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Chew on this: Amoebic trogocytosis and host cell killing by *Entamoeba histolytica*

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Abstract

Entamoeba histolytica was named "histolytica" (*histo-:* tissue; *lytic-:* dissolving) for its ability to destroy host tissues. Direct killing of host cells by the amoebae is likely to be the driving factor that underlies tissue destruction, but the mechanism was unclear. We recently showed that after attaching to host cells, amoebae bite off and ingest distinct host cell fragments, and that this contributes to cell killing. Here we review this process, termed "amoebic trogocytosis" (*trogo-:* nibble), and how this process interplays with phagocytosis, or whole cell ingestion, in this organism. "Nibbling" processes have been described in other microbes and in multicellular organisms. The discovery of amoebic trogocytosis in *E. histolytica* may also shed light on an evolutionarily conserved process for intercellular exchange.

Keywords

Entamoeba; phagocytosis; trogocytosis; cytotoxic; cell death

Amoebiasis

Entamoeba histolytica is a protozoan parasite and the causative agent of amoebiasis in humans (Figure 1). *E. histolytica* cysts are found in contaminated food or water sources, and following ingestion and excystation, motile amoeboid trophozoites colonize the colon (Fig. 1). This can be asymptomatic or result in diarrheal symptoms. Trophozoites can invade the intestine, resulting in amoebic colitis with profound ulceration that is associated with bloody diarrhea (Fig. 1). They can also disseminate and cause abscess formation in other sites in the body, most commonly in the liver (Fig. 1). While it is difficult to obtain an exact measurement of the burden of disease, *E. histolytica* is remarkably common in developing nations, and is responsible for an estimated 50,000,000 diarrheal infections per year [1]. Amoebic liver abscess results in an estimated 100,000 deaths annually [1]. Birth cohort studies indicate that in the first year of life, approximately 50% of infants in an urban slum

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in Dhaka, Bangladesh are infected [2]. Malnourishment and stunting are associated with repeated infections in children [3]; hence children are a vulnerable group.

There is no vaccine, though acquired resistance to infection is associated with mucosal IgA directed to the trophozoite surface D-galactose/N-acetyl-D-galactosamine (Gal/GalNAc)-specific lectin, suggesting that the Gal/GalNAc lectin represents a vaccine candidate [4]. Vaccination with fragments of the Gal/GalNAc lectin heavy chain has been shown to be protective in animal models [5–12]. Treatment with metronidazole is the standard [13, 14], though it has toxic side effects [13] and *E. histolytica* can develop resistance *in vitro* [15, 16]. Some second line drugs are available [13, 14], and the re-purposed drug auranofin holds promise as a potential new therapeutic option [17]. Given the paucity of available drugs, resistance is a concern [15, 16]. Improved understanding of disease pathogenesis and development of new therapeutics are key priorities.

Host cell killing and pathogenesis

The organism was named “histolytica” (*histo-:* tissue; *lytic-:* dissolving) for its ability to damage tissue. *E. histolytica* trophozoites are profoundly cytotoxic, making it likely that direct killing of host cells underlies the ability of trophozoites to invade and destroy host tissues [18–22]. Despite the fundamental importance in pathogenesis, the precise mechanism by which *E. histolytica* trophozoites kill host cells has been unclear. In studies where *E. histolytica* trophozoites were incubated with a combination of living and pre-killed host cells, they appeared to preferentially ingest pre-killed cells, which suggested that the trophozoites kill host cells prior to ingestion [23]. Thus the prevailing model has been that trophozoites first kill host cells, prior to phagocytosis of dead cell corpses [18, 23, 24].

Cell killing is an active process

Host cell killing by *E. histolytica* is contact-dependent and requires the trophozoite surface Gal/GalNAc lectin for host cell attachment [25, 26]. Gal/GalNAc lectin engagement might also transduce signals that initiate the cell-killing mechanism [26]. An intact, viable trophozoite is required and there does not appear to be a secreted toxin, since neither trophozoite extracts, supernatants, nor killed trophozoites are cytotoxic [19, 20, 27]. Killing is an active process since amoebic cytoskeletal rearrangements are also necessary [25]. Additionally, trophozoite acidic intracellular vesicles have also been implicated in cell killing, since the addition of weak bases raises the vesicular pH and blocks cytotoxicity [28]. The precise role of these vesicles in killing is unknown. In host cells, calcium becomes elevated shortly after contact with an amoebic trophozoite [27]. Global dephosphorylation of tyrosine residues has also been reported to occur in host cells following contact [29].

Putative cell killing effectors

It has been hypothesized that the pore-forming “amoebapore” proteins mediate cell killing by acting as secreted toxins [30], though the lack of killing activity in amoebic lysates or supernatants is not supportive of the presence of a toxin [19, 20]. The three amoebapores, A, B and C, have sequence similarity to the mammalian membrane-permeabilizing proteins NK-lysin and granulysin [31]. All three amoebapores induce pore formation in synthetic liposomes [32]. However, there is no experimental evidence to demonstrate secretion and transfer of amoebapores to host cells. The amoebapores require pH ~5.2 for pore-forming activity [32], due to pH-dependent dimerization [33], therefore a low pH environment would be needed for activity on host cell membranes. Amoebapore A has been epigenetically silenced, and this led to a decrease in liver abscess diameter in mice [34, 35], but silenced trophozoites were not defective in the SCID-hu-int model of intestinal amoebiasis (in which human intestinal xenografts are established in severe combined immunodeficident mice) [36], and they were not defective in tissue invasion in an *ex vivo* human intestine model [37]. Hence amoebapore A does not appear to be absolutely required for tissue destruction *in vivo*, though it was required for monolayer disruption *in vitro* [35].

