Brief Definitive Report

USE OF CHINESE HAMSTER OVARY CELLS WITH ALTERED GLYCOSYLATION PATTERNS TO DEFINE THE CARBOHYDRATE SPECIFICITY OF *ENTAMOEBA HISTOLYTICA* ADHESION

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Entamoeba histolytica is an enteric protozoan parasite that causes disease in humans by disruption and invasion of the colonic mucosa. A number of studies have shown that various soluble saccharide inhibitors will interfere with amoebic adherence to mammalian target cells in vitro (1), suggesting that amoebic lectins mediate amoebic adherence to mammalian cells. Based on this approach, two mechanisms of adherence of intact amoeba to mammalian cells have been described, one sensitive to inhibition with the monosaccharides *N*-acetylgalactosamine or galactose and a second inhibited by *N*-acetylglucosamine oligosaccharides.

Studies on the binding specificity of various lectins have shown that sugars that are good lectin inhibitors in solution may not reflect the carbohydrate structure of receptors found on cell surfaces (2). Furthermore, among lectins that interact with monosaccharides, several exhibit a pronounced preference for more complex oligosaccharides, suggesting that these lectins have extended binding sites (2). To obtain more information on the nature of the glycoconjugate receptors for *E. histolytica,* we measured the adhesion of *E. histolytica* to wild-type Chinese hamster ovary (CHO) cells and three lectin-resistant somatic cell mutants, WGA^R 1021, WGA^R 13, and RIC^R 15B (see Fig. 1). WGA^R 1021 is deficient in membrane-bound sialic acid and has increased galactose residues at the nonreducing termini (3). WGA^R 13 is deficient in both membrane bound sialic acid and galactose and has increased *N*-acetylglucosamine residues at the nonreducing termini (3). As a consequence of a deficiency in *N*-acetylglucosaminyltransferase I, RIC^R 15B cells lack Asn-linked complex (*N*-acetyllactosamine) units and accumulate Asn-linked oligomannosyl units in their glycoproteins (4, 5). We report here our analysis of *E. histolytica* trophozoite adherence to this panel of cells.

Materials and Methods

Cells. *E. histolytica* HM1-IMSS were kindly provided by Cheryl Murphy and Dr. Jonathan Ravdin. *E. histolytica* strains HM1-IMSS and HK-9 were obtained from the Amer-

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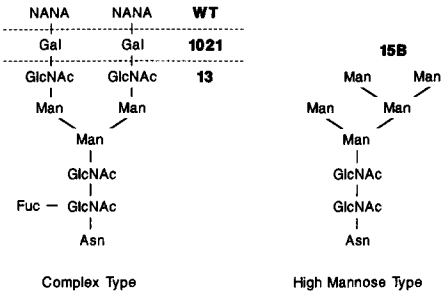

FIGURE 1. Carbohydrate structure alterations in the lectin-resistant cell lines. To illustrate the alterations in carbohydrate structures found in WGA^R 1021 and WGA^R 13 cells compared with wild-type (WT) cells the structure of a biantennary Asn-linked complex-type oligosaccharide is shown on the left. Shown on the right is the structure of the predominant oligosaccharide found on RIC^R 15B cells (5).

ican Type Culture Collection (Rockville, MD) The amoeba were grown in TYI-S-33 medium as described previously (6).

The wild-type and lectin-resistant CHO cells were kindly provided by Dr. Stuart Kornfeld and were grown as described previously (3).

Adherence of [^{3}H]thymidine-labeled E. histolytica Trophozoites to Monolayers of CHO Cells. 48-h cultures of amoebic trophozoites were labeled overnight with 100 μCi of [^{3}H]thymidine (73 Ci/mmol, ICN) in TYI-S-33 medium with 15% heat-inactivated bovine serum. They were harvested by chilling for 5 min in ice, and centrifuging for 3 min at 150 *g*. They were washed four times with test media (MEM-α, 1% BSA, 5.7 mM cysteine, 10 mM Hepes, pH 7.5) at 4°C, and resuspended in the same medium at a desnity of 4 $\times$ 10^5 cells/ml.

CHO cells were seeded at 10^6 cells/well in 24-well plates and incubated overnight to produce confluent monolayers. They were washed two times with MEM-α at 4°C. The washed monolayers were incubated with 0.25 ml of amoebic trophozoites (suspended in test medium at 4 $\times$ 10^5 cells /ml) for 20 min at 4°C. The monolayers were washed three times with MEM-α at 4°C. The bound amoeba were solubilized in 0.5 ml of 2% Triton-X-100, 0.2% sodium azide and were transferred to scintillation vials containing 10 ml of Scintiverse I (Risher Scientific, Fairlawn, NJ). Under the conditions of this assay, 10–20% of the radioactive counts associated with the amoeba were released into the medium. The specific activity of the amoeba were corrected for this release of counts. 1 cpm represented three to eight amoeba. Duplicate wells were used in each assay.

