Entamoeba histolytica: parasite-host interactions

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Entamoeba histolytica remains a significant cause of morbidity and mortality worldwide. E. histolytica causes two major clinical syndromes, amebic colitis and amebic liver abscess. Recent advances in the development of in vitro and in vivo models of disease, new genetic approaches, the identification of key E. histolytica virulence factors, and the recognition of crucial elements of the host response to infection have led to significant insights into the pathogenesis of amebic infection. E. histolytica virulence factors include 1) a surface galactose binding lectin that mediates E. histolytica binding to host cells and may contribute to amebic resistance to complement, 2) amebapores, small peptides capable of lysing cells, which may play a role in killing intestinal epithelial cells, hepatocytes, and host defense cells, and 3) a family of secreted cysteine proteinases that play a key role in E. histolytica tissue invasion, evasion of host defenses, and parasite induction of gut inflammation. Amebae can both lyse host cells and induce their suicide through programmed cell death. The host response is also an important factor in the outcome of infection, and neutrophils may play a key role in contributing to the tissue damage seen in amebiasis and in controlling amebic infection.

FEW MICROORGANISMS are more aptly named than Entamoeba histolytica, the “tissue-lysing” ameba. This invasive intestinal protozoan parasite infects ~50,000,000 people worldwide and kills more than 50,000 people yearly. The two major clinical manifestations of E. histolytica infection are amebic colitis and amebic liver abscess. The pathological picture of amebic colitis ranges from multiple discrete ulcers separated by regions of normal-appearing colonic mucosa to a diffusely inflamed and edematous mucosa (22). These findings may strongly resemble those seen with inflammatory bowel disease. Microscopic studies have shown that early in the disease, amebic trophozoites cause thickening of the colonic mucosa and a mild to moderate infiltration of neutrophils around capillaries and within the epithelium (22). Over time, neutrophil infiltration increases and lymphocytes and macrophages can be seen in the lamina propria, progressing to mucosal ulceration with E. histolytica trophozoites invading through the colonic mucosa and undermining the submucosal tissues, creating a “flask-shaped” ulcer. Neutrophils are present at the submucosal layer but are less common in the exudate overlying the ulcer.

Amebic trophozoites also cause liver abscesses with well-circumscribed lesions containing dead hepatocytes and cellular debris without a preceding phase of hepatitis. A rim of connective tissue, some inflammatory cells, and a few amebic trophozoites surround the lesion, whereas the adjacent liver parenchyma is usually completely normal.

The goal of this themes article is to summarize recent studies directed at understanding the molecular basis for E. histolytica induction of gut inflammation, tissue damage, and cell death. Because of space limitations, many important contributions could not be cited. The reader is referred to other recent reviews for a more comprehensive look at amebic infection (11, 21).

PATHOPHYSIOLOGY OF AMEBIC COLITIS

The first step in bowel invasion is adherence of E. histolytica trophozoites to colonic epithelial cells. The primary molecule implicated in the adhesive process is a galactose/N-acetylgalactosamine-specific lectin, which consists of noncovalently linked 170-kDa and...
35- or 31-kDa subunits (40). The heavy subunit contains the carbohydrate binding domain and a short cytoplasmic tail that could be involved in signaling by the lectin (40). The function of the light subunit is unknown, but inhibition of light subunit synthesis by the episomal expression of an antisense message significantly reduced *E. histolytica* virulence while leaving trophozoite adherence properties intact (1). Mutant CHO cells lacking NH2-terminal galactose or N-acetyl-galactosamine were relatively resistant to amebic trophozoite adherence and *E. histolytica*-mediated killing, confirming a physiological role for the lectin in the adhesive process (16). Contact between amebic trophozoites and the extracellular matrix protein fibronectin triggers protein kinase C (PKC) and protein kinase A (PKA) signaling cascades within the parasite, resulting in actin rearrangements that facilitate adhesion and possibly invasion (20).