E. histolytica possesses at least 50 cysteine protease genes, some of which are secreted [38]. It has been hypothesized that secreted amoebic cysteine proteases are also involved in cell killing [39–41], but again the lack of killing activity in amoebic lysates or supernatants is not supportive of a role for proteases in cell killing [19, 20]. The assay that was used to examine the potential contribution of cysteine proteases to cell killing was a measurement of the total dye remaining in a methylene-blue stained monolayer after exposure to trophozoite extracts or cysteine protease-overexpressing trophozoites. The assay does not specifically measure cell death and is complicated by the monolayer-disrupting activity of amoebic cysteine proteases. Amoebic cysteine proteases are capable of acting on a variety of host substrates including mucin, collagen, and an extracellular matrix (ECM) from vascular smooth muscle [42–46]. Studies employing *ex vivo* human intestine [37] suggest that amoebic cysteine proteases, particularly CP-A5, are likely to play a critical role in tissue invasion and damage independent of cell killing [37, 47, 48].

Amoebic trogocytosis

To improve understanding of the mechanisms underlying host cell killing, we recently employed live imaging studies to examine cell killing in real time. Unexpectedly, we found that following host cell attachment, *E. histolytica* trophozoites ingested distinct "bites" of host cells (Figure 2a), which we termed "amoebic trogocytosis" (Greek, *trogo–:* nibble) [49]. Within one minute of host cell contact, amoebic trogocytosis was initiated. Host cells were alive when this process began, but eventually died as evidenced by loss of membrane integrity (Figure 2b) [49]. Interestingly, once host cells had been killed, amoebic ingestion ceased and trophozoites detached from dead cell corpses [49]. When trophozoites were incubated with a combination of living and pre-killed host cells, the live host cells were ingested by amoebic trogocytosis, while the pre-killed host cells were ingested whole (Figure 2c – 2d). The ingestion of pre-killed cells is consistent with previous studies [23]. It is possible that pre-killed host cells have different surface characteristics from cells directly killed by the amoebae, and that these surface characteristics determine the type of ingestion that occurs (see below).

Combined use of pharmacological, biochemical and genetic approaches demonstrated that amoebic trogocytosis requires physiological temperature, amoebic actin rearrangements, Gal/GalNAc lectin, EhC2PK and PI3K signaling [49]. Although these proteins also have roles in phagocytosis in *E. histolytica* (Table 1), amoebic trogocytosis is predominant with living host cells. Therefore, interference with actin, Gal/GalNAc lectin, EhC2PK or PI3K quantitatively reduced amoebic trogocytosis of living human cells, as measured by imaging flow cytometry. In all cases, when amoebic trogocytosis was quantitatively reduced, there was a corresponding reduction in host cell death. Cell death following amoebic trogocytosis might be due to the accumulation of physical damage in the nibbled cell. Host cells retained membrane integrity for an average of ~ 27 minutes [49], suggesting that either numerous bites are needed to precipitate cell death, or that following the initial damage, a cell death program is initiated that takes some time to complete.

Trogocytosis in other organisms

Morphologically similar processes occur in a variety of amoebae. In some cases, these processes have been termed “trogocytosis,” but the extent to which the mechanisms are similar is not yet known. The term was first coined in studies of the interactions between the pathogenic amoeba *Naegleria fowleri* and host cells [50]. Prior to this, there were reports of “nibbling, piecemeal” ingestion of red blood cells by *N. fowleri* and *Hartmanella* [51, 52]. A process termed “nibbling” has also been described in *Dictyostelium caveatum* during predation of other *Dictyostelium* species [53, 54]. Since amoebae do not form a taxonomic group, it is notable that nibbling processes have been observed in numerous amoebae from at least two eukaryotic supergroups, the Amoebozoa and Excavates.

In addition to the occurrence of nibbling processes in amoebae, a morphologically similar process, also termed trogocytosis, occurs in multicellular organisms [55]. This was first described at the immunological synapse in mammalian immune cells, where lymphocytes obtain plasma membrane fragments and surface molecules from antigen-presenting cells [56–59]. Trogocytosis is now recognized to occur between a variety of different immune cell types [60]. A key difference between this processes in multicellular organisms and in amoebae, is that trogocytosis in multicellular organisms does not appear to result in cell death. The reason for this distinction is not yet apparent, but this may be because the described examples of trogocytosis in multicellular organisms involve the exchange of fewer bites, and these bites are primarily fragments of cell membrane. In contrast, in amoebic trogocytosis, ingested bites commonly contain target cell cytoplasm and can also contain organelles [49].

Amoebic trogocytosis versus phagocytosis in *E. histolytica*

An important question is whether amoebic trogocytosis is mechanistically distinct from phagocytosis in *E. histolytica*. Since the underlying mechanistic differences are not yet apparent, we will refer here to “trogocytosis” as ingestion in which bites of cellular material are internalized, and “phagocytosis” as ingestion in which an entire cell is internalized. In other organisms, the mechanistic basis for trogocytosis is not known and specific signaling processes have not been well defined. Notably in T cell trogocytosis, two small GTPases have been identified that are involved in its regulation, TC21 and RhoG [61]. RhoG has an established role in phagocytosis [61]. Additionally, trogocytosis by CD4+ T cells has been shown to involve actin rearrangements, PI3K, Src and Syk signaling [61, 62]. Thus the relationship between trogocytosis and phagocytosis in not understood in any organism, though it appears that the two processes share some features.

