Int. J. Biol. Chem. Sci. 1(2): 186-196, 2007

ISSN 1991-8631

International Journal of Biological and Chemical Sciences

Review

http://indexmedicus.afro.who.int/Journals/Indexj.htm

Polymorphism and virulence factors in the pathogenesis of amoebiasis

Wellington A. OYIBO [1*], Oladele T. OJUROMI [2], Oladipo OLADOSU [1], C. O. AGOMO [1,3], Adetayo F. FAGBENRO-BEYIOKU [1] and C. C. ANUNOBI [4]

[1] *Tropical Disease Research Laboratory, College of Medicine, University of Lagos, Idi-araba, Lagos, Nigeria*
[2] *Department of, Faculty of Science, Lagos State University, Ojo, Lagos , Nigeria.*
[3] *Department of Zoology Nigeria Institute of Medical Research, Yaba, Lagos, Nigeria.*
[4] *Department of Morbid Anatomy, College of Medicine, University of Lagos, Idiaraba, Lagos, Nigeria*
[*] *Corresponding author, E-mail: wellao@yahoo.com, Tel: 234-8035374004*

ABSTRACT

Amoebiasis is one of the world's most prevalent infectious diseases of developing world. *E. histolytica* and *E. dispar* are two morphologically identical but genetically distinct species. Infection with *E. histolytica* may be symptomatic and asymptomatic. *E. dispar* is non-pathogenic. Both innate and acquired immune responses limit amoebic infection while different strains of *E. histolytica* and its virulence have been described and virulence factors of *E. histolytica* such as cysteine proteinases, Gal/GalNAc-inhibitable lectin and ameobapore are known to be involved in *E.histolytica* pathogenesis. Proteolytic enzymes and cysteine proteases facilitate tissue invasion while Gal/GalNAc-inhibitable lectin aids adherence and amoebapores are involved in lysis of target cells. Three new strains of *E. histolytica* (Rahman, HK-9, and 200: NIH) have been described as well as the previously known strain (HM 1 IMSS). This review highlights the newly described strains and virulent factors involved in the pathogenesis of *E. histolytica.*

Keywords: Amoebiasis, *E histolytica, E. dispar*, Gal/GalNAc-inhibitable lectin, Cysteine proteinases, Ameobapore, Rahman, HK-9, and 200: NIH, HM 1 IMSS.

INTRODUCTION

Amoebiasis is an intestinal or extraintestinal infection with the protozoan parasite *Entamoeba histolytica.* More than 50 million people worldwide are infected, and up to 100,000 of these die every year (WHO, 1997). Amoebiasis is one of the world's most prevalent and fatal infectious diseases and a primary problem of developing world.

There are four species of the protozoan genus *Entamoeba* which are commonly found in the human gastrointestinal tract; these are: *E. dispar, E. coli, E. hartmanni* and *E. histolytica. E. histolytica* has been reclassified into two morphologically identical but genetically distinct species; *E. histolytica,* which is potentially invasive, and *E. dispar* which is non–pathogenic and non invasive.

Infection is normally initiated by ingestion of *E. histolytica* cysts .Immediately after ingestion, excystation occurs in the bowel lumen, where motile and potentially invasive trophozoites are formed. The trophozoites are capable of penetrating colonic mucosa and can sometimes form cyst in the colon. The cyst(s) (infective stage) are excreted in the stool into the environment to renew the life cycle.

Invention of molecular work such as amoebic antigen and DNA detection enzyme immunoassay (EIA) and polymerase chain reaction (PCR), have made the diagnosis of *E. histolytica* from *E. dispar* possible (Evangelopoulos et al., 2000; Tanyuksel and Petri, 2003).

Parasite invasion of the intestinal epithelium is characterized by extensive degradation of the extracellular matrix by amoebic secreted proteases, human cell cytolysis, and phagocytosis. Highly motile trophozoites gain access to the bloodstream in region of ulcer formation and eventually disseminate to other organs, causing amoebic liver abscesses (Ravdin, 1995).Virulence factors such as cysteine proteinases, galactose and N-acetyl galactosamine inhibitable surface lectin (Gal/Ga/Nac) and amoebapore are known to have contributed to the pathogenesis of *Entamoeba.* The objective of this review is to discuss the different virulence factors in *Entamoeba* and to examine the virulence and diversity in different strains.

SPECTRUM OF INFECTION WITH *E. histolytica* AND *E. dispar*

There is convincing evidence that shows that what is currently identified as *E. histolytica* actually comprises two morphologically identical species differing genetically and in their capacity to cause disease. The acceptance of *E. dispar* as a distinct but closely related protozoan species has had profound implication for epidemiology of amoebiasis, since most asymptomatic infections found world wide are considerably attributed to non-invasive amoeba.

Asymptomatic Colonization

In up to 90% of *E.histolytica* infections, the symptoms are absent or very mild (Gatti et al., 2002). The patients have normal rectosigmoidoscopic findings and without a history of blood in stool samples, cyst and trophozoite lacking ingested red blood cells may be visible on microscope (Garcia and Bruckner, 1997).

E. dispar infection do not show evidence of disease or a serum anti-amoebic antibody response, while symptomatic *E.histolytica* intestinal infection does show a systemic immune response (Gathiram and Jackson, 1987). Many questions on why majority of individuals infected with *E.histolytica* do not develop symptomatic disease (invasive amoebiasis) have been asked by a number of authors. Two hypotheses have been previously proposed (a) there are diversity strains of *E. histolytica* that differ in pathogenicity, and (b) there are underlying host factors that make an individual more susceptible to disease (Stanley, 2001).

