**Tissue destruction and invasion by **Entamoeba histolytica**

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*Entamoeba histolytica* is the causative agent of amebiasis, a disease that is a major source of morbidity and mortality in the developing world. The potent cytotoxic activity of the parasite appears to underlie disease pathogenesis, although the mechanism is unknown. Recently, progress has been made in determining that the parasite activates apoptosis in target cells and some putative effectors have been identified. Recent studies have also begun to unravel the host genetic determinants that influence infection outcome. Thus, we are beginning to get a clearer picture of how this parasite manages to infect, invade and ultimately inflict devastating tissue destruction.

**Overview of amebiasis**

*Entamoeba histolytica* is a protozoan parasite and the causative agent of amebiasis in humans. Parasite cysts are transmitted through contaminated food and water, making the incidence of disease high in areas of poor sanitation. *E. histolytica* is responsible for an estimated 35 to 50 million cases of symptomatic disease and approximately 100,000 deaths annually [1]. Parasite destruction of host tissue appears to be the basis of disease (Figure 1). The majority of morbidity and mortality occurs in Asia, Central and South America and Africa. Children are especially vulnerable as they can suffer malnourishment and stunting as the result of repeated infection [2]. Acquired resistance to infection is associated with interferon-γ production by peripheral blood mononuclear cells and mucosal IgA directed to the parasite surface lectin [3]; hence there is an ongoing effort to develop an effective vaccine. Nitroimidazoles such as metronidazole are available to treat invasive amebiasis, although shortcomings include toxic side effects and a need for additional drugs in 40–60% of patients to cure infection [4]. The continued morbidity and mortality indicate that current therapies are insufficient.

Besides *E. histolytica*, there are two related human-infectious species, *E. dispar* and *E. moshkovskii*, that are virtually identical in morphology, with the exception that *E. histolytica* trophozoites are more likely to contain ingested erythrocytes (Figure 1(c)) [5]. Combined, it is estimated that they are responsible for infecting approximately 500 million people, or almost 10% of the world [5]. Co-infection with the three species is common: of 109 stool specimens from preschool children in Bangladesh, 21% were positive for *E. moshkovskii* infection, and 73% of these also carried *E. histolytica* or *E. dispar* [6]. Similar observations have been made in India [7]. Although there is extensive evidence of the nonpathogenicity of *E. dispar*, even in individuals with HIV/AIDS, it remains to be seen if *E. moshkovskii* is solely a commensal.

**Variable outcome of disease**

*E. histolytica* infection occurs after the ingestion of cysts. Parasite excystation in the small intestine produces eight trophozoites per cyst, which then colonize the large intestine, existing both in the lumen and attached to mucus and epithelial cells [3]. Infection has a variable outcome, manifesting in asymptomatic colonization, diarrhea, invasive colitis, liver abscess or metastatic infection. Invasive disease pathologies, namely colitis, abscess and metastatic disease, are associated with massive host tissue destruction. For example, the hallmarks of amebic colitis are flask-shaped ulcers, with invasion of parasites into the lamina propria (Figure 1(a and b)) [8]. Amebic liver abscesses are massive fluid-filled cavities that result from extra-intestinal spread of the parasite; and can be fatal if untreated [9].

The outcomes of infection are not mutually exclusive and patients can progress from one clinical manifestation to another. Colitis develops in 10–25% of cases, whereas liver abscess occurs in only about 1% of cases and most commonly in adult males [10]. Hence, before considering the mechanistic basis of host tissue destruction in invasive disease, it is important to consider what determines infection outcome. Recently, clear roles for human and parasite genetics and environmental factors have begun to emerge.

**Human genetics**

One clear finding from prospective observation of a cohort of children in Bangladesh is that not all children are equally susceptible to infection. That is, although *E. histolytica* infection is extraordinarily common, some children resist infection [11]. It has also become clear that malnutrition substantially increases susceptibility [2]. Therefore, a recent study tested for genetic polymorphisms that influence nutritional status and susceptibility [12]. Malnourished children are known to have low levels of the hormone leptin that signals satiety and also influences the immune system. Genetic variants in leptin and the leptin receptor were evaluated for association with infection, and increased susceptibility to intestinal *E. histolytica* infection was found to be associated with a single amino acid polymorphism in the
leptin receptor [12]. Children carrying the 223R allele were nearly four times more likely to have an infection than those homozygous for the 223Q allele (Figure 2(a)). This association was also true among adults with liver abscess and in mice segregating for the 223R allele (Figure 2(b) and Box 1) [12].

