Carbohydrate Research, 213 (1991) 331–338
Elsevier Science Publishers B.V., Amsterdam

Note

The D-galactose-inhibitable lectin of *Entamoeba histolytica**

Barbara J. Mann[†,‡], David Mirelman[**], and William A. Petri, Jr.[†]
[†]*Departments of Medicine, Microbiology, and the Cancer Center, University of Virginia, Charlottesville, Virginia 22908 (U.S.A.) and* [**]*Department of Biophysics and Unit for Molecular Biology of Parasitic Diseases, Weizmann Institute, Rehovot (Israel)*

(Received January 17th, 1990; accepted for publication, February 6th, 1990)

Adherence to host tissue and cell surfaces is an essential aspect of the pathogenesis of many viral, bacterial, and parasitic infections. A growing number of lectins have been identified in pathogenic organisms that appear to play key roles in the adherence process. The interaction of influenza virus with target cells is one of the best characterized examples of lectin–carbohydrate interactions. The hemagglutinin (HA) on the viral surface reacts with sialic acid-containing carbohydrates on the surface of target cells[1–3]. A large body of evidence has accumulated which demonstrates that the HA is the viral component to which protective antibody is directed. Removal of sialyl groups from the surface of target cells eliminates binding, preventing subsequent fusion of the viral and cellular membranes and infection of the cell. Another example of a lectin that shows a strong correlation with pathogenicity is the P adhesin that is associated with fimbriae in uropathogenic strains of *Escherichia coli*. The P adhesin binds to α-D-galactopyranosyl-(1→4)-D-galactose (Gal→Gal) groups[4]. One hundred percent of the strains isolated from pyelonephritis patients had Gal→Gal binding activity[5]. Adherence by uropathogenic *E. coli via* the P adhesin appears to be a requirement for successful colonization of the urogenital tract, and experiments with animal models indicated that the Gal→Gal P adhesin is probably essential for the production of pyelonephritis in women[6]. A third system where one or several lectin(s) play a critical role in adherence is the enteric parasite *Entamoeba histolytica*. Amebic adherence is likely to be multifactorial since several surface proteins have been identified that appear to mediate adherence to target cells. Two of these proteins have been identified as lectins. This review will discuss the adherence proteins of *E. histolytica* and focus on the D-galactose-inhibitable lectin.

Entamoeba histolytica. — *E. histolytica* infects approximately 10% of the world's population and is the third leading cause of death due to parasitic disease[7]. Invasive amebiasis results in amebic colitis which can be complicated by intestinal perforation, peritonitis, or liver abscess[8,9]. Identification and characterization of amebic proteins

* Dedicated to Professors Toshiaki Osawa and Nathan Sharon.
‡ To whom correspondence should be addressed.

0008-6215/91/$03.50

essential for pathogenesis may prove central to the control of the disease *via* the development of a vaccine.

The life cycle of *E. histolytica* consists of an invasive trophozoite and an infective cyst[10]. Infection occurs when cysts are ingested *via* fecally contaminated material. Excystation occurs in the large bowel where cysts divide to form trophozoites. The trophozoites colonize the large bowel where encystation or tissue invasion occurs. Only the trophozoite has the potential to invade the host; cysts have never been found in tissue, but are excreted to start a new round of infection.

Adherence of the trophozoite to the colonic mucosa of the large bowel is an important step in pathogenesis *in vivo*. Colonic biopsies of patients with amebiasis have amebas in the lumen and adherent to the mucosal layer of the colon[11,12]. Trophozoites have been found associated with focal ulcerations of the colonic epithelium[13,14]. By use of organ culture systems, trophozoites were found to adhere *in vitro* to the mucosa prior to penetration[15,16]. *E. histolytica* has a cytopathic effect on cells both *in vivo*, as evidenced by the amorphous eosinophillic debris surrounding trophozoites[11,14], and *in vitro*[17]. The *in vitro* cytopathic effect could be separated into steps of adherence, adherence-dependent cell lysis, and phagocytosis[18,19]. A number of methods including cinemicroscopy[17], kinetic analysis of amebas killing in a pellet system[17], and studies of adherence in high-molecular-weight Dextran[20] have demonstrated a necessity for cell–cell contact prior to amebic lysis of the target cell *in vitro*.