Rafael and Adriana (2005) in their study concluded that the most relevant host factor is the innate immunity, which either controls the infection and avoids the invasion, leading to asymptomatic colonization, or is unable to recognize and eliminate the parasite, leading to invasive amoebiasis.

Symptomatic Infection

Depending on the affected organ, the clinical manifestation of amoebiasis is intestinal or extraintestinal. There are four clinical forms of invasive intestinal amoebiasis, all of which are generally acute: dysentery or bloody diarrhoea, fulminating colitis, amoebic appendicitis, and amoeboma of the colon. The lack of faecal leukocytes and presence of blood are the most common stool findings in acute stage. The development of fulminant colitis, amoeboma, cutaneous amoebiasis and rectovaginal fistulas can occur as complications of intestinal amoebiasis (Renmert and Ray, 2000).

The damage caused by *E. histolytica* is restricted in the majority of symptomatic cases to the intestinal mucosa, while in extraintestinal invasion there appears to be no means available for the host to limit the pathology induced by the parasite. The fact that dysentery is the most dominant symptom found, would suggest that the host has defense mechanisms that restrict the penetration of the parasite to the intestinal mucosa (Gilter and Mirelman, 1986). Liver abscess is the most common manifestation of extra-intestinal amoebiasis. Unusual sites or complications of extraintestinal amoebiasis include direct extension from the liver to the pleura and/or pericardium brain abscess and occasionally to the lung, brain, skin, and genitourinary amoebiasis (Mayhew et al., 2000).

POLYMORPHISM IN *E. histolytica*

The extent of genetic diversity among *E.histolytica* clinical isolates is still unclear. Some studies have analyzed a small number of highly repetitive and polymorphic genetic loci by various techniques, such as randomly amplified polymorphic DNA (RAPD), RNA arbitrarily primed PCR, and restriction fragment length polymorphism (RFLP) (Valle

et al., 2000; Ayeh-Kumi et al., 2001; Haghighi et al., 2003). Pathogenic and non-pathogenic strains differ in the ability to cause invasive disease according to several biochemical and molecular criteria. The marked differences in the levels of both lipophosphoglycan-like (LPG) and lipophosphopeptidoglycan (LPPG) molecules between pathogenic and non-pathogenic strains of *E. histolytica* suggest that genetic differences exist in those loci. Polymorphisms in the structure of LPG and LPPG may contribute to polymorphism in the pathogenicity of *E. histolytica* (Zaki and Clark, 2001).

Shah et al (2005) carried out a study in which four *E. histolytica* and two *E. dispar* laboratory strains were genotyped. Three strains of *E. histolytica* (Rahman, HK-9, and 200: NIH) were discovered apart from *E. histolytica* strain (HM 1 IMSS) which have been known. Two *E. dispar* strains (SAW 1734 and SAW 760) were also isolated. The report of their study showed that *E. histolytica* strain (Rahman) was less virulent as assessed by decreased monolayer destruction in animal models and cytotoxicity, and the *E. histolytica* strain was the only strain isolated from asymptomatic individual. Another study using Randomly amplified polymorphic DNA (RAPD) also identified *E. histolytica* strain (Rahman) as distinct from virulent *E. histolytica* strains (Valle et al., 2000). The clone that aligned closest with *E. histolytica* strain (HM-1: IMSS) was (HK-9), which upon original isolation was invasive, but it is now considered to possess attenuated virulence (Shah et al., 2005).

IMMUNE MECHANISM

Little is known about protective immunity to amoebiasis, but apparently both innate and acquired response limit amoebic infection. The importance of innate immunity in amoebiasis was supported by a model of amoebic liver abscess produced by intraperitonial inoculation of *E. histolytica* trophozoites in hamsters. In absence of T and B cells specific for *E. histolytica* antigens, hamsters can mount a protective response against *E. histolytica* (Shibayama et al., 2000). Amoebic infection was reported to be more common, severe and prolonged in children with serum anti-lectin IgG (Hague et al., 2001). Innate immunity was associated with the lack of serum anti-parasite IgG, however, the resistance to infection was seen despite the fact that children were infected with genetically diverse strains of *E. histolytica* (Hague et al., 2001). Children who lack serum anti-amoebic IgG probably have a more robust innate immune response that prevents parasite invasion into the intestinal epithelium, thereby preventing a systemic IgG anti-parasitic immune response (Duggal et al., 2004). Genetic differences in susceptibility to *E. histolytica* infection may manifest themselves in the ability of the innate immunity mechanisms to control this parasite (Duggal et al., 2004).

Inspite of being infected with genetically distinct strains of *E. histolytica*, possibly with different virulence, an individual can remain asymptomatic. Rafael and Adriana (2005) hypothesized that the immune mechanisms of such a resistant host recognize LPG and LPPG on different strains of *E. histolytica* and thus avoid tissue invasion, whereas those mechanisms in a susceptible host only recognize LPG and LPPG on *E. dispar* and non-pathogenic strains of *E. histolytica*.

