INFECTION AND IMMUNITY, May 1985, p. 292–297
0019-9567/85/050292-06$02.00/0

Vol. 48, No. 2

Adherence of *Entamoeba histolytica* Trophozoites to Rat and Human Colonic Mucosa

JONATHAN I. RAVDIN,[1]* JAMES E. JOHN,[1] LEAH I. JOHNSTON,[1] DONALD J. INNES,[2] AND RICHARD L. GUERRANT[1]

Divisions of Infectious Diseases, Clinical Pharmacology, and Geographic Medicine, Department of Internal Medicine,[1] and Department of Pathology,[2] University of Virginia School of Medicine, Charlottesville, Virginia 22908

Received 13 November 1984/Accepted 28 January 1985

We studied the adherence of [^{3}H]thymidine-labeled axenic *Entamoeba histolytica* (strain HM1-IMSS) to in vitro preparations of rat and human colonic mucosa. Studies were performed with fixed or unfixed rat colonic mucosa, unfixed rat mucosa exposed to trypsin, unfixed rat submucosa, and fixed human colonic mucosa. Twenty percent of the amebae adhered to fixed rat colonic mucosa; adherence was specifically inhibited by *N*-actetyl-D-galactosamine (GalNAc), galactose, and asialofetuin. The adherence of amebae to fixed human colonic mucosa was also GalNAc inhibitable. Greater adherence was found with unfixed rat colonic mucosa (40.9%) and was not GalNAc inhibitable unless the tissue was first exposed to trypsin. However, GalNAc did inhibit the adherence of amebae to unfixed rat submucosa. Glutaraldehyde fixation of amebae inactivates known amebic adhesin proteins; there was a markedly decreased adherence of fixed amebae to trypsin-exposed mucosa or fixed rat colonic mucosa. However, fixed or viable amebae had equal levels of adherence to unfixed rat colonic mucosa, suggesting the presence of a host adhesin protein that binds to receptors on amebae. Human (10%) and rabbit (5%) immune sera reduced the adherence of viable amebae to fixed rat colonic mucosa. We concluded that the GalNAc-inhibitable adhesin protein on the surface of *E. histolytica* trophozoites mediated adherence to fixed rat mucosa, fixed human colonic mucosa, trypsin-exposed unfixed rat mucosa, and unfixed rat submucosa. The surface of unfixed rat colonic mucosa contained a glutaraldehyde- and trypsin-sensitive host adhesin protein, perhaps in the overlying mucus blanket, which bound viable or fixed *E. histolytica* trophozoites.

The pathogenic protozoan *Entamoeba histolytica* causes disease in humans by disruption and invasion of the colonic mucosa. Studies utilizing in vivo or in vitro intestinal models have indicated that amebae may adhere to the colonic mucosa before invasion (9, 13, 20). The purpose of this study was to explore the biochemical basis of the adherence of amebae to the relevant target tissue, mammalian colonic mucosa.

E. histolytica contains at least two surface adhesin molecules. The adherence of axenic *E. histolytica* trophozoites to Chinese hamster ovary (CHO) cells, human erythrocytes, and human neutrophils is specifically inhibited by *N*-acetyl-D-galactosamine (GalNAc) or galactose (23; J. I. Ravdin, C. F. M. Murphy, R. A. Salata, R. L. Guerrant, and E. L. Hewlett, J. Infect. Dis., in press). Amebae lyse target mammalian cells only on direct contact (10, 22, 23); GalNAc inhibits the amebic cytolysis of target cells by inhibiting amebic adherence (23). The GalNAc-inhibitable amebic adhesin was subsequently found to mediate the adherence of amebae to bacteria that were either opsonized with antibacterial antibody or contained GalNAc or galactose in their lipopolysaccharide (3, 4). A soluble GalNAc-inhibitable lectin from *E. histolytica*, which may be identical to the GalNAc-inhibitable adhesin protein on the surface of amebae, has recently been partially purified (Ravdin et al., in press). A chitotriose (*N*-acetyl-D-glucosamine trisaccharide)-inhibitable amebic adhesin can, under certain buffer conditions, mediate amebic adherence to Henle cell monolayers (12) and may be identical to the chitotriose-inhibitable soluble lectin described by Kobiler and Mirelman (11).

* Corresponding author.

The Wistar rat strain has been successfully used in models of colonic amebiasis (24, 26); we studied the carbohydrate sensitivity of the in vitro adherence of amebae to preparations of rat colonic mucosa. Based on these findings we also studied amebic adherence to human tissue.