Neuraminidase Treatment of the Monolayers. The monolayers were washed two times with MEM-α and incubated for 1 h with 0.1 U/ml of *V. cholerae* neuraminidase (Calbiochem-Behring Corp., La Jolla, CA) in MEM-α containing 1 mg/ml BSA. Control monolayers were incubated with MEM-α containing 1 mg/ml BSA over the same time period. The monolayers were washed twice with MEM-α before incubation with the amoeba.

Inhibitors of Adhesion. Saccharides were purchased from Sigma Chemical Co. (St. Louis, MO) and Pfahnstiehl Laboratories (Waukegan, IL). The amoeba were preincubated with the inhibitor dissolved in test medium for 5 min at 4°C before incubation with the monolayer. The adherence of treated amoeba was compared with that of control amoeba suspended in test medium lacking inhibitors.

Results and Discussion

To examine the carbohydrate specificity of *E. histolytica* trophozoite adherence to mammalian cells, we measured the adherence of [^{3}H]thymidine-labeled trophozoites (HM1-IMSS) to a confluent monolayer of live (unfixed) CHO cells. The relative adherence of trophozoites to monolayers of wild type, WGA^R 1021, WGA^R 13 and RIC^R 15B cells is shown in Figs. 2 and 3. Approximately twice as many trophozoites adhered to the WGA^R 1021 cells compared with the wild-type cells. In contrast, adherence to WGA^R 13 and RIC^R 15B cells was reduced to 31% and 12% of the adherence observed with wild-type cells respectively.

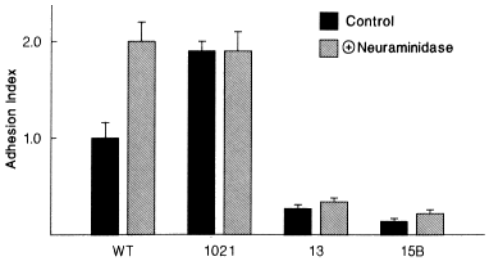

FIGURE 2. Relative adherence of [^{3}H]thymidine-labeled amoebic trophozoites (strain HM1-IMSS) to monolayers of wild-type (WT), WGA^R 1021 (1021), WGA^R 13 (13), and RIC^R 15B (15B) cells, with and without neuraminidase pretreatment. Adherence was measured as described in Materials and Methods. The adhesion index was expressed as the number of amoeba bound to the test monolayer/number of amoeba bound to wild-type cell monolayers. The values shown are an average of three experiments ± SD. 15–20% of the trophozoites were adherent to wild-type CHO cells. Binding was complete after 10–20 min of incubation.

Only an occasional trophozoite could be seen attached to WGA^R 13 monolayers (Fig. 3 *c*) and virtually no trophozoites were observed attached to the RIC^R 15B monolayers (Fig. 3 *d*).

The relative adhesion of the amoeba to wild-type, WGA^R 1021, and WGA^R 13 cells correlates directly with the presence of terminal galactose residues. This was confirmed by neuraminidase treatment of wild-type cells resulting in levels of amoebic adherence comparable to that observed with the sialic acid–deficient WGA^R 1021 cells (see Fig. 2). Treatment with neuraminidase had little or no effect on amoebic adherence to the mutant cell lines. Furthermore, amoeba adhere very poorly to RIC^R 15B cells at levels consistently lower than observed for WGA^R 13 cells although the total membrane-bound galactose content of RIC^R 15B cells and of WGA^R 13 cells are 51% and 15% of wild-type levels, respectively. RIC^R 15B cells have a selective deficiency in Asn-linked complex-type units and contain wild-type levels of membrane-bound *N*-acetylgalactosamine (4, 5). WGA^R 13 cells have decreased incorporation of galactose in both glycoproteins and glycolipids due to a defect in the transport of UDP-galactose into the Golgi apparatus (7). The poor adherence of amoeba to RIC^R 15B cells,

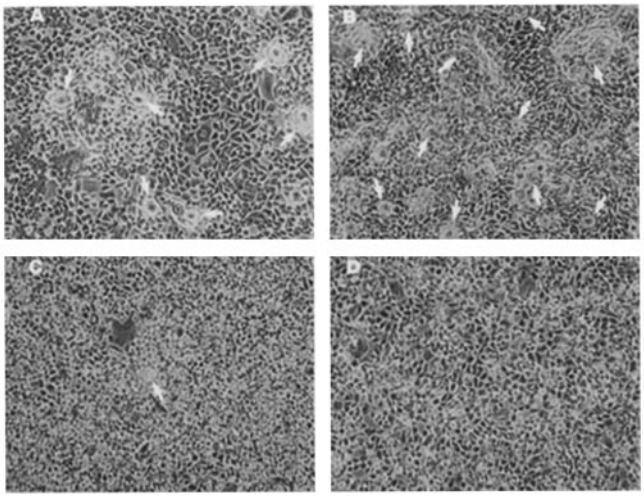

FIGURE 3. Phase-contrast photomicrographs of amoebic trophozoites (HM1-IMSS strain) bound to monolayers of A, wild-type; B, WGA^R 1021; C, WGA^R 13; and D, RIC^R 15B cells. The arrows point to some of the bound amoeba. Cells were photographed on Polaroid type 107 film through a Nikon phase-contrast microscope. Magnification is 44 diameters.