Adherence molecules of E. histolytica. — The study of amebic adherence to target cells has evolved into a complex process involving a number of different molecules. Four surface proteins that may play a role in adherence of amebic trophozoites to target cells have been identified and characterized in *E. histolytica* (Table I). Recently, Rodriguez *et al.*[21] have identified with anti-amebic antisera potentially four additional amebic proteins of 24, 50, 70, and 90 kDa molecular masses, associated with red blood cells after interaction with amebas. The relationship and interaction of these adherence molecules has not yet been examined. Possible associations with virulence and specific target cells also need to be further investigated.

The four better-characterized adherence molecules appeared to be distinct molecules located on the surface of ameba. A 220-kDa amebic glycoprotein that binds to

TABLE I

Adherence proteins of *E. histolytica*

Adherence protein	*Ligand*	*Target cells*	*Ref.*
Gal–GalNAc lectin (260-kDa)	Gal–GalNAc	Human colonic mucins, CHO cells	27
220-kDa Protein	Chitotriose, hyaluronic acid	Erythrocytes, MDCK cells	22
112-kDa Protein	Unknown	Erythrocytes	24
Fibronectin receptor (37-kDa)	Fibronectin	Extracellular matrix	25

Madin–Darby canine kidney cell (MDCK) monolayers and agglutinates erythrocytes[22] has been purified. Hemagglutination mediated by the 220-kDa protein was inhibited by chitotriose and hyaluronic acid. Antibodies specific for the 220-kDa protein inhibited adherence of ameba to erythrocytes and MDCK cells up to 50% (ref. 23). A 112-kDa protein has been identified by two monoclonal antibodies that react strongly with strain HM1:IMSS but weakly with adherence-deficient mutants of *E. histolytica*[24]. Monoclonal antibodies specific for the 112-kDa protein partially blocked adherence and ingestion of red blood cells, and inhibited cytopathic effects on cell culture monolayers. Adherence of ameba to red blood cells mediated by the 112-kDa protein appeared to be temperature dependent. Increased amounts of 112-kDa protein were found on the surface of erythrocytes incubated with amebas at 37°, as compared to erythrocytes incubated at 0° or room temperature[21]. A 37-kDa fibronectin receptor has also been identified[25]. The fibronectin receptor may play a role in the attachment to the extracellular matrix and tissue invasion. The D-galactose and *N*-acetyl-D-galactosamine-inhibitable surface lectin has a native molecular weight[26] of 260 000. Antibodies specific for the 260-kDa lectin block amebic adherence to Chinese hamster ovary cells (CHO)[27] and human colonic mucins[28]. The role of the Gal–GalNAc lectin in binding of amebas to colonic mucin is especially interesting, as this is the most relevant target for amebic adhesion.

D-Galactose-inhibitable lectin. — A D-galactose–*N* acetyl-D-galactosamine (Gal–GalNAc)-inhibitable amebic adhesin was first described by Ravdin and Guerrant[20] in adherence of amebic strain HM1:IMSS trophozoites to trypsinized CHO cells (Fig. 1). Pretreatment of amebas with GalNAc or Gal (55mM) resulted in the complete inhibition of adherence to CHO cells. Other carbohydrates, such as D-GlcNAc, neuraminic acid, maltose, dextrose, chitotriose, methyl mannoside, D-xylose, and D-mannose, had no effect[20]. The glycoproteins containing nonreducing D-galactosyl groups, such as asialofetuin (9 terminal galactosyl groups) and asialoorosomucoid (12–16 terminal galactosyl groups), were 1000-fold more potent than GalNAc in inhibiting amebic adherence to CHO cells[27]. The adhesin was demonstrated to be present on the amebas rather than on the CHO cells, because only pretreatment of the amebas with GalNAc or asialofetuin resulted in inhibition of adherence. Pretreatment of CHO cells had no effect on adherence[20,29]. The Gal–GalNAc amebic adhesin also mediated adherence to baby hamster kidney cell monolayers[30], human erythrocytes[20], human neutrophils[29], certain bacteria[30,31], human colonic mucosa[29], and rat and human colonic mucin glycoproteins[28]. Cytolysis is also contact dependent. If adherence is blocked with Gal–GalNAc, amebic cytolysis of CHO cells, human neutrophils, macrophages, and lymphocytes is also blocked[20,29,32,33].

