Galactose/N-acetylgalactosamine lectin: the coordinator of host cell killing

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Entamoeba histolytica is an enteric parasite that can kill host cells via a contact-dependent mechanism. This killing involves the amoebic surface protein referred to as the Gal/GalNAc lectin. The Gal/GalNAc lectin binds galactose and N-acetylgalactosamine allowing the adherence of amoebas to host cells. Involvement of the lectin in the pathogenesis of *E. histolytica* infection will be reviewed in this paper. The lectin has been shown to have very specific and substantial effects on adherence, cytotoxicity, and encystation. There is also possible involvement of the lectin in phagocytosis and caspase activation in host cells.

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1. Introduction

Entamoeba histolytica is the causative enteric parasite of amebiasis. Each year it is believed to be responsible for 50 million cases of colitis and/or liver abscess and 100,000 deaths worldwide (Petri and Singh 1999). *E. histolytica* has only been found in human hosts, and the infection is attributable to contamination of food or drinking water. This disease is endemic to poor areas in developing nations (Haque *et al* 2001).

The life cycle of *E. histolytica* is simple (figure 1). The protozoan can be either a cyst or a trophozoite. The cyst is a metabolically reduced tetra-nucleated cell. This cyst is resistant to desiccation as well as other environmental stresses. The cyst form of this parasite is spread through a typical fecal-oral route. Once a cyst enters a new host, a transition to the trophozoite form of the organism begins. This occurs by division of the nuclei, followed by division of the cytoplasm, thus yielding 8 trophozoites from one cyst. Colonization of the intestine then ensues, by adherence to the epithelium of the host.

Many individuals exposed to *E. histolytica* do not present with invasive disease. In fact, it is estimated that only 10% of those infected ever develop invasive amebiasis (Haque *et al* 2001). Host factors have a role in susceptibility to amebiasis. Some of these factors may include the bacterial flora of the gut (Mirelman 1987), mucin polymorphisms present in the host (Vinall *et al* 1998), and the specific immune response elicited.

Several amoebic proteins have been identified as virulence determining factors in *E. histolytica* including amoebapore, cysteine proteases and the galactose/N-acetylgalactosamine (Gal/GalNAc) lectin. Amoebapore has been shown to form pores in the membranes of host cells at μM concentrations (Leippe *et al* 1991, 1995). Cysteine proteases have previously been implicated in the digestion of the extra-cellular matrix (DeMeester *et al* 1990). The goal of this review is to summarize the role of the Gal/GalNAc lectin in amebiasis.

2. *Entamoeba histolytica* adhesion to host cells

E. histolytica kills host cells via a contact-dependent mechanism (Ravdin *et al* 1980). Therefore, adherence of the amoeba to the host cell is a pivotal step in the

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Abbreviations used: CRD, carbohydrate recognition domain; Gal/GalNAc, galactose/N-acetylgalactosamine.

progression of disease. *E. histolytica* adhere to most cells, including human erythrocytes and colonic epithelial (Guerrant *et al* 1981). Adherence can be effectively blocked by the addition of Gal or GalNAc to media (Chadee *et al* 1987). This indicates that the ligands on the host cells are galactose terminal, N- or O-linked glycoconjugates. CHO cell glycosylation mutants lacking N- and O-linked Gal/GalNAc residues are resistant to both adherence and cytolysis (Li *et al* 1988, 1989). The only *E. histolytica* molecules discovered that bind these residues are the Gal/GalNAc heavy and intermediate subunits of the lectin.

3. Lectin structure

The discovery that *E. histolytica* adherence could be inhibited by the presence of galactose has led to affinity purification of the Gal/GalNAc lectin (Petri *et al* 1987, 1989). It is comprised of a 260 kDa heterodimer comprised of a heavy subunit and light subunit that non-covalently associate with an intermediate subunit (figure 2). This lectin binds to both host galactose and N-acetyl-D-galactosamine. The Gal/GalNAc lectin has a high cysteine content (Tannich *et al* 1991; Mann *et al* 1991), which gives it resistance to proteases. It has been shown that antibodies to the heavy subunit can block adherence, whereas light subunit antibodies do not (McCoy *et al* 1994). There is also evidence that the heavy subunit is predominantly responsible for binding of galactose and N-acetyl-D-galactosamine (Adler *et al* 1995).

The heavy subunit (hgl) is the most conserved of the subunits. It is coded for by a single orf, hgl. There are 5 unlinked genes in the hgl family (Ramakrishnan *et al* 1996), but these are between 85 and 95% identical. The predicted size of hgl (by amino acid sequence) is 143 kDa, although the observed mass of the protein is 170 kDa, reflecting carbohydrate modifications which make up 6% of the protein. Sequence analysis shows that it contains a 15 amino acid signal peptide, a 41 amino acid cytoplasmic domain, and a 26 amino acid transmembrane domain. The remainder of the protein, approximately 1209 amino acids, is extracellular.