TABLE I
Inhibition of Amoebic Adherence to WGA^R 1021 Cells

Compound (50 mM)	Adherence (percent of control)		Number of experiments	Concentration for 50% inhibition
	Mean ± SD	Range		
Gal	75 ± 6	70–82	3	mM
Man	114 ± 29	95–148	3	
GalNac	80 ± 16	67–98	3	
GlcNac	100 ± 13	89–114	3	
NANA	76 ± 8	71–81	2	
Galβ1→4Glc (*N*-acetyllactosamine)	22 ± 5	16–28	4	25
Galβ1→4Glu (lactose)	42 ± 18	26–66	4	50
Galα1→6Glu (melibiose)	104 ± 4	101–107	2	
GlcNacβ1→4GlcNAc (chitobiose)	99 ± 1	98–100	2	
NANAβ2→3Galβ1-4Glu (sialylactose)	70 ± 12	58–83	3	

Assays were performed as described in Materials and Methods.

therefore, suggests that the receptors for amoebic adherence are located on the Asn-linked complex-type chains.

We examined the effect of various soluble monosaccharide and disaccharide inhibitors on amoebic adherence to monolayers of WGA^R 1021 cells (see Table I). Of the disaccharides tested, *N*-acetyllactosamine appeared to be the most effective. Lactose at 50 mM also inhibited adherence. However, 50 mM melibiose and 50 mM chitobiose had no effect on adherence. Minimal inhibition was observed with sialyllactose, galactose, and *N*-acetylgalactosamine at 50 mM.

Inhibition of amoebic adherence to WGA^R 1021 by *N*-acetyllactosamine confirmed that the differences in amoebic adhesion to the different cell lines were due to differences in carbohydrate structure. The observation that galactose and melibiose are relatively poor inhibitors suggests that the amoebic adhesin has an extended binding site. The carbohydrate specificity of amoebic adherence is similar to that reported for the *Erythrina cristagalli* lectin (8).

The concentrations of inhibitors required in this assay are higher than those reported using the rosetting technique of Ravdin and coworkers (1, 9). This may be because the conditions for binding in this assay are more stringent. In our assay, we were unable to demonstrate a chitobiose-inhibitable mechanism of adhesion. This may relate to the fact that the target cells used in this assay were not fixed with glutaraldehyde, unlike the target cells used in studies describing the chitobiose-inhibitable mechanism of adherence (1, 10). The effects of glutaraldehyde on cell surface glycoconjugates are not well understood.

Our studies indicate that terminal *N*-acetyllactosamine units on Asn-linked complex-type oligosaccharide chains provide the major receptors for amoebic adhesion to CHO cells. CHO cells contain a diverse population of complex-type oligosaccharide chains that differ not only in the arrangement of sugars in the peripheral branches but also in the substitution of the common trimannosyl core. In addition to the biantennary structure with nonrepeating *N*-acetyllactosamine units shown in Fig. 1, a significant proportion of galactose residues are

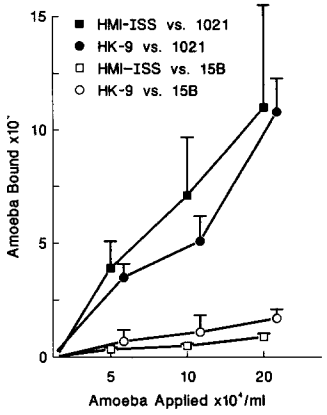

FIGURE 4. Adherence of [^{3}H]thymidine-labeled HM1-IMSS and HK-9 trophozoites to monolayers of WGA^R 1021 and RIC^R 15B cells. Adherence was measured as described in Materials and Methods. The values shown are an average of three separate experiments ± SD.

contained in oligosaccharides containing repeating disaccharide (Galβ1→4G1cNacβ1→3) units (11). The structure of the major complex-type chain serving as the receptor for amebic adhesion remains to be defined. Although the receptor on CHO cells appears to be restricted to Asn-linked complex type chains, this does not mean that this is the case for the receptors on colonic mucosa. For example, *N*-acetyllactosamine units are also found on *O*-linked oligosaccharide chains in colonic mucin (12).