MATERIALS AND METHODS

Cultivation and radiolabeling of *E. histolytica*. Axenic trophozoites, strain HM1-IMSS, were obtained from the American Type Culture Collection (Rockville, Md.) and have been maintained in our laboratory for 2 years. The amebae were grown in TYI-S-33 medium (Trypticase, yeast extract, iron, serum) as developed by Diamond et al. (7), containing 100 U of penicillin per ml and 100 μg of streptomycin sulfate per ml (GIBCO Laboratories, Grand Island, N.Y.). Axenic cultures were maintained and harvested after 48 to 72 h of growth as described before (22). Amebae (approximately 10^6) were suspended in 4 ml of TYI-S-33 medium in 15-ml sterile glass tubes to which was added 10 μCi of [^{3}H]thymidine (New England Nuclear Corp., Boston, Mass.). The culture tubes were incubated upright at 36°C for 24 h, and the TYI-S-33 medium with [^{3}H]thymidine was removed (amebae adhered to the glass tubes) and replaced with 12 ml of nonradioactive TYI-S-33 medium. The tubes were incubated at 36°C in a horizontal position for an additional 24 h. To decrease the spontaneous release of [^{3}H]thymidine before use in adherence assays, amebae were washed twice in TYI-S-33 and were placed in fresh medium for an additional 2 h. The amebae were then washed, counted in a hemocytometer chamber, and adjusted to 2×10^5 amebae per ml in TYI medium (without serum).

Preparation of colonic tissue for study. After a 24-h fast, 21-day-old Wistar rats (Hilltop Laboratories, Dublin, Va.)

TABLE 1. Inhibition by GalNAc or asialofetuin of the adherence of amebae to fixed rat colonic mucosa

Medium	%[^{3}H]thymidine-labeled amebae adherent after 30 min at 37°C (total no. of amebae tested)	% Inhibition
Control	20.0 ± 1.16 (48)	
With GalNAc (g%)		
0.01	17.9 ± 1.78[a] (15)	10.5
0.05	13.1 ± 2.04[b] (15)	34.5
0.10	12.9 ± 1.56[c] (16)	35.5
0.50	11.3 ± 1.25[d] (16)	43.5
1.00	11.6 ± 0.85[d] (28)	42.0
Control[e]	20.5 ± 2.13 (5)	
With asialofetuin (g%)		
0.01	16.9 ± 1.89[f] (5)	17.6
0.05	12.2 ± 0.95[g] (5)	40.5

[a] $P = 0.630$ compared with control medium.
[b] $P = 0.038$ compared with control medium.
[c] $P = 0.0023$ compared with control medium.
[d] $P < 0.001$ compared with control medium.
[e] Paired control for asialofetuin studies.
[f] $P = 0.034$ compared with control medium.
[g] $P = 0.0054$ compared with control medium.

had laparotomies performed under penthrane (Abbott Laboratories, North Chicago, Ill.) anesthesia. A 15-cm midline incision was made, the cecum was mobilized, and a clamp was placed on the cecum-colon junction. The entire colon was removed, cut into 15-mm sections, and placed in a cold Dulbecco phosphate-buffered saline (PBS; GIBCO). Rats were immediately sacrificed by overdosage with penthrane after colectomy.

The colon was everted by grasping one end with an eye dressing forceps and inserting the forceps through the lumen, taking care to handle only the terminal edge of the tissue and not to disturb the mucosa. The everted colonic section was swirled in a beaker of PBS (20 ml) to remove fecal debris and then cut into 3-mm lengths. Terminal edges of tissue that had been handled were cut off and discarded, and 3-mm sections were either placed in cold PBS for immediate use as unfixed colonic mucosal sections or put in PBS containing 1% glutaraldehyde for fixation over 24 h at 4°C before use as fixed rat colonic mucosa. In some studies, unfixed tissue was placed in 0.25% trypsin (GIBCO) in PBS at 37°C for 30 min and washed, and then trypsin inhibitor (Sigma Chemical Co., St. Louis, Mo.) was added. The tissue was washed in PBS before use. Unfixed rat submucosa was prepared by stripping mucosa from colonic samples, which were used directly without everting so as to be submucosal side out, and cutting the tissue into 3-mm sections.

