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# HOST TISSUE DESTRUCTION BY *ENTAMOEBA HISTOLYTICA:* MOLECULES MEDIATING ADHESION, CYTOLYSIS, AND PROTEOLYSIS

ROLF D. HORSTMANN; MATTHIAS LEIPPE & EGBERT TANNICH

Bernhard Nocht Institute for Tropical Medicine, Bernhard Nocht St 74, 2000 Hamburg 36, FR Germany

*Entamoeba histolytica, the protozoan parasite causing human amoebiasis, has recently been found to comprise two genetically distinct forms, potentially pathogenic and constitutively nonpathogenic ones. Host tissue destruction by pathogenic forms is believed to result from cell functions mediaed by a lectin-type adherence receptor, a pore-forming peptide involved in host cell lysis, and abundant expression of cysteine proteinase(s). Isolation and molecular cloning of these amoeba products have provided the tools for structural analyses and manipulations of cell functions including comparisons between pathogenic and nonpathogenic forms.*

Key words: taxonomy – parasite pathogenicity – amoebiasis

*Entamoeba histolytica* is the causative agent of human amoebiasis. The amoebae are protozoa, eucaryotic unicellular organisms. The reproductive forms are called trophozoites, which inhabit the cavity of the lower intestine. They multiply and give rise to the transmissible forms of cysts, which are excreted with the faeces and eventually are ingested by a new host. Under conditions which are completely unknown the amoebic trophozoites become aggressive, invade the host tissues and cause ulcerative colitis or extra-intestinal abscesses. The disease states result worldwide in approximately 50,000 fatalities annually (Walsh, 1986).

At present, research in amoebiasis concentrates on two subjects (1) the dual manifestation of the infection as harmless colonization of the intestinal cavity or pathogenic tissue invasion and (2) the molecular analysis of functions of *E. histolytica* that are considered essential for pathogenicity.

(1) The dual behaviour is still a matter of discussion (Mirelman, 1987; Sargeaunt, 1987). Recent genetic analyses, however, together with epidemiological observations strongly suggest that nonpathogenic forms do exist which are not capable of becoming invasive and causing disease. Initial evidence for genetic differences between pathogenic and nonpathogenic forms came from DNA probes derived from repetitive genes, which were used for the differentiation of pathogenic and nonpathogenic "stages" (Garfinkel et al., 1989). At the same time, several proteins of the amoebae were characterized in our laboratory by sequencing the corresponding genes. In all of them we found significant differences in the DNA sequences between pathogenic isolates on one hand and nonpathogenic ones on the other (Tannich et al., 1989; Tannich et al., 1991a, c). Within each of the two groups of amoebae, however, the sequences appeared highly conserved (Tannich et al., 1989; Tannich & Burchard, 1991). These observations indicated that pathogenic forms differ genetically from nonpathogenic ones and suggested that they represent two different species. Very recently, *E. histolytica* was studied with regard to the genomic sequences for ribosomal RNA, which are established markers of phylogenetic relationships. Following these analyses, pathogenic and nonpathogenic *E. histolytica* are two different species, which have developed separately for 50-100 million years (Clark & Diamond, 1990).

The apparent existence of two separate species poses a variety of questions concerning the biology of the parasite. It must be emphasized that pathogenic forms, too, undergo a nonpathogenic stage, i.e. a stage of commensal parasitism in the intestinal cavity with cyst formation. This stage must be considered the reservoir of the pathogen because pathogenic behaviour itself seems to be a dead end street in the development of the parasite since tissue forms are considered not to be able to transform into cysts. This is most evident in cases of amoebic abscesses when the amoebae

loose connection to the intestinal cavity. If indeed man is the only relevant host, the separation of *E. histolytica* into two species would substantiatlly reduce the prevalence of the pathogen and the size of its reproductive population and reservoir.

(2) The second subject of current research is the analysis of functions of the amoebae that are involved in pathogenic tissue invasion. Pathogenicity is currently viewed as a result mainly of (a) adherence of the amoebae to host cells, (b) killing of host cells, and (c) proteolysis of the host's extracellular matrix. Above all it must be emphasized that in none of these functions qualitative differences have been observed between pathogenic and nonpathogenic forms. Thus, the key to pathogenicity might not have become evident yet.

(a) Adherence of *E. histolytica* to colonic mucins and to host cells is considered essential for the colonization of the large intestine and for pathogenic tissue invasion, respectively (Ravdin, 1989). Both forms of adherence appear to be primarily mediated by a surface lectin of the amoebae which is inhibitable by galactose and N-acetyl-galactosamine (Chadee et al., 1987; Ravdin et al., 1985). Its carbohydrate binding specificity was reported to be similar to that of the lectin of the coral tree *Erythrina cristagalli* in that terminal N-acetyllactosamine units provide the major binding determinants (Lit et al., 1988). The structure, function, and antigenicity of the lectin have been studied in considerable detail (for review see Ravdin, 1989). The lectin is a membrane-associated glycoprotein with a molecular mass of 170 kDa (Petri et al., 1987). It is disulfide-linked to one or more units of a 35 kDa Mr protein which was reported to function as a fibronectin receptor (Petri et al., 1989; Petri, 1990). Five functionally relevant epitopes of the lectin have been defined by studying the binding of monoclonal antibodies and their influence on adherence: antibody binding to two epitopes decreased adherence to mucins and to target cells, binding to another epitope inhibited adherence to target cells but not to mucins, and binding to two further epitopes enhanced adherence to both mucins and target cells (Petri et al., 1990). Similar effects were observed when antisera of patients with invasive amoebiasis were studied. The antisera either reduced or enhanced the adherence reactions (Petri et al., 1990). Interestingly, vaccination studies with gerbils using as an antigen the isolated lectin also revealed two contrary groups of responders; some animals were protected whereas the others showed an increase in morbidity (Petri & Ravdin, 1991). It was concluded that selected epitopes of the lectin molecule may be candidates for a vaccine against invasive amoebiasis.