Once adherence occurs, *E. histolytica* may kill colonic epithelial cells after direct contact. The cytolytic capabilities of *E. histolytica* have been recognized for decades, but the molecular mechanisms are now being elucidated. *E. histolytica* contact with neutrophils causes a rapid (within minutes) reduction in neutrophil motility, loss of cytoplasmic granules and structures, and nuclear swelling followed by the eventual disappearance of the nucleus. Similar changes have been reported for CHO cells in contact with *E. histolytica* trophozoites, and Jurkat cells showed a loss of membrane integrity (by trypan blue exclusion) within 30 min of contact with amebae (4). The critical effector molecules for *E. histolytica*-mediated cytosis are amebapores, a family of three 77-amino acid peptides capable of forming pores in lipid bilayers (14). The addition of purified amebapores to Jurkat cells resulted in lysis of >75% of the cells within 2 h (4). Inhibition of amebapore synthesis by the expression of an antisense message to the amebapore A molecule significantly reduced the cytolytic capabilities of *E. histolytica* trophozoites (5). Amebapores are found within cytoplasmic granules of *E. histolytica* and may exocytose to the target cell membrane on contact (14).

*E. histolytica* may also kill colonic epithelial cells by inducing them to undergo apoptosis. Studies using a murine myeloid cell line demonstrated DNA ladder formation in cells after contact with *E. histolytica* trophozoites, consistent with induction of the apoptotic pathway (24). As discussed in *Pathophysiology of Amoeba Liver Abscess*, *E. histolytica* causes hepatocyte apoptosis in an animal model of amebic liver abscess, suggesting that intestinal epithelial cells could suffer the same fate (34). It is possible that amebapores may play a critical role in both the lytic and apoptotic pathways, because sublytic concentrations of pore-forming proteins can induce apoptosis in target cells (4).

The early effects of *E. histolytica* on the intestine extend beyond the lysis of individual epithelial cells. Analysis of *E. histolytica* interactions with polarized human intestinal epithelial Caco-2 cells demonstrated that amebic trophozoites added to the apical surface induced a rapid decrease in transepithelial resistance that preceded any morphological disruption of the monolayer integrity (17). The resistance response was not associated with changes in short-circuit current but was associated with an increase in mannitol flux across the monolayer consistent with a nonselective increase in intestinal permeability. More recently, studies of *E. histolytica* interactions with T-84 cells have shown that the alterations in permeability are associated with disruption of tight junction proteins, dephosphorylation of ZO-2, and loss of ZO-1 (15). The *E. histolytica* molecules responsible for these effects remain unknown, but studies in the Caco-2 model suggest that these changes are dependent on *E. histolytica*-target cell contact rather than secreted factors (17).

The next phase of amebic colitis may center on the intestinal cell response to *E. histolytica* infection. Intestinal epithelial cells can serve as sensors for infection and initiate host inflammatory responses (9). Intestinally derived transformed epithelial cell lines produce a variety of proinflammatory mediators in response to intracellular infection with bacteria (9). These include interleukin (IL)-1, IL-8, cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS). Several of these mediators can attract and activate neutrophils and macrophages at the site of pathogen invasion. *E. histolytica*, an extracellular pathogen, can also induce the production of cytokines and chemokines from intestinally derived cell lines (10). Coincubation of *E. histolytica* with cultured epithelial cell lines caused epithelial cell secretion of IL-8, growth-regulated oncogene (GRO)α, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-1α, and IL-6 (10). IL-8 production by HeLa cells was mediated primarily through the effects of IL-1α that had been released from epithelial cells lysed by *E. histolytica* trophozoites (10).

