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INTESTINAL INVASION BY *ENTAMOEBA HISTOLYTICA*

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Introduction

Entamoeba histolytica is a protozoan parasite that infects humans and causes the disease amebiasis. The spectrum of intestinal amebiasis varies from colonization without symptoms to fulminating diarrhea and intestinal hemorrhage. The dissemination of the parasite via invasion of the intestinal epithelium allows the trophozoites to invade extra-intestinal sites, most usually the liver. Without treatment, the amebic liver abscesses may continue to enlarge and, if ruptured, cause mortality owing to acute peritonitis. Cases of clinical amebiasis have been reported worldwide, in particular in under-developed and developing counties in Africa, South America, the Indian subcontinent, and Mexico. It has been estimated that approximately 500 million individuals are infected with *E. histolytica* and about 100,000 people die of invasive amebiasis annually, making it the third leading parasitic cause of death, after malaria and schistosomiasis.[1] The host-parasite interaction in human amebiasis is very complicated, and different aspects of innate immunity of the human host against the parasite still are unknown. New insights into the pathogenesis of amebic infections have come from development of *in vitro* and *in vivo* models of disease, new molecular and genetic approaches, the identification of key factors in *E. histolytica* pathogenesis, recognition of the mechanisms of evasion from the host's harmful responses, and detection of crucial elements of the host immune responses both innate and acquired. In this chapter, we discuss the innate immunity of human hosts against the parasite and the most important parasite virulence factors and survival strategies that are implicated in pathogenesis.

Innate mechanisms of host resistance to *E. histolytica*

Ameba encounter natural "barriers" in both the intestine and systemic circulation after extra-intestinal invasion. Although the exact role of these "natural barriers" and their potential roles are still undefined, in the gut, for example, these innate "barriers" prevent potential pathogens and antigens from gaining access to the underlying epithelium, a process called non-immune exclusion.

Intestinal mucins are highly glycosylated molecules and consist of a core protein (apomucin) joined to oligosaccharides. Mucin glycoproteins line the surface epithelium of respiratory, urogenital, and digestive tracts from the nasal cavity/oropharynx to rectum.[2] Member of the mucin family can differ considerably in size. Some are small, whereas others contain several thousands of residues and are among the largest known.[3] However, gastrointestinal mucins consist solely of high molecule mass glycoproteins and their size varies from 0.5×10^6 to 25×10^6 Dalton.[4] Mucins consist of a peptide backbone containing alternating glycosylated and nonglycosylated domains, with *O*-linked glycosylated regions comprising 70–80% of the polymer. *N*-Acetylglucosamine, *N*-acetylgalactosamine, fucose, and galactose are the 4 primary mucin oligosaccharides.[5,6] Mucin oligosaccharide chains are often terminated with

For Parasite Invasion book (Eds Burleigh B & Soldati D)

sialic acid or sulfate groups, which account for the polyanionic nature of mucins at a neutral pH.[5] Gastrointestinal mucins are the first line of host defense against enteric pathogens, including *E. histolytica*. Binding sites of mucins have been shown to compete with those of underlying epithelium, preventing attachment of pathogens to the intestinal wall. To establish colonization, *E. histolytica* needs to bind to colonic mucin oligosaccharides via the 170kDa heavy subunit of the parasite's Gal/GalNAc lectin. The Gal/GalNAc lectin binds to rat and human colonic mucins with a very high affinity.[7] The ability of rat and human colonic mucins to inhibit amebic attachment and cytolysis of target epithelial cells demonstrate the protective role of mucin against *E. histolytica*. In the gerbil model of amebic colitis, it has been shown that goblet cell mucin stores has been depleted before invasion of *E. histolytica* to colonic epithelium.[8] Although the cause of such depletion is still unknown, it is speculated that parasite-derived secretagogues are responsible.[9] In addition to the direct protective role of intestinal mucins, intestinal bacterial flora compete for attachment to mucin and may prevent amebic lodgment.[10] The protective key role of mucus blanket in natural immunity against *E. histolytica* infections has been verified both *in vitro* and *in vivo*. For example, the mucus–producing colonic cell line, LS174T, inhibits amebic adherence to Chinese hamster ovary (CHO) cells, showing the ability of these mucus-producing cells to compete with attachment sites that are of the utmost importance for *E. histolytica* colonization.[11]. Also, the protective role of the mucus layer has been detected *in vitro* by the finding that *E. histolytica* trophozoites obliterate epithelial cell monolayers without a mucus barrier more quickly and easily than those protected by a mucus cover.[11] Additionally, studies by using the mouse model of intestinal amebiasis showed *E. histolytica* induced the expression of cyclooxygenase-2 in epithelial cells and macrophages, and the resultant prostaglandins enhance epithelial permeability, mediating neutrophils responses.[12]

