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# MONOCLONAL ANTIBODIES DIRECTED AGAINST THE GALACTOSE-BINDING LECTIN OF *Entamoeba histolytica* ENHANCE ADHERENCE[1]

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**The *Entamoeba histolytica* galactose-binding lectin is a surface glycoprotein composed of 170- and 35-kDa subunits. Inhibition of this lectin with galactose or anti-170 kDa subunit polyclonal antibody blocks amebic adherence to target cells and colonic mucin glycoproteins. We describe the properties of 10 mAb with specificity for the 170-kDa subunit. Based on competitive binding studies, six nonoverlapping antigenic determinants on the lectin were identified. The effect of the mAb on adherence of amebic trophozoites to both Chinese hamster ovary (CHO) cells and human colonic mucins was measured. Antilectin antibodies directed against epitopes 1 and 2 enhanced adherence, with the number of amebae having at least three adherent CHO cells increasing with the addition of epitope 1 mAb from 26 ± 9 to 88 ± 2% and the binding of colonic mucins innreasing from 34 ± 1 to 164 ± 3 pg/$10^5$ amebae. Antibody-enhanced adherence remained 90 to 100% galactose inhibitable, occurred at 4°C and was not Fc mediated. Univalent Fab fragments of epitope 1 mAb augmented mucin binding by 238% and CHO cell adherence by 338%. The binding of purified lectin to CHO cells was increased from 1.1 ± 0.1 to 2.4 ± 0.3 ng/$10^3$ CHO cells by mAb directed to epitope 1, demonstrating that enhanced adherence was due to direct activation of the lectin. mAb to epitope 3 bound to the lectin only upon its solubilization from the membrane and had no effect on adherence. Adherence to CHO cells and mucins was inhibited from 50 to 75% by mAb to epitopes 4 and 5; epitope 6 mAb inhibited amebic adherence to CHO cells but not mucins. The pooled sera from 10 patients with amebic liver abscess blocked the binding to the 170-kDa subunit of mAb directed to all six epitopes. Striking individual variations in the effects of immune sera on adherence were observed. Although the sera of all 44 South African patients with amebic liver abscess had high titer anti-lectin antibodies, 16 patients' sera significantly (more than 3 SEM) enhanced adherence whereas 25 patients' sera significantly inhibited adherence. Antilectin antibodies exert profound functional effects on the interaction of *E. histolytica* with target cells and human colonic mucins. Exploration of the clinical consequences of adherence-enhancing and inhibitory antibody responses may give insight into the role of antilectin antibodies in immunity to invasive amebiasis.**

Amebiasis is a prevalent worldwide disease caused by the cytolytic protozoan *Entamoeba histolytica*. Currently there is no means available to prevent the approximately 100,000 deaths per year resulting from complications of amebic liver abscess and colitis. *E. histolytica* adherence to host cells is an attractive area for therapeutic intervention in this disease. Colonic biopsies of patients with amebiasis have shown amebae adherent to the mucus layer of the colon and associated with focal ulcerations of the colonic epithelium in areas of mucus depletion (1–4). Animal models have shown that adherence of *E. histolytica* trophozoites to intestinal mucus and epithelial cells is followed by invasion of the colonic epithelium (5, 6). Amebic adherence to human colonic mucins and CHO[3] cells in vitro is mediated by a galactose and N-acetyl-D-galactosamine-inhibitable cell surface lectin. Inhibition of the adherence lectin with galactose prevents amebic contact-dependent killing of host cells (7).

The adherence lectin has been purified from a pathogenic strain of *E. histolytica* by either galactose or mAb affinity chromatography (8). It is a heterodimer of heavy (170 kDa) and light (35 kDa) glycoproteins linked by disulfide bonds (9). The purified adherence lectin retains the ability to bind to CHO cells in a galactose-inhibitable manner and competitively inhibits amebic adherence to CHO cells. The 170-kDa subunit is an antigenically conserved and immunodominant Ag, whereas an immune response has not been detected to the 35-kDa subunit (10).

