HOST TISSUE DESTRUCTION BY \textit{ENTAMOEBA HISTOLYTICA}: MOLECULES MEDIATING ADHESION, CYTOLYSIS, AND PROTEOLYSIS

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\textit{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 mediated 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

\textit{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 \textit{E. histolytica} that are considered essential for pathogenicity.

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, \textit{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 \textit{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 sep-
aration of *E. histolytica* into two species would
substantially reduce the prevalence of the
pathogen and the size of its reproductive popu-
lation and reservoir.

(2) The second subject of current research
is the analysis of functions of the amoebae
that are involved in pathogenic tissue inva-
sion. 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 patho-
genicity might not have become evident yet.

(a) Adherence of *E. histolytica* to colonic
mucins and to host cells is considered essen-
tial for the colonization of the large intestine
and for pathogenic tissue invasion, respectively
(Ravdin, 1989). Both forms of adherence ap-
ppear 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 carbohy-
drate 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 bind-
ing determinants (Lit et al., 1988). The struc-
ture, 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 mo-
lecular 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 func-
tion 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 anti-
sera either reduced or enhanced the adherence
reactions (Petri et al., 1990). Interestingly, vac-
cination studies with gerbils using as an anti-
gen the isolated lectin also revealed two con-
trary groups of responders; some animals were
protected whereas the others showed an in-
crease in morbidity (Petri & Ravdin, 1991). It
was concluded that selected epitopes of the
lectin molecule may be candidates for a vac-
cine 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 tropho-
zoites. The cDNA deduced amino acid se-
quence 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 cytoplas-
mic domain with potential phosphorylation
sites, a transmembrane region, and a large
extracellular portion with nine potential aspar-
agine-linked glycosylation sites. The extracel-
lular portion may be separated into a cysteine-
poor domain and a cysteine-rich domain,
the latter of which shows in part a repetitive struc-
ture 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 bind-
ing site nor did they result in reasonable as-
sumptions 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 activ-
ity 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-form-
ing molecule has a molecular mass of 8 kDa
and that its N-terminal amino acid sequence
may form an amphiphilic alpha helix with simi-
larity to melittin, the membrane damaging
peptide of bee venom. The overall primary
structure of the amoebic polypeptide, in par-
ticular 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 protease 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 protease in pathogenic forms causes an overflow of the enzyme from vacuolar/lyososomal 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*.

REFERENCES


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