Effect of target cell deformability

In the case of amoebic trogocytosis, the occurrence of this process or phagocytosis appears to depend on qualities of the target cell, including its deformability, whether it is viable, and its size (Figure 3). Target cell deformability appears to be important during *E. histolytica* ingestion, since cell distortion during phagocytosis by *E. histolytica* has been previously reported [63, 64]. During ingestion of Chinese Hamster Ovary (CHO) cells, a "tunnel" of CHO cell material was stretched into the trophozoite, which sometimes preceded ingestion of the entire CHO cell, and sometimes persisted indefinitely [64]. A similar tunnel of material, referred to as "suction" or "micro-phagocytosis," occurred during ingestion of human red blood cells, where 90% of human red blood cells were ingested in this manner and the remaining 10% were directly ingested as a single unit [63]. We have observed similar tunnels during amoebic trogocytosis, and have sometimes detected both the appearance of a tunnel and bites (*e.g.*, Figure 2a), suggesting that the observed bites may potentially fragment off of the stretched tunnel of intracellular material. In the case of red blood cells, increasing the rigidity by pre-exposing red blood cells to increasing concentrations of fixative prior to co-incubation with trophozoites led to a reduction in micro-phagocytosis [65]. Therefore, natural differences in the deformability of different cell types could influence the extent of fragmentation that occurs during ingestion, and whether phagocytosis or amoebic trogocytosis occurs.

Effect of target cell viability

The viability of the target cell may also be an important determinant for the occurrence of amoebic trogocytosis or phagocytosis (Figure 3). When trophozoites were co-incubated with a combination of living host cells, and host cells that had been pre-killed, the living cells were ingested by amoebic trogocytosis and the pre-killed cells were ingested by phagocytosis (Figure 2d) [49]. The dependence of amoebic trogocytosis on living host cell targets may again suggest that the deformability of the target cell influences its fate during amoebic ingestion, since dead cells are likely to be less deformable than living cells. Alternatively, differences in amoebic surface proteins that bind to living vs. dead cells (see below) may activate different downstream signaling pathways in the trophozoite leading to amoebic trogocytosis of live cell targets, and phagocytosis of dead cell targets. An additional possibility is that living host cells actively contribute to amoebic trogocytosis in some way, making amoebic trogocytosis only possible with living host cell targets.

Effect of target cell size

Finally, whether amoebic trogocytosis or phagocytosis occurs also depends the size of the target cell. With smaller cells, such as human red blood cells (diameter ~ 7 µm, thickness ~ 2 µm), both micro-phagocytosis and phagocytosis were reported to occur, and we have also detected the occurrence of amoebic trogocytosis [49, 63, 65]. Very little red blood cell material remains extracellular following ingestion [49, 63, 65], reflecting that either the entire red blood cell has been ingested in successive bites, or that phagocytosis has occurred.

Slightly larger cells such as human Jurkat T cells (diameter ~ 12 μm, thickness ~ 12 μm) are also ingested by both amoebic trogocytosis and phagocytosis, although the balance is shifted toward amoebic trogocytosis in this case [49]. We detected up to 20% of Jurkat cells that are ingested by phagocytosis, with the remainder ingested by amoebic trogocytosis [49]. Significantly more material remains extracellular in this case, including the prominent, undigested Jurkat cell nuclei [49].

Common features of amoebic trogocytosis and phagocytosis

Since no unique signaling pathways that define trogocytosis have been defined in any organism, it has only been possible to test whether proteins with known roles in phagocytosis in *E. histolytica* also play a role in amoebic trogocytosis. By defining that amoebic trogocytosis requires physiological temperature, amoebic actin rearrangements, Gal/GalNAc lectin, EhC2PK and PI3K signaling (Figure 3), thus far all tested proteins that are required for phagocytosis [23, 66, 67] are also required for amoebic trogocytosis [49]. Additionally, it appears that amoebic trogocytosis is under "feed-forward" regulation, as has been demonstrated during amoebic phagocytosis of beads [68]. During amoebic phagocytosis of beads, trophozoites that had previously been exposed to beads upregulated a number of genes and were "primed" to undergo enhanced ingestion of beads relative to trophozoites that had not been exposed to beads [68]. Similarly, trophozoites that had previously undergone amoebic trogocytosis were primed to undergo more ingestion and more cell killing than trophozoites that had not undergone amoebic trogocytosis [49].

Phagocytosis in *E. histolytica*

As outlined above, there are features that are common to both amoebic trogocytosis and phagocytosis. It is not yet clear whether there are also mechanistic distinctions between the two processes. Here we summarize the current paradigms for phagocytosis in *E. histolytica,* highlighting aspects that could potentially be relevant to amoebic trogocytosis.