The observation that a polymorphism in the leptin receptor affected susceptibility to amebiasis led to the

Figure 1. Tissue destruction associated with E. histolytica infection. (a) An example of amebic colitis, showing the presence of multiple ulcers. (b) A side-view of the classical flask-shaped ulcer seen in amebic colitis. (c) E. histolytica trophozoites (arrows) taken from an ulcer, showing the presence of numerous ingested red blood cells. Panels (a–b) are reproduced from [83], with permission.

Figure 2. Leptin signaling influences tissue destruction during amebic infection. (a) Chart illustrating how leptin receptor polymorphisms at position 223 influence susceptibility to infection. Shown is Kaplan-Meier survival free of E. histolytica infection of children (n=185) with Gln/Gln (QQ), Gln/Arg (QR) or Arg/Arg (RR) genotypes. (b) Mice carrying one or two copies of the arginine allele at the 223 codon were more susceptible to intestinal infection with E. histolytica than those homozygous for the glutamine (QQ) allele (QQ versus QR p = 0.009, QQ versus RR p = 0.001 by Chi-square test). (c) Mice deficient for leptin (ob/ob) or the functional leptin receptor (db/db) exhibit dramatic destruction of the mucosal epithelium upon intracecal E. histolytica infection. Shown is the normal epithelium in an infected wild-type B6 mouse for comparison. Panels (a–b) are reproduced from [12] and panel (c) is reproduced from [13], with permission.
hypothesis that the enhanced susceptibility results from diminished leptin signaling. In a murine model, leptin signaling was found to have a protective role, because mice that lacked the functional leptin receptor developed devastating mucosal destruction after intracecal *E. histolytica* challenge (Figure 2(c)) [13]. The site of leptin action was subsequently localized to the gut because an intestinal epithelium-specific deletion of the leptin receptor rendered mice susceptible to infection and mucosal destruction [13].

Mutation of tyrosines in the intracellular domain of the receptor that mediate signaling through the Src homology-2 domain-containing protein-tyrosine phosphatase (SHP2)/extracellular signal-regulated kinase (ERK) and signal transducer and activator of transcription 3 (STAT3) pathways demonstrated that both were important for mucosal protection [13]. Therefore, leptin-mediated resistance to amebiasis appears to function via its actions on the intestinal epithelium, and requires leptin receptor signaling through both the STAT3 and SHP2/ERK pathways.

There are certainly additional genetic determinants of susceptibility. There is evidence that class II human leukocyte antigen (HLA) alleles influence infection, with the DQB1*0601 HLA allele having an association with protection from infection [14]. There are also gender differences in susceptibility because liver abscesses are much more common in men than women. The genetic basis is unknown, but mouse studies suggest that increased interferon-γ and functional natural killer T cells in females might underlie resistance to liver abscess [15], and human serum from men and women has been reported to differ in its ability to lyse *E. histolytica in vitro* [16]. Additional studies that move to whole genome associations will be of great value, and will likely uncover novel genes involved in susceptibility and infection outcome (Box 2).

### Box 2. Outstanding questions

- How do leptin polymorphisms determine susceptibility to infection? How does leptin signaling result in protection from mucosal tissue destruction when infection does occur?
- What other human genetic polymorphisms contribute to differences in susceptibility to amebic infection? Do differences in the makeup of the gut microbiome play a role?
- What is the basis of parasite strain-specific differences in virulence and invasiveness? Does this tie in to differences in cytotoxic properties?
- How do calcium influx and tyrosine dephosphorylation precipitate target cell death?
- What are the amebic effectors of cell killing and how are they delivered? Are amoebapores or acid vesicles required? Does lectin engagement initiate the cytotoxic program?
- How exactly do parasite proteases and pro-inflammatory host immune reactions augment tissue invasion and extra-intestinal spread?
- What is the role of calcium flux in modulating parasite invasiveness? Are other mechanisms of environmental sensing and chemotaxis employed by the parasite *in vivo*?
the parasite genotype in virulence, and further suggest that there is a genetic bottleneck where only certain genotypes are capable of causing abscesses. More work will be needed to identify the specific genes involved and their contributions to virulence. Although the HM1:IMSS strain of *E. histolytica* has been fully sequenced [20] and is now accessible through AmoebaDB [21], ongoing efforts in sequencing clinically relevant strains should prove useful in this regard.