The human complement system is an important early host defense against amebic infection. *E. histolytica* that disseminates from the bowl through the blood stream is exposed to complement, another component of innate mechanism of host resistance against invading pathogens. Earlier, it had been demonstrated that non-immune sera lysed axenic strains of *E. histolytica*, implying a role of complement against the parasite in blood circulation.[13] Axenic *E. histolytica* isolates activate both the alternative and classical pathways.[14,15] Additionally, this event has been shown to occur in patients with ALA by finding the high concentrations of serum or plasma concentrations of components of the classical (C1q, C4) and alternative (C3, factor B) pathways, regulatory protein factor H, and one of the C3 products of degradation, C3d, in patients with amebic liver abscess.[16] It has been shown that the major *E. histolytica* extracellular proteinase, a 56kDa neutral cysteine proteinase, activates complement by *E. histolytica* in the fluid phase.[17] It is generally believed that both pathogenic *E. histolytica* and nonpathogenic *E. dispar* are susceptible to human complement. It was shown that more than 90% of *E. histolytica* trophozoites were lysed after exposure to the alternative pathway components.[18] However, even though human complement-mediated cytolysis of *E. histolytica* has an effective amebicidal activity *in vitro*, a considerable number of ameba can survive and escape human complement activity *in vivo* (see below). Reed et al.[19], for the first time, showed that both serum-sensitive and serum-resistant stains of the parasite could activate complement. The increased frequency, rate, and severity of amebic liver abscess (ALA) in complement-depleted hamsters treated with cobra venom factor (CoF) reinforced the fact that complement components may play an important role in innate immunity against amebic infections.[20] The Gal/GalNAc lectin molecule of *E. histolytica* has sequence homology and antigenic cross-reactivity with CD59, a membrane inhibitor of C5b-9 in human erythrocytes, suggesting the *E. histolytica* adhesin has both molecular mimicry and shared complement-inhibitory functions.[21] The Gal/GalNAc lectin bound C8 and C9 and efficiently, preventing membrane attack complex (C5b-9) formation and subsequent cell lysis. A definite role for the Gal/GalNAc lectin was confirmed by abrogation of amebic complement resistance following treatment with a monoclonal antibody to Gal/GalNAc lectin molecule.[21]

Different mechanisms of pathogenesis in *E. histolytica*

Several factors contribute to the pathogenicity of *E. histolytica*, and some may still await identification. However, three pathogenic factors of the parasite have been investigated extensively and characterized at molecular levels. These three virulence factors are: the Gal/GalNAc adhesin, mediating adherence to host cells and contributing to amebic resistance to complement, the amoebapores, small peptides that produce pores in target cell membranes, and the cysteine proteinases that play a key role in *E. histolytica* tissue invasion, evasion of host defenses, and parasite induction of gut inflammation.

Gal/GalNAc lectin

The Gal/GalNAc lectin is a novel multifunctional virulence factor of *E. histolytica*, participating in adherence, cytolysis, invasion, resistance to human complement, and also perhaps encystation.[22] Perhaps, the most important part in amebic pathogenesis and pathology is to adhere to the colonic wall. Adhesion of the parasite occurs mainly through the Gal/GalNAc lectin, which binds to exposed terminal Gal/GalNAc residues of target cell glycoproteins.[23] Other molecules thought to be involved in part in adhesion of the parasite are: a 220-kDa lectin, a 112-kDa adhesin, and a surface lipophosphoglycan.[24]

The Gal/GalNAc adhesin is a novel multifunctional protein composed of a heterodimer of heavy (170-kDa), light (35/31 kDa) [23], with a non-covalently-linked intermediate (150-kDa) subunit.[24] The 170-kDa heavy subunit (*hgl*) is a type I transmembrane protein with a small intracellular domain and a carbohydrate recognition domain (CRD) contained in its extracellular domain.[25] The 30-kDa light subunit (*lgl*) is covalently attached to the heavy subunit through disulfide linkages. The light subunit has been shown to have several isoforms [23,26], although the significance of the different isoforms is not clear yet. The intermediate subunit (*igl*) of the Gal/GalNAc has been cloned and characterized recently, and it has been shown that it lacks a carbohydrate recognition domain (CRD) [24]. The Gal/GalNAc lectin mediates adherence of trophozoites to human colonic glycoproteins, human colonic epithelium, human neutrophils, and erythrocytes, and to certain bacteria.[27,28] Evidence for the participation of this molecule in the adhesion event of the parasite has been detected by decreased amebic adherence to target cells when the lectin is inhibited by galactose[29], by inhibition of adherence with monoclonal antibodies (mAbs) directed against the carbohydrate recognition domain (CRD) of the lectin, and finally by the lack of amebic adherence to Chinese hamster ovary (CHO) cell mutants lacking Gal/GalNAc[30]. In addition to its role in adherence, the Gal/GalNAc lectin also participates in the cytolytic events, since contact-dependent target cell lysis is reduced in the presence of galactose as well as by a monoclonal antibody against the heavy subunit is able to inhibit cytolysis in part without blocking adherence.[31,32] Interestingly, the purified lectin, even at high concentrations, has no cytotoxic effect, suggesting that this protein may be involved in signaling of cytolysis most likely via stimulation of active polymerization.[32,33] Furthermore, it has been suggested that this lectin is implicated in amebic resistance to human complement. The adhesin binds to purified c8 and c9 components of complement and prevents formation of the membrane-attack complex (C5b-9).[21]

Amoebapore

Once *E. histolytica* establishes contact with mammalian cells, a rapid cytolytic event takes place that result in swelling, surface blebbing, and lysis of the target cell and leaving the parasite intact. The similarity of this phenomenon to perforin-mediated lysis of target cells by cytotoxic T lymphocytes[34] suggested the possible presence of a channel-forming protein, amoebapore, in *E. histolytica*.[35,36] Amoebapores are a family of small peptides contained in cytoplasmic vesicles in the trophozoites with maximum activity at acidic pH. The amebapore of *E.*

histolytica is a channel-forming peptide of 77 amino acid residues; these proteins have now been purified, sequenced, and the relevant genes have been cloned.[37] Three amoebapore isoforms, A, B, and C, at a ratio of 35:10:1, respectively, have been characterized; these peptides showing 35 to 57% deduced amino acids sequence identity and are encoded by a family of three genes.[37,38]