Within the extracellular portion of hgl is the carbohydrate recognition domain (CRD). Although this is widely accepted, there is controversy over exactly which amino acids account for this activity. The region between amino acids 895 and 998 has putatively been designated the CRD (Dodson *et al* 1999) based on its ability to bind galactose. This region is cysteine rich and is recognized by adherence inhibiting monoclonal antibodies (Mann *et al* 1993). Using peptide fragments, Kain and his colleagues have mapped two separate sequences responsible for the binding to CHO cells: residues 356–480 and 900–1143 (Pillai *et al* 1999). This suggests that there may be more than one CRD or that folding of the lectin is not accounted for in these models.

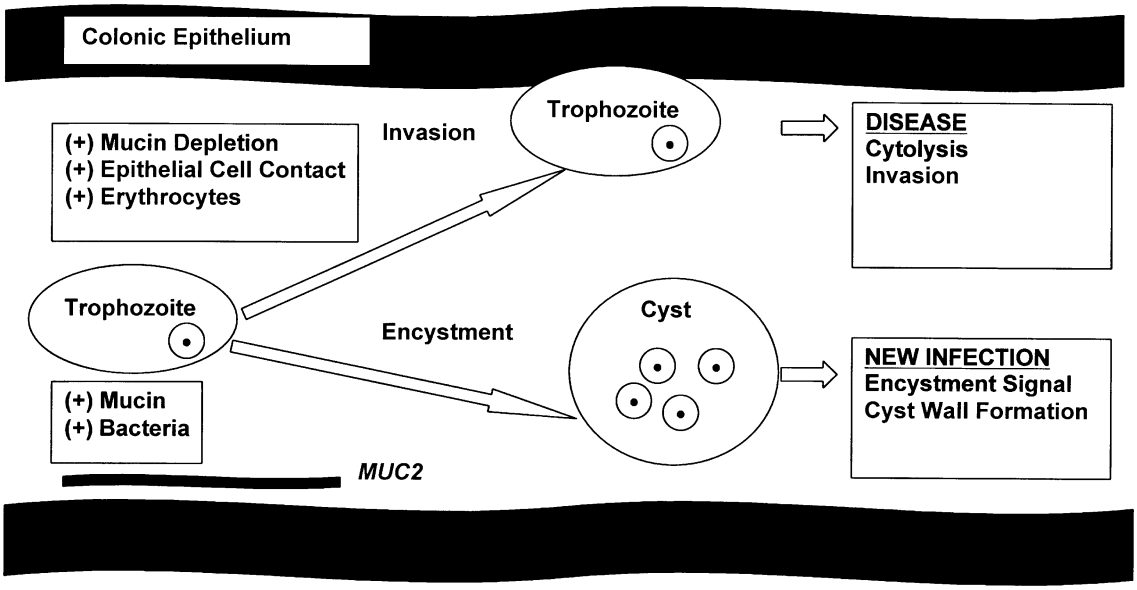

Figure 1. The life cycle of *E. histolytica* is carried out by its two forms encystation and invasive trophozoites. Local environmental cues signal via the Gal/GalNAc lectin and trigger encystment or invasion. Encystation may require bacterial flora and mucin binding. This form allows the cyst to leave the host and spread the infection to new individuals. The trophozoites cause tissue damage by use of the lectin to adhere and kill host cells. This figure is based on material accepted for publication in the *Annual Review of Microbiology*, volume 56, 2002 by Annual Reviews, www.AnnualReviews.org. Used with permission.

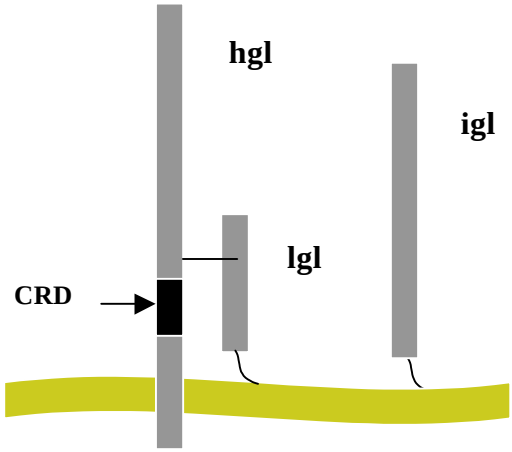

Figure 2. Gal/GalNAc adherence lectin of *E. histolytica*. The Gal/GalNAc lectin IS present of the surface amoeba and is comprised of three parts: htl, lgl, and igl. Hgl and lgl are covalently linked and non-covalently associate with igl. Within hgl is the carbohydrate recognition domain (CRD). This region is believed to be responsible for the binding of hgl to host cells. Figure from Petri *et al* 2002 *Annu. Rev. Microbiol.* **56** 39–64, printed with permission.