Toll-like receptors (TLRs) have essential role in the innate recognition of pathogen-associated molecular patterns (PAMPs) and in triggering of innate and adaptive immunity. These receptors activate signal-transduction pathways, which induce the expression of immune-response genes that promote the inflammatory response as well as the recruitment and activation of macrophages, dendritic cells and antigen-specific lymphocytes (Underhill and Ozinsky, 2002; Akira, 2003). TLRs could initiate innate/inflammatory responses that either contribute to the invasion and liver damage or eliminate the parasite, mainly through the activation of macrophages and the production of macrophages and nitric oxide (Kammanadiminti et al., 2004). Cell-mediated responses have also been described in patients with amoebic liver abscess, characterized by lymphocyte proliferation and lymphokine secretion that is amoebicidal *in vitro* (Reed et al., 1995).

PATHOGENICITY AND VIRULENCE

Virulence of a given isolate of *Amoeba* depends on the intrinsic properties of

trophozoites, the growth condition, and the test system used to evaluate its pathogenicity *in vivo* or *in vitro* (Gilter and Mirelman, 1986) .Virulence has mainly been studied by growing pathogenic *E. histolytica* (zymodeme II) in Diamond's axenic medium (i.e. in the absence of bacteria or other living organisms) and testing for the Amoeba's capacity to induce abscess when injected directly into the liver of newborn or one to two-month-old hamsters (Gilter and Mirelman, 1986). Under these conditions the *Amoebae* are weakly to moderately virulent.

Numerous possible virulence factor of *E. histolytica* such as cysteine proteinase, Gal/Gal NAc–inhibitable lectin and amoebapore are known to be involved in *E. histolytica* virulence. *E. histolytica* contains proteolytic enzymes (collagenase and neutral proteases) and cysteine proteinases, which presumably facilitate its tissue invasion (Gillchrist and Petri, 1999). Trophozoites of *E. histolytica* adhere to the intestinal epithelium by interaction of the parasite Gal/Gal/NAc- inhibitable lectin with host–derived glycoproteins, which have high affinity ligands for amoebic lectin (Stanley, 2000).

E. histolytica is characterized by its extraordinary capacity to destroy human tissue leading to massive and sometimes lethal pathological alternations such as ulcerative colitis or abscesses of various organs, most commonly the liver (Ravdin, 1995). It has been shown that incubation of *E. histolytica* trophozoites with activated macrophages in ratio 1:500 (amoeba:target cells) would practically kill all macrophages within minutes (Tannich, 1998).

The surface properties of *E. histolytica* and *E. dispar* has also revealed that pathogenic action of the amoebas mainly depends on direct contact with host cells (McCoy et al., 1994) and thus their pathogenicity may be related to the composition and properties of the surface coat components. Several studies have shown that the cell surface structures of *E. dispar* and *E. histolytica* are different, as *E. histolytica* has a thicker and more uniform surface coat (Espinosa-Cantellano and Martinez-Palomo, 2000).

The surface coat contains the amoebic antigens recognized as foreign by both the innate and acquired immune system. The more-extensively studied molecules on the surface of the amoebae are the galactose and N-acetylgalactosamine-inhibitable lectin, the serine–rich surface protein, and the 29-KDa putative surface antigen (Mann, 2002).

GENERAL PROPERTIES OF MOLECULES INVOLVED IN VIRULENCE

Various mechanisms that are involved in tissue destruction and in identification of various virulence proteins have become possible to study axenically (Gilter and Mirelman, 1986). Moreover, biochemical, molecular and immunological methods have made it possible to characterize and differentiate these virulence proteins. These virulence proteins include (a) galactose inhibitable lectin (b) the pore-forming protein and the various proteinases.

The galactose and N-acetyl galactosamine inhibitable surface lectin (Gal/GalNAe) - for Attachment

Adhesion of the parasites occurs mainly through a surface Gal/GalNAc lectin that binds to exposed terminal Gal/GalNAc residues of target cell glycoprotein (Petri et al.,1989) Other molecules in adhesion include a 220-KDa lectin (Rosales-Eucina et al., 1987) a 112-KDa adhesin and a lipophosphoglycan (Arroyo and Orozo,1987).

The Gal/GalNAc adhesion is a multifunctional protein composed of heterodimer of heavy (170-KDa) and light (35/31-KDa) subunits (Petri et al., 1989). Evidence for participation of this molecule in adhesion event of the parasite have been demonstrated by reduced amoebic adherence to human erythrocytes, neutrophils, colonic mucins, and epithelia and to certain bacteria when the lectin is inhibited by galactose (Burchard and Bilke, 1992). Adhesion also participates in cytolytic processes since contact-dependent target cell lysis is reduced in the presence of galactose and a monoclonal antibody against the heavy subunit is capable of partially inhibiting cytolysis without blocking adherence (Saffer and Petri, 1991). Furthermore adhesin binds to purified C8 and C9 components of complement and blocks the assembly of the complement membrane attack complex on the amoebic plasma membrane,

this probably suggests a role in mediating amoebic resistance to complement lysis through components C5b through C9 (Braga et al., 1992).

Evidence suggested that even when the lectin is blocked with high concentration of galactose or GalNAc monomers, complete inhibition of *E.histolytica* adherence to target cells or colonic mucins was not observed. It has been suggested that the spacing of multiple GalNAc residues on the surface of target cells is important for optimal lectin binding (Adler et al., 1995) and in addition, molecules mentioned above could participate in adhesion event.