In our study 10 mAb prepared against the purified amebic lectin were used to map antigenic sites on the heavy subunit. Adherence to CHO cells and human colonic mucins (the relevant receptor in the initial patho-

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[3] Abbreviations used in this paper: CHO cell, Chinese hamster ovary cell; M199s, medium M199 supplemented with HEPES, cysteine, BSA, and serum; BSA PBS-T, BSA in PBS, pH 7.2, containing 0.05% Tween 20.

genesis of invasive amebiasis) were measured in the presence of the mAb. The results show that the mAb bound to six epitopes and defined distinct functional domains of the heavy subunit, and that a human antibody response was mounted to these domains during amebic infection. An unexpected result was the enhancement of adherence by mAb directed against epitopes 1 and 2 of the heavy subunit of the lectin. Dramatic individual variations in the effects of immune sera on adherence were seen in patients with amebic liver abscess.

## MATERIALS AND METHODS

*Cultivation and harvesting of E. histolytica and CHO cells.* Axenic *E. histolytica*, pathogenic strain HM1-IMSS, were grown in medium TYI-S-33 (trypticase yeast extract, iron, and serum) with 100 U/ml of penicillin and 100 μg/ml of streptomycin sulfate (Pfizer, Inc., New York, NY) at 37°C in 250-ml plastic tissue culture flasks (11). Amebae were harvested after 72 h of growth by centrifugation at 150 × *g* for 5 min at 4°C and washed twice in ice-cold 75 mM Tris (Sigma Chemical Co., St. Louis, MO) 65 mM NaCl, pH 7.2 (7).

CHO cells cultured included the wild type Gat-[2] parent and the Lec2 glycosylation mutant (12). The CHO cells were grown in MEM α-medium (GIBCO, Grand Island, NY) supplemented with 10% FBS (GIBCO), penicillin (100 U/ml), and streptomycin (100 μg/ml). For adherence assays CHO cells were released from the monolayer by trypsinization (7).

*Adherence of E. histolytica trophozoites to CHO cells.* The measurement of *E. histolytica* trophozoite adherence to CHO cells was performed as previously described (7). Briefly, trophozoites ($1 \times 10^4$) and CHO cells ($2 \times 10^5$) were suspended together at 4°C in M199 media (GIBCO) containing 25 mM Hepes pH 6.8 (Sigma), 5.7 mM cysteine, 0.5% BSA, and 10% heat-inactivated adult bovine serum (M199s). The trophozoites and CHO cells were centrifuged together at 150 × *g* for 5 min and then incubated at 4°C for 2 h. Adherence was measured as the number of amebae having at least three adherent CHO cells upon vortex resuspension of the cellular pellet with at least 50 amebae counted per tube. Adherence was expressed as the percent of adherence in paired studies performed in control medium. Galactose was obtained from Pfanstiehl Laboratories, Waukegan, IL. The effect of antibodies on CHO cell adherence was measured by preincubating amebae on ice with purified mAb (10 μg/$10^4$ amebae) or human sera (1/100 dilution) in M199s for 60 min before measuring CHO cell adherence.

*Purification of human colonic mucin glycoproteins.* Colonic mucins were purified as previously described (13). Human colonic tissues (left and transverse colons) were obtained from patients requiring colectomy for localized colonic carcinoma. Mucosal scrapings were taken 5 to 10 cm on either side of the carcinomas, and histologic examination confirmed that only normal mucosa was used. Specimens were received within 60 min after removal and placed on ice. After vigorous scraping of the colonic mucosa in cold (4°C) PBS, the mucus glycoproteins were solubilized by vigorous vortexing for 10 min in cold PBS. The preparation was centrifuged three times (30,000 × *g*) at 4°C for 15 min and dialyzed (1000 Da exclusion) extensively against deionized water. The crude mucus preparation was applied to Sepharose 4B (1.5 × 30 cm column) (Bio-Rad Laboratories, Richmond, VA) equilibrated in 0.01 M Tris HCl.