Human tissue was obtained from surgical specimens of grossly normal colon (which were to be discarded) from patients requiring colectomy for colonic carcinoma; the carcinoma was not contiguous to the experimental sections. The full-thickness colonic sections were placed in cold PBS, and the colonic mucosa was immediately stripped from the underlying submucosa after careful dissection of the two tissue planes. Stripped human mucosa was placed on wet filter paper (Whatman no. 1; Whatman, Inc., Clifton, N.J.) and cut into circles 8 mm in diameter with a specially constructed cylindrical steel blade through which a piston was pushed. Tissue was deposited (with filter paper attached to the submucosal side) in a glutaraldehyde (1%)-PBS solution for fixation at 4°C over 24 h.

Adherence of [^{3}H]thymidine-labeled amebae to mammalian colonic mucosa. In initial studies with fixed everted sections of rat colon, the tissue was placed at the bottom of a plastic tube in TYI medium without serum, amebae were added, and the tube was centrifuged at 50 × *g* for 5 min, and then incubated at 37°C for 30 min. Using more delicate unfixed rat tissue, amebae were allowed to settle on the mucosa during incubation at 37°C for 60 min. After inoculation, the tissue was removed with forceps from the test medium and washed twice by dipping in 1.5 ml of fresh TYI medium and then placed in 3 ml of scintillation fluid in 5-ml scintillation vials. The original test supernatant, both washes, and the tissue were then all counted separately for ^{3}H activity in a scintillation counter. [^{3}H]thymidine-labeled amebae without tissue were concurrently studied under identical test conditions to provide spontaneous release data. Percent adherent amebae was calculated by the formula: % adherent amebae = [(tissue counts) × (1 + fractional spontaneous release)/(tissue counts) + (supernatant and wash counts)] × 100.

Studies with human tissue were performed by using semisolid warm TYI medium with agar (5.0%) to create a flat base in the round-bottomed plastic tubes; circular pieces of tissue were placed submucosal side down in semisolid warm agar, which was allowed to cool, thus the tissue was mounted mucosal side up. TYI medium plus amebae was placed on top of the tissue, and the amebae were allowed to settle for 60 min at 37°C.

In some studies carbohydrates were added to the test medium, including GalNAc, galactose, *N*-acetyl-D-glucosamine, α-methyl mannoside, D(+)fucose, lactose, asialofetuin, and fetuin (all from Sigma). The other sugars tested included D-mannose (Difco) and dextrose (Fisher Scientific Co., Pittsburgh, Pa.). Amebae were incubated with the carbohydrates at 4°C for 20 min before exposure to colonic mucosal tissue.

Human immune serum was kindly provided by George Healy, Parasitology, Centers for Disease Control, which had an antiamebic antibody titer of 1:1,024 by indirect hemagglutination. Rabbit immune serum was prepared by immunization with 100 μg of lyophilized, partially purified, amebic GAlNAc-inhibitable lectin obtained by gel filtration of a soluble fraction of amebic sonicate (Ravdin et al., in press). New Zealand White rabbits (University of Virginia Vivarium) were injected intramuscularly with 100 μg of amebic protein suspended in Freund complete adjuvant (1 ml) (GIBCO); subsequent booster injections at 2, 4, and 8 weeks were in Freund incomplete adjuvant (GIBCO). Rabbits were bled at 10 weeks to obtain rabbit immune serum; control serum was obtained before initial immunization. Amebae were incubated in immune or nonimmune serum at 4°C for 20 min, then were vortexed to disrupt aggregates of amebae (confirmed microscopically) before being placed on the colonic mucosal tissue.

Statistics. Studies are expressed as the mean ± the standard error of the mean and were compared by the paired or unpaired Student *t* test as appropriate.

RESULTS

[^{3}H]thymidine-labeled axenic amebic trophozoites (strain HM1-IMSS) adhered to 3-mm sections of glutaraldehyde (1.0%)-fixed rat colonic mucosa (20.0% adherent after 30 min at 37°C). Optimal adherence of labeled amebic trophozo-

TABLE 2. Inhibition by GalNAc of the adherence of amebae to fixed human colonic mucosa

Medium	%[^{3}H]thymidine-labeled amebae adherent after 60 min at 37°C (total no. of studies)	% Inhibition
Control	19.0 ± 1.71 (6)	
With GalNac (%)		
0.1	12.5 ± 1.56[a] (6)	34.2
1.0	8.3 ± 2.20[b] (6)	56.3

[a] $P = 0.039$ compared with control medium.
[b] $P < 0.001$ compared with control medium.