Haematophagous trophozoites are found only in the faeces of patients with invasive disease caused by *E. histolytica in vivo*, even though *in vitro* both *E. dispar* and non-pathogenic *E. coli* ingest erythrocytes (Ackers, 1996). The benefit which *E. histolytica* derived from ingestion of red cells could be source of iron, which is absolute requirement for the metabolic activities of *E. histolytica* and microaerophilic condition (Diamond et al., 1995).

The attachment of the amoebic trophozoite to target cells appears to be the first step in pathogenesis, be it erythrocytes or nucleated host erythrocyte, polymorph or lymphocyte. Attachment to the erythrocyte is followed by microphagocytosis (suction with cell deformation) if the cell is easily deformable (Lejeune and Gicquand, 1992).

Cysteine proteinases

Cysteine proteinases released by *E. histolytica* trophozoites play a key role in gut invasion and inflammation, and are the most abundant proteases in the parasite. A total of 20 cysteine proteinases have been isolated (Brachhaus et al., 2003). They are known to be active against different substrates and increased activity has been reported in clones of high virulence (Espinosa-Cantellano and Martinez Palomo, 2000). Cysteine proteinases are responsible for the detachment of tissue culture monolayers, the most widely used assay for amoebic toxins and other virulence factors. Cysteine proteinases also interfere with the function of the host immune system by cleaving complement component (C3) by a unique mechanism which enables *E. histolytica* to activate complement in the fluid phase (Reed and Gilgi, 1990). The proteinase also degrades immunoglobulin A (IgA) and anaphylaxtoxins C3a and C5a, which may explain the relative paucity of neutrophils noted in amoebic liver abscesses (Reed et al., 1995) Cysteine proteinases of *E. histolytica* play crucial roles in the interactions between parasite and host, including the acquisition of nutrients, facilitation of tissue invasion, and defense against immune attack. Therefore amoebic cysteine proteinases are important targets for novel chemotherapeutic strategies. Various inhibitors have been identified in a variety of parasitic protozoa, including *Leshmania, Trichomonas, Trypanosoma* and *Schistosoma* as reported by Que and Reed (2000).

Out of the extracellular matrix (ECM) components that *E.histolytica* encounter during colonic invasion laminin, collagen types I, III, IV and VI, and fibronectin (FN) are good targets for EhCP 1, EhCP 2, EhCP5 membrane-bound protease, and the neutral 56-KDa proteases. If *E. dispar* indeed lacks several of the potent *E. histolytica* cysteine proteases (EdCP1, EdCP5 and the neutral protease), it is possible that this difference could partially explain its non-invasive nature (Espinosa-Cantellano and Martinez-Palomo, 2000). Based on protein and RNA analyses, previous studies suggested that EhCP1, EhCP2, and EhCP5 are the most abundantly expressed cysteine proteases in culture of *E. histolytica* trophozoites, whereas EdCP3 was expressed most in *E. dispar*.

Though all cysteine proteinases are found within amoeba's granules, EhCp5 is exceptionally localized on the amoeba surface (Jacobs et al., 1998) .Some researchers have hypothesized that EhCP5 is an important factor for amoeba pathogenicity. This was based on assumption that EhCp5 is currently the only structurally characterized member of the amoebic cysteine proteinases family that is exclusively present in *E. histolytica* and it appears to be functionally unique (Tannich, 1998) though, more studies are still needed to prove or disprove this hypothesis.

A study showed that culture fibroblast monolayers are disrupted by purified *E. histolytica* cysteine proteinases and this could probably be due to their ability to degrade ECM components (Keene et al., 1990). Cysteine proteases are important factor of

various infectious agent and the main proteolytic enzymes in many protozoan parasites (Sajid and Mackerrow, 2002).

Amoebapores

Once *E. histolytica* establishes contact with mammalian cells *in vitro*, a rapid cytolytic event takes place that results in swelling, surface bleeding, and lysis of the inadvertent target cell, including lymphocyte polymorphonuclear leuckocytes, and macrophages, leaving the parasite unharmed. This initially suggested the participation of a channel–forming protein called the amoebapore, whose activity had been identified in *E. histolytica* lysates (Young et al., 1982). They are pore forming proteins secreted by *E. histolytica* and they develop in Amoeba initially as a cytoplasmic granule.

The amoebapore of *E. histolytica* is a channel-forming peptide of 77 amino acid residues, which has now been purified; the protein has been sequenced and the respective genes have been cloned (Leippe et al., 1992). Structural modeling suggests a compact tertiary of 4-amphopathic alpha-helics which are structures stabilized by three disulfide bonds. The molecule is able to bind to and insert into membranes, where the amoebapore monomers tend to oligomerise and form water-filled channels through which ions, water and other small molecules can pass and thus lysing the target cell (Leippe et al., 1994). Three isoforms, amoebapore A, B and C are known at a ratio 35:10:1, respectively with genes showing 35 to 75% deduced amino acid sequence identity and amoebapore C has been reported to be the most efficient in lysing erythrocytes (Leippe et al., 1994). Similar amoebapores have been identified in *E. dispar* but the specific activity is 60% lower than that of the one *E. histolytica (*Leippe et al., 1993). The activity of amoebapore is optimally expressed at acidic pH, which is consistent with observation that lysis of target cells by *E.histolytica* required a pH of 5.0 within amoebic vesicles (Leippe et al., 1992).