The physiological relevance of epithelial cell-derived mediators in *E. histolytica*-induced gut inflammation was established by studies in a severe combined immunodeficient mouse-human intestinal xenograft (SCID-HU-INT) model of amebic colitis (31). Introduction of *E. histolytica* trophozoites, but not a nonvirulent ameba, *E. moshkovskii*, into human intestinal xenografts caused marked gut inflammation and tissue damage over a 24-h period that was indistinguishable from that seen in patients with amebic colitis. The chimeric nature of the SCID-HU-INT mouse, with intestinal epithelial cells in the xenograft of human origin and inflammatory cells of murine origin, allowed the isolation of the contribution of human intestinal epithelial cells to the gut inflammatory response. Human intestinal epithelial cells produce IL-1β and IL-8 in response to amebic infection, as measured by ELISA assays specific for human cytokines or immunohistochemistry with human-specific reagents (31). Intestinal epithelial cells removed from the site of *E. histolytica* contact also produce IL-8, indicating that a soluble factor produced either by the damaged cells or *E. histolytica* trophozoites contributes to intestinal epithelial cell activation in human intestine (31).
Both IL-1β and IL-8 gene expression are regulated by the transcription factor nuclear factor (NF)-κB. Specific inhibition of human NF-κB synthesis in human intestinal cells (by introducing an antisense oligonucleotide to the p65 subunit of human NF-κB into the lumen of the human intestinal xenografts) blocked the increase in IL-1β and IL-8 production seen in response to *Entamoeba histolytica* infection (32). Blockade of intestinal cell IL-1β and IL-8 production significantly reduced *E. histolytica*-induced gut inflammation (as measured by neutrophil influx into the intestine) and tissue damage (measured histologically and by quantifying changes to the intestinal permeability barrier). These were the first in vivo studies to link the intestinal epithelial cell cytokine response to *E. histolytica* to the subsequent inflammatory process and resultant tissue damage seen in amebic colitis. *E. histolytica* infection also induces COX-2 production in human intestinal epithelial cells in vivo (37a). Specific blockade of COX-2 significantly reduced IL-8 production, neutrophil influx, and tissue damage in *E. histolytica*-infected human intestinal xenografts.

Neutrophils are a key effector cell in this process, and depletion of neutrophils from SCID-HU-INT mice significantly reduced the gut tissue damage seen early in *E. histolytica* infection (32). Mediators released by neutrophils may contribute to both diarrhea and tissue damage. *E. histolytica* can lyse neutrophils, and this can contribute to mediator release as well as explain the relative paucity of neutrophils seen in direct contact with amebic trophozoites (22). In addition, the transmigration of neutrophils across the epithelial border in response to IL-8 and other chemoattractants may contribute to the increase in intestinal permeability seen with *E. histolytica* infection. Although inflammation clearly contributes to tissue damage early in disease in this model, ultimately, the inflammatory response may be key to controlling and resolving amebic colitis. This is consistent with the clinical experience that individuals with amebic colitis who mistakenly receive corticosteroids (potent inhibitors of NF-κB) develop more severe disease with a higher incidence of perforation.

What molecules are required for the induction of cytokine production and tissue damage in amebic colitis in SCID-HU-INT mice? Although direct evidence is not yet available, amebapores are obvious candidates, because intestinal epithelial cells lysed by amebapores could release IL-1α that could then stimulate cytokine production from neighboring cells (10). Alternatively, intestinal epithelial cells undergoing *E. histolytica*-induced apoptosis could release human precursor IL-1β (pIL-1β), which could also contribute to activation of cytokine production in adjacent cells. The GaI/GaINAc lectin may play a role in inducing cytokine production as well, because in vitro studies have shown that macrophages exposed to certain regions of the lectin produce tumor necrosis factor (TNF)-α (30).

Cysteine proteinases released by *E. histolytica* trophozoites play a key role in gut invasion and inflammation. To date, seven distinct genes encoding prepro forms of papain family proteinases have been identified in *E. histolytica* (reviewed in Ref. 23). Proteinases are secreted by *E. histolytica* trophozoites, and one of the distinct differences between *E. histolytica* and the closely related but nonvirulent *Entamoeba dispar* is that *E. histolytica* trophozoites can secrete 10- to 1,000-fold higher levels of cysteine proteinases than those of *E. dispar* (27). Most of the proteinase activity seen in *E. histolytica* lysates can be attributed to four proteinases: amebic cysteine proteinase (ACP)1, [E. histolytica cysteine proteinase (EhCP)3], ACP2 (EhCP2), ACP3 (EhCP1) and EhCP5 (7, 25). *E. histolytica* cysteine proteinases can cleave components of the extracellular matrix, and the cytopathic effect of amebic lysates on cell monolayers (rounding and detachment of cells from the underlying substrate) is blocked by specific cysteine proteinase inhibitors (12). *E. histolytica* trophozoites with reduced levels of proteinase activity were generated by the episomal expression of an antisense message to the ehcp5 gene (2). Total thiol-dependent proteinase activity in these trophozoites was reduced to 10% of the levels seen in control trophozoites. The proteinase-deficient trophozoites were less virulent in a hamster model of amebic liver abscess and displayed a defect in phagocytosis (2, 3).