Recently, the lectin was cloned and its primary structure was deduced from the cDNA sequence (Tannich et al., 1991b; Mann et al., 1991). Southern blot analyses suggested that multiple genes may encode for the lectin or closely related proteins in pathogenic trophozoites. The cDNA deduced amino acid sequence revealed an N-terminal signal peptide and a mature protein of 1270 amino acids corresponding to a molecular mass of 143 kDa, which comprises a short C-terminal cytoplasmic domain with potential phosphorylation sites, a transmembrane region, and a large extracellular portion with nine potential asparagine-linked glycosylation sites. The extracellular portion may be separated into a cysteine-poor domain and a cysteine-rich domain, the latter of which shows in part a repetitive structure with sequence homology to wheat germ agglutinin and to pDd63, a developmentally expressed protein of *Dictyostelium discoideum*. However, these structural similarities neither allowed the allocation of the carbohydrate binding site nor did they result in reasonable assumptions as to the tertiary structure of the molecule.

(b) The powerful activity of *E. histolytica* to kill host cells has been attributed to a pore-forming molecule termed amoebapore (Lynch et al., 1982; Young et al., 1982). Recently an amoebic polypeptide with pore-forming activity has been purified and studied in detail (Leippe et al., 1991; Leippe et al., manuscript submitted). Activity was optimally expressed at low pH, and the polypeptide preferentially inserted into negatively charged membranes. Structural analyses indicated that the pore-forming molecule has a molecular mass of 8 kDa and that its N-terminal amino acid sequence may form an amphiphilic alpha helix with similarity to melittin, the membrane damaging peptide of bee venom. The overall primary structure of the amoebic polypeptide, in particular the number and positions of cysteine residues, showed some degree of similarity to saposins and surfactant-associated protein B, mammalian polypeptides of similar size which interact with phospholipids and proteins.

(c) Proteolytic enzymes are believed to be part of the armament that enables *E. histolytica* to invade host tissues. Proteases may damage both the cells and the extracellular matrix of the host (Munoz et al., 1982; Lushbaugh et al., 1985; Keene et al., 1986; Luaces & Barrett, 1988). A variety of proteinases have been attributed to *E. histolytica,* several of which have been characterized as cysteine proteinases (Lushbaugh et al., 1985; Keene et al., 1986; Luaces & Barrett, 1988; Scholze & Schulte, 1988). The enzymes were found to abolish adhesion of tissue culture cells and to degrade matrix proteins such as collagens, laminin, and fibronectin. Higher activities of the enzymes were measured in pathogenic compared to nonpathogenic *E. histolytica* (Reed et al., 1989; Keene et al., 1990). These findings correspond well to the invasive properties of pathogenic amoebae and suggest an important role for the enzymes in pathogenicity. Three laboratories reported on the purification of a cysteine proteinase from *E. histolytica,* the source was in each case amoebae of the pathogenic isolate HM-1:IMSS. The purified enzymes resembled each other in substrate specificities and pH optima. Their molecular weights were estimated to be 56 kDa, 27 kDa, and 26 kDa, respectively. The N-terminal amino acid sequences were determined for the 26 kDa and the 27 kDa enzymes; they showed a high degree of similarity, i.e. 12 out of 15 residues were identical.

Using an antiserum raised against the 27 kDa enzyme (Schulte & Scholze, 1989), we isolated a cDNA clone representing a cysteine proteinase of pathogenic forms (Tannich et al., 1991c). The clone was used to identify the homologous clone in a cDNA library from nonpathogenic forms. cDNA sequence analysis and comparison of the predicted amino acid sequences revealed an evolutionary divergence of 16% (Tannich et al., 1991c). The differences in primary structure were of interest with regard to possible functional differences between the two enzymes. The relationship between the structure and the function of cysteine proteinases has been studied in great detail with the plant enzyme papain (Lowe, 1976), which corresponds to the amoebic enzymes in 34% of the residues. X-ray crystallography revealed that papain consists of two domains separated by a deep cleft. A number of residues facing the cleft were identified as being essential for the proteolytic function. All of them were found to be conserved in the enzymes from pathogenic and nonpathogenic *E. histolytica.* Moreover, alignments of the sequences with the one of papain presented in tertiary structure indicated that all amino acids forming and neighbouring the putative catalytic site are conserved (Tannich et al., 1991c). This finding suggested that the structural divergence of the homologous enzymes from pathogenic and nonpathogenic *E. histolytica* did not result in functional differences.

In contrast, the quantitative difference in the expression of these two genes was striking. Northern blot analysis indicated that the expression of the respective mRNA was consistently 10 to 100-fold higher in pathogenic than in nonpathogenic isolates. This estimate is in good agreement with functional studies (Reed et al., 1989). In the light of these findings, recent data on the expression and sorting of the yeast proteinase A are of interest (Rothman et al., 1986). This enzyme is transported into vacuoles. When it was expressed in excessive amounts, however, it was secreted into the medium suggesting that the intracellular sorting system had been saturated. It may be hypothesized that, assuming that similar sorting mechanisms exist in *E. histolytica,* overexpression of the cysteine proteinase in pathogenic forms causes an overflow of the enzyme from vacuolar/lysosomal to secretory pathways. Thus, a quantitative difference in expression might result in a profound alteration of cellular properties, in this case the *de novo* secretion of a proteolytic enzyme. One might speculate that such an event would elicit pathogenicity of *E. histolytica.*

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