Target cell attachment in phagocytosis

There are a number of *E. histolytica* surface proteins with roles in attachment to host cells, including some with roles in attachment that are specific to live or dead cells, which may be relevant to the specificity of amoebic trogocytosis for living cells (Figure 3). Engagement of different surface receptors by live and dead host cells could potentially trigger different ingestion processes. The amoebic Gal/GalNAc lectin plays a more significant role in attachment to living cells than apoptotic cells or calcium ionophore-treated erythrocytes [23, 69]. The rhomboid protease EhRom1 can cleave the Gal/GalNAc lectin heavy subunit [70] and knockdown of EhRom1 reduces attachment [71] as well as cell motility [72]. The attachment defect in the EhRom1 knockdown mutant was specific to live host cells, and attachment to apoptotic host cells was normal [71]. These data together imply a significant role for the Gal/GalNAc lectin in recognition of living cells. There is some evidence that suggests signaling downstream of the lectin might regulate amoebic trogocytosis. Blocking antibody studies previously suggested that lectin engagement plays a role in initiating the cell-killing program [26], and lectin signaling also appears to be critical in regulating amoebic trogocytosis, since the same blocking antibody reduced amoebic trogocytosis [49].

Hence it is possible that engagement of the Gal/GalNAc lectin by living cells triggers ingestion via amoebic trogocytosis.

In the recognition of dead cells, amoebic binding to host cells that have been chemically induced to undergo apoptosis has been the most characterized. The surface metalloprotease EhMSP-1 was shown to have a role in attachment to both live and apoptotic cells [73]. A blocking antibody directed to the serine-rich *E. histolytica* protein SREHP, reduced attachment to apoptotic host cells, but had a much smaller effect on attachment to live host cells [74]. *E. histolytica* trophozoites can bind to phosphatidylserine (PS) [69]. Opsonization of apoptotic cells with C1q or collectin family members enhanced uptake by *E. histolytica* [75], and C1q has been shown to bind to trophozoite surface calreticulin [76]. Together, these findings suggest that both PS and additional physiological ligands present on apoptotic cells may be important determinants for amoebic attachment.

Additional proteins with roles in attachment include the transmembrane kinase family members TMK39 [77], TMKb1-9 [78] and PATMK [79]. There are ~ 90 such TMK genes in the *E. histolytica* genome [80, 81], making it likely that other TMKs are involved in recognition and attachment to different ingestion targets. Most of the TMKs await functional characterization. Another gene family involved in attachment is the family of predicted transmembrane serine-, threonine, and isoleucine rich proteins, known as EhSTIRP [82]. Additionally, the 112 kDa cysteine protease and adhesin complex, EhCPADH, contributes to attachment [83]. Finally, the amoeba protein KERP1 may also be involved in host cell attachment [84, 85]. Notably, the recent cell surface proteome of *E. histolytica* identified 693 candidate membrane proteins, but strikingly, 49% of the identified proteins lack conserved surface association domains or motifs [86]. Hence there are far more proteins present on the trophozoite surface than previously understood, and it is likely that at least some of these proteins contribute to attachment.

Initiation of phagocytosis

As in other organisms, phagocytosis in *E. histolytica* requires actin and myosin [87]. In the process of initiating phagocytosis and regulating actin rearrangements, there are roles for a family of calcium-binding proteins (CaBPs) unique to *Entamoeba* (Figure 3) [88]. There are 27 CaBPs with multiple EF-hand calcium-binding domains in the *E. histolytica* genome [88]. Characterized CaBPs do not have conserved actin binding or lipid binding domains, yet many of them functionally interact with actin or lipids, making it possible that along with actin remodeling, CaBPs are also involved in initiating membrane deformation, which is also a necessary event in initiating ingestion. Some of the CaBPs represent independent regulators of ingestion, making it possible that differential triggering of CaBPs could influence the ingestion mechanism engaged by different host cell targets. Additional complexity to CaBP signaling comes from the fact that some key protein-protein and protein-lipid interactions are calcium independent, while others are dependent on calcium.

Calcium-binding protein 1 (EhCaBP1), together with EhC2PK, is part of a signaling pathway that initiates ingestion. EhC2PK binds amoebic PS in the presence of calcium and recruits EhCaBP1 to the cell membrane [67]. EhCaBP1 binds F-actin and is crucial for F-actin dynamics, as its loss affects cell proliferation, phagocytosis, and fluid-phase endocytosis [89, 90]. EhCaBP1 also recruits the alpha kinase EhAK1, which was recently shown to directly phosphorylate G-actin [91]. The interaction of EhCaBP1 and EhAK1 is calcium dependent [91], while the interaction of EhCaBP1 and EhC2PK is calcium independent [67]. Such behavior of EhCaBP1 may be responsible for giving rise to mechanistic differences in fluid-phase endocytosis versus phagocytosis and spatial regulation of actin dynamics. Another calcium-binding protein, EhCaBP3 interacts with lipids directly and may function in initiation of phagocytosis independent of the EhCaBP1/EhC2PK pathway [92]. EhCaBP3 also binds actin directly and influences bundling and polymerization, and may regulate closure of phagocytic cups since phagocytosis is slowed when EhCaBP3 expression is knocked down [92]. Finally, another calcium-binding protein, EhCaBP5, was recently shown to interact with myosin 1B in a calcium independent manner and may represent a myosin light chain [93].

Intracellular trafficking in phagocytosis

PI3K signaling is important in the early stages of phagosome formation, and there is a role for FYVE-domain proteins [64, 94]. Following initiation of ingestion and the formation of a phagosome, intracellular trafficking in *E. histolytica* appears to be complex. The Rab, Arf, Rho and Rac GTPases gene families are all greatly expanded in *E. histolytica* [81, 95]. Many of the small GTPases, and other candidate phagosome proteins, have been identified in proteomic analyses of *E. histolytica* phagosomes and await functional characterization [79, 96–98]. It is an intriguing possibility that the expansion of small GTPases reflects the complexity of ingestion in *E. histolytica*, with potentially different intracellular trafficking occurring in phagocytosis and amoebic trogocytosis. However, how the small GTPases intersect with amoebic trogocytosis is not yet clear.