Proteinase-deficient amebic trophozoites were also markedly less virulent in infections of human intestinal xenografts (41). They induced less gut inflammation (reduced levels of IL-1 and IL-8 and decreased neutrophil influx) and caused significantly less damage to intestinal tissue. Proteinase-deficient amebic trophozoites appeared to be less invasive and were rarely detected in the submucosal tissues of the human intestinal xenograft (41). The reduced virulence of protease-deficient *E. histolytica* trophozoites may be multifactorial. An inability to cleave extracellular matrix proteins could reduce trophozoite invasiveness, and the unexplained phagocytosis defect in protease-deficient ameba could have physiological effects as well (2). Cysteine proteinases may have important functions within the parasite (digestion of ingested bacteria and red blood cells, activation of other enzymes, etc.), and inhibition of these functions may impact on virulence in subtle ways. *E. histolytica* lysates or purified amebic cysteine proteinases are capable of mimicking the activity of IL-1-converting enzyme (ICE or caspase 1) and can cleave pIL-1β to form the active mature cytokine (41). Intestinal epithelial cells lysed by *E. histolytica* trophozoites may release pIL-1β, which could then be activated by extracellular amebic cysteine proteinases and further amplify the inflammatory process in amebic colitis.

As *E. histolytica* trophozoites invade through the mucosa and submucosal tissues, they will encounter additional host defenses, including the complement system and serum antibodies. *E. histolytica* trophozoites are covered by a complex lipophosphoglyconjugate, a glycosphatidylinositol (GPI)-anchored molecule, which may also contain a peptide moiety (36). This molecule has been convincingly linked to amebic virulence and may serve as a physical barrier to com-
plem components (19). *E. histolytica* cysteine proteinases activate complement by cleaving the α-chain of C3, generating functionally active C3b (28). However, amebic cysteine proteinases can also cleave and destroy the anaphylatoxins C3a and C5a, thus circumventing that component of the immune response (26). The Gal/GalNAc binding lectin of *E. histolytica* contains a region with antigenic cross-reactivity with CD59, a membrane inhibitor of the C5b-9 attack complex found in human red blood cells (6). This region of the lectin could confer trophozoite resistance to lysis by the membrane attack complex. Cysteine proteinases may provide defense against host antibody as well, because cleavage of both human IgA and IgG by *E. histolytica* cysteine proteinases has been described (13, 38).

The contact-dependent cytolytic capabilities of *E. histolytica* may provide protection against host defense cells, but neutrophils and macrophages activated by interferon-γ can kill *E. histolytica* trophozoites in vitro (8). *E. histolytica* produces a small peptide, monocyte locomotion inhibitory factor (MLIF), which reportedly specifically inhibits the motility of monocytes and macrophages and also suppresses monocyte and neutrophil nitric oxide (NO) production (29). All of these factors may contribute to the ability of *E. histolytica* trophozoites to survive within the host and, in some cases, to establish prolonged infection.

PATHOPHYSIOLOGY OF AMEBIC LIVER ABSCESS

It is estimated that 10% of individuals with amebic colitis will develop an amebic liver abscess after dissemination of amebic trophozoites from the bowel through the portal circulation. To reach the liver intact, amebic trophozoites must be capable of surviving host defenses against blood-borne pathogens, including resistance to complement-mediated lysis. Our understanding of what happens once *E. histolytica* trophozoites reach the liver is derived primarily from rodent models of amebic liver abscesses. Studies using direct intraportal inoculation of amebic trophozoites into hamsters have shown that trophozoites are first seen within liver sinusoids and are rapidly (within 60 min) surrounded by neutrophils (39). Neutrophils may be important both in the pathogenesis of amebic liver abscess and in the host’s ability to control infection. Lysis of neutrophils by *E. histolytica* trophozoites may release mediators that lead to hepatocyte death and extend damage to distant hepatocytes (39). However, neutrophils are also involved in host defense against liver abscess in the murine model of disease, because depletion of neutrophils from mice led to significantly larger amebic liver abscesses (35). At least two other host molecules are involved in controlling experimental amebic liver abscesses in mice, interferon-γ and NO. SCID mice with targeted disruption of the interferon-γ receptor gene showed increased susceptibility to amebic liver abscess, with abscesses that were significantly larger than those seen in control SCID mice (33). Mice with targeted disruption of the gene encoding inducible nitric oxide synthase (iNOS) had significantly larger amebic liver abscesses than the parent strain (33). NO has been linked to macrophage killing of *E. histolytica* trophozoites but also could play a role in liver regeneration and hepatocyte resistance to apoptosis.