**Environmental factors**

Malnutrition substantially increases susceptibility to amebiasis [2]. Hence, obvious environmental factors influencing susceptibility include nutritional status and food availability. There is also an emerging view that the gut microbiome in a given individual could potentially influence *E. histolytica* infection. There are many reports of *E. histolytica* virulence changing depending on the presence of co-cultured bacteria *in vitro* [22,23]. Whether this will be borne out *in vivo*, and whether specific differences in microbiomes translate to differences in the outcome of *E. histolytica* infection awaits determination.

**Mechanism of host tissue destruction**

*E. histolytica* infection has a variable outcome, but when invasive disease does occur, the potent cytotoxic activity of the parasite is likely to be a major contributor to the extensive tissue damage observed (Figure 1). The parasite is able to kill and ingest host cells in a contact-dependent manner and within minutes [24]. Other factors are likely to augment tissue destruction, such as parasite proteases and inflammatory host immune reactions. Thus, as outlined below, a combination of cytotoxicity and additional factors result in host tissue damage. A better understanding of these processes will be necessary to get a clearer picture of the mechanism of disease and to enable the identification of new targets for therapeutic intervention.

**Cytotoxicity**

*E. histolytica* is cytotoxic to a variety of cell types, including neutrophils, T lymphocytes, macrophages and a variety of tissue culture lines. Although cytotoxic activity is what the parasite was named for, the precise mechanism remains an enigma. In a stepwise process, *E. histolytica* adheres to the target, induces its death, and then ingests the killed cell. Killing of the target cell appears to be primarily via activation of apoptosis; that is, the parasite ‘tricks’ the host cell into killing itself.

**Adherence**

The parasite surface Gal/GalNAc lectin mediates binding to host carbohydrate determinants that contain galactose (Gal) and/or N-acetyl-D-galactosamine (GalNAc) [3]. Other proteins also contribute to host cell-binding, however, the lectin is critical because the addition of Gal or GalNAc abolishes the majority of binding [25]. The lectin is composed of a heterodimer of heavy (170 kDa) and light (35/31 kDa) subunits, which are disulfide-linked, together with a non-covalently linked intermediate subunit (150 kDa). The heavy subunit contains the carbohydrate recognition domain (CRD) [3]. The functions of the light and intermediate subunits are unknown; although there appears to be some requirement for the light subunit in cytotoxicity [26] and the intermediate subunit could be the antigen that has been characterized as an integrin-like fibronectin-binding protein [27]. There are multiple genes encoding each subunit that are not identical [3], however, whether the different copies have different specificities or functionalities is unknown.

Lectin-mediated adherence to target cells is required for cytotoxicity, because excess Gal or GalNAc prevents adherence and killing [25], and mutants that lack surface glycosylation are resistant to killing [28]. In some cases, the lectin is transferred to the target cell [29], but the significance is presently unclear. Besides mediating adherence, the lectin may also trigger the initiation of a cytotoxic program. Strong evidence comes from the finding that when parasites and host cells are combined in the presence of Gal or GalNAc and forced together via centrifugation, the parasites contact host cells but are not able to kill [24]. Anti-Gal/GalNAc lectin monoclonal antibodies have also been identified that block cytotoxicity without blocking adherence, suggesting that the lectin has separable roles in both processes [30].

**Calcium influx**

Inhibitors and chelators of calcium block cytotoxicity, suggesting that influx of extracellular calcium is critical for target cell killing [31,32]. Following parasite contact, there is a dramatic calcium elevation in the target cell (Figure 3(a–c)) [33] that is irreversible and precedes death. Interestingly, cells adjacent to the targeted cell also demonstrate a reversible calcium increase that does not lead to death, thus transient calcium elevation alone is not sufficient for killing [33]. Fixed amebae are able to induce partial, unsustained calcium elevation in contacted host cells, suggesting that parasite surface molecules might be able to trigger calcium influx [33]. It is possible that this activity may be attributable to the Gal/GalNAc lectin because the addition of affinity-purified lectin to target cells also results in reversible calcium elevation [33].