Small GTPases that have been characterized to have roles in phagocytosis in *E. histolytica* include the Rac protein p21RacA [66]. Among Rab proteins, EhRabB localizes to the phagocytic cup during phagocytosis [99–101] and appears to interact with a candidate G-protein coupled receptor, EhGPCR-1 [102]. EhRab5 does not appear to be involved in endocytosis as in other organisms, and together with a Rab7 homologue, EhRab7A, it localizes to a pre-phagosomal vacuole [103]. These pre-phagosomal vacuoles appear to arise *de novo* and distinct from phagosomes [103]. Following dissociation of EhRab5, the pre-phagosomal vacuole fuses with the phagosome, and EhRab7A dissociates [103]. EhRab7B appears to play a role in late endosome-lysosome fusion [104]. EhRab7A may also be involved in secretion [105]. Finally, additional Rabs with likely roles in the *E. histolytica* secretory pathway include EhRab11B, EhRab8, and EhRabA [40, 106, 107].

Amoebic trogocytosis in tissue invasion and destruction

An important question is how amoebic trogocytosis and/or phagocytosis influence tissue invasion and damage *in vivo*. Amoebic trogocytosis occurs in the context of *ex vivo* mouse intestinal tissue [49]. Perhaps in the context of the intestinal epithelium, with the tight intercellular connections between cells, phagocytosis of entire cells is difficult or impossible. Amoebic trogocytosis of cells, on the other hand, may allow trophozoites to ingest portions of intestinal epithelial cells, with the consequence of ultimately leading to cell death and localized tissue damage. This could potentially facilitate a subsequent opportunity for trophozoites to breach the epithelial barrier and invade. Amoebic trogocytosis might provide an opportunity for environmental sensing by allowing amoebic trophozoites to sample different cell types, or it could serve a nutritional role by providing amoebae with macromolecules that are costly to synthesize. Given the finding that amoebic trogocytosis occurs during tissue invasion, and that inhibition of amoebic trogocytosis quantitatively reduces invasion depth of *ex vivo* mouse intestinal tissue [49], there appears to be a role for this process in invasive pathology. Further suggesting a role for amoebic trogocytosis in tissue damage, in a 3-D liver culture model, trophozoites invading the upper layer of liver sinusoidal endothelial cells (LSEC) were observed to contain fragments of the LSEC, potentially reflecting the occurrence of amoebic trogocytosis [108].

Concluding remarks

Tissue lysis underlies pathogenesis of invasive amoebiasis and is the feature for which the pathogen was named. Direct killing of host cells is likely to be a major contributor to tissue damage. With the discovery of amoebic trogocytosis, we have a new model for how amoebae kill host cells. With this new model, there are many questions about how amoebic trogocytosis interplays with phagocytosis, and whether there are distinct pathways for each process (Box 1). Given the abundance of amoebic receptors for host cell attachment, and the precedence for receptors that are specific for living vs. dead host cells, it is likely that engagement of different receptors triggers amoebic trogocytosis or phagocytosis. Additionally, with the expansion of genes involved in vesicle trafficking, and the large number of calcium-binding proteins that regulate ingestion in this organism, it is possible that distinct intracellular machinery is engaged for each process.

Box 1

Outstanding questions

- Does engagement of different amoeba surface receptors by live and dead cells dictate whether amoebic trogocytosis or phagocytosis occurs?
- What are the ligands of the large family of TMKs?
- Are there more receptors for host attachment among the large number of recently discovered *E. histolytica* membrane proteins?
- Do distinct mechanisms occur in phagocytosis and amoebic trogocytosis?
- Are there shared mechanisms for cell nibbling processes that are seen in other organisms?
- Is trogocytosis a more widespread form of intercellular exchange than we currently appreciate?

While amoebic trogocytosis occurs in a tissue model, it will be of interest to better define how this process impacts pathogenesis *in vivo.* Additionally, amoebic trogocytosis in *E. histolytica* may be relevant beyond amoebiasis, as a cell biological process that also appears to be relevant to many organisms. “Nibbling” processes occur in a variety of amoebae as well as multicellular organisms. It will be of interest to better understand why trogocytosis in multicellular organisms does not appear to result in cell death, but it is associated with killing of nibbled cells by microbes. One possibility is that a common pathway for intercellular exchange has been taken to the extreme in the case of cytotoxic microbes, which appear to ingest more cellular material during nibbling, both in terms of cellular contents and sheer amount of ingested material. Studies of amoebic trogocytosis in *E. histolytica* may shed light on a potentially evolutionarily conserved process that can result in cellular communication or death. It is certainly "food for thought."

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Highlights

- Host cell killing is likely to underlie the pathogenesis of amoebiasis.
- Amoebic trogocytosis (*trogo-:* nibble) by *E. histolytica* is a recently discovered mechanism for host cell killing.
- We place amoebic trogocytosis in the context of previous studies of cell killing by *E. histolytica.*
- Amoebic trogocytosis and phagocytosis are compared, and potential mechanistic differences highlighted.
- “Nibbling” processes by other organisms are discussed.