All of these three peptides have a common six cysteine residues at identical positions and also a histidine residue near the C terminus. Significant similarities have been determined between these peptides in both structural and functional basis and NK-lysin, pore-forming peptide occurring in natural killer (NK) cells and porcine T cells.[39] Structural modeling with the use of genetic algorithm, suggests a compact tertiary structure composed of four α-helix bundles stabilized by three disulfide bonds, a structure that is also present in NK-lysin.[40]

Amebapores are now believed that aggregate through the arrangement of their amphipathic α-helices and finally they form a channel within the plasma membrane through which water, ions, and other small molecules pass and thus the target cell lyses. Amebapores have cytolytic activity against several human cell lines including human Jurkat T cells;[40] these peptides also are effective in forming pores in gram-positive bacterial membrane.[38,40] However, causing damage to thick gram-negative bacterial shield requires high concentrations of amebapore or removal of thick wall in advance with lysosome.[40]

A homologous peptide to *E. histolytica* has been determined in non-pathogenic *E. dispar* trophozoites.[41] This peptide has significant shared structural and functional properties to that of *E. histolytica*, including highest activity at acidic pH, presence in cytoplasmic granules, and a 95% identity of primary structures. However, irrespective of these similarities, the activity of *E. dispar* amebapore is 60% lower than that from *E. histolytica*. It has been suggested that this lower activity may be related to shortened putative amino-terminal α-helix of the *E. dispar* porin.[41] Irrespective of all advances in the molecular biology and biochemistry of amebapores, their exact roles in *E. histolytica* cytolytic events have not been yet known. Amebapores are not continuingly secreted from viable trophozoites in vitro,[42] suggesting that these peptides may be secreted upon stimulus, including target cell contact, and play a role in lysis of host cells during invasion.[39] However, more recently, transcriptional silencing of the gene encoding amoebapore isoform A (ap-a) was detected in *E. histolytica* when trophozoites were transfected with a hybrid plasmid construct containing the ap-a gene flanked by the upstream and downstream segments of the original Ehap-a gene.[43] According to *invitro* investigations, it also proposed that trophozoites without amoebopore A were unable to produce pathological effects as well as the to adhere to lyse bacteria.[43] *In vivo*, it has been demonstrated that the G3 strain of *E. histolytica*, an avirulent strain of the parasite derived from virulent strain HM1:IMSS that lacks amoebapore A, had behaviors comparable to avirulent Rahman avirulent strain of the parasite and it may be consider as a vaccine candidate in the future.[44] The presence of pore-forming activity in non-pathogenic *E. dispar* suggests that the primary role of these peptides is likely to destroy phagocyted bacteria, the main source of *Entamoeba* sp. in the gut[39,45].

Cysteine proteinases

Cysteine proteinases occur in a wide range of organism including bacteria, plants, invertebrates, and vertebrates.[46] In mammals, these enzymes are involved in protein turnover within lysosomes. In addition, extracellular cysteine proteases have been implicated in various physiological and pathophysiological processes, including tumor invasion and metastasis.[47] In protozoan parasites, different types of cysteine proteases have been characterized widely and shown to have a variety of functions (including evasion from the host immune responses

and roles in developmental cycle of the parasite) and cytopathic effects (including induction of apoptosis).[48]

Several cysteine proteinases with molecular weight between 16 to 96 kDa have been observed in *E. histolytica* extracts. Previously, investigators purified two distinct cysteine proteinases from *E. histolytica* and designated them as amoebapain (now know as *ehcp3*) [49] and histolysin (now known as *ehcp1*).[50] However, three different main isoforms of cysteine proteinases are produced in *E. histolytica* of about 30kDa (27-to 30-kDa) and are encoded by a family of more than 30 genes. [51,52] *E. histolytica* trophozoites show strong proteolytic activity and release large amounts of cysteine proteinases into growth media.[50,53] Previous studies showed that *E. histolytica* secreted 10- to 1,000-fold more cysteine proteinase activity than did *E. dispar* isolates.[54] It appears that *ehcp5* is the only cysteine proteinase knows that is present on ameba surface.[55] In addition to *ehc5*, it has been suggested that *ehp1* is also important in *E. histolytica*-induced pathogenesis.[51] The non-pathogenic *E. dispar* has four cysteine proteinase genes (*edcp2, edcp3, edcp4, edcp6*), with the highest expression belonging to *edcp3*. Using *ehcp* sequences as cross-hybridizing probes, it was revealed that functional orthologs corresponding to *ehcp1* and *ehcp5* are absent in *E. dispar*.[56,57] Apparently, only two of *E. dispar* cysteine proteinase genes are expressed,[56] and this may explain why *E. dispar* has low levels of cysteine proteinase activity. Additionally, *E. dispar* lacks several of the most important *E. histolytica* cysteine proteinases, and this again might explain in part its non-invasive nature.