ites to fixed rat colon mucosa occurred at 2×10^5 amebae per ml. GalNAc (0.1 to 1.0 g% or 4.5 to 45 mM) inhibited amebic adherence to fixed rat colonic mucosa (Table 1). Galactose (1.0 g% or 56 mM, the only concentration studied) was also inhibitory (by 43.3%, $P < 0.01$). Other monosaccharides studied (all at 1.0 g%), including *N*-acetyl-D-glucosamine, fucose, mannose, α-methyl mannoside, lactose, and dextrose, were not inhibitory. Asialofetuin, which has three terminal β-1-4-linked galactose residues, inhibited amebic adherence to fixed rat colonic mucosa at concentrations as low as 0.01 g% ($P \leq 0.034$; Table 1). The terminal galactose residues appear to be responsible for this inhibitory effect, since fetuin (in which the galactose is linked to terminal sialic acid residues) had no effect on the adherence of [^{3}H]thymidine-labeled amebae to fixed rat colonic mucosa (0.5 g%, 99.9% of control adherence, 9 studies).

The adherence of [^{3}H]thymidine-labeled amebae to human colonic mucosa was performed by taking 8-mm-diameter punched out circles of normal human colonic mucosa stripped from the underlying serosa and embedding fixed tissue mucosal side up in TYI medium with 5% agar. GalNAc (0.1 and 1.0 g%) inhibited amebic adherence to glutaraldehyde (1.0 g%)-fixed human colonic mucosa ($P \leq 0.039$; Table 2), confirming the relevance of the rat model.

The adherence of amebae to unfixed rat colonic mucosa was studied by immediately placing sections of everted rat colon in TYI medium, adding [^{3}H]thymidine-labeled amebae and studying adherence after 60 min at 37°C (Fig. 1). Rat colonic tissue remained morphologically intact during this period. In paired studies, GalNAc (1.0 g%) had no effect on the adherence of amebae to unfixed rat colonic mucosa (91.4% of control, 27 studies, $P > 0.1$). However, if the rat colonic mucosa was exposed to trypsin (0.25%) for 30 min at 37°C, followed by the addition of trypsin inhibitor and washing, the adherence of amebae to the trypsin-exposed rat mucosa was equal to that seen with nonexposed, unfixed tissue and was GalNAc-inhibitable (0.1 to 1.0 g%, $P \leq 0.021$; Table 3). The adherence of amebae to intact, unfixed rat submucosa was also inhibitable by GalNAc (1.0 g%) (37.8% inhibition, $P < 0.05$).