The carbohydrate receptors for the Gal–GalNAc lectin of CHO cells have been investigated by use of CHO cell mutant strains with altered glycosylation. Li *et al.*[34] found that CHO cells deficient in *N*-linked glycans containing *N*-acetyllactosamine had only 12% of the level of adherence observed for wild-type CHO cells. Ravdin *et al.*[35] found amebic adherence was almost undetectable with CHO cells deficient in both *N*-linked and *O*-linked glycans containing Gal and GalNAc units, suggesting that both

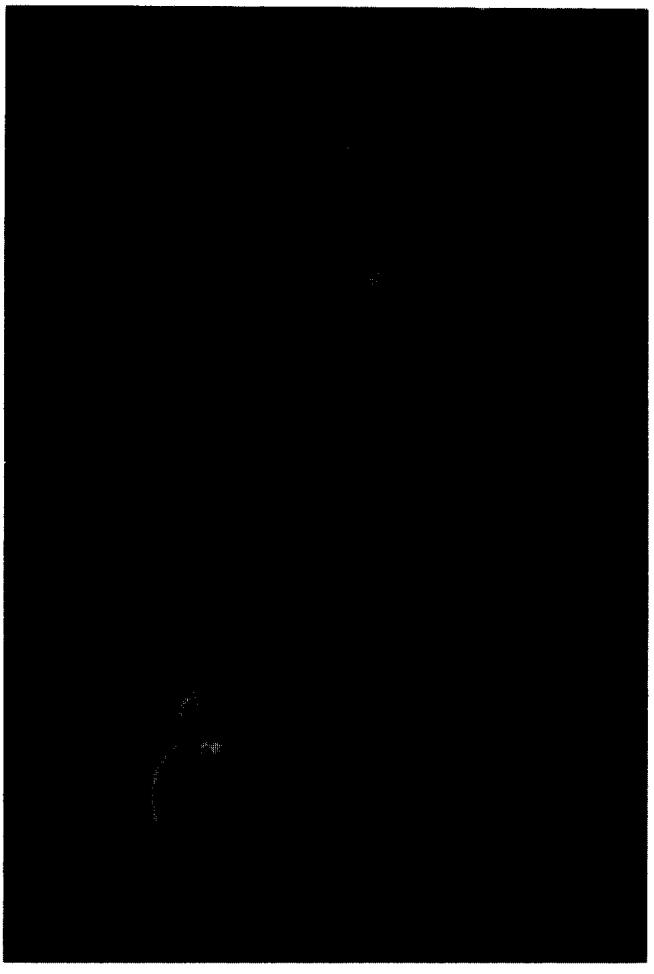

Fig. 1. Adherence of CHO cells to *E. histolytica* trophozoites. Amebic trophozoites were mixed with CHO cells at a ratio of 1:20 and centrifuged off. After incubation on ice, the pellet was resuspended (Vortex). Arrows indicate CHO cells.

N- and *O*-linked carbohydrates may function as receptors for the Gal–GalNAc lectin. In addition, amebic adherence was enhanced in CHO mutants with increased terminal Gal groups and in wild-type CHO cells treated with neuraminidase to expose Gal residues[35].