Recently a galactose-binding 170-kD lectin, which inhibits amoebic adherence to CHO cells, has been purified from amoebic trophozoites by Petri et al. (9). Based on our observations, we suggest that this protein may bind *N*-acetyllactosamine with much higher affinity than galactose. The role played by a 220-kD protein, which displays chitin inhibitable agglutination of glutaraldehyde fixed erythrocytes, described by Rosales-Encina et. al. (10), and a 112-kD protein described by Arroyo and Orozco (13) are less clear.

We compared the adherence of a less cytopathogenic strain of trophozoites, HK-9 (14), with that of HM1-IMSS to monolayers of WGA^R 1021 and RIC^R 15B (see Fig. 4) and found no significant difference in the adherence properties of these two strains of amoeba. Although adherence is probably important in colonizing the colonic mucosa, adherence may not be directly involved in cytopathogenicity. We are currently using this panel of cells to investigate the role of glycoconjugates in mediating cytopathogenicity.

Summary

We compared the adherence of *E. histolytica* trophozoites with a panel of lectin-resistant CHO mutants with altered glycosylation patterns. Our results coupled with data from saccharide inhibition studies indicate that terminal *N*-acetyllactosamine units on Asn-linked complex type oligosaccharides provide the major determinants on the cellular receptor for *E. histolytica* adhesion.

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References

1. Mirelman, D., and J. I. Ravdin. 1986. Lectins in *Entamoeba histolytica. In* Microbial Lectins and Agglutinins: Properties and Biological Activity. D. Mirelman, editor. John Wiley and Sons, New York, 319–334
2. Goldstein, I. J., and R. D. Poretz. 1986. Isolation, physicochemical characterization, and carbohydrate-binding specificity of lectins. *In* The Lectins Properties, Functions and Application in Biology and Medicine. I. E. Liener, N. Sharon, and I. J. Goldstein, editors. Academic Press, Inc. Orlando. 35–247
3. Briles, E. B., E. Li, and S. Kornfeld. 1977. Isolation of wheat germ agglutinin-resistant clones of Chinese hamster ovary cells deficient in membrane sialic acid and galactose. *J. Biol. Chem.* 252:1107
4. Gottlieb, C., A. Skinner, and S. Kornfeld. 1974. Isolation of a clone of Chinese hamster ovary cells deficient in plant lectin-binding sites. *Proc. Natl. Acad. Sci. USA.* 71:1078
5. Li, E., and S. Kornfeld. 1978. Structural studies of the altered oligosaccharide present in glycoproteins from a clone of Chinese hamster ovary cells deficient in N-acetylglucosaminyltransferase activity. *J. Biol. Chem.* 253:6426
6. Diamond, L. S., D. Harlow, and C. C. Cunnick. 1978. A new medium for the axenic cultivation of *Entamoeba histolytica* and other *Entamoeba. Trans. R. Soc. Trop. Med. Hyg.* 4:431
7. Deutcher, S. L., and C. Hirscherg, 1986. Mechanism of galactosylation in the Golgi apparatus. A Chinese hamster ovary cell mutant deficient in translocation of UDP-galactose across Golgi vesicle membranes. *J. Biol. Chem.* 216:96
8. Iglesias, J. I., H. Lis, and N. Sharon. 1982. Purification and properties of a D-galactose/ N-acetyl-D-galactosamine-specific lectin from *Erythrina cristagalli. Eur. J. Biochem.* 123:247
9. Petri, W. A. Jr., R. D. Smith, P. H. Schlesinger, and J. I. Ravdin. 1987. Isolation of the galactose-binding lectin which mediates the *in vitro* adherence of *Entamoeba histolytica. J. Clin. Invest.* 80:1238
10. Rosales-Encina, J. L., I. Meza, A. López-DeLeón, P. Talamás-Rohana, and M. Rojkind. 1987. Isolation of a 220-kilodalton protein with lectin properties from a virulent strain of *Entamoeba histolytica. J. Infect. Dis.* 1981. 144:539
11. Li, E., R. Gibson, and S. Kornfeld. 1980. Structure of an unusual complex-type oligosaccharide isolated from Chinese hamster ovary cells. *Arch. Biochem. Biophys.* 199:393
12. Podolsky, D. K. 1985. Oligosaccharide structures of isolated human colonic mucin species. *J. Biol. Chem.* 260:15510
13. Arroyo, R. and E. Orozco. 1987, Localization and identification of an *Entamoeba histolytica* adhesin. *Mol. Biochem. Parasitol.* 15:49
14. Mattern, C. F. T., and D. B. Keister. 1977. Experimental amebiasis. III. A rapid *in vitro* assay for virulence of *Entamoeba histolytica. Am. J. Trop. Med. Hyg.* 27:882