Cysteine proteinases have been shown to be involved in host invasion by some other parasites including *Trypanosoma cruzi*, *Plasmodium falciparum*, *Cryptosporidium parvum*, and *Toxoplasma gondii*. [58] Cysteine proteinases have been implicated in the cytopathic effects of *E. histolytic* upon target cells, resulting in the release of adherent cells from monolayers, presumably by degradation of the components of the extra-cellular matrix, including fibronectin, laminin, and collagens as well as an extra-cellular matrix from vascular smooth muscles.[59] These cytopathic effects correspond with the amount of CP activity secreted and can be inhibited by some specific peptide inhibitors.[60] For example, using exogenous laminin or cysteine proteinase-specific inhibitor L-*trans*-epoxysuccinyl-leucylamido- (4-guanidino) butane (E-64) to neutralize cysteine proteinase reduced the formation of ALA significantly in the severe combined immunodeficient (SCID) mouse.[61] In the recent years, new generations of cysteine proteinase-specific inhibitors have been introduced which have been effective against *E. histolytica* CPs, [60] suggesting the potential of these specific inhibitors as novel antiamebic chemotherapy.[62]

The role of signal transudation in *E. histolytica* pathogenicity

Interaction of *E. histolytica* trophozoites with diverse external stimuli, such as exposure to extracellular matrix (ECM) proteins, appears to activate signaling pathways through G-protein-coupled receptors. *In vitro* studies indicate that the parasite releases proteases and/or toxin-like molecules when in contact with several types of cultured cells.[56] (Meza, 2000). Adhesion to fibronectin (FN) and its proteolytic fragments is known to induce a wide variety of cellular responses, such as expression of genes encoding proteases, secretion of proteins, activation of lymphocytes and differentiation of neural, endothelial, myoblastic and many other types of cells.[63,64]; an early reaction to fibronectin binding is formation of actin adherence plates and focal contact in trophozoites. It has been shown that FN action is mainly dependent on the influx of external Ca^{2+}. [65] It has been shown that the protein kinase C (PKC) pathways are activated in amebas by information transduced as a result of trophozoite binding to FN.[66] Adherence of *E. histolytica* trophozoites to another extracellular component, collagen, is a known stimulus for parasite activation, leading to subsequent tissue destruction and invasion. *In vitro* interaction of *E. histolytica* with collagen induces intracellular formation and release

of electron-dense granules (EDGs) and stimulation of collagenolytic activity.[67] Additionally, results of one study showed that tyrosine phosphorylation is involved in collagen signaling in amoebas and that pp125FAK and p42MAPK homologs may play an active role in turning on the genetic program that enables the parasite to invade its host.[68] There is also evidence suggesting that adhesion to collagen and activation of EDGs secretion are integrin-dependent events and the involvement of actin, vimentin, and tubulin in restructuring cytoskeleton during EDGs secretion are evident. [69] More recent experimental studies attempting to identify and characterize the gene(s) that are upregulated by the human collagen type I and Ca2+ interactions showed that interaction of *E. histolytica* with human collagen type I and Ca^{2+} triggers the transcriptional activation of at least two important genes responsible for pathogenesis of amebiasis.[70] It was demonstrated that there were similarities between mechanisms of phagocytosis of bacteria and erythrocytes by ameba and macrophages, support the idea of coincidental selection of amebic genes encoding proteins that mediate destruction of host cells.[71] *Rho* family GTPases regulates many features of the cell behavior in eukaryotic cells and the members of the *rac* superfamily have critical role in regulating a wide range of cellular processes such as cellular growth, differentiation, vesicle transport, nuclear transport, and actin cytoskeleton regulation. [72] Genes for these components of signaling pathway have been identified in *E. histolytica*.[73] More recently, a family of over 80 putative transmembrane kinases (TMKs) has been recognized in *E. histolytica*.[74] In addition to their role in antigenic variations, it seems that they are involved in signal transduction via sensing the E. histolytica ambient.

Parasite evasion of innate and acquired immune responses

a. Degradation of antibodies by parasite's proteases

Amebic granules contain copious amounts of tissue-destructive activities, such as hydrolytic enzymes and strong cysteine proteinases, whose secretion contributes to the damage of host cells and tissues. These proteases, mainly cysteine proteinases, can escape the antibody-mediated humoral immune responses by degrading IgA and IgG antibodies. It has been well documented that human serum and secretory IgA was degraded completely when exposed to viable axenic trophozoite of *E. histolytica* (HM1:IMSS strain), parasite lysates, and medium that had been conditioned by incubation with viable trophozoites.[75] This phenomenon could be more important for ameba since *E. histolytica* trophozoites must conquer the destructive act of secretory IgA (sIgA) and the serum IgG antibodies during intestinal colonization and extra-intestinal dissemination.

b. Shedding of immune complexes by capping

Interaction of *E. histolytica* trophozoites with sera from patients with invasive amebiasis results in initial clustering of bound antibodies into small patches, followed by a rapid mobilization of these patches to the posterior pole of the cell, where the uroid is formed.[76] It is believed that the uroid, the posterior appendix that forms during the movement of trophozoite, plays a role in the escape of ameba from the host immune response. In one study, it was shown that myosin II is of the utmost importance in capping and formation of uroid in *E. histolytica* parasite, in such a way that myosin II was three times more concentrated within the uroid compared with the rest of the cell, suggesting that the release of caps may depend upon mechanical contraction driven by myosin II activity.[77] This results in the remarkable ability of *E. histolytica* to rapidly regenerate substantial amounts of plasma membrane. The properties of surface receptor redistribution (capping), liberation of caps, and plasma membrane regeneration, may contribute to the survival of the parasite in the host during infection.