The amebic GalNAc-inhibitable adhesin protein is known

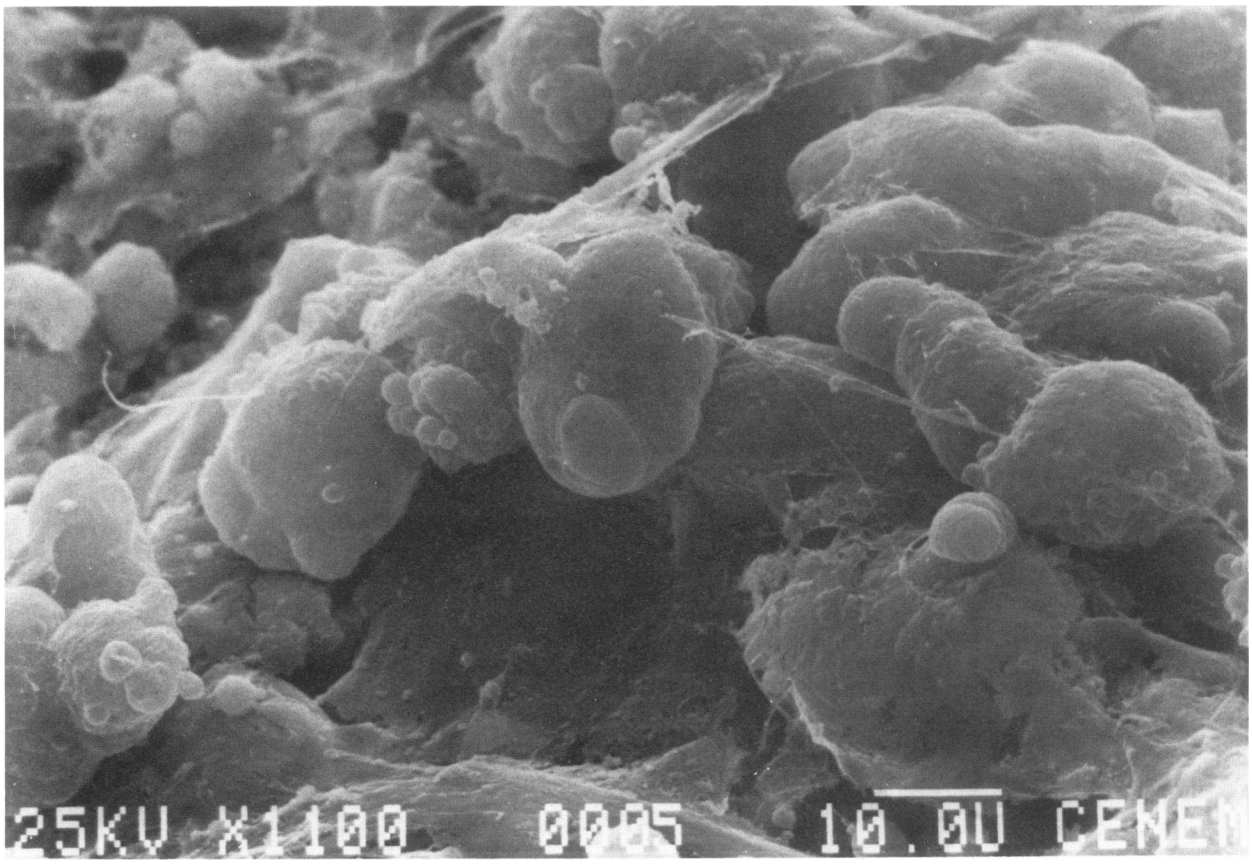

FIG. 1. Scanning electron micrograph of amebic trophozoites adherent to unfixed rat mucosa. Note strands of material, possibly mucus, adherent to amebae.

to be glutaraldehyde sensitive (4; J. I. Ravdin, unpublished). The adherence of amebae fixed with 1% glutaraldehyde to unfixed rat colonic mucosa was equal to that of control viable amebae (92.3%, 6 studies). However, glutaraldehyde-fixed amebae exhibited markedly decreased adherence to trypsin-treated, unfixed tissue or fixed colonic mucosa (36.9 and 50.9% of control, respectively, $P \leq 0.01$). These studies indicated that there is an adhesin on the surface of the rat colonic mucosa, which is both glutaraldehyde and trypsin sensitive, and which binds either fixed or unfixed amebae by a GalNac-insensitive mechanism. The amebic GalNAc-inhibitable adhesin was operative in adherence to fixed rat mucosa, trypsin-treated rat mucosa, and unfixed rat submucosa.

Axenic amebae have been shown at 37°C to rapidly and repeatedly shed or ingest attached antiamebic antibody (5). We evaluated whether antiamebic antibody could inhibit amebic adherence to rat colonic mucosa in our in vitro model. Heat-inactivated human immune serum was used from a patient with amebic liver abscess, who had an antiamebic indirect hemagglutination antibody titer of 1:1,024. Rabbit immune serum was prepared by injection with partially purified preparations of the soluble GalNAc-inhibitable amebic lectin prepared by gel filtration chromatography of a soluble fraction of amebic sonicate. Rabbit immune serum was positive by Ouchterlony immunodiffusion and direct immunofluorescence with amebic trophozoites. Both human (10%) and rabbit (5%) immune sera inhibited amebic adherence to glutaraldehyde-fixed rat colonic mucosa ($P < 0.01$; Table 4), suggesting that antibody may have a role in decreasing the adherence of amebae to the colonic mucosa in vivo.

DISCUSSION

The study indicated that under certain conditions the amebic GalNAc-inhibitable adhesin is operative in the in vitro adherence of amebae to their in vivo target tissue, the colonic mucosa. We found evidence for a glutaraldehyde- and trypsin-sensitive host adhesin on rat colonic mucosa, probably in mucus, that binds amebae independently of the amebic GalNAc-inhibitable adhesin molecule. In addition, serum containing antiamebic antibody can reduce amebic adherence to fixed rat colonic mucosa.

TABLE 3. Inhibition by GalNAc of adherence of amebae to trypsin-exposed unfixed rat colonic mucosa

Tissue	Medium	%[^{3}H]thymidine-labeled amebae adherent after 60 min at 37°C (total no. of studies)	% Inhibition
Normal	Control	40.9 ± 2.83 (15)	
	With GalNAc (1.0%)	36.7 ± 3.27 (15)	10.3
Trypsin-exposed (0.25%)	Control	39.9 ± 3.02 (15)	
	With GalNac (%)		
	0.1	32.4 ± 7.15[a] (8)	18.8
	0.5	29.1 ± 9.56[b] (8)	27.1
	1.0	28.9 ± 3.04[b] (15)	27.6

[a] $P = 0.021$ compared with control medium.
[b] $P < 0.001$ compared with control medium.

