INFECTION AND IMMUNITY, July 1990, p. 2389–2391
0019-9567/90/072389-03$02.00/0

Vol. 58, No. 7

Roles of Target Cell Membrane Carbohydrate and Lipid in *Entamoeba histolytica* Interaction with Mammalian Cells

GORDON B. BAILEY,* JEFFREY R. GILMOUR,† AND NORMAN E. McCOOMER

Morehouse School of Medicine, Atlanta, Georgia 30310

Received 15 February 1990/Accepted 19 April 1990

Latex beads and liposomes carrying glycoproteins with carbohydrate sequences recognized by an *Entamoeba histolytica* galactose-specific binding protein were assessed for their ability to adhere to trophozoites and to stimulate amoeba actin polymerization. Glycoprotein-conjugated beads bound significantly to amoebae but did not stimulate actin polymerization. Glycoprotein-bearing liposomes bound to amoebae and did enhance actin polymerization, as do recognized glycosphingolipid-bearing liposomes (G. B. Bailey, E. D. Nudelman, D. B. Day, C. F. Harper, and J. R. Gilmour, Infect. Immun. 58:43–47, 1990). Liposome-stimulated actin polymerization occurred only if the vesicle contained negatively charged phospholipid. It was concluded that both glycoprotein and glycosphingolipid glycans on the target cell surface are involved in attachment to *E. histolytica* but do not themselves induce the transmembrane signals that lead to cytoskeleton activation and target destruction. This requires interaction with lipids of the target membrane bilayer.

There is considerable evidence that an initial event in the destructive interaction of *Entamoeba histolytica* with mammalian cells in vitro is binding of an amoeba membrane lectin to terminal galactose (Gal) or *N*-acetylgalactosamine (GalNAc) residues of oligosaccharides on the surface of the target cell (8, 9). Both glycoprotein (4–6, 10) and glycosphingolipid (3) glycans are recognized. The earliest biochemical response that has been detected after contact with the target cell is polymerization of amoeba actin (1); this is associated with extension of phagocytic membranes. Cytochalasin inhibition studies have shown that the action of the amoeba cytoskeleton is required for effective parasite attachment and destruction of target cells (1, 7, 9). Therefore, contact-induced actin polymerization may be an essential event of the target cell destructive process.

E. histolytica actin polymerization is also stimulated by contact with liposomes prepared from target cell membrane lipids and by negatively charged synthetic liposomes (2). The stimulating activity of the latter is greatly enhanced by inclusion in the vesicles of glycosphingolipids with glycans recognized by the amoeba Gal-binding protein (3). The following questions concerning the roles of carbohydrate ligands and of membrane lipids in eliciting the cytoskeleton response have not been thoroughly answered. Will binding of recognized glycans alone, if presented as a multivalent target of suitable size, trigger actin polymerization? Does the parasite distinguish between glycoprotein and glycolipid glycans on the target membrane surface in the mechanism of this response? We have addressed these questions using target models bearing glycans recognized by the amoeba lectin and without or with a lipid membrane.

Latex beads (Polysciences, Inc., Warrington, Pa.) with the same mean diameter as human erythrocytes were covalently conjugated with fetuin (*N*-acetylneuraminate [NeuAc]-Galβ1-4*N*-acetylglucosamine [GlcNAc] termini) and asialofetuin (Galβ1-4GlcNAc termini) at a ratio of 80 μg of protein per 10^8 beads, by using dimethylcarbodiimide (Pierce Chemical Co., Rockford, Ill.) as described by the vendor. Agalactosyl-asialofetuin-conjugated beads (GlcNAc termini) were prepared by treatment of the asialofetuin beads with jack bean β-galactosidase (Sigma Chemical Co., St. Louis, Mo.; 0.1 U of enzyme per 10^8 beads in 0.1 M citrate, pH 4, for 4 h at 37°C) followed by washing with phosphate-buffered saline, pH 6.3 (PBS). The effectiveness of glycoprotein conjugation and galactose hydrolysis was estimated by fluorescence cytometry of beads after binding of 0.1 μg of the β-galactoside-specific lectin, fluorescein isothiocyanate-Allo-A (E-Y Laboratories, Inc., San Mateo, Calif.), per ml and washing with 0.01% sodium dodecyl sulfate to remove nonspecifically bound lectin. A fluorescence signal much more intense than that of the unconjugated beads was obtained with all glycoprotein-conjugated beads. The integrated signal of the asialofetuin-conjugated beads was strongest; this was reduced 17% by treatment with β-galactosidase ($P < 0.001$).