c. Anergy of T cells and suppression of macrophages

Tissue invasion by *E. histolytica* has been associated with suppression of cell-mediated immunity. It has been known that *E. histolytica* exerts different modulatory effects on macrophages, and T cells especially T helper type 1(Th1).[78] T cells are important sources of macrophages-activating cytokines, and can be directly cytotoxic to amoeba, although the mechanisms involved are unknown. There is some evidence that amebic infections are associated with T cell modulation. Reduced delayed-type hypersensitivity reaction during the acute phase of the disease is one index of functional suppression of T cell during human amebiasis.[79] Similarly, in the mice models, intestinal amebiasis has been associated with a cyclic suppression in immune responses, and it has been proposed that these alterations, observed at the cellular level, might facilitate invasion of the host by the parasite.[80] The cytotoxic ability of macrophages and their potential as antigen-presenting and cytokines-releasing elements are reduced during the acute phase of ALA. Exposure of human mononuclear phagocytes to the monocyte locomotion-inhibitory factor produced by *E. histolytica* led to a swift increase in the intracellular concentration of adenosine 3':5' cyclic monophosphate (cAMP) and inhibits the respiratory burst in human macrophages.[81] This study suggests that like other leukotactic inhibitors, the monocyte locomotion-inhibitory factor produced by *E. histolytica* operates through modulations of intracellular cAMP. In addition, the parasite exerts suppression that appears to be a local event mediated by direct exposure of macrophages to *E. histolytica* directly or its by-products. For examples, the treatment of murine macrophages with amebic antigens *in vitro* reduces the production of molecules associated to the I region of the MHC induced by INF-γ.[82] This study also showed that *E. histolytica* subverted critical macrophage accessory function in part via prostaglandin E2 (PGE2) biosynthesis. Similarly, amebic liver abscess-derived macrophages produced low basal levels of TNF in response to stimulation with lipopolysaccharide (LPS), whereas peritoneal and spleen macrophages as well as Kupffer cells from infected animals did not release TNF constituently *in vitro*.[83]

d. Complement resistance

It has been shown that ameba develop complement resistance after repeated exposure to active human serum.[84] In this study, it also was shown that susceptibility to complement-dependent lysis was regained 6 weeks after serum treatments were terminated, suggesting that resistance to lysis was an acquired rather than a genetic property. It was also shown that both complement resistant and complement sensitive strains of *E. histolytica* could activate complement but only the latter could survive.[19] It has been suggested that complement resistance is necessary for *E. histolytica* tissue invasion. Braga et al. [21] showed that the Gal/lectin molecule had sequence homology and antigenic cross reactivity with CD59, a membrane inhibitor of C5b-9 in human erythrocytes, suggesting that the Gal/lectin also has complement-inhibitory functions. It seems that Gal/lectin bound C8 and C9, preventing formation of the membrane attack complex and subsequent cell lysis.[21] Recently, it has been shown that different strains of the mouse model of intestinal amebiasis and also genetically deficient mice for IL-12, IFN-gamma, or inducible NO synthase are resistance against intracecal inoculation of *E. histolytica*.[85] This study also demonstrated the important role of host immune responses to resistance to infection as well as the fact that depletion of $CD4^+$ cells significantly diminished both parasite burden and host's inflammatory responses.

REFERENCES

1. World Health Organization: Amebiasis. WHO Weekly Epidemiological Records. 1997. p. 97-100.
2. Lamont JT. Mucus: the front line of intestinal mucosal defense. Ann NY Acad Sci 1992;664:190–201. [PubMed: 1456650]

3. Perez-Vilar J, Hill RL. The structure and assembly of secreted mucins. J Biol Chem 1999;274(45): 31751–31754. [PubMed: 10542193]
4. Moncada DM, Kammanadiminti SJ, Chadee K. Mucin and Toll-like receptors in host defense against intestinal parasites. Trends Parasitol 2003;19(7):305–311. [PubMed: 12855381]
5. Forstner, JF.; Oliver, MG.; Sylvester, FA. Production, structure, and biologic relevance of gastrointestinal mucins. In: Blaser, MJ.; Smith, PD.; Ravdin, JI.; Greenberg, HB.; Guerrant, RL., editors. Infections of the gastrointestinal tract. New York: Raven Press; 1995. p. 71-88.
6. Deplancke B, Gaskins HR. Microbial modulation of innate defense: goblet cells and the intestinal mucus layer. Am. J. Clinical Nutrition 2001;73(6):1131S–1141S. [PubMed: 11393191]
7. Chadee K, Petri WA Jr, Innes DJ, et al. Rat and human colonic mucins bind to and inhibit adherence lectin of *Entamoeba histolytica*. J Clin Invest 1987;80(5):1245–1254. [PubMed: 2890655]
8. Chadee K, Meerovitch E. *Entamoeba histolytica*: early progressive pathology in the cecum of the gerbil (*Meriones unguiculatus*). Am J Trop Med Hyg 1985;34:283–291. [PubMed: 2858986]
9. Belley A, Keller K, Gottke M, et al. Intestinal mucins in colonization and host defense against pathogens. Am J Trop Med Hyg 1999;60:10–15. [PubMed: 10344672]
10. Ravdin JI. Immunobiology of human infection by *Entamoeba histolytica.* Pathol Immunopathol Res 1989;8(3–4):179–205. [PubMed: 2549533]
11. Belley A, Keller K, Grove J, et al. Interaction of LS174T human colon cancer cell mucins with *Entamoeba histolytica*: an in vitro model for colonic disease. Gastroenterology 1996;111(6):1484–1492. [PubMed: 8942726]
12. Stenson WF, Zhang Z, Riehl T, et al. Amebic infection in the human colon induces cyclooxygenase-2. Infect Immun 2001;69(5):3382–3388. [PubMed: 11292761]
13. Huldt G, Davies P, Allison AC, et al. Interactions between *Entamoeba histolytica* and complement. Nature 1979;277(5693):214–216. [PubMed: 233113]18
14. Meri S, Richaud G, Linder E. Complement activation by antigenic fractions of *Entamoeba histolytica*. Parasite Immunol 1985;7(2):153–164. [PubMed: 2860628]
15. Calderon J, Schreiber RD. Activation of the alternative and classical complement pathways by *Entamoeba histolytica*. Infect. Immun 1985;50:560–565. [PubMed: 2865212]
16. Munoz LE, Salazar OG. Complement activation in patients with amebic liver abscess. J Hepatol 1987;5(1):30–36. [PubMed: 2888804]
17. Reed SL, Keene WE, McKerrow JH, et al. Cleavage of C3 by a neutral cysteine proteinase of *Entamoeba histolytica*. J. Immunol 1989;143:189–195. [PubMed: 2543700]
18. Walderich B, Weber A, Knobloch J. Sensitivity of *Entamoeba histolytica* and *Entamoeba dispar* patient isolates to human complement. Parasite Immunol 1997;19(6):265–271. [PubMed: 9364556]
19. Reed SL, Curd JG, Gigli I, et al. Activation of complement by pathogenic and nonpathogenic *Entamoeba histolytica*. J Immunol 1986;136(6):2265–2270. [PubMed: 2869084]
20. Capin R, Capin NR, Carmona M, et al. Effect of complement depletion on the induction of amebic liver abscess in the hamster. Arch Invest Med (Mex) 1980;11(1 Suppl):173–180. [PubMed: 7469638]
21. Braga LL, Ninomiya H, McCoy JJ, et al. Inhibition of the complement membrane attack complex by the galactose-specific adhesion of *Entamoeba histolytica*. J Clin Invest 1992;90(3):1131–1137. [PubMed: 1381719]
22. Mann BJ. Structure and function of the *Entamoeba histolytica* Gal/GalNAc lectin. Int Rev Cytol 2002;216:59–80. [PubMed: 12049210]
23. Petri WA Jr, Chapman MD, Snodgrass T, et al. Subunit structure of the galactose and *N*-acetyl-*D*-galactosamine-inhibitable adherence lectin of *Entamoeba histolytica*. J Biol Chem 1989;264(5): 3007–3012. [PubMed: 2536731]
24. Cheng XJ, Hughes MA, Huston CD, et al. Intermediate subunit of the Gal/GalNAc lectin of *Entamoeba histolytica* is a member of a gene family containing multiple CXXC sequence motifs. Infect Immun 2001;69(9):5892–5898. [PubMed: 11500468]
25. Stanley SL Jr, Huizenga H, Li E. Isolation and partial characterization of a surface glycoconjugate of *Entamoeba histolytica*. Mol Biochem Parasitol 1992;50(1):127–138. [PubMed: 1542307]