The high m.w. mucin glycoprotein carbohydrate containing fractions from the Sepharose 4B column chromatography was digested with 100 μg/ml bovine DNase I and bovine RNase III (Sigma) to remove nucleic acid contaminants. After 14-h digestion at room temperature in 5.0 ml PBS (pH 7.4, containing 0.02% (w/v) $NaN_3$ and 1 mM $MgSO_4$), the digest mixture was centrifuged at 15,000 × *g* for 30 min to remove the resultant flocculent precipitate and the supernatant then dialyzed against PBS (4°C) for 24 h. The dialyzed material was then diluted with PBS and cesium chloride (Sigma) added to make a 59% (w/v) solution (starting density, 1.42 g/ml). The sample (10 ml) was distributed in polyallomer centrifuge tubes (14 × 89 mm; Beckman Instruments, Inc., Palo Alto, CA) and ultracentrifuged in a SW41 rotor at 250,000 × *g* for 48 h at 4°C. After centrifugation, the bottoms of the tubes were pierced and eight fractions of equal volume were collected in preweighed test tubes for density determination. Fractions were dialyzed (10,000 Da exclusion) exhaustively against deionized water for 24 h and then assayed for protein (14), nucleic acid absorbance at 260 nm, and carbohydrate by the phenol-$H_2SO_4$ method (15). Fractions 6 and 7 from the bottom of the CsCl gradient, containing 1000-fold enriched human colonic mucin glycoproteins, were used to measure amebic adherence to mucins (13).

*Adherence of E. histolytica to human colonic mucins.* Purified mucin glycoproteins (CsCl fraction 6 and 7 human mucin) were radiolabeled with $Na^{125}I$ (New England Nuclear, Boston, MA) using iodo-beads (Pierce Chemical Co., Rockford, IL) at room temperature for 15 min. The iodinated mucins were isolated by chromatography on Sephadex G25 columns (Isolab Inc., Akron, OH). TCA (Sigma) (10%) precipitable $^{125}I$ counts were >90%. Binding of colonic mucin to amebae was studied in M199s at $10^5$ amebae/ml at 4°C. After incubation with the labeled mucin, amebae were sedimented (300 × *g*, 5 min at 4°C), the supernatant discarded, and 100 μl M199s added. The cells were layered over oil (0.25 ml, four parts silicon oil (Accumetric Inc., Elizabeth, KY), one part mineral oil (Sigma)) and microfuged for 1 min (9000 × *g*). The tips of the tubes containing the cellular pellet were cut off with a scalpel and $^{125}I$ activity counted in a gamma counter (Beckman Instruments, Inc., Fullerton, CA). Specific binding of mucin to amebae was determined by adding galactose (55 mM). The effect of mAb was measured by preincubating amebae on ice with purified mAb (1 μg antibody/$10^4$ amebae) for 60 min before measuring mucin binding.

*Purification of lectin.* Amebic trophozoites harvested from a 72-h culture (four 250-ml flasks) were preincubated on ice in 5 ml of 75 mM Tris, 65 mM NaCl with a 1/1000 dilution of diisopropylfluorophosphate (Sigma) before solubilization in 10 ml of 150 mM NaCl, 50 mM Tris, pH 8.3, 0.5% Nonidet P-40 (Sigma), 5 mM EDTA (Sigma), and 2 mM PMSF. Extreme care was taken with the use of diisopropylfluorophosphate, including use only in a fume hood with other laboratory personnel present and the antidote (atropine) immediately available. The solubilized amebae were microfuged for 10 min and the supernatant applied at 4°C to a mAb affinity column consisting of 2 mg each of protein A-purified anti-lectin mAb H85, 7F4, 5B8, 3F4, and 6D2 immobilized on 1 to 2 ml of Affi-Gel 10 (Bio-Rad). The supernatant was recirculated through the column with a peristaltic pump overnight, and the column then washed with 15 ml of solubilization buffer followed with 15 ml of PBS (pH 7.5). The bound amebic lectin was eluted with 10 ml of 0.2 N acetic acid, pH 2.5, immediately neutralized by collecting eluted fractions into a 50-ml tube containing 5 ml of 1.5 M Tris pH 8.8, dialyzed overnight against $dH_2O$, frozen and lyophilized.