MODE OF *E. histolytica* INVASION

The local depletion of the intestinal mucus and disruption of the epithelial barrier is first indication of amoebic pathology and this is due to degradation of the ECM, which occurs in part as action of cysteine proteinases. Trophozoite finally attach to the colonic mucus and epithelial cells by a galactose–inhibitable lectin (Petri et al., 1989) . The invasion of luminal parasites to the liver are divided into adherence, target cell killing (cytotoxicity), dissolution of membrane, ingestion of cell fragment and erythrocytes (Phagocytosis) and establishment of foci of *infection* in the liver.

Adherence

One of the molecules which mediate attachment to enterocytes is galactose/N-acetylgalactosamine inhibitable lectin (GIL). Adherence of the parasite to the epithelial cell makes GIL a key virulence factor. In gerbil model, mucus depletion precedes epithelial erosion and heat stable secretagogue detected in axenic cultures of *E. histolytica* is known to be involved in the invasion process by facilitating adherence of trophozoites to the mucosa (Chadee and Mecrovitch, 1985).

In vitro adherence assays using carbohydrates inhibitors have proven the pivotal role of glycoprotein binding proteins (lectins) in amoebic adherence to target tissues (McCoy et al., 1993). Adherence of *E. histolytica* trophozoites to human erythrocytes and Chinese hamster ovary (CHO) cells is inhibited approximately 90% by millimole concentrations of N-acetyl-D-galactosamine (GalNAc) and D-galactose (Gal), but not other sugars (Ravdin and Guerrant, 1981). Most importantly, the Gal/GalNAc inhibitable lectin mediates adherence to human neutrophils, colonic mucins, and epithelial cells which are the *in vivo* targets of *E. histolytica*. Moreover virulence of different *E. histolytica* strains correlates with lectin mediated adherence (Ravdin and Guerrant, 1981).

The adherence properties of this protein are further shown by monoclonal antibodies directed against certain regions of the Gal/GalNAc lectin resulting in inhibition of amoebic adherence to Chinese hamster ovary cells (Ravdin and Guerrant, 1981), while monoclonal antibodies to other epitopes actually enhance ameobic adhesion to the same cells (Petri et al., 1990). All monoclonal antibodies to Gal/Gal/NAc lectin that affect adherence appear to bind to the cysteine–rich region of the heavy subunit (Petri et al., 1990).

Some molecules mediating adherence have been studied apart from galactose

inhibitable lectin. A 112 KDa adhesion, 220 KDa lectin and galactose inhibitable lectin have been found to mediate the binding of *E. histolytica* to red blood cells and erythrophagocytosis. However, binding of 220KDa can be inhibited by polymers of N-acetyl/glucosamine (Rosales-Eucina et al., 1987). It is important to note that not all avirulent *E. histolytica* mutants with a reduced ability to bind Chinese hamster ovary cells are deficient in adherence to red blood cells (Orozco et al., 1987).

Target cell killing

The ability of *E. histolytica* to destroy tissues is one of the most studied properties of the parasite and this gives it its name (Ackers, 1996). Killing of nucleated mammalian cells is the first step in this process, though; *E. dispar* largely lacks the ability to destroy cells (Burchard et al., 1992).

Although, cytotoxicity is contact dependent (Ackers, 1996), galactose inhibitable lectin (GIL) is involved in killing as well as adherence. The fact that contact has been established presumably causes the *Amoeba* to activate a specific killing mechanism. It has been suggested that target cells may be ordered to commit suicide. There is an indication for preliminary evidence that *E. histolytica* induces apoptosis (Ragland et al., 1994) and GIL is transferred to the target cells following adherence (Leroy et al., 1995).

Best study of the amoeba's killing mechanism is the family of pore forming peptides known collectively as amoebapores. They are channel-forming pepetides of 77 amino acid residue and their protein has been sequenced and respective genes cloned. Three isoforms, amoebapores A, B and C are present in the ratio 35:10:1 respectively (Leippe et al., 1992). Higher concentration particularly of the C isoforms is capable of killing tumor cell lines. It is reasonable to assume that the primary function of amoebaopore is to destroy phagocytosed bacteria, thus having a similar function to defensin found in mammalian phagocytes that kill bacteria and fungi within digestive vacuole (Ackers 1996; Tannich, 1998).

Proteolytic activity

E. histolytica has been considered to play a role in tissue invasion through enzymatic degradation of ECM components, basement membrane proteins and connective tissue (Ackers, 1996). Most of the reports from *in vitro* studies are mediated by collagenase and cysteine proteinases, and levels of expression have been correlated widely with virulence (Keene et al., 1990).

Levels of cysteine proteinase expression and secretion are widely regarded as key virulence determinants (Stanley et al., 1995). It has been hypothesized that over production of primarily intracellular proteins by *E. histolytica* could activate a secretory pathway, thereby adventitiously conferring on it the ability to degrade extracellular tissue components and become an invasive parasite (Tannich et al., 1991). Cysteine proteinases are responsible for the detachment of tissue culture monolayer, the most widely used assay for study of amoebic toxins and other virulence factors (Que and Reed, 2000). Cell lysis by *E. histolytica in vitro* is a more complex process, involving attachment via the GIL and lysis by the amoebapore (Petri et al., 1989).

Greater activity of amoebic cysteine proteinases against a variety of substrates has been reported in clones of high virulence *E. histolytica* (Navarro-Garcia et al., 1995). Of the ECM that *E. histolytica* encounters during colonic invasion, laminin, collagen type I and IV, and FN are good targets for EhCp5 membrane-bound protease as reviewed by Espinosa-Cantellano and Martinez-Palomo , 2000).