Asialoglycophorin (Galβ1-3GlcNAc termini) liposomes were prepared by dialysis of lipid-protein suspensions as follows. Membrane lipids (Sigma), formulated as previously described (3) and containing 0.1 μCi of [^{14}C]phosphatidylcholine to monitor the total lipid concentration, were dried on the wall of a glass test tube. Human asialoglycophorin A (Sigma; 0.1 mg/mg of lipid) was dissolved in 0.1% Triton X-100 (1.8 ml/mg of protein, containing 5 mg of carboxyfluorescein per ml if required), and then 1/10 volume of 10× PBS was added. The protein was suspended with the lipids by brief sonication and dialyzed for 48 h against several changes of PBS containing Biobeads SM-2 (Bio-Rad Laboratories, Rockville Centre, N.Y.). The liposomes were collected and washed once with PBS by centrifugation at 100,000 × *g* for 40 min and finally suspended in PBS at a concentration of 4.5 mg of total lipid per ml (3). Asialoglycophorin incorporation into liposomes was determined by protein assays and ranged from 60 to 75% of the calculated maximum in the preparations used. Protein-free liposomes used in this study were prepared by the same procedure.

Trophozoites of axenically cultured *E. histolytica* HM1-IMSS were used (1). Adherence and phagocytosis of beads and erythrocytes were determined by microscopic counts after challenge with 20 targets per amoeba, as described elsewhere (1). Actin polymerization was quantitated by

* Corresponding author.
† Present address: Department of Biochemistry, Brandeis University, Waltham, MA 02154.

TABLE 1. Adherence and phagocytosis by *E. histolytica* of protein-conjugated latex beads and erythrocytes

Glycoprotein conjugate (glycan terminal sequence)	Mean ± SEM[a]		
	Adherence	Phagocytosis	Adherence + phagocytosis
None	55.8 ± 3.9	0.0	55.8
Fetuin (NeuAc-Galβ1-4GlcNAc-)	82.6 ± 3.2	10.0 ± 2.8	92.6
Asialofetuin (Galβ1-4GlcNAc-)	147.8 ± 5.0[b]	15.6 ± 3.0	163.4[c]
Agalactosylasialofetuin (GlcNAc-)	95.8 ± 3.9	8.0 ± 1.9	103.8
Whole erythrocytes	14.4 ± 3.8	268.0 ± 8.3[d]	282.4[d]

[a] Number of beads attached and phagocytized per 100 amoebae, determined for five assays. Significance was calculated with Student's *t* test.
[b] Significantly greater than all other bead target values ($P < 0.01$).
[c] Significantly greater than values for unconjugated and fetuin-conjugated beads ($P < 0.01$).
[d] Significantly greater than all other bead target values ($P < 0.001$).

counting the number of stimulated amoebae after rhodamine-phalloidin staining (2).