26. Dodson JM, Lenkowski PW Jr, Eubanks AC, et al. Infection and immunity mediated by the carbohydrate recognition domain of the *Entamoeba histolytica* Gal/GalNAc lectin. J Infect Dis 1999;179(2):460–466. [PubMed: 9878032]
27. McCoy JJ, Weaver AM, Petri WA Jr. Use of monoclonal anti-light subunit antibodies to study the structure and function of the *Entamoeba histolytica* Gal/GalNAc adherence lectin. Glycoconj J 1994;11(5):432–436. [PubMed: 7696848]
28. Ravdin JI, John JE, Johnston LI, et al. Adherence of *Entamoeba histolytica* trophozoites to rat and human colonic mucosa. Infect Immun 1985;48(2):292–297. [PubMed: 2580787]
29. Bracha R, Mirelman D. Adherence and ingestion of *Escherichia coli* serotype 055 by trophozoites of *Entamoeba histolytica*. Infect Immun 1983;40(3):882–887. [PubMed: 6303959]
30. Petri WA, Haque R, Mann BJ. The bittersweet interface of parasite and host: lectin-carbohydrate interactions during human invasion by the parasite *Entamoeba histolytica*. Annu Rev Microbiol 2002;56:39–64. [PubMed: 12142490]
31. Petri WA Jr, Joyce MP, Broman J, et al. Recognition of the galactose-or *N*-acetylgalactosamine-binding lectin of *Entamoeba histolytica* by human immune sera. Infect Immun 1987;55(10):2327–2331. [PubMed: 2888730]
32. Saffer LD, Petri WA Jr. Role of the galactose lectin of *Entamoeba histolytica* in adherence-dependent killing of mammalian cells. Infect Immun 1991;59(12):4681–4683. [PubMed: 1937828]
33. Mann BJ, Lockhart LA. Molecular analysis of the Gal/GalNAc adhesin of *Entamoeba histolytica*. J Eukaryot Microbiol 1998;45(2):13S–16S. [PubMed: 9561778]
34. Tschopp J, Nabholz M. Perforin-mediated target cell lysis by cytolytic T lymphocytes. Annu Rev Immunol 1990;8:279–302. [PubMed: 2188665]
35. Lynch EC, Rosenberg IM, Gitler C. An ion-channel forming protein produced by *Entamoeba histolytica*. EMBO J 1982;1(7):801–804. [PubMed: 6329705]
36. Rosenberg I, Gitler C. Subcellular fractionation of amoebapore and plasma membrane components of *Entamoeba histolytica* using self-generating Percoll gradients. Mol Biochem Parasitol 1985;14(2):231–248. [PubMed: 2581133]
37. Leippe M, Tannich E, Nickel R, et al. Primary and secondary structure of the pore-forming peptide of pathogenic *Entamoeba histolytica*. EMBO J 1992;11(10):3501–3506. [PubMed: 1396552]
38. Leippe M, Andra J, Nickel R, et al. Amoebapores, a family of membranolytic peptides from cytoplasmic granules of *Entamoeba histolytica*: isolation, primary structure, and pore formation in bacterial cytoplasmic membranes. Mol Microbiol 1994;14(5):895–904. [PubMed: 7715451]
39. Leippe M. Amoebapores. Parasitol Today 1997;13(5):178–183. [PubMed: 15275088]
40. Leippe M, Andra J, Muller-Eberhard HJ. Cytolytic and antibacterial activity of synthetic peptides derived from amoebapore, the pore-forming peptide of *Entamoeba histolytica*. Proc Natl Acad Sci U S A 1994;91(7):2602–2606. [PubMed: 8146160]
41. Leippe M, Bahr E, Tannich E, et al. Comparison of pore-forming peptides from pathogenic and nonpathogenic *Entamoeba histolytica*. Mol Biochem Parasitol 1993;59(1):101–109. [PubMed: 8515772]
42. Leippe M, Sievertsen HJ, Tannich E, et al. Spontaneous release of cysteine proteinases but not of pore-forming peptides by viable *Entamoeba histolytica*. Parasitology 1995;111(Pt 5):569–574. [PubMed: 8559589]
43. Bracha R, Nuchamowitz Y, Mirelman D. Transcriptional silencing of an amoebapore gene in *Entamoeba histolytica*: molecular analysis and effect on pathogenicity. Eukaryot Cell 2003;(2):295–305. [PubMed: 12684379]
44. Bujanover S, Katz U, Bracha R, et al. virulence attenuated amoebapore-less mutant of *Entamoeba histolytica* and its interaction with host cells. Int J Parasitol 2003 Dec;33(14):1655–1663. [PubMed: 14636681]
45. Tannich E. *Entamoeba histolytica* and *E. dispar*: comparison of molecules considered important for host tissue destruction. Trans R Soc Trop Med Hyg 1998;92(6):593–596. [PubMed: 10326098]
46. Berti PJ, Storer AC. Alignment/phylogeny of the papain superfamily of cysteine proteases. J. Mol. Biol 1995;246:273–283. [PubMed: 7869379]
47. Sloane BF, Rozhin J, Hatfield JS, et al. Plasma membrane-associated cysteine proteinases in human and animal tumors. Exp. Cell Biol 1987;55:209–224. [PubMed: 3315769]