CONCLUSION

E. histolytica shows a wide spectrum of manifestation ranging from asymptomatic colonization to amoebic colitis and dysentery, and extraintestinal amoebiasis. All genetic evidences show unequivocally two different species: *E. histolytica* which is invasive and *E. dispar* which is not. One strain of *E. histolytica* (Rahman) out of four strains identified has been reported to be less virulent as assessed by its mode of invasion and disease causation.

The pathogenecity and virulence of *E. histolytica* is well documented from identification of different virulence proteins that are involved in invasion and destruction of the host tissues. Amoebic proteins potentially associated with virulence include

surface antigens Gal/GalNAc lectin which is responsible for adherence of the amoeba to enterocytes of the intestine and signaling cytolysis. Cysteine proteinases play a major role in aiding attachment by digesting ECM, while pore-forming peptides, termed amoebapores, are involved in target cell killing.

Essentially, the mode of *E. histolytica* invasion occurs by adherence of the parasites to the host cell, mediated by the GIL. Pore-forming peptides known as amoebapores are also involved in invasion by killing the host cell, though, the main causes of cell death are yet to be reported and proteolytic activity of cysteine proteinases shows that, the release of these molecules during amoebic invasion is a major virulence determinant in amoebiasis. In addition, interesting mechanisms of the parasite modulation of host immune response are being unraveled. The main targets of this modulation appear to be neutrophils and macrophages, which are recruited at the site of the lesion but are unable to abort infection. Finally, much work needs to be done especially in developing countries where the disease is endemic. Efforts are needed to be geared towards proper identification of pathogenic from non-pathogenic strains based on microscopy and serological techniques and understanding the complex pathways of the parasite and host immune response. More studies are needed to elucidate *E. histolytica* in the asymptomatic carrier group vis-à-vis polymorphism and the virulence factors.

REFERENCES

Ackers JP. 1996. The invasiveness of *Entamoeba histolytica* – a continuing enigma. *J. Clin. Pathol. Mol. Biol.*, **49**: 192-198.

Adler P, Wood SJ, Lee YCRT, Petri WA, Schnaar RI. 1995. High affinity binding of *Entamoeba histolytica* lectin to polyvalent N-acetygalactosamines. *J. Biol. Chem.*, **270**: 5164-5171.

Akira S. 2003. Mammalian toll like receptors. *Curr. Opin. Immunol.*, **15**: 5-11.

Arroyo R,Orozco E. 1987. Localization and identification of an *Entamoeba histolytica* adhesion. *Mol. Biochem. Parasitol.*, **23**: 151-158.

Ayeh-Kumi PE, Ali IM, Lockhart A, Gillchrist CA, Petri WAJr, Haque R. 2001. *Entamoeba histolytica* diversity of clinical isolate from Bangladesh as demonstrated by polymorphisms in the serine-rich gene. *Exp. Parasitol.*, **99**: 80-88

Brachhaus I, Loftus BJ, Hall N, Tannich E. 2003. The intestinal protozoan parasite *Entamoeba histolytica* contain 20 cysteine protease genes of which only a small subset is expressed during *invitro* cultivation. *Eukaryotic Cell.*, 501-509.

Braga L, Ninomiya H, McCoy JJ, Eacker S, Wiedmer T, Pham C, Wood S, Sims PJ Petri WAJr. 1992. Inhibition of complement membrane attack complex by the galactose specific adhesin of *Entamoeba histolytica. J. Clin. Investig.*, **90**: 1131-1137.

Burchard GD, Bilke R. 1992. Adherence of pathogenic and non-pathogenic *Entamoeba histolytica* strains to neutrophils. *Parasitol. Res.*, **78**: 146-153.

Burchard GD, Prange G, Mirelman D. 1992. Interaction of various *Entamoeba histolytica* strains with human intestinal cell lines. *Arch. Med. Res.*, **23**: 193-195.

Chadee K, Mecrovitch E. 1985. *Entamoeba histolytica* early progressive pathology in the caecum of gerbil (*Meriones unguiclatus*). *Am. J. Trop. Med. Hyg.*, **34**: 283-291.

Diamond LS, Clark CG, Cunnick CC. 1995. YI-S, a casein-free medium for axenic cultivation of *Entamoeba histolytica*, related *Entamoeba, Giardia intestinalis and Trichomonas vaginalis. J. Euka. Microbiol.*, **42**: 277-278.

Duggal P, Haque R, Roy S, Mondal D, Sack RB, Farr BM, Beauty TA, Petri WA Jr. 2004. Influence of human leukocyte antigen class II alleles on susceptibility to *Entamoeba histolytica* infection in Bangladeshi. *J. Infect. Dis.*, **189**: 520-526.

Espinosa–Cantellano M, Martinez–Palomo A. 2000. Pathogenesis of intestinal amoebiasis from molecules to disease. *Clin. Microbiol. Rev.*, **13**: 318-331.

Evangelopoulos A, Spanakos G, Patsoula E, Vakatis N, Legakis N. 2000. A nested multiple, PCR assay for the simultaneous detection and differentiation of *Entamoeba histolytica* and *E.dispar* in

faeces. *Ann. Trop. Med. Parasitol.*, **94**: 233-240.

Garcia IS, Bruckner DA. 1997. *Diagnostic Medical Parasitology* (3rd edn). ASM Press: Washington D. C.