TABLE 4. Inhibition by human and rabbit immune serum of adherence of amebae to fixed rat colonic mucosa

Medium	%[^{3}H]thymidine-labeled amebae adherent after 60 min at 37°C (total no. of studies)	% Inhibition
Control	22.7 ± 1.59 (11)	
Human serum (control) (10%)	24.5 ± 2.25 (11)	−7.9
Human immune serum (10%)	16.4 ± 1.86[a] (11)	27.8
Control[b]	19.3 ± 2.15 (8)	
Rabbit serum (control) (5%)	19.0 ± 1.17 (8)	2.0
Rabbit immune serum (5%)	15.1 ± 0.96[a] (8)	22.1

[a] $P < 0.01$ compared with control serum.
[b] Paired controls for rabbit serum studies.

This is the first report of a truly quantitative in vitro model of adherence of radiolabeled amebae to a mammalian colonic mucosa, especially including human tissue. Previous studies of in vitro adherence events by *E. histolytica* utilized rosetting techniques with CHO cells, human erythrocytes (11, 23), human neutrophils (Ravdin et al., in press) the adherence of [^{35}S]cystine-labeled amebae to fixed Henle cell monolayers (12), the adherence of amebae to MDCK cells or guinea pig intestine judged by microscopic counts (20), and the adherence or ingestion of bacteria by amebae after separation in a Percoll gradient (3). Axenic amebic trophozoites were easily labeled with [^{3}H]thymidine with low levels of spontaneous release, providing a quantitative method of measuring adherence with relatively small numbers of amebae. This methodology was applied to the first report of the adherence of amebae to fixed human colonic mucosa stripped from the underlying serosa. Such studies will be applicable to future investigations utilizing viable human tissue to evaluate the efficacy of more specific antiamebic antibodies and the importance of human nonimmune host defenses such as mucus.

The inhibition of amebic adherence to fixed rat colonic mucosa by GalNAc monomers and by even lower concentrations of asialofetuin indicated that adherence was mediated by the amebic adhesin, which is inhibited by GalNAc or galactose residues; this surface adhesin has been described in previous studies of amebic adherence to CHO cells and human leukocytes (Ravdin et al., in press). The relevance of the rat model was supported by identical findings with fixed human tissue. The effective inhibition observed with low concentrations of asialofetuin is consistent with studies of the adherence of amebae to CHO cells (Ravdin et al., in press) and indicates a greater specificity of the amebic adhesin for the three-terminal galactose residues as presented on a large molecule (asialofetuin) compared with GalNAc or with galactose monomers. The exact receptor for the amebic adhesin on the fixed colonic mucosa, trypsin-treated unfixed mucosa, and unfixed rat submucosa was not determined by these studies. Both human and rat mucus is rich in GalNAc (21); in addition, studies utilizing lectin probes have indicated the presence of GalNAc on the surface of rat intestinal mucosal cells and human rectal cells (2, 8). Although amebae were rinsed before use and studied in antibiotic-containing medium, bacteria adherent to the surface of the colonic mucosal samples may theoretically have enhanced amebic adherence (3, 4). GalNAc or asialofetuin provided only partial inhibition with the various tissue preparations studied. We believe this is due to nonspecific factors accounting for a portion of the adherence observed in

our in vitro tests. This hypothesis is supported by the observation that after gluteraldehyde fixation of the amebae, which inactivates known amebic adhesins (4, 12, 15), adherence to trypsin-treated or fixed rat mucosa was at 30 to 50% of the levels observed with viable amebae.