The Gal–GalNAc lectin that is responsible for adherence to CHO cells and colonic mucin has been purified by two methods: by a D-galactose- or a D-galactose-terminal-glycoprotein-affinity column, and by affinity columns prepared with monoclonal antibodies that inhibit amebic adherence[27]. The purified lectin has an apparent mol. wt. of 260 000 on sodium dodecyl sulfate (SDS)–poly(acrylamide) gels, and is a heterodimer consisting of 170-kDa and 35-kDa subunits linked by disulfide bonds (Fig. 2). The amino termini of both subunits have been sequenced by sequential Edman degradation[26], and do not share any close sequence identity with any sequenced protein

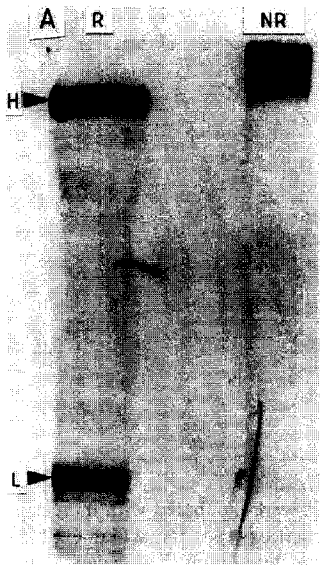

Fig. 2. Coomassie Blue-stained SDS-PAGE of Gal–GalNAc lectin purified by monoclonal-antibody affinity. The Gal–GalNAc lectin was reduced (R) with 10% β-mercaptoethanol or nonreduced (NR). Apparent mol. wts. of the subunits are: H, 170 000; and L, 35 000. Reprinted by permission from Petri *et al.*[26].

Heavy (170-kDa) subunit

Gly-Lys-Leu-Asn-Glu-Phe-Ser-Ala-Asp-Ile-Asp-Tyr-Tyr-Asp-Leu

Light (35-kDa) subunit

Lys-Thr-$^{Asn}_{Gln}$-Asp-$^{Asn}_{Gly}$-$^{Arg}_{Lys}$-Asp-Gln-$^{Phe}_{Leu}$-Ser-Pro-Asn-Tyr-Pro-Tyr-Gly-Lys

Scheme 1. Amino terminal amino acid sequences of the subunits of the Gal–GalNAc adherence lectin. The electroeluted heavy chain ($\sim$40 pmol) and the electroeluted light chain ($\sim$100 pmol) were subjected to sequence determination. The yield of PTH-derivatives for the heavy chain at each step was 8 ± 2, for the light chain 21 ± 3 pmol ($x \pm SD$) (Reprinted by permission from Petri *et al.*)[26].

in the National Biomedical Research Foundation data bank. The light subunit exhibited microheterogeneity in a 1:1 molar ratio at several residues, thus suggesting that more than one gene encodes the light subunit (Scheme 1).

The affinity-purified Gal–GalNAc lectin was tested in functional assays to demonstrate that the purified lectin was the molecule that mediated adherence[27]. CHO cells were pretreated with purified Gal–GalNAc lectin in the presence or absence of D-galactose or *N*-acetyl-D-galactosamine. The purified Gal–GalNAc lectin competitively inhibited amebic adherence to pretreated CHO cells, and the lectin-mediated inhibition of adherence was blocked by the presence of D-galactose. The purified lectin was also shown to retain its ability to bind target cells in a D-galactose-inhibitable manner (Fig. 3).

Monoclonal antibodies specific for the Gal–GalNAc lectin and polyclonal anti-

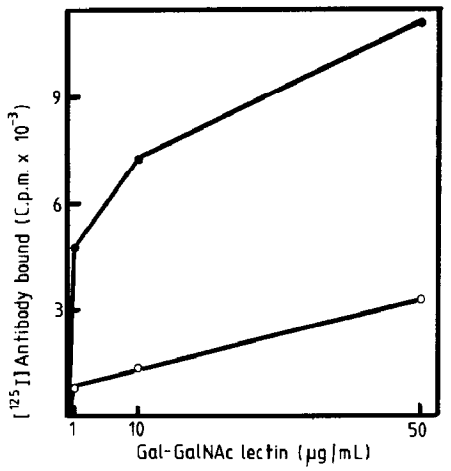

Fig. 3. Binding of purified Gal–GalNAc lectin to CHO cells. CHO cells were incubated with increasing amounts of affinity-purified Gal–GalNAc lectin in the presence (○) or absence (●) of 55mM Gal–GalNAc at 4°. Cells were washed and centrifuged off. Bound lectin was measured by adding ^{125}I-labeled, lectin-specific monoclonal antibody. After incubation, cells were washed and counted in a gamma counter.