Gathiram V, Jackson TF. 1987. A longitudinal study of asymptomatic carriers of pathogenic zymodemes of *Entamoeba histolytica S. Afri. Med. J.*, **72**: 669-672.

Gatti S, Swierczynski O, Robinson F, Anseni M, Corrales J, Moreira J, Montalvo G, Bruno A, Mascrati R, Binson Z, Scaglia M. 2002. Amoebic infections due to the *Entamoeba histolytica-E.dispar* complex: a study of the incidence in a remote rural area of Ecuador. *Am. J. Trop. Med. Hyg.*, **67**: 123-127.

Gillchrist CA, Petri WAJr. 1999. Virulence factors of *Entamoeba histolytica. Curr. Opin. Micro.*, **2**: 433-437.

Gilter C, Mirelman D. 1986. Factors contributing to the pathogenic behaviour of *Entamoeba histolytica. Ann. Rev. Microbiol.*, **40**: 237-261.

Haghighi A, Kobayashi S, Takeuchi T, Thammapalerd N, Nozaki T. 2003. Geographic diversity among genotypes of *Entamoeba histolytica* field isolates. *J. Clin. Micro.*, **41**: 3748-3756.

Hague R, Ali M, Sack RB, Farr BM, Ramakrishnan G, Petri WAJr. 2001. Amoebiasis and mucosal IgA antibody against the *Entamoeba histolytica* adherence lectin in Bangladeshi children. *J. Infect. Dis.*, **183**: 1787-1793.

Jacobs T, Bruchhaus I, Dandekar T, Tannich E, Leippe M. 1998. Isolation and molecular characterization of a surface-bound proteinases of *Entamoeba histolytica. Mol. Microbiol.*, **27**: 269-276.

Kammanadiminti SJ, Mann BJ, Dutil L, Chadee K. 2004. Regulation of toll like receptor-2 expression by the Gal-lectin of *Entamoeba histolytica. FASEB. J.*, **18**: 155-157.

Keene WE, Hidalgo ME, Orozco E, Mckerrow JH. 1990. *Entamoeba histolytica*; correlation of the cythopathic effect of virulent trophozoites with secretion of a cysteine proteinases. *Exp. Parasitol.*, **71**: 199-206.

Leippe MJ, Andra,J, Nickei R, Tannich E, Muller-Eberhard HJ. 1994. Amoebapore, a family of membranolytic from cytoplasmic granules of *Entamoeba histolytica* isolation, primary and pore formation in bacterial cytoplasmic membrane *Mol. Microbiol.*, **14**: 895-904.

Leippe M, Bahr E, Tannich E, Horstmann RD. 1993. Comparison of pore forming peptides from pathogenic and non-pathogenic *Entamoeba histolytica. Mol. Biochem. Parasitol.*, **59**: 101-110.

Leippe M, Tannich E, Nickel R, VanderGrot G, Pattus F, Horstmann RD, Muller-Eberhard HJ. 1992. Primary and secondary structure of the pore-forming peptide of pathogenic *Entamoeba histolytica .EMBO. J.*, **11**: 3501-3506.

Lejeune A, Gicquaud C. 1992. Target cell deformability determines the type of phagocytic mechanism used by *Entamoeba histolytica*-like, Laredo strain. *Biol. Cell.*, **74**: 211-216.

Leroy A, Debruyne G, Mareel M, Nokkaew C, Bailey G, Nells H. 1995. Contact–dependent transfer of the galactose-specific lectin of *Entamoeba histolytica* to the lateral surface of enterocytes in culture. *Infect. Immun.*, **63**: 4253-4260.

Mann BJ. 2002. Structure and function of the *Entamoeba histolytica* Gal/GalNAc lectin. *Int. Rev. Cytol.*, **216**: 59-80.

Mayhew KM, Dundoo M, Dune FE, Dwinell BG, Stephen JK. 2000. Inguinal lymphadenitis caused by *Entamoeba histolytica*: case report and literature review. *Mayo. Clin. Proc.*, **75**: 513-516.

McCoy JJ, Mann BJ, Petri WA Jr. 1994. Adherence and cytotoxicity of *Entamoeba histolytica* or how lectins let parasites stick around. *Infect. Immun.*, **62**: 3045-3050.

McCoy JJ, Mann BJ, Vedvick TS, Oak Y, Hemark DB, Petri WA. 1993. Structural analysis of the light subunit of the *Entamoeba histolytica* galactose-specific adherence lectins. *J. Biol. Chem.*, **268**: 24223-24231.

Navarro-Garcia F, Charevez-Duenas L, Tsutsumi V, Posadas del Rio F, Lopez Revilla. 1995. *Entamoeba histolytica*; increase of enterotoxicity and of 53-and 75 KDa Cysteine proteinases in a clone of higher virulence. *Exp. Parasitol.*, **80**: 361-372.

Orozco E, Rodriquez MA, Murphy GF, Salata RA, Petri WAJr, Smith RD. 1987.

Entamoeba histolytica cytopathogenicity and lectin activity of avirulent mutants. *Exp. Parasitol.*, **63**: 157-165.

Petri WA, Chapman MD, Snalgras T, Mann BJ, Broman J, Ravdin JI. 1989. Subunit structure of the galactose and N-acetyl-D-galactosamine-inhibitable adherence lectin of *Entamoeba histolytica*. *J. Biol. Chem.*, **264**: 3007-3012.