*Production of mAb.* Detailed protocols for the production of murine anti-lectin mAb have been previously reported (9, 16). The mAb were obtained from two fusions using spleen cells from BALB/c mice receiving injections with purified lectin in complete and then IFA followed by a final boost intrasplenically. Spleen cells from the immunized mice were fused to Sp2/0-Ag14 myeloma cells with polyethylene glycol. Thirty-six mAb were produced from the two fusions that bound to the purified lectin as determined by an ELISA using lectin-coated (1 μg/well) microtiter plates. The 10 mAb that were Western blot positive for the lectin heavy subunit were selected for use in our study; none of the 36 mAb produced recognized the light subunit on Western blot. The isotypes of the mAb were determined by an ELISA using anti-mouse subclass antisera (HyClone Laboratories, Logan, UT). Selected mAb were purified from 50% ammonium sulfate fractions of ascites by preparative isoelectric focusing or protein A affinity chromatography (17). Purified mAb were labeled with $^{125}I$ using the chloramine T technique to a sp. act. of 20 to 40 μCi/μg (18).

*Epitope analysis using mAb.* The epitope specificity of murine mAb was compared by cross-inhibition RIA using $^{125}I$ labeled mAb in a modification of described techniques (17). Polyvinyl chloride microtiter plates (Dynatceh, Alexandria, VA) were coated with 1 μg/well of mAb 3F4 in 0.1 M bicarbonate buffer, pH 9.6, overnight at 4°C, and residual binding sites were "blocked" with 0.1 ml 1% BSA PBS-T. Each well was inbubated with 0.1 ml of a 200 μg/ml solubilized amebic membrane fraction for 2 h, washed five times with PBS-T, and then incubated with cold mAb together with 2 to 5 ng $^{125}I$ labeled mAb for 4 h. After further washing, the plates were dried, and individual wells were counted in a gamma counter (Micromedic 4/200; Horsham, PA). For assays using $^{125}I$ 3F4, lectin was directly bound to microtiter wells or coupled to the solid phase using 1 μg/well 7F4. All assays were carried out in duplicate, and the sample diluent was 1% BSA PBS-T. Uninhibited binding of $^{125}I$ mAb to lectin was calculated from the mean cpm of four wells incubated with PBS-T. The percent inhibition of each of six $^{125}I$ mAb by different cold mAb was calculated as:

$$\frac{\text{mean cpm of PBS control} - \text{mean cpm of cold mAb}}{\text{mean cpm of PBS control}} \times 100\%$$

*Cross-inhibition studies using human sera.* Pooled human immune sera from individuals recovered from amebic liver abscess were obtained from Dr. George Healy at the Centers for Disease Control, Atlanta, GA. Pooled human control sera were from eight individuals who were seronegative for *E. histolytica* as determined by indirect hemagglutination or counter-immunoelectrophoresis

(19). Final dilutions of the control and immune sera were tested for their ability to block the binding of $^{125}$I-labeled mAb to the lectin. Percent inhibition of mAb binding was expressed as described above, except that the control used control sera instead of PBS.

*RIA of lectin.* Polyvinyl chloride microtiter plates (Dynatech, Alexandria, VA) were coated with 1 μg/well of anti-lectin mAb 3F4 in 0.1 M bicarbonate buffer, pH 9.6, overnight at 4°C and residual binding sites blocked with 1% BSA PBS-T. Affinity-purified lectin (1 to 50 ng/well) or amebic extracts were incubated in the antibody-coated wells for 2 h at room temperature. After washing the wells, immobilized lectin was quantified by adding $10^5$ cpm/well of $^{125}$I-labeled anti-lectin mAb 7F4 for 4 h at room temperature. The amount of radioactive 7F4 bound per well was linearly related to lectin concentration from 1 to 25 ng/well (9). Protein concentrations were determined by the BCA protein assay (Pierce Chemical Co., Rockford, IL) using BSA as the standard.