Recognition-specific binding of glycoprotein-conjugated beads was demonstrated by the results shown in Table 1. The known target cell carbohydrate specificity of *E. histolytica* predicts that the Galβ1-4GlcNAc terminal sequence of the asialofetuin beads would be the best recognized. Adherence of asialofetuin beads to *E. histolytica* was significantly greater than that of unconjugated or fetuin- or agalactosylasialofetuin-conjugated beads ($P < 0.01$). This implied that the terminal Gal residues of the asialofetuin-conjugated beads were specifically recognized by the amoeba lectin. Failure of the recognized-glycoprotein-conjugated beads to stimulate *E. histolytica* actin polymerization is shown in Table 2. No increase in amoeba-polymerized actin was detected after challenge for 2, 4, and 10 min (1, 2) with asialofetuin-conjugated beads (Table 2). Thus, lectin binding of recognized carbohydrates, even when carbohydrates are presented on the surface of particulate target of suitable size, is not sufficient to stimulate the amoeba cytoskeleton response.

Only a small percentage of the attached asialofetuin beads were phagocytized (Table 1). Under identical assay conditions, erythrocyte phagocytosis occurred quickly after contact. If it is assumed that adherence to the Gal-specific lectin precedes phagocytosis, then the total number of adhered erythrocytes was approximately twice that of asialofetuin beads, while the number phagocytized was 17-fold greater. Thus, while phagocytosis may be enhanced by lectin binding (2, 3), it is not a direct consequence of adherence.

The abilities of glycoprotein-bearing liposomes and liposomes with various negative phospholipid contents to stimulate actin polymerization are shown in Table 2. Liposomes constructed with asialoglycophorin, an erythrocyte integral membrane glycoprotein with Galβ1-3GlcNAc termini, stimulated significant amoeba actin polymerization (Table 2) compared with the carbohydrate-free liposomes with the same phospholipid composition (Table 2; $P < 0.001$). We have shown previously that liposomes lacking a negatively charged phospholipid did not stimulate actin polymerization, even when they contained a recognized glycosphingolipid (3). Likewise, asialoglycophorin liposomes lacking a negatively charged phospholipid did not stimulate actin polymerization (Table 2). They did, however, bind to the galactose lectin on the amoeba surface. This was demonstrated by using carboxyfluorescein-loaded liposomes. Under the fluorescence microscope, carboxyfluorescein-loaded asialoglycophorin liposomes lacking phosphatidylserine, but not similar glycoprotein-free liposomes, were clearly seen to bind to the amoeba surface when added under the cover slip of a fresh mount of *E. histolytica* trophozoites washed and suspended in PBS. This attachment was completely eliminated by prior addition to the amoeba suspension of 50 mM lactose, a recognition-specific inhibitor (3), but not by addition of the same concentration of the nonspecific sugar glucose. Therefore, the failure of the uncharged asialoglycophorin liposomes to stimulate amoeba actin polymerization was not due to a failure to bind to the amoeba lectin.

Increasing the negatively charged phospholipid concentration of liposomes partially obscured the enhancing effect of incorporated Gal-terminal glycans on amoeba actin polymerization. Carbohydrate-free liposomes containing 8.6 mol% phosphatidylserine (Table 2) had about one-fourth the stimulating activity of the same liposomes containing, in addition, 10 mol% paragloboside (Table 2). The activity of the glycan-free liposomes was increased by increasing the concentration of negatively charged phospholipid (Table 2).

We interpret these results as follows. Target cell surface glycan binding to the *E. histolytica* galactose lectin is responsible for attachment of target cells but is not directly involved in the mechanism of amoeba cytoskeleton activation. Both glycoprotein and glycolipid glycans participate in target binding. The transmembrane signals that lead to *E. histolytica* actin polymerization following target contact are triggered, at least in part, by negatively charged lipids of the target plasma membrane. Glycan binding enhances the efficiency of interaction with lipids and therefore the degree of cytoskeleton response, possibly by facilitating closer or longer association of target lipids with reacting sites on the amoeba surface. Increasing the concentration of negatively charged lipid in the glycan-free liposomes mimicked the effect of glycans simply by increasing the percent saturation

TABLE 2. Stimulation of *E. histolytica* actin polymerization by various target models