The adherence of amebae to unfixed rat colonic mucosa was not GalNAc inhibitable; however, after trypsin treatment of the mucosal surface, amebic adherence was GalNAc inhibitable. *E. histolytica* characteristically causes flask-shaped colonic ulcerations by extension into the submucosa; the adherence of amebae to unfixed rat submucosa was also mediated by the amebic GalNAc-inhibitible adhesin. These studies suggested that a gluteraldehyde- and trypsin-sensitive substance on the rat colonic mucosal surface, probably in mucus, binds amebae in a GalNAc-insensitive manner. This substance may be similar to the fucose-sensitive lectin in guinea pig intestinal mucus that binds bacteria as described by Mirelman and co-workers (19). Fucose (1.0%) did not inhibit amebic adherence to intact rat colonic mucosa. It is unlikely that an additional amebic adhesin, distinct from the GalNAc-inhibitable adhesin, was operative in adherence to intact rat colonic mucosa, since neither *N*-actyl-D-glucosamine nor glutaraldehyde fixation of amebae inhibited this adherence event. If the trypsin- and glutaraldehyde-sensitive binding protein on rat colonic mucosa was in mucus, it may have served as host defense mechanism preventing amebae from directly contacting the mucosal cell. The increased binding of other intestinal parasites by mucus has been associated with more rapid expulsion in animal models (14). Leitch et al. (13) observed that when amebae were injected into loops of rat colon, they appeared to be rapidly absorbed by the colonic mucus blanket, became immobile, and were sloughed off with mucus fragments. It is possible that only amebae that evade this host defense mechanism or inhibit the production of mucus are able to attach to the mucosa by their GalNAc-inhibitable adhesin and then initiate cytolytic activity (23) or release various contact-dependent or -independent proteases (6, 16, 18) to damage the mucosa. In addition, the ability of amebae to initiate an inflammatory response and to lyse neutrophils (10; Ravdin et al., in press) may also contribute to the disruption of the colonic mucosa by releasing toxic neutrophil products (28). Determination of the presence of such a binding moiety in humans awaits studies with viable human tissue and human mucus.

The adherence of amebae at 37°C to fixed rat colonic mucosa was partially inhibited by heat-inactivated human and rabbit immune serum. Both sera contained immunoglobulin G antibodies which bound to the surface of amebae by immunofluorescence. Despite the well described ability of amebae to shed or ingest bound antibody at 37°C (1, 5), there was still an inhibitory effect. Coproantibodies to *E. histolytica* have been demonstrated in individuals with amebic colitis, although they may be of a different antibody class (immunoglobulin A) than serum antibodies (17, 25). However, our findings suggest that the development of antiamebic antibodies in the gut may contribute to the prevention of recurrent invasion of the colonic mucosa by *E. histolytica* trophozoites.

Further in vitro study of the mechanisms of amebic adherence to the in vivo target tissue, the human colonic mucosa, will aid in elucidating the parasite adhesin molecules operative in the pathogenesis of human amebiasis and the nonimmune and immune mechanisms of host defense in the colon. The eventual development of a means to augment host defenses and prevent invasive amebiasis is a potential result of such studies.

ACKNOWLEDGMENTS

This work was supported by a grant from the Edna McConnell Clark Foundation and grants to J. I. Ravdin from the National Institute of Allergy and Infectious Diseases (TMP R23 AI 18841) and the John A. Hartford Foundation. J. I. Ravdin is a Hartford Foundation fellow. The Division of Geographic Medicine is supported by the Rockefeller Foundation.

We thank M. C. Wilhelm for obtaining surgical specimens of human tissue, and Barbara Norden, Nancy Malone, and Kathy May for secretarial assistance.