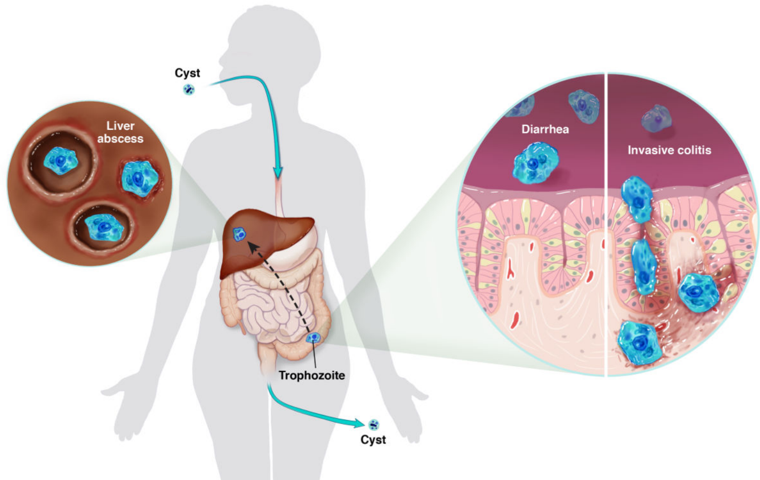

Figure 1. Amoebiasis in humans

Model for the *Entamoeba histolytica* life cycle and pathogenesis of disease in humans. Infection occurs following the ingestion of *E. histolytica* cysts that are found in contaminated water or food sources. Following excystation, motile amoeboid trophozoites colonize the large intestine. Encystation can occur to produce new cysts. Both cysts and trophozoites are found in the feces of infected individuals. Colonization with *E. histolytica* trophozoites can be asymptomatic or lead to diarrheal symptoms, and the trophozoites are thought to be noninvasive in these situations. Trophozoites can also invade and damage the large intestine, resulting in ulceration and dysentery symptoms. Less commonly, trophozoites can spread to other tissues in the body, and they most often spread to the liver. Trophozoites that have spread outside of the intestine result in abscesses that can be fatal.

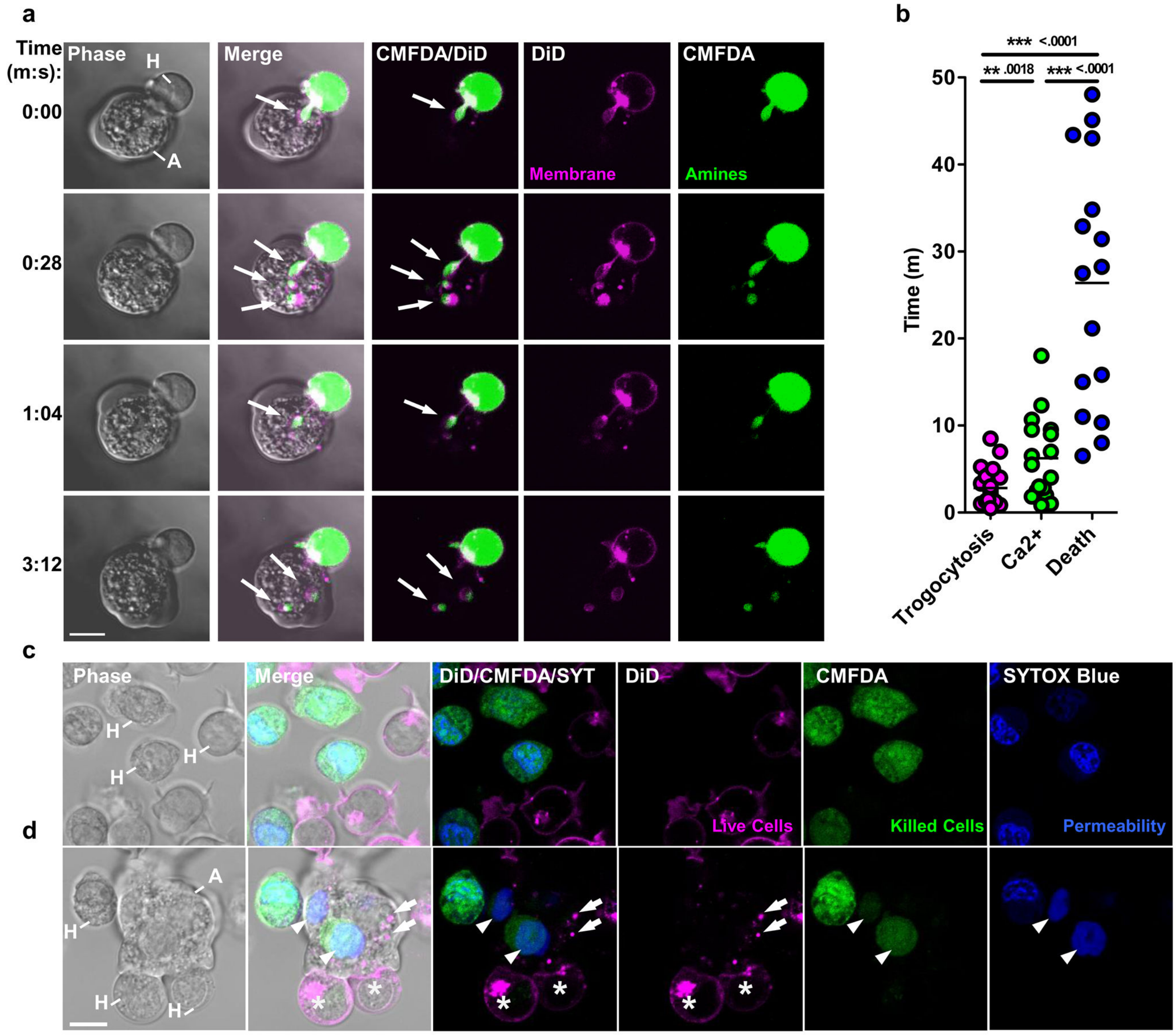

Figure 2. Amoebic trogocytosis is specific to live human cells and occurs prior to human cell death