Calcium flux in the parasite also appears to be important. Pre-treatment of amebae with calcium channel blockers inhibits killing [32]. Amebae also possess a calcium-dependent phospholipase activity that appears to contribute to cytotoxicity [34]. However, amebic calcium levels do not change upon target cell contact [33]. There might be a role for calcium in regulating virulence genes. The calcium-regulated transcription factor, URE3-BP, modulates the expression of known virulence factors and URE3-BP mutants that constitutively bind DNA result in enhanced parasite invasion *in vivo* [35]. Thus there are many potential roles of amebic calcium flux that warrant further study.

**Dephosphorylation**

*E. histolytica* alters host cell tyrosine phosphorylation (Figure 3(d)). Global tyrosine dephosphorylation occurs upon parasite contact and host phosphatases appear to be responsible [36]. This is required for killing because pretreatment of host cells with a protein tyrosine phosphatase (PTPase) inhibitor prevents death [36]. Dephosphorylation
Figure 3. *E. histolytica* induces calcium influx, tyrosine dephosphorylation and caspase-3 dependent host cell death. (a–c) Time series of Fura-2-loaded CHO cells before contact (top panels) and 30 s after contact (bottom panels) with an *E. histolytica* (Eh) trophozoite. (a) Phase images and (b) digitized R340/380 images are shown. Bar, 10 μm. (c) Color bar indicating intensity from background (dark blue) to a maximal R340/380 (red) of Fura-2 fluorescence. (d) FACS analysis of *E. histolytica*-induced protein dephosphorylation in Jurkat cells. Cells were incubated with or without *E. histolytica*, fixed, permeabilized and stained with FITC-pTyr-MAb PT-66. The data express the fluorescence intensities from Jurkat cells; cells in medium alone are shown with the dotted line; cells that were incubated with amebae are shown with the continuous line and shaded area. (e) *E. histolytica* activates caspase-3 in a contact-dependent manner in vitro. A time series of *E. histolytica* and Jurkat T cells pre-loaded with the fluorescent caspase-3 substrate rhodamine-G1D2-rhodamine (PhiPhiLux). Images at 0, 8, 16 and 20 minutes are shown. One Jurkat cell is already positive for active caspase-3 (showing green fluorescence) at time zero. The other Jurkat cells (arrows) show active caspase-3 only following contact with the trophozoite. (f) TUNEL staining in a mouse model of amebic colitis. TUNEL positive apoptotic cells surround *E. histolytica* trophozoites (arrows) in the infected caecum. (g) Pre-treatment with the pan caspase inhibitor zVAD-fmk reduces the formation of liver abscesses in a mouse model. TUNEL staining of liver sections is shown from mice that were pre-treated with zVAD-fmk or left untreated prior to amebic infection. The tissue has a normal appearance and is mostly TUNEL negative in the treated example, whereas in the untreated sample the tissue is TUNEL positive, appears dead and has numerous detectable *E. histolytica* trophozoites (arrows). Panels (a–d) are reproduced from [33], panel (d) is reproduced from [36], panels (e) and (f) are reproduced from [41], and panel (g) is reproduced from [43], with permission.
may be linked to calcium flux because a particular calcium-regulated PTPase, PTPase 1B (PTP1B), appears to be involved. PTP1B is known to be proteolytically activated by calpain when intracellular calcium becomes elevated. The *E. histolytica*-induced tyrosine dephosphorylation is inhibitable with the calpain inhibitor calpeptin, and moreover, cleaved PTP1B is detected and this can be blocked with calpeptin [36]. However, PTP1B is probably not the only PTPase activated by *E. histolytica* because calpeptin is only able to inhibit dephosphorylation at extremely low parasite:host ratios [36]. Additional studies have shown that amebae appear to induce cleavage of the calpain inhibitor calpastatin that contributes to calpain activation [37], and there might be roles for the PTPases SHP1 and SHP2 [38].