48. Aly AS, Matuschewski K. A malarial cysteine protease is necessary for *Plasmodium* sporozoite egress from oocysts. J Exp Med 2005;202(2):225–230. [PubMed: 16027235]
49. Scholze H, Schulte W. On the specificity of a cysteine proteinase from *Entamoeba histolytica*. Biomed Biochim Acta 1988;47(2):115–123. [PubMed: 2902852]
50. Luaces AL, Barrett AJ. Affinity purification and biochemical characterization of histolysin, the major cysteine proteinase of *Entamoeba histolytica*. Biochem J 1988;250(3):903–909. [PubMed: 2898937]
51. Bruchhaus I, Loftus BJ, Hall N, et al. The intestinal protozoan parasite *Entamoeba histolytica* contains 20 cysteine protease genes, of which only a small subset is expressed during in vitro cultivation. Eukaryot Cell 2003;2(3):501–509. [PubMed: 12796295]
52. Loftus B, Anderson I, Davies R, et al. The genome of the protist parasite *Entamoeba histolytica*. Nature 2005;433(7028):865–868. [PubMed: 15729342]
53. Keene WE, Hidalgo ME, Orozco E, et al. *Entamoeba histolytica*: correlation of the cytopathic effect of virulent trophozoites with secretion of a cysteine proteinase. Exp Parasitol 1990;71(2):199–206. [PubMed: 2373188]
54. Reed SL, Keene WE, McKerrow JH. Thiol proteinase expression and pathogenicity of *Entamoeba histolytica*. J Clin Microbiol 1989;27(12):2772–2777. [PubMed: 2556432]
55. Jacobs T, Bruchhaus I, Dandekar T, et al. Isolation and molecular characterization of a surface-bound proteinase of *Entamoeba histolytica*. Mol. Microbiol 1998;27:269–276. [PubMed: 9484883]
56. Bruchhaus I, Jacobs T, Leippe M, et al. *Entamoeba histolytica* and *Entamoeba dispar*: differences in numbers and expression of cysteine proteinase genes. Mol Microbiol 1996;22(2):255–263. [PubMed: 8930910]
57. Willhoeft U, Hamann L, Tannich E. A DNA sequence corresponding to the gene encoding cysteine proteinase 5 in *Entamoeba histolytica* is present and positionally conserved but highly degenerated in *Entamoeba dispar*. Infect. Immun 1999;67:5925–5929. [PubMed: 10531249]
58. Forney JR, Yang S, Healy MC. Protease activity associated with excystation of *Cryptosporidium parvum* oocysts. J Parasitology 1996;82(6):889–892.
59. Keene WE, Petitt MG, Allen S, et al. The major neutral proteinase of *Entamoeba histolytica*. J Exp Med 1986;163(3):536–549. [PubMed: 2869098]
60. Reed S, Bouvier J, Pollack AS, et al. Cloning of a virulence factor of *Entamoeba histolytica*: pathogenic strains possess a unique cysteine proteinase gene. J Clin Invest 1993;91(4):1532–1540. [PubMed: 8473498]
61. Li E, Yang WG, Zhang T, et al. Interaction of laminin with *Entamoeba histolytica* cysteine proteinases and its effect on amebic pathogenesis. Infect Immun 1995;63(10):4150–4153. [PubMed: 7558332]
62. Que X, Reed SL. Cysteine proteinases and the pathogenesis of amebiasis. Clin Microbiol Rev 2000;13 (2):196–206. [PubMed: 10755997]
63. Juliano RL, Haskill S. Signal transduction from the extracellular matrix. J Cell Biol 1993;120(3): 577–585. [PubMed: 8381117]
64. Meza I. Extracellular matrix-induced signaling in *Entamoeba histolytica*: its role in invasiveness. Parasitol Today 2000;16(1):23–28. [PubMed: 10637584]
65. Carbajal ME, Manning-Cela R, Pina A, et al. Fibronectin-induced intracellular calcium rise in *Entamoeba histolytica* trophozoites: effect on adhesion and the actin cytoskeleton. Exp Parasitol 1996;82(1):11–20. [PubMed: 8617326]
66. Santiago A, Carbajal ME, Benitez-King G, et al. *Entamoeba histolytica*: PKC transduction pathway activation in the trophozoite-fibronectin interaction. Exp Parasitol 1994;79(3):436–444. [PubMed: 7957762]
67. Munoz ML, Lamoyi E, Leon G, et al. Antigens in electron-dense granules from *Entamoeba histolytica* as possible markers for pathogenicity. J Clin Microbiol 1990;28(11):2418–2424. [PubMed: 2174899]
68. Perez E, Munoz ML, Ortega A. *Entamoeba histolytica*: involvement of pp125FAK in collagen-induced signal transduction. Exp Parasitol 1996;82(2):164–170. [PubMed: 8617343]
69. de Lourdes Munoz M, Das P, Tovar R. *Entamoeba histolytica* trophozoites activated by collagen type I and Ca^{2+} have a structured cytoskeleton during collagenase secretion. Cell Motil Cytoskeleton 2001;50(1):45–54. [PubMed: 11746671]

