/c mice exposed to *E. histolytica* proteins increase the expression of c-*fos* and TNF-α mRNA via the PKC pathway. However, both mRNAs are rapidly degraded and no TNF protein is secreted, suggesting a mechanism whereby the parasite can suppress macrophage function (142).

Surface expression of class II major histocompatibility complex (MHC) molecules is necessary for the effective participation of macrophages as antigen-presenting cells to $CD4^+$ helper T lymphocytes. Transcription of class II MHC genes in macrophages is induced by IFN-γ. This molecule also stimulates the production of TNF-α, which, together with IFN-γ, induces L-arginine-dependent cytotoxic effector mechanisms through the production of nitric oxide (NO) (110). During the development of amebic liver abscess in gerbils, macrophages isolated from the lesions are refractory to IFN-γ and LPS stimulation and show decreasing levels of TNF-α production as the infection progresses (173, 174). Thus, these macrophages could be impaired in their antigen-presenting function (see also below) and in the production of cytotoxic molecules like NO.

Prostaglandin E_2 (PGE_2) is a modulator of the immune response, having proinflammatory effects that enhance edema and leukocyte infiltration while inhibiting some macrophage functions like IL-1 and TNF-α production and class II MHC gene complex expression (118). In the gerbil model, amebic liver abscess-derived macrophages display high basal levels of PGE_2 production, which can be further enhanced upon stimulation with live trophozoites or amebic proteins (172). In addition, naive peritoneal and bone marrow-derived macrophages are induced to secrete PGE_2 following incubation with *E. histolytica* proteins (172, 175), which results in decreased TNF-α production in response to lipopolysaccharide stimulation (173). Production of enhanced levels of PGE_2 by macrophages in response to amebic proteins is in part responsible for the suppression of IFN-γ-induced macrophage surface Ia antigen synthesis and IAb-mRNA expression from the class II MHC gene complex, since cotreatment with the cyclooxygenase inhibitor indomethacin partially restores surface Ia antigen expression (175). Further support for the participation of PGE_2 in the downregulation of macrophages has been obtained in vivo. Although the effect of PGE_2 has not been tested in animal intestine models, results obtained from rodents inoculated intrahepatically with amebas suggest a role for this molecule in the induction of amebic liver abscess. Thus, hamsters treated with indomethacin show decreased levels of PGE_2 in plasma and reduction in size of abscess lesions by 30% (136).

How can the in vivo macrophage anergy state be reconciled with the in vitro enhanced ability of activated macrophages to kill *E. histolytica*? As detailed above, amebic proteins inhibit naive macrophage activation (39, 173–175). However, if macrophages are activated prior to exposure to live trophozoites, there is a 10-fold increase in TNF-α production (173), explaining the enhanced amebicidal activity observed in vitro.

Another topic of interest in the late invasive lesion is the presence of trophozoites in the internal muscular layer in the absence of tissue damage, which seems to occur quite frequently in biopsy samples from patients with invasive amebiasis (91). It has been speculated that tissues might have different susceptibilities to the aggressive mechanisms of the parasite. However, amebas can invade and destroy virtually every tissue in the organism. It has also been suggested that there may be a requirement for a minimal number of trophozoites to produce tissue damage. This could be a possibility, since histopathological sections of intestinal tissue from humans with amebiasis show that in the muscle layer amebas tend to be isolated rather than forming aggregates. However, this does not explain the absence of the inflammatory infiltrate, because in experimental models strikingly large foci of acute inflammation are elicited by very few parasites (163).

Granulating Ulcer

The formation of granulation tissue is characterized by the proliferation of small vessels and usually indicates healing of a lesion. Since the reports describing this stage of amebic lesions in humans do not provide data on the clinical status of the patient, it is difficult to interpret whether this repair process occurs spontaneously or is secondary to antiamebic chemotherapy. It is known that if properly treated, invasive amebic lesions in humans, whether localized in the large intestine, liver, or skin, almost invariably heal without the formation of scar tissue. The mechanism of this striking repair process is still unknown.

CONCLUSIONS

Lysis of the colonic mucosa in intestinal amebiasis has been related to a variety of molecules produced by *E. histolytica*: adhesins, amebapores, and proteases. A multifunctional adherence lectin allows the parasite attachment to the colonic mucus blanket, thereby avoiding elimination through the intestinal stream. The lectin is also involved in signaling cytolysis and in blocking the deposition of the harmful membrane attack complex of complement, and it could participate in the anchorage of the ameba to proteoglycans during the invasion process. The amebapores of *E. histolytica*, small but potent peptides, destroy ingested bacteria that serve as the main nutrients for the parasite in the otherwise nutrient-scarce colonic environment. Their participation in the cytolytic event has not yet been proven. Proteases can be used to degrade the extracellular matrix during invasion and aid in the lysis of target cells. In addition, interesting mechanisms of parasitic modulation of the host immune response are starting to be unravelled. The main targets of this modulation appear to be neutrophils and macrophages, which, although recruited at the site of the lesion, are unable to abort infection.

In spite of these advances, many questions must be answered before we can fully understand the sequence of events during amebic invasion of the colon. To name but a few, the signal for the initiation of the invasion process, the mechanism of elimination of the mucus barrier, the role of EDG, and the in vivo participation of the different molecules in this multifactorial process have not been demonstrated. The recent advances in amebic transfection technology (56, 98, 104, 106, 122, 168) will undoubtedly shed new light on the involvement of specific molecules in the pathogenic mechanism. The uncertainty of the ploidy of *E. histolytica* has hindered the production of gene knockout clones like those produced for the study of the pathogenicity of *Toxoplasma gondii* and *Leishmania* spp. (44, 46). However, inhibition of expression of a surface antigen has already been achieved through transfection and transcription of its antisense RNA (4). In vivo infections with these modified trophozoites in current or in newly developed animal models (knockout or SCID mice) would also help our understanding of the process. Finally, much work needs to be done to clarify the complex signalling pathways of the parasite and to explain the modulation of the host immune response that results in the establishment and continuation of infection.

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