(A) Example of amoebic trogocytosis. Time-lapse confocal microscopy demonstrating the ingestion of fluorescently labeled "bites" of human cell material by an amoebic trophozoite. Human Jurkat T cells were pre-labeled with 1,1′-Dioctadecyl-3,3,3′,3′-Tetramethylindodicarbocyanine, 4-Chlorobenzenesulfonate (DiD) and 5-chloromethylfluorescein diacetate (CMFDA). DiD (shown in pink) labels the plasma membrane, while CMFDA (shown in green) labels amines. H, human cell; A, amoeba. Arrows, ingested "bites." Time is indicated in minutes:seconds. Bar, 10 μm. **(B)** Timing of the first occurrence of events as detected by live confocal microscopy, relative to the time that trophozoites were combined with human Jurkat T cells. Human Jurkat T cells were pre-labeled with DiD and pre-loaded with the calcium indicator fluo4. SYTOX blue was present in the media during imaging. Amoebic trogocytosis was detected by the appearance of DiD-labeled human cell bites with the trophozoites. Calcium elevation was assessed by the appearance of a sustained increase in intensity of Fluo4. Cell death was assessed by the uptake of SYTOX blue, reflecting loss of membrane integrity. 60 cells from 15 independent experiments were quantified; shown are the individual data points, means and standard deviations. P-values from statistical analyses are indicated. **(C – D)** Human Jurkat T cells were either alive or pre-killed via heat treatment, and separately labeled. Live human cells were labeled with DiD (pink). Heat-killed human cells were labeled with CMFDA (green). SYTOX blue was present in the media during imaging. **(C)** Pre-killed and live human cells were combined at an equal ratio in the absence of *Entamoeba histolytica.* SYTOX blue labeling demonstrates that heat-killed human cells are dead. **(D)** Pre-killed and live human cells were combined with *E. histolytica* at a ratio of 1 amoeba to 5 pre-killed and 5 living human cells. Pre-killed cells were ingested whole (arrowheads, ingested pre-killed cells). Live cells (asterisks) were ingested by amoebic trogocytosis (arrows, ingested bites of live cells). Bar, 10 μm. Reprinted with permission from [49].

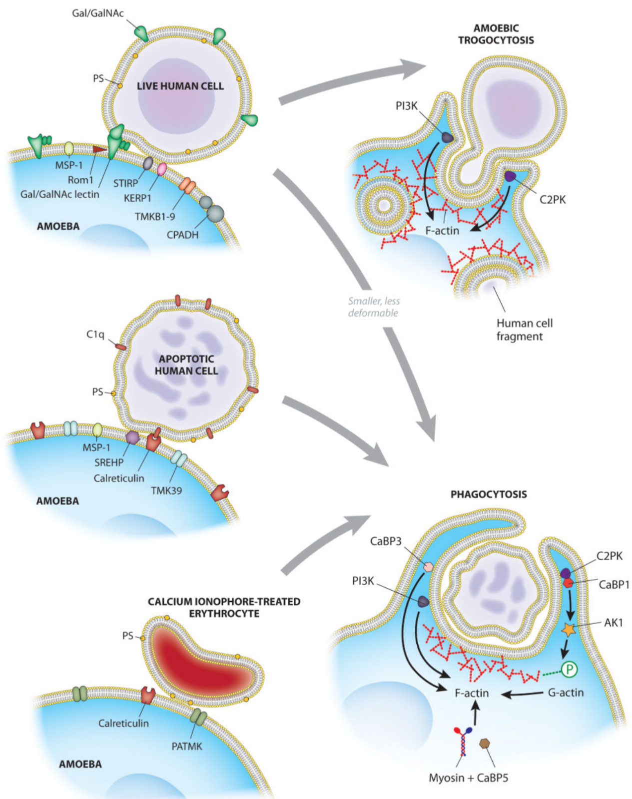

Figure 3. Amoebic trogocytosis and phagocytosis

Model for amoebic attachment to different host cell types, and signal transduction during the initiation of amoebic trogocytosis or phagocytosis. **(A)** Attachment to live host cells is mediated by the amoebic Gal/GalNAc lectin that binds to Gal or GalNAc residues on host surface proteins. The Gal/GalNAc lectin consists of a heavy chain that binds Gal or GalNAc, a covalently associated light chain and a non-covalently associated intermediate chain. The rhomboid protease EhRom1 can cleave the Gal/GalNAc lectin heavy chain. Other amoebic proteins that are involved in attachment to live cells include the family of predicted transmembrane serine-, threonine, and isoleucine rich proteins, known as EhSTIRP, the transmembrane kinase family member TMKb1-9, and the 112 kDa cysteine protease and adhesin complex CPAdh. KERP1 may also be involved in attachment. **(B)** Attachment to host cells that have been induced to undergo apoptosis involves the serine-rich *Entamoeba histolytica* protein SREHP, and the transmembrane kinase family member TMK39. Attachment to host cells that have been induced to undergo apoptosis cells and have subsequently been opsonized with C1q or collectin family members involves amoebic calreticulin. **(C)** Attachment to calcium ionophore-treated erythrocytes involves the transmembrane kinase family member PATMK. Exposed phosphatidyl serine (PS) appears to be a ligand for amoebic binding. (**D–E)** Larger or more deformable cells are more likely to be ingested by amoebic trogocytosis, while smaller or less deformable cells are more likely to be ingested by phagocytosis. Pre-killed cells that are killed via heat treatment are ingested by phagocytosis (Figure 2c), making it likely that host cells that have been induced to undergo apoptosis and calcium-treated erythrocytes are also ingested by phagocytosis.