Target (PS[a] concn, mM)	Relative stimulation (mean ± SEM)[b]
Asialofetuin beads	0.00
Asialoglycophorin liposomes (0.6 mM PS)	0.75 ± 0.12[c]
Asialoglycophorin liposomes (no PS)	0.00
Glycolipid-free liposomes (0.6 mM PS)	0.26 ± 0.07
Glycolipid-free liposomes (0.12 mM PS)	0.45 ± 0.09[d]
Glycolipid-free liposomes (0.18 mM PS)	0.52 ± 0.08[d]
Paragloboside liposomes (0.6 mM PS)	0.90 ± 0.11[c]
Erythrocyte membrane liposomes	1.00 ± 0.16[c]

[a] PS, Phosphatidylserine.
[b] Relative fraction of stimulated amoebae in 100 cells counted compared with amoebae challenged with erythrocyte membrane liposomes.
[c] Significantly greater than value in line 4 ($P < 0.001$).
[d] Significantly greater than value in line 4 ($P < 0.01$).

of lipid-reacting sites on the amoeba surface. Further elucidation of the role of target cell membrane lipids in the mechanism of *E. histolytica* cytoskeleton activation should provide useful information about the molecular mechanism of *E. histolytica* cytopathogenicity.

This work was funded by Public Health Service grants AI23523 and RR08248 from the National Institutes of Health and contract DAMD 17-88-C-8117 from the U.S. Army Medical Research and Development Command.

LITERATURE CITED

1. **Bailey, G. B., D. B. Day, and J. W. Gasque.** 1985. Rapid polymerization of *Entamoeba histolytica* actin induced by interaction with target cells. J. Exp. Med. **162**:546–558.
2. **Bailey, G. B., D. B. Day, C. Nokkaew, and C. C. Harper.** 1987. Stimulation by target cell membrane lipid of actin polymerization and phagocytosis by *Entamoeba histolytica*. Infect. Immun. **55**:1848–1853.
3. **Bailey, G. B., E. D. Nudelman, D. B. Day, C. F. Harper, and J. R. Gilmour.** 1990. Specificity of glycosphingolipid recognition by *Entamoeba histolytica* trophozoites. Infect. Immun. **58**:43–47.
4. **Chadee, K., W. A. Petri, Jr., D. J. Hines, and J. I. Ravdin.** 1987. Rat and human colonic mucins bind to and inhibit adherence lectin of *Entamoeba histolytica*. J. Clin. Invest. **80**:1245–1254.
5. **Li, E., A. Becker, and S. L. Stanley.** 1988. Use of Chinese hamster ovary cells with altered glycosylation patterns to define the carbohydrate specificity of *Entamoeba histolytica* adhesion. J. Exp. Med. **167**:1725–1730.
6. **Li, E., A. Becker, and S. L. Stanley, Jr.** 1989. Chinese hamster ovary cells deficient in *N*-acetylglucosamine transferase I activity are resistant to *Entamoeba histolytica*-mediated cytotoxicity. Infect. Immun. **57**:8–12.
7. **Lopez-Revilla, R., and R. Cano-Mancera.** 1982. Adhesion of *Entamoeba histolytica* trophozoites to human erythrocytes. Infect. Immun. **37**:281–285.
8. **Ravdin, J. I.** 1989. *Entamoeba histolytica*: from adherence to enteropathy. J. Infect. Dis. **159**:420–429.
9. **Ravdin, J. I., and R. L. Guerrant.** 1981. The role of adherence in cytopathogenic mechanisms of *Entamoeba histolytica*. Study with mammalian tissue culture cells and human erythrocytes. J. Clin. Invest. **68**:1305–1313.
10. **Ravdin, J. I., P. Stanley, C. F. Murphy, and W. A. Petri, Jr.** 1989. Characterization of cell surface carbohydrate receptors for *Entamoeba histolytica* adherence lectin. Infect. Immun. **57**:2179–2186.