Petri WA, Snodgrass TL, Jackson TFHG, Osathiran V, Simjee AE, Chadee K, Chapman MD. 1990. Monoclonal antibodies directed against the galactose-binding lectin of *Entamoeba histolytica* enhance adherence *J. Immunol.*, **144**: 4803-4809.

Que. XC, Reed SL. 2000. Cysteine proteinases and the pathogenesis of amoebiasis. *Clin. Microbiol. Rev.*, **13**: 196-206.

Rafael C, Adriana JL. 2005. The pathogenicity of *Entamoeba histolytica* is related to the capacity of evading innate immunity. *Parasitol.*bbbbb *Immunology.*, **27**: 1-8.

Ragland BD, Ashley LS, Vanx DL, Petri WA Jr. 1994. *Entamoeba histolytica*; target cells killed by trophozoites undergo DNA fragmentation which is not blocked by Bcl-2 *Exp. Parasitol.*, **79**: 460-467.

Ravdin JI. 1995. Amebiasis. *Clin. Infect. Dis.*, 1453-1466.

Ravdin JI, Guerrant RL. 1981. Role of adherence in cytopathic mechanism of *Entamoeba histolytica.* Study with mammalian tissue culture cells and human erythrocytes. *J. Clin. Investig.*, **68**: 1305-1313.

Reed SL, Ember JA, Herdman DS, Di-Seipio RG, Hugli TE, Gilgi I. 1995. The extracellular neutral cysteine proteinase of *Entamoeba histolytic*a degrades anaphylatoxins C3a and C5a. *J. Immunol.*, **55**: 266-274.

Reed SL, Gilgi I. 1990. Lysis of complement sensitive *Entamoeba histolytica* by activated terminal complement. Initiation of complement activation by an extracellular neutral cysteine proteinase. *J. Clin. Investig.*, **86**: 1815-1822.

Renmert W, Ray C. 2000. Fulminant amoebic colitis in a ten day old infant. *Pediatr. Infect. Dis. J.*, **19**: 1111-1112.

Rosales –Eucina JL, Meza T, Lopez De Leon A. 1987. Isolation of 220 KDa protein with lectin properties from a virulent strain of *Entamoeba histolytica. J. Infect. Dis.*, **156**: 790-797.

Saffer LD, Petri WAJr. 1991. Role of Galactose lectin of *Entamoeba histolytica* in adherence-dependent killing of mammalian cells. *Infect. Immun.*, **59**: 4681-4683.

Sajid M, Mackerrow JH. 2002. Cysteine proteinases of parasitic organisms. *Mol. Biochem. Parasitol.*, **120**: 1-21.

Shah PH, Macfarlane RC, Bhattacharya D, Matese JC, Demeter J, Stroup SE, Singh U. 2005. Comparative genomic hybridizations of *Entamoeba* strains reveal unique genetic fingerprints that correlate with virulence. *Eukaryotic. Cell.*, **4**(3): 504-515.

Shibayama M, Campos-Rodriguez R, Ramirez C, Pacheco Yepez, Tsutsum V. 2000. Studies on the natural immunity in harmsters using the intraperitonial model of amebic liver abscess. *Arch. Med. Res.*, **31**: 578-588.

Stanley SLJr. 2000. Prevention and potential of new interventions. In *Amoebiasis,* Ravdin JI (ed). Imperial College Press: London, United Kingdom; 137-162.

Stanley SL, Zhang T, Rubin D, Li E. 1995. Role of *Entamoeba histolytica* cysteine proteinase in amoebic liver abscess formation in severe combined Immunodeficient mice *Infect. Immun.*, **63**: 1587-1590.

Stanley SLJr. 2001. Protective immunity to amoebiasis: new insights and new challenges *J. Infect. Dis.*, **184**: 504-506.

Tannich E. 1998. *Entamoeba histolytica* and *E. dispar* comparison of molecules considered important for host tissue destruction. *Trans. Roy. Soc. Trop. Med. Hyg.*, **92**: 593-596.

Tannich E, Scholze A, Nickel R, Horstmann RD. 1991. Homologous cysteine proteinases of pathogenic and non-pathogenic *Entaemoba histolytica*: differences in structure and expression. *J. Biol. Chem.*, **266**: 4798-4803.

Tanyuksel M, Petri WA Jr. 2003. Laboratory diagnosis of amoebiasis. *Clin. Microbiol. Rev.*, **16**(4): 713-729.

Underhill R, Ozinsky A. 2002. A toll like receptors: key mediators of microbe detection. *Curr. Opin. Immunol.*, **14**: 103-110.

Valle P R, Souza MB, Pires EM, Silva EF, Gomes MA. 2000. Arbitrarily primed PCR fingerprinting of RNA and DNA in *Entamoeba histolytica. Rev. Inst. Med. Trop.* Sao Paulo., **42**: 249-253.

WHO (World Health Organization). 1997. Amoebiasis *WKLY. Epidemic. Rec.*, **72**: 97-100.

Young JDE, Young MT, Lu L P, Unkeless J C, Cohn ZA. 1982. Characterization of a membrane pore-forming protein from *Entamoeba histolytica. J. Exp. Med.*, **156**: 1677-1690.

Zaki M, Clark CG. 2001. Isolation and characterization of polymorphic DNA from *Entamoeba histolytica. J. Clin.. Microbiol.*, **39**: 897-905.