**Activation of apoptosis**

Ultimately, the target cell succumbs to death. The cumulative evidence supports caspase-3 dependent apoptotic death as the major mechanism both *in vitro* and *in vivo*; however other forms of cell death should not be excluded. This was initially controversial and *in vitro* studies employing DNA degradation, TUNEL staining, and/or morphology to assay for apoptotic death reported contradictory findings [39,40]. However, later studies demonstrated that caspase-3 is activated in host cells in a contact-dependant manner (Figure 3(e)), and that killing is blocked by the caspase-3 inhibitor AC-DEVD-CHO, hence it is caspase-3 dependent [41]. Inhibition of caspase-8 or -9 via genetic or pharmacological means did not inhibit cytotoxicity; therefore

![Figure 4. Model for the mechanism of cytotoxicity. *E. histolytica* (blue) is shown adhered to the targeted host cell (tan) via the action of the parasite Gal/GalNAc lectin (green rectangles). Parasite acidic vesicles (purple circles) that are hypothesized to play a role in cytotoxicity are shown; it is possible that their contents are released at a localized synapse between host and parasite. It is unknown whether any parasite effector molecules are transferred to the target cell, but candidates include KERP1 (pink rectangles), the amoebapores and other members of the SAPLIP family (orange donuts). Note that there are additional proteins besides the Gal/GalNAc lectin that influence host cell binding (orange rectangles, turquoise rectangles) such as TMKB1-9 and the EhSTIRPs. The Gal/GalNAc lectin may itself contribute to the cytotoxic mechanism, either by triggering the initiation of the cytotoxic program and/or by triggering calcium influx. Note also that the intermediate and light chains of the lectin (igl, lgl) might play some role in host cell killing. A dramatic influx of extracellular calcium (Ca$^{2+}$, pink circles) is required for target cell killing; the mechanism for calcium entry is unknown, although it could involve the amoebapores and other members of the SAPLIP family. Calcium influx appears to activate calpain, which proteolytically activates PTP1b, which in turn results in tyrosine dephosphorylation (tyrosine de-P). However, PTP1b is unlikely to be the only PTPase responsible for the observed tyrosine dephosphorylation. Ultimately, caspase-3 becomes activated (caspase-3$^{*}$) and the target cell succumbs to apoptotic death; however the specific upstream signals that activate caspase-3 are unknown.]
activation of host cell apoptosis is independent of these caspases [41].

In animal models, TUNEL staining is observed in both liver abscesses and intestinal colitis (Figure 3(f-g)) [41,42]. This is not dependent on signaling through Fas or TNFα receptors because liver abscesses and DNA degradation are still detected in mutant mice lacking these receptors [42]. The caspase inhibitor zVAD-fmk reduces the formation of liver abscesses (Figure 3(g)) [43], and caspase-3 knockout mice resist intestinal amebiasis [44]. Together, these data implicate parasite-induced apoptosis as central to tissue destruction. However, one remaining enigma is how the parasite is able to effect apoptosis within minutes, which typically requires hours to complete. This observation, together with the fact that killing is independent of Fas and TNFα receptors, caspase-8 and -9 and perhaps Bcl2 [40-42,44], emphasize that E. histolytica appears to induce a non-classical mechanism of caspase-3 dependent killing.

What are the cytotoxic effectors? How does E. histolytica elicit host cell death? The cumulative data demonstrate that target cell killing requires lectin-mediated contact, calcium influx, tyrosine dephosphorylation and caspase-3 activation (Figure 4). Killing is an active process because it requires parasite cytoskeletal remodeling [25] and fixed parasites do not induce sustained calcium influx [33]. There is not an isolated toxin because parasite sonicate does not induce killing [24]. These observations support a model where cytotoxic effectors are secreted in a regulated, contact-dependent manner. As outlined below, several candidate cytotoxic effectors have been identified but thus far no bona fide effectors are known.

Amoebapores
The first group of proteins implicated as effectors are the pore-forming proteins (amoebapores) that have sequence similarity to the mammalian membrane-permeabilizing proteins NK-lysin and granulysin [45]. All three amoebapores (A, B and C) can induce pore formation in synthetic liposomes and are maximally active at pH 5.2 [45], which seems to result from a pH-dependent dimerization event [46]. However, the conundrum is whether they are physiologically active on gut bacteria, host cells or both. Purified amoebapores are bactericidal to gram positive bacteria at low nM concentrations [45]. In contrast, they are cytotoxic to eukaryotic cells at more than 10–100 μM [47]. Host cell DNA degradation is not observed [47], suggesting that purified amoebapores act by a different mechanism than intact amebae, or that they are not sufficient to induce caspase-dependent killing. Partial colocalization of amoebapore A with ingested bacteria has been observed [45]. There are currently no data that demonstrate transfer of amoebapores to host cells, and this demands investigation.