In experimental amebic liver abscesses in mice, hepatocytes die from both apoptosis and necrosis. DNA obtained from sections of amebic liver abscesses in mice showed the classic ladder formation characteristic of apoptosis as soon as 1 h after *E. histolytica* inoculation (34). The inflammatory cells adjacent to *E. histolytica* trophozoites and many nearby hepatocytes had TUNEL-positive nuclei. *E. histolytica*-induced apoptosis may have a unique mechanism. Studies looking at *E. histolytica* killing of the murine myeloid cell line FDC-P1 showed that Bcl-2 expression could not prevent *E. histolytica*-induced apoptosis (24). In vivo studies using C57Bl6.gld and C57BL6.lpr mice demonstrated that *E. histolytica*-induced apoptosis in hepatocytes is independent of the Fas-Fas ligand pathway (34). Induction of amebic liver abscesses in mice with targeted disruption of TNF-α receptor-1 (TNFR-1) indicated that amebic-induced apoptosis can occur in the absence of TNFR-1 as well (34).

To date, a search for hepatocyte apoptosis in liver abscesses from the other major rodent models, gerbils and hamsters, or from human specimens has not been performed. However, necrosis appears prominent in both gerbil and hamster abscesses, with central necrotic foci surrounded by a relatively thin wall of fibrous tissue and scattered inflammatory cells (39). In amebic liver abscesses from humans and in animal models, amebic trophozoites are most often at the periphery of the abscess and there is a relative paucity of ameba given the size of the abscess. This latter finding is consistent with the idea that some hepatocytes may die without direct contact with *E. histolytica* trophozoites.

Multiple *E. histolytica* molecules have been implicated in the pathogenesis of amebic liver abscess. Inhibition of amebic cysteine proteinase activity by treatment with the specific cysteine proteinase inhibitor trans-epoxysuccinyl-l-leucylamido-(4-guanidino)butane (E-64) significantly reduced liver abscess size in the murine model of disease (37). Cysteine-proteinase-deficient ameba, expressing the antisense message to ehcp5 (2), caused significantly smaller amebic liver abscesses in the hamster, as did *E. histolytica* trophozoites expressing lower levels of the amebapores (5). Reduced expression of the light chain of the Gal/GalNAc lectin as well as overexpression of the cytoplasmic tail of the heavy chain of the lectin (presumably acting as a dominant negative to inhibit lectin-mediated signaling) have each been associated with decreased liver abscess formation in rodent models of disease (1, 40). Antibodies to the amebic lipophosphoglyconjugate (19), the serine-rich *E. histolytica* protein (SREHP) (37), or portions of the heavy chain of the Gal/GalNAc binding lectin can completely inhibit amebic liver abscess formation in mice (18). Whether these molecules...
play a direct role in hepatocyte or inflammatory cell death, are required for basic parasite functions (e.g., phagocytosis or motility), or subvert some component of the host immune response to infection remains to be determined.

OUTLOOK

*E. histolytica* is a remarkable pathogen, with an impressive repertoire of virulence factors. Significant progress has been made in developing in vitro and in vivo models for amebic disease. From these models with newly developed genetic tools, we have learned that both amebic colitis and amebic liver abscess arise from complex interactions between *E. histolytica* trophozoites and human cells and tissues and have begun to define key host and parasite molecules in these processes. Despite this progress, serious obstacles to studying this parasite remain, including the lack of some of the powerful genetic tools available for other microbial pathogens, such as the ability to perform targeted disruption of specific *E. histolytica* genes. Further understanding of the pathophysiology of amebiasis will require continued analyses of both sides of the host-pathogen interface, and these studies will need to apply new genetic and proteomic tools. The *E. histolytica* genome project will undoubtedly give new leads into amebic pathogenesis and may provide new paradigms for understanding this important disease.

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