LITERATURE CITED

1. **Aust-Kettis, A., and K. G. Sundquist.** 1978. Dynamics of the interaction between *Entamoeba histolytica* and components of the immune response. Scand. J. Immunol. **7**:35–44.
2. **Boland, C. R., C. K. Montgomery, and Y. S. Kim.** 1982. Alterations in human colonic mucin occurring with cellular differentiation and malignant transformation. Proc. Natl. Acad. Sci. U.S.A. **279**:2051–2055.
3. **Bracha, R., D. Kobiler, and D. Mirelman.** 1982. Attachment and ingestion of bacteria by trophozoites of *Entamoeba histolytica*. Infect. Immun. **36**:396–406.
4. **Bracha, R., and D. Mirelman.** 1983. Adherence and ingestion of *Escherichia coli* serotype 055 by trophozoites of *Entamoeba histolytica*. Infect. Immun. **40**:882–887.
5. **Calderon, J., M. A. de Lourdes Munoz, and H. M. Acosta.** 1980. Surface redistribution and release of antibody-induced caps in *Entamoeba*. J. Exp. Med. **151**:184–193.
6. **De Lordes-Munoz, M., J. Calderon, and M. Rojikind.** 1982. The collagenase of *Entamoeba histolytica*. J. Exp. Med. **155**:42–51.
7. **Diamond, L. S., D. R. 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. **72**:431–432.
8. **Essner, E., J. Schreiber, and R. A. Griewski.** 1978. Localization of carbohydrate components in rat colon with fluoresceinated lectins. J. Histochem. Cytochem. **26**:452–458.
9. **Galindo, J. M., A. Martinez-Palomo, and B. Chavez.** 1978. Interacion entre *Entamoeba histolytica* y el epitelio cecal del cobayo. Arch. Invest. Med. **9**(Suppl. 1):261–274.
10. **Guerrant, R. L., J. Brush, J. I. Ravdin, J. A. Sullivan, and G. L. Mandell.** 1981. Interaction between *Entamoeba histolytica* and human polymorphonuclear neutrophils. J. Infect. Dis. **143**:83–93.
11. **Kobiler, D., and D. Mirelman.** 1980. Lectin activity in *Entamoeba histolytica* trophozoites. Infect. Immun. **29**:221–225.
12. **Kobiler, D., and D. Mirelman.** Adhesion of *Entamoeba histolytica* trophozoites to monolayers of human cells. J. Infect. Dis. **144**:539–546.
13. **Leitch, G. J., A. D. Dickey, I. A. Udezulu, and G. B. Bailey.** 1985. *Entamoeba histolytica* trophozoites in the lumen and mucus blanket of rat colons studied in vivo. Infect. Immun. **47**:68–73.
14. **Levy, D. A., and C. Frondoza.** 1983. Immunity to intestinal parasites: role of mast cells and goblet cells. FASEB Monogr. **42**:1750–1755.
15. **Lopez-Revilla, R., and R. Cano-Mancera.** 1982. Adhesion of *Entamoeba histolytica* trophozoites to human erythrocytes. Infect. Immun. **37**:281–285.
16. **Lushbaugh, W. B., A. B. Kairalla, J. R. Cantey, A. F. Hofbauer, and F. E. Pittman.** 1979. Isolation of a cytotoxin-enterotoxin from *Entamoeba histolytica*. J. Infect. Dis. **139**:9–17.
17. **Mahajan, R. C., S. C. Agarwal, P. N. Chuttani, and W. L. Chitkarn.** 1972. Coproantibodies in intestinal amoebiasis. Indian J. Med. Res. **60**:547–550.
18. **McGowan, K., C. F. Deneke, G. M. Thorne, and S. L. Gorbach.** 1982. *Entamoeba histolytica* cytotoxin: purification, characterization, strain virulence, and protease activity. J. Infect. Dis. **146**:616–625.
19. **Mirelman, D., M. Ishar, and Y. Eshdat.** 1983. Carbohydrate recognition mechanisms which mediate microbial adherence to mammalian mucosal surfaces. Tokai J. Exp. Clin. Med. **7**: 177–183.

20. **Orozco, M. E., G. Guarneros, A. Martinez-Palomo, A. G. Robles, and J. M. Galindo.** 1982. Las interacciones entre lectina y receptor median la adherencia de *E. histolytica* a celulas epitheliales. Relacion de la adhesion con la virulencia de las cepas. Arch. Invest. Med. **13**(Suppl. 3):159–167.
21. **Podolsky, D. K., and K. J. Isselbacher.** 1983. Composition of human colonic mucin. J. Clin. Invest. **72**:142–153.
22. **Ravdin, J. I., B. Y. Croft, and R. L. Guerrant.** 1980. Cytopathogenic mechanisms of *Entamoeba histolytica*. J. Exp. Med. **152**:377–390.
23. **Ravdin, J. I., and R. L. Guerrant.** 1981. Role of adherence in cytopathogenic mechanisms of *Entamoeba histolytica*. J. Clin. Invest. **68**:1305–1313.
24. **Ross, G. W., and R. Knight.** 1973. Dietary factors affecting the pathogenicity of *Entamoeba histolytica* in rats. Trans. R. Soc. Trop. Med. Hyg. **67**:560–567.
25. **Shaalan, M., and R. P. Baker.** 1970. Detection of coproantibodies in amebiasis of the colon: a preliminary report. Am. J. Clin. Pathol. **54**:615–617.
26. **Singh, B. N., R. V. N. Srivastava, and G. P. Dutta.** 1971. Virulence of strains of *Entamoeba histolytica* to rats and the effect of cholesterol, rat caecal and hamster liver passage on the virulence of noninvasive strains. Indian J. Exp. Biol. **9**:21–27.
27. **Takeuchi, A., and B. P. Phillips.** 1975. Electron microscope studies of experimental *Entamoeba histolytica* infection in the guinea pig. I. Penetration of the intestinal epithelium by trophozoites. Am. J. Trop. Med. Hyg. **23**:34–48.
28. **Tsutsumi, V., R. Mena-Lopez, R. Anaya-Velazquez, and A. Martinez-Palomo.** 1984. Cellular bases of experimental amebic liver abscess formation. Amer. J. Pathol. **117**:81–91.