70. Debnath A, Akbar MA, Mazumder A, et al. *Entamoeba histolytica*: Characterization of human collagen type I and Ca^{2+} activated differentially expressed genes. Exp Parasitol 2005;110(3):214–219. [PubMed: 15955315]
71. Ghosh SK, Samuelson J. Involvement of p21racA, phosphoinositide 3-kinase, and vacuolar ATPase in phagocytosis of bacteria and erythrocytes by *Entamoeba histolytica*: suggestive evidence for coincidental evolution of amebic invasiveness. Infect Immun 1997;65(10):4243–4249. [PubMed: 9317033]
72. Aguilar-Rojas A, Almaraz-Barrera Mde J, Krzeminski M, et al. *Entamoeba histolytica*: inhibition of cellular functions by overexpression of EhGEF1, a novel Rho/Rac guaninenucleotide exchange factor. Exp Parasitol 2005;109(3):150–162. [PubMed: 15713446]
73. Lohia A, Samuelson J. Molecular cloning of a *rho* family gene of *Entamoeba histolytica*. Mol Biochem Parasitol 1993;58(1):177–180. [PubMed: 8459832]
74. Beck DL, Boettner DR, Dragulev B, et al. Identification and gene expression analysis of a large family of transmembrane kinases related to the Gal/GalNAc lectin in *Entamoeba histolytica*. Eukaryot Cell 2005;4(4):722–732. [PubMed: 15821132]
75. Kelsall BL, Ravdin JI. Degradation of human IgA by *Entamoeba histolytica*. J Infect Dis 1993;168 (5):1319–1322. [PubMed: 8228372]
76. Espinosa-Cantellano M, Martinez-Palomo A. *Entamoeba histolytica*: mechanism of surface receptor capping. Exp Parasitol 1994;79(3):424–435. [PubMed: 7957761]
77. Arhets P, Gounon P, Sansonetti P, et al. Myosin II is involved in capping and uroid formation in the human pathogen *Entamoeba histolytica*. Infect Immun 1995;63(11):4358–4367. [PubMed: 7591071]
78. Zambrano-Villa S, Rosales-Borjas D, Carrero JC, et al. How protozoan parasites evade the immune response. Trends Parasitol 2002;(6):272–278. [PubMed: 12036742]
79. Ortiz-Ortiz L, Zamacona G, Sepulveda B, et al. Cell-mediated immunity in patients with amebic abscess of the liver. Clin Immunol Immunopathol 1975;4(1):127–134. [PubMed: 165023]
80. Ghosh PK, Castellanos-Barba C, Ortiz-Ortiz L. Intestinal amebiasis: cyclic suppression of the immune response. Parasitol Res 1995;81(6):475–480. [PubMed: 7567905]
81. Rico G, Diaz-Guerra O, Kretschmer RR. Cyclic nucleotide changes induced in human leukocytes by a product of axenically grown *Entamoeba histolytica* that inhibits human monocyte locomotion. Parasitol Res 1995;81(2):158–162. [PubMed: 7731924]
82. Wang W, Chadee K. *Entamoeba histolytica* suppresses gamma interferon-induced macrophage class II major histocompatibility complex Ia molecule and I-A beta mRNA expression by a prostaglandin E2-dependent mechanism. Infect Immun 1995;63(3):1089–1094. [PubMed: 7868232]
83. Wang W, Keller K, Chadee K. Modulation of tumor necrosis factor production by macrophages in *Entamoeba histolytica* infection. Infect Immun 1992;60(8):3169–3174. [PubMed: 1639488]
84. Calderon J, Tovar R. Loss of susceptibility to complement lysis in *Entamoeba histolytica* HM1 by treatment with human serum. Immunology 1986;58(3):467–471. [PubMed: 2874111]
85. Houpt ER, Glembocki DJ, Obrig TG, et al. The mouse model of amebic colitis reveals mouse strain susceptibility to infection and exacerbation of disease by CD4+ T cells. J Immunol 2002 Oct 15;169 (8):4496–4503. [PubMed: 12370386]