Amoebapore A has been silenced by antisense and epigenetic approaches [48–51]. However both approaches work by unknown mechanisms and have unanticipated ‘off-target’ effects [48]; thus it is not possible to definitively attribute phenotypes to the silencing of the targeted gene. Additionally, high levels of drug selection were required for the antisense approach [49] that may have created artifacts. Nonetheless, both the antisense and epigenetically silenced strains caused diminished liver abscess pathology in vivo [49,50]. The epigenetically silenced strain was not attenuated in the SCID-HU-INT model of intestinal disease [52]. Defects in cytotoxicity were noted in vitro using trypan blue exclusion, but detailed measurements of caspase-3 activation were not done [48–51]. However, because the approaches that were used to silence amoebapore A affected other genes [48], the precise functions of the amoebapores remain unknown.

Other saposin-like proteins
Amebapores belong to the family of saposin-like proteins (SAPLIPs), which associate with lipids. Sixteen additional SAPLIPs have been identified in the genome [53,54]. One of these proteins shares a high percent identity with amoebapore A [53], and might be another member of the amoebapore family. Currently, little is known about the SAPLIPs, although they are all transcribed in vitro [53,54]. Recombinant SAPLIP 3 did not exhibit bactericidal activity and tests of cytotoxic activity were not done [53]. The recombinant protein possessed membrane-fusogenic but not pore-forming activity [53]; thus the putative SAPLIPs cannot be assumed to possess pore-forming activity. Any potential roles as effectors are presently unknown.

Cysteine proteases
E. histolytica cysteine proteases act on a variety of host substrates [55–59] and appear to function in tissue invasion by degrading mucus and ECM. Several studies have concluded that cysteine proteases contribute to cytotoxicity [60–62], but these data should be interpreted with caution. Monolayer destruction was assayed [60–62], but this does not distinguish between monolayer release (reflecting ECM degradation) and cell death (reflecting cytotoxicity). Therefore studies using readouts such as caspase-3 activation are needed.

Membrane proteins
Several membrane proteins and predicted membrane proteins have been characterized as potential effectors. At least two of these proteins associate with the Gal/GalNAc lectin, hence it can be difficult to distinguish between roles in adhesion versus killing. The two lectin-associated proteins are the light subunit of the lectin [26], and the surface-localized thiol-dependent peroxidase [63]. Antisense inhibition of the light subunit did not inhibit adhesion but did inhibit killing and resulted in decreased liver abscess size in hamsters [26]. Antisense inhibition of the surface-localized thiol-dependent peroxidase also inhibited killing and decreased liver abscess size in hamsters, but adhesion was not examined, hence it is possible that the antisense-inhibited cells have adhesion defects that prevent killing [63].

Several membrane proteins not known to be associated with the Gal/GalNAc lectin have also been characterized as effectors. The family of five predicted membrane-localized serine-, threonine-, and isoleucine-rich proteins (EhSTIRP) genes was silenced using dsRNA directed to a common sequence [64]. Defects in both adhesion and cytotoxicity were noted [64], thus the EhSTIRPs may be required for adhesion, killing, and/or both processes. Similarly, when transmembrane kinase B1-9 (EhTMKB1-9) was inhibited
by antisense expression, defects in both adhesion and monolayer destruction were noted [65]. Therefore, like the EhSTIRPs, EhTMKB1-9 may function in adhesion. Finally, lysine- and glutamic acid-rich protein 1 (KERP1) is a parasite membrane protein that also binds host cell membranes [66,67]. Antisense silencing of KERP1 did not result in a significant reduction in mRNA or protein [68]. A decrease in liver abscess formation in a hamster model was noted, but given the lack of inhibition of KERP1 [66], the significance of this phenotype is unclear. Although a potential role in cytotoxicity is implied by the affinity of KERP1 for host cell membranes, no specific role in killing has yet been experimentally demonstrated.

