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Use of monoclonal anti-light subunit antibodies to study the structure and function of the *Entamoeba histolytica* Gal/GalNAc adherence lectin

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Adherence of *Entamoeba histolytica* trophozoites to host cells is mediated by a galactose (Gal) and *N*-acetylgalactosamine (GalNAc)-specific surface lectin. The lectin is a heterodimeric protein composed of heavy (170 kDa) and light (35–31 kDa) subunits linked by disulfide bonds. Polyclonal and monoclonal antibodies (mAb) raised against a light subunit-glutathione-*S*-transferase fusion protein were used to probe its structure and function. Four light subunit-specific mAb were produced which recognized distinct epitopes on five different light subunit isoforms. Immunoblots with these mAb demonstrated co-migration of light and heavy subunits when nonreduced trophozoite proteins were analysed by SDS-PAGE, indicating that the subunits do not exist free of the heterodimer in significant quantities. While anti-heavy subunit antibodies had previously been shown to alter adherence, anti-light subunit antibodies did not, suggesting that the heavy subunit contains the carbohydrate recognition domain.

Keywords: lectin; *Entamoeba histolytica*; *Entamoeba dispar*; adherence; galactose; amebiasis.

Introduction

Glycoconjugate-lectin interactions play an important role in the pathogenesis of amebiasis. Amebiasis is a common worldwide parasitic infection that results annually in 40 to 50 million cases of amebic colitis and liver abscess. Trophozoites adhere to human colonic mucins and colonic epithelial cells to initiate colonization and invasion of humans. Adherence *in vitro* is mediated by a surface lectin that binds to terminal galactose (Gal) and *N*-acetylgalactosamine (GalNAc) [1, 2]. *Entamoeba histolytica* kills human macrophages, monocytes, neutrophils and T lymphocytes in a contact-dependent process that also requires the activity of this lectin. Mere apposition of amebic and target cell plasma membranes, as can be achieved by centrifuging target cells and amebae together into a pellet, will not lead to cytolysis if the amebic lectin is inhibited with Gal or GalNAc, indicating that the lectin either signals the initiation of cytolysis or directly participates in the cytolytic event.

Evasion of serum lysis also involves the Gal/GalNAc lectin: evasion of the host complement system is critical for survival of the extracellular trophozoite. *Entamoeba histolytica* trophozoites are resistant to the complement C5b-9 complexes which form the membrane attack complex. Monoclonal antibodies directed against amino acids 895–1082 of the cysteine-rich domain (epitopes 6 and 7) of the lectin heavy subunit greatly increase the sensitivity of *E. histolytica* to lysis by human sera and by purified human C5b-9. The lectin binds to purified human C8 and C9, and the binding is inhibited by anti-lectin mAb which block serum resistance. The purified lectin confers C5b-9 resistance when reconstituted into C5b-9 sensitive amebae, a direct demonstration of its C5b-9 inhibitory activity [3, 4].

The amebic Gal/GalNAc lectin is a 260 kDa heterodimeric glycoprotein consisting of heavy (170 kDa) and light (35 and 31 kDa) subunits linked by disulfide bonds [5]. The 170 kDa subunit is encoded by a gene family of which three members (89–95% identical) from *E. histolytica* strain HM1:IMSS have been sequenced [6]. The 170 kDa subunit sequences contain a carboxy-terminal putative cytoplasmic and transmembrane domain followed by an extensive extracellular cysteine-rich domain. This cysteine-rich domain is recognized by adherence-inhibitory anti-lectin monoclonal antibodies (mAb) [4].

The light subunit has been resolved into 35 and 31 kDa isoforms by SDS-PAGE. The 35 and 31 kDa isoforms have

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