The light subunit gene (lgl) is more variable than the heavy subunit. At minimum, six different genes encode for light subunits with altered glycosylation patterns (McCoy *et al* 1993b). Proteins of 31 and 35 kDa represent the dominant isoforms (McCoy *et al* 1993a). Isoforms of lgl are very similar, showing 79–85% amino acid identity. Distinctions exist in the post-translational processing of these proteins. The 35 kDa subunit is highly glycosylated, but has no GPI anchor. However, the 31 kDa subunit is the converse. Each subunit can bind to the hgl subunit to form the heterodimer. Typically, the light subunit contains a 13 amino acid signal sequence and a 7 amino acid GPI anchor addition motif (Ramakrishnan *et al* 2000). The use of a GPI anchor and lack of a CRD clearly distinguish lgl from hgl (McCoy *et al* 1994).

A relationship between isoforms of lgl and virulence is evident. In the Rahman strain there is a reduced virulence as well as a reduction in the expression of the 35 kDa light subunit. However, this phenotype could not be overcome by over-expression of lgl in this strain. This was illustrated again by both incubation of *E. histolytica* with *Escherichia coli* serotype 055 (Padilla-Vaca *et al* 1999) and antisense inhibition of lgl (Ankri *et al* 1999). Interestingly, antibodies that bind the light subunit do not block adherence, but a depletion of the lgl gene has been shown to reduce cytotoxicity. This decrease in cytotoxicity may be due to a reduction in the amount of heterodimeric lectin that is formed. Hence, lgl still has an undefined role in the virulence of *E. histolytica*.

Finally, an intermediate subunit (igl) of 150 kDa mass non-covalently associates with the lectin heterodimer (Cheng *et al* 2001). Two copies of igl are present in *E. histolytica* which share an 84% identity (Cheng *et al* 1998). Antibodies to the intermediate subunit have been shown to block adherence of *E. histolytica* to host cells. An analysis of the igl gene shows that although it lacks a CRD, it contains CXXC protein–protein interaction domains (Cheng *et al* 2001).

4. Lectin function in host cell death

As mentioned earlier, *E. histolytica* kill host cells in a contact-dependent manner (Ravdin *et al* 1980). This is a calcium-dependent event (Ravdin *et al* 1982, 1985) that leads to the appearance of apoptotic features in the host cell. *In vitro* experiments have shown that purified lectin alone can cause a calcium flux in host cells, but will not recapitulate the apoptotic death. This indicates that there must be more requirements for killing than lectin alone. Research into lectin involvement in signalling has identified *b*2 integrin motifs in the cytoplasmic tail of hgl (Vines *et al* 1998), but little is known of any role of lectin in signal transduction. There are also many calcium independent factors working in cytolisis. The discovery of amoebapore (Berninghausen and Leippe 1997) and the activity of the amoeba cytoskeleton cannot be ignored (Arhets *et al* 1998).

The apoptotic phenotype has been a very intriguing element of cell death. Dead host cells are clearly TUNEL positive, and display both cytoplasmic blebbing and chromosome condensation. However, this cell death could not be countered by overexpression of bcl-2 in cultured cells (Ragland *et al* 1994). Host cell apoptosis is not Fas-dependent (Seydel and Stanley 1998), and does not occur following the addition of amoeba lysate. However, amoebic liver abscess is blocked by addition of Z-VAD-FMK, a pan caspase inhibitor (Yan and Stanley 2001). Recently, caspase 3 activation was found in host cells as a result of amoeba directed apoptotic death. Inhibition of caspase 3 consequently offered protection to host cells from this killing (Huston *et al* 2000). Surprisingly, caspase 8 and caspase 9 were not required for killing by the amoeba. This may be a novel entry into the caspase cascade.

Transfer of the lectin from amoeba to host epithelial cells has been noted. The lectin seems very efficiently transferred to these cells (within 5 min of co-incubation), even prior to cell death (Leroy *et al* 1995). This may present another separate killing technique in the *E. histolytica* repetoire that is specific to epithelial cells.

5. Role of lectin in encystation

Due to the inability to induce encystation *in vitro*, little is known about encystation of *E. histolytica*. Recently, studies in *Entamoeba invadens* have begun to give an idea of how this process occurs (Cho and Eichinger 1998). In *E. invadens*, encystation begins by nutrient deprivation. Multicellular aggregates form by means of binding to mucins (Coppi and Eichinger 1999) or other galactose terminal glycoproteins (Eichenger 2001b). This process can be blocked by the addition of free galactose and gives strong evidence of the importance of the Gal/GalNAc lectin (Eichenger 2001a).

6. Conclusions

Although the Gal/GalNAc lectin is not the only known virulence factor in *E. histolytica*, it has been the most dynamic factor identified. With roles in nearly every process of infection, it is a natural target for research and therapy.

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