Acid vesicle components
E. histolytica possesses a class of acidic intracellular vesicles (pH 5.4) that appear to play a role in cytotoxicity [68]. Treatment of amebae with weak bases or lysosomotropic drugs blocks cytotoxicity substantially, such that up to 70% of host cells remain viable [68]. Neither base treatment nor drug treatment appeared to alter amebic viability or adherence [68]. Hence it is possible that amebic cytotoxicity is analogous to mammalian cytotoxic T lymphocytes, which possess acidic intracellular granules pre-loaded with pore-forming proteins and proteases that are released upon target cell contact. However, more work will be needed to test this hypothesis, specifically focusing on identifying vesicle contents and determining if they are involved in target cell death.

Other factors contributing to tissue invasion
Because caspase inhibitors reduce liver abscess formation and caspase-3 knockout mice resist intestinal amebiasis, parasite-induced apoptosis is central to tissue destruction. However, other factors are likely to augment invasiveness, such as parasite motility, proteases and pro-inflammatory host immune reactions.

Motility and chemotaxis
E. histolytica trophozoites are motile, and because parasites can invade the intestinal epithelium and spread to distal tissues, a role for self-driven motility in tissue invasion is implied. Trophozoite motility is myosin-dependent and bleb-based, driven by dynamic instability of the intracellular hydrostatic pressure [69]. Both random and chemotactic behaviors have been observed. E. histolytica may generate its own chemotactic factors because conditioned medium drives negative chemotaxis [70]. E. histolytica also responds to TNF-α as a chemoattractant in vitro [71]. The in vitro molecules that guide chemotactic behavior and the exact role of motility in extra-intestinal spread in vivo remain to be determined.

Proteases
E. histolytica possesses 50 cysteine protease genes [72]. These proteases have been demonstrated to act on a variety of host substrates in vitro [55–59]. At least some are secreted and a few have been characterized as surface-localized, hence they have the potential to contribute to host tissue breakdown in vivo. More than 80% of amebiasis patients express antibody to amebic cysteine proteases [73], further supporting their extracellular localization in vivo. Strong support for the importance of proteases in tissue destruction comes from studies where they were inhibited with E64 or antisense expression, and this resulted in diminished liver abscess formation in SCID mice and hamsters [74,75]; antisense expression also inhibited gut inflammation in the SCID-HU-INT mouse model [76]. Cysteine proteases are therefore being exploited as potential targets for therapeutic intervention, and rational drug design approaches have recently been used to develop several new inhibitors that block the activity of specific amebic proteases in vitro and also reduce disease pathogenesis in animal models [77,78].

Immune response
Pro-inflammatory reactions may contribute to tissue damage that facilitates invasion. Co-culture of trophozoites with epithelial cell lines in vitro has been shown to induce production of pro-inflammatory cytokines [79]; this is also seen in the SCID-HU-INT model of intestinal disease [80]. Amebic cysteine proteases also stimulate pro-inflammatory signaling; they possess IL-1β converting activity [76] and a surface-localized protease can activate the alternative complement pathway [81]. A recent report also suggests that EhCP5 binds integrin and stimulates NF-κB dependent pro-inflammatory responses [82].

Conclusions
E. histolytica is a scourge in the developing world, with children bearing an enormous burden of developmental consequences resulting from infection. Pathogenesis appears to result from the potent cytotoxic activity of the parasite, as emphasized by the fact that inhibition of apoptosis in vivo markedly inhibits both invasive colitis and liver abscess formation. It will be of great interest to determine how the parasite effects apoptosis; namely the identities of the effectors and how they are delivered. There are also many open questions regarding other factors that contribute to tissue destruction. A better understanding of these processes will be necessary to get a clearer picture of the basic mechanism of disease and to begin to develop new therapeutics.

It has also become clear that not everyone is equally susceptible to infection, and when infection does occur, the outcome is highly variable. Clear roles for human genetics are coming into view, with leptin receptor polymorphisms influencing infection susceptibility. There is also an emerging understanding that parasite genetics contribute significantly to virulence, and in particular, not all strains are capable of causing liver abscess. Roles for environmental factors, such as the gut microbiome, await determination. Because there is an emerging appreciation that E. histolytica infection is extraordinarily common, and is a major cause of morbidity and mortality in developing countries, future studies of the determinants of invasive disease have promise to positively impact global health.

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