Dead cells are also likely to be less deformable. **(D)** Signal transduction in the initiation of amoebic trogocytosis includes PI3K and EhC2PK, both of which influence actin polymerization. **(E)** Signal transduction in the initiation of phagocytosis includes EhCaBP3 and PI3K, both of which influence actin polymerization. EhC2PK also recruits EhCaBP1, which recruits EhAK1. EhAK1 phosphorylates G-actin, and thereby impacts actin dynamics. EhCaBP5 appears to be a light chain of myosin.

Table 1

Amoebic molecules with roles in amoebic trogocytosis and phagocytosis in *E. histolytica*.[a]

Amoebic molecule	Process	Function (or subcellular location)	Host cell types	Reference
Gal/GalNAc lectin	Amoebic trogocytosis and phagocytosis	Attachment to Gal or Gal/NAc, initiation of amoebic trogocytosis	Live cells (numerous cell lines and cell types)	[4, 23, 26, 49, 69]
EhRom1	Phagocytosis	Attachment, cleavage of Gal/GalNAc lectin and unknown substrates	Live CHO cells, apoptotic CHO cells, erythrocytes	[70, 71]
EhMSP-1	Phagocytosis	Attachment, cleavage of unknown substrates	Live Jurkat cells, apoptotic Jurkat cells	[73]
SREHP	Phagocytosis	Attachment	Apoptotic Jurkat cells	[74]
Calreticulin	Phagocytosis	Attachment to C1q and unknown substrates	Apoptotic Jurkat cells, apoptotic Jurkat cells opsonized with C1q, ionophore-treated erythrocytes	[76]
TMK39	Phagocytosis	Attachment	Apoptotic Jurkat cells	[77]
TMKb1-9	Phagocytosis	Attachment	Fixed CHO monolayers	[78]
PATMK	Phagocytosis	Attachment	Ionophore-treated erythrocytes	[79]
EhSTIRP	Phagocytosis	Attachment	Live CHO cells	[82]
EhCPADH	Phagocytosis	Attachment	Erythrocytes	[83]
KERP1	Phagocytosis	Attachment	Fixed Caco2 monolayers, fixed CHO monolayers	[84, 85]
EhC2PK	Amoebic trogocytosis and phagocytosis	Initiation of ingestion, binding to amoebic PS and recruitment of EhCaBP1	Live Jurkat cells, erythrocytes	[49, 67]
EhCaBP1	Phagocytosis	Initiation of ingestion, recruitment of EhAK1	Erythrocytes	[89, 90]
EhAK1	Phagocytosis	Phosphorylation of G-actin	Erythrocytes	[91]
EhCaBP3	Phagocytosis	Initiation of ingestion, binding to amoebic membrane and actin, actin remodeling	Erythrocytes	[92]
EhCaBP5	Phagocytosis	Myosin light chain	Erythrocytes	[93]
Myosin	Phagocytosis	Generation of force, shape changes in ingestion, intracellular trafficking	Likely numerous cell types and both live and dead cells	[87]
Actin	Amoebic trogocytosis and phagocytosis	Generation of force, shape changes in ingestion, intracellular trafficking	Likely numerous cell types and both live and dead cells	[49, 87]
PI3K	Amoebic trogocytosis and phagocytosis	Generation of phosphoinositides, leading to phagosome formation and actin remodeling	Live Jurkat cells, erythrocytes	[49, 66, 94]
EhFP4 and other FYVE-domain proteins	Phagocytosis	Phosphatidylionositol 3- phosphate-binding	Live CHO cells, erythrocytes	[64, 94]
p21RacA	Phagocytosis	Likely regulates actin remodeling	Erythrocytes	[66]
EhRabB	Phagocytosis	(Phagocytic cup)	Erythrocytes	[99–101]
EhRab5	Phagocytosis	(Pre-phagosomal vacuole)	Erythrocytes	[103]
EhRab7A	Phagocytosis	Pre-phagosomal vacuole- phagosome fusion	Erythrocytes	[103]
EhRab7B	Phagocytosis	Late endosome-lysosome- lysosome fusion	Erythrocytes	[104]

[a]Likely functions of each protein are summarized, or in cases where functional information is not available, subcellular localization is indicated. Host cell types that have been characterized are listed.

Abbreviations as follows: D-galactose/N-acetyl-D-galactosamine-specific lectin, Gal/GalNAc; Chinese hamster ovary cell, CHO; transmembrane kinase, TMK; serine-, threonine, and isoleucine rich protein family, EhSTIRP; cysteine protease and adhesin complex, CPADH; phosphatidyl serine, PS.

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