lectin antisera raised against native or denatured lectin recognize only the 170-kDa subunit[36]. The polyclonal antisera inhibit amebic adherence to CHO cells by 100% and block the binding of [^{125}I]mucin to amebic trophozoites[28]. These results suggested but do not prove that the 170-kDa subunit is primarily responsible for amebic adherence to target cells. The presence of D-galactose-binding activity only in the native heterodimer has made it impossible so far to confidently assign the lectin activity to an individual subunit. The 35-kDa subunit did not appear to be immunogenic in mice, rabbits, or human[36]. A search for its function is currently an area of investigation in our laboratories.

Pooled human sera, from patients treated for amebic liver abscesses, recognized the purified 170-kDa Gal–GalNAc lectin subunit[36]. Immunoprecipitation of [^{35}S]methionine-metabolically-labeled amebas with a monoclonal antibody to the Gal–GalNAc lectin and the pooled human sera demonstrated that the 170-kDa protein was the major antigen recognized by the human sera. The Gal–GalNAc lectin also exhibited antigenic stability. Immunoprecipitation of *E. histolytica* proteins with the sera of patients, recovered from amebic liver abscess and colitis from different geographical locations, demonstrated that the Gal–GalNAc lectin was recognized by all patients' sera regardless of the site of infection (liver abscess or colitis) or geographic region[37].

Conclusions. — Lectin-carbohydrate interactions are a common theme in the pathogenesis of infectious diseases. Glycoproteins and glycolipids are prevalent on the surface of eukaryotic cells[38], so lectins provide a logical and efficient means of adherence and initiation of infection. Adherence of *E. histolytica* to target cells involves a complex array of molecules. The Gal–GalNAc and chitotriose lectins, along with other adherence proteins, may augment each other or have different functions in adherence to

different cell types, in virulence, and phagocytosis. Many questions remain about the function of the Gal–GalNAc lectin, including characterization of its binding domains, the function of the light subunit, and regulation of it's surface expression. Characterization of the Gal–GalNAc lectin and other adherence proteins at the molecular level should lead to a greater understanding of mechanisms of amebic adherence and cell lysis, and a possible means of controlling one of the world's most common infections.

REFERENCES

1 N. Sharon and H. Lis, *Science*, 246 (1989) 227–246.
2 J. C. Paulson, in P. M. Conn (Ed.), *The Receptors*, Academic Press, New York, 1985, Vol. 2, pp. 131–219.
3 D. C. Wiley and J. J. Skehel, *Annu. Rev. Biochem.*, 56 (1987) 365–394.
4 H. Hoschutzky, F. Lottspeich, and K. Jann, *Infect. Immun.*, 57 (1989) 76–81.
5 P. O'Hanley, D. Low, I. Romero, D. Lark, K. Vosti, S. Falkow, and G. K. Schoolnik, *New Engl. J. Med.*, 313 (1985) 414–420.
6 P. O'Hanley, D. Lark, S. Falkow, and G. K. Schoolnik, *J. Clin. Invest.*, 75 (1985) 347–360.
7 J. A. Walsh, *Rev. Infect. Dis.*, 8 (1986) 228–238.
8 J. I. Ravdin and T. C. Jones, in G. L. Mandell, R. G. Douglas, and J. E. Bennett (Eds.), *Principles and Practice of Infectious Disease*, Wiley, New York, 1984, pp. 1506–1510.
9 B. Sepulveda and A. Martinez-Polomo, in K. S. Warren and A. A. F. Mahmoud (Eds.), *Tropical and Geographic Medicine*, McGraw-Hill, New York, 1984, pp. 305–318.
10 H. W. Brown, *Basic Clinical Parasitology*, Appleton-Century-Crofts, New York, 1969, pp. 17–44.
11 H. Brandt and R. Perez-Tamayo, *Hum. Pathol.*, 1 (1970) 351–385.
12 K. Juniper, V. W. Steele, and C. L. Chester, *South. Med. J.*, 51 (1958) 545–553.
13 F. E. Pittman, W. K. El-Hashimi, and J. C. Pittman, *Gastroenterology*, 65 (1973) 588–603.
14 K. Prathap and R. Gilman, *Am. J. Pathol.*, 60 (1970) 229–239.
15 J. M. Galindo, A. Martinez-Polomo, and B. Chavez, *Arch. Invest. Med.*, 9 (Suppl. 1) (1978) 261–274.
16 E. Orozco, A. Martinez-Polomo, G. Guarneros, D. Kobiler, and D. Mirelman, *Arch. Invest. Med.*, 13 (Suppl. 3) (1982) 177.
17 J. I. Ravdin, B. Y. Croft, and R. L. Guerrant, *J. Exp. Med.*, 152 (1980) 377–390.
18 W. A. Petri, Jr., and J. I. Ravdin, *Eur. J. Epidemiol.*, 3 (1987) 123–136.
19 C. Gitler, E. Calef, and R. Rosenberg, *Philos. Trans. R. Soc. London, Ser. B*, 307 (1984) 73–85.
20 J. I. Ravdin and R. L. Guerrant, *J. Clin. Invest.*, 68 (1981) 1305–1381.
21 M. A. Rodriguez, F. Hernandez, L. Santos, A. Valdez, and E. Orozco, *Mol. Biochem. Parasit.*, 37 (1989) 87–100.
22 J. L. Rosales-Encina, I. Meza, A. Lopez-de-Leon, P. Talamas-Rohana, and M. Rojkind, *J. Infect. Dis.*, 156 (1987) 790–797.
23 I. Meza, F. Cazares, J. L. Rosales-Encina, P. Talamas-Rohana, and M. Rojkind, *J. Infect. Dis.*, 156 (1987) 798–805.
24 R. Arroyo and E. Orozco, *Mol. Biochem. Parasit.*, 23 (1987) 151–158.
25 P. Talamas-Rohana and I. Meza, *J. Cell. Biol.*, 106 (1988) 1787–1795.
26 W. A. Petri, Jr., M. D. Chapman, T. Snodgrass, B. J. Mann, J. Broman, and J. I. Ravdin, *J. Biol. Chem.*, 264 (1989) 3007–3012.
27 W. A. Petri, Jr., R. D. Smith, P. H. Schlesinger, C. F. Murphy, and J. I. Ravdin, *J. Clin. Invest.*, 80 (1987) 1238–1244.
28 K. Chadee, W. A. Petri, Jr., D. J. Innes, and J. I. Ravdin, *J. Clin. Invest.*, 80 (1987) 1245–1254.
29 J. I. Ravdin, C. F. Murphy, R. A. Salata, R. L. Guerrant, and E. L. Hewlett, *J. Infect. Dis.*, 151 (1985) 804–815.
30 R. Bracha and D. Mirelman, *J. Exp. Med.*, 160 (1984) 353–386.
31 R. Bracha and D. Mirelman, *Infect. Immun.*, 40 (1983) 882–887.
32 R. A. Salata, R. D. Perason, and J. I. Ravdin, *J. Clin. Invest.*, 76 (1985) 491–499.
33 R. A. Salata and J. I. Ravdin, *J. Infect. Dis.*, 154 (1986) 19–26.
34 E. Li, A. Becker, and S. L. Stanley, Jr., *Infect. Immun.*, 57 (1989) 8–12.
35 J. I. Ravdin, P. Stanley, C. F. Murphy, and W. A. Petri, Jr., *Infect. Immun.*, 57 (1989) 2179–2186.

36 W. A. Petri, Jr., M. P. Joyce, J. Broman, R. D. Smith, C. F. Murphy, and J. I. Ravdin, *Infect. Immun.*, 55 (1987) 2327–2331.
37 W. A. Petri, Jr., J. Broman, G. Healy, T. Quinn, and J. I. Ravdin, *Am. J. Med. Sci.*, 296 (1989) 164–165.
38 G. M. Cook, *J. Cell Sci.*, 4 (Suppl.) (1986) 45.