*Adherence of purified lectin to CHO cells.* Lec2 CHO cells were trypsinized, washed in ice-cold MEM α-medium containing 10% FCS followed by Dulbecco's PBS pH 7.4 (Mediatech, Washington, DC). The CHO cells were resuspended to $2.5 \times 10^6$ cells/ml in PBS in presence or absence of 20 mg/ml galactose. Affinity purified lectin (20 μg/ml final concentration) was added to 0.3 ml of the CHO cells on ice for 60 min. The cells were then washed twice with PBS and resuspended in 0.3 ml PBS. Bound lectin was determined by adding $5 \times 10^6$ cpm/ml of $^{125}$I-labeled mAb 7F4 to the CHO cells and separating bound from free $^{125}$I-7F4 by pelleting CHO cells through a 4/1 silicon oil:mineral oil solution (12). The amount of $^{125}$I-7F4 bound to the CHO cells was corrected for $^{125}$I-7F4 binding to CHO cells that had not been incubated with lectin (background binding of $^{125}$I-7F4 was 10% of binding to CHO cells incubated with lectin) and converted to ng lectin by RIA. The effect of mAb 3F4 on lectin binding to CHO cells was measured by including either 5 or 50 μg/ml of protein A purified mAb 3F4 in the incubation mixture.

*Production of Fab fragments of mAb.* Protein A purified mAb (1 mg in 1 ml) were digested with immobilized papain (Pierce) in 20 mM $NaH_2PO_4$, pH 7.0, 20 mM cysteine and 10 mM EDTA at 37°C overnight, with the immobilized papain kept in solution with a rocking platform. After digestion, 3 ml of 10 mM Tris pH 7.5 was added and the supernatant applied to a 5 ml immobilized protein A column. The unbound protein collected in the effluent contained the Fab fragments. No undigested antibody H chain could be detected by SDS-PAGE after digestion with papain.

*Subjects.* Sera were obtained with informed consent within three days of hospitalization from 44 individuals with amebic liver abscess and from 17 uninfected individuals from the Durban and Gazankulu areas of South Africa. The diagnosis of amebic liver abscess was established by clinical presentation, bacteriologically sterile abscess aspirates, positive serologic studies for antiamebic antibodies and by response to specific therapy with metronidazole. All 44 of the amebic liver abscess patients' sera and none of the 17 uninfected individuals' sera contained antibodies to the *E. histolytica* galactose-binding lectin as determined by ELISA and Western blots with affinity-purified lectin (20).

## RESULTS

*Specificity of mAb.* Ten IgG mAb obtained from the two fusions recognized the heavy subunit of the purified lectin from the pathogenic HM-1 strain of *E. histolytica* on Western blots. The lack of mAb that recognized the light subunit reflected that the sera from the mice immunized with the native lectin also recognized solely the heavy subunit (9). These mAb did not bind to components of the TYI-S-33 amebic growth medium nor to the CHO cells used for adherence assays (data not shown). These mAb have previously been demonstrated to immunoprecipitate metabolically labeled lectin (19) and have formed the basis for the radioimmunoassay and the affinity purification of the lectin (9).

*Epitope mapping of lectin heavy subunit.* Epitope specificity was assessed by cross-inhibition RIA using a panel of $^{125}$I-labeled, purified mAb. In the cross-inhibition experiments, lectin was "presented" to the $^{125}$I-labeled mAb by another mAb coated on plastic microtiter wells, and the binding of the radiolabel to lectin competitively inhibited using cold mAb. The results demonstrated that binding of each $^{125}$I-labeled mAb was inhibited by 94 to 98% by cold mAb from the same clone whereas mAb from other epitopes generally inhibited binding by less than 20% (Fig. 1). Epitope 1 included the greatest number of mAb: 3F4, 4F4, 3H6, and 5B8 that were all $IgG_1$ isotypes. Epitope 4 included mAb 8C12 and 6H3 which were also both $IgG_1$ isotypes. The other four epitopes were represented by a single mAb clone.

*Cross-inhibition experiments using human antiamebic immune sera.* To determine if the human antigenic sites on the H chain of the adherence lectin included the six mAb-defined epitopes described, we tested the ability of human immune sera to block $^{125}$I-labeled mAb binding to the adherence lectin. The human immune sera used from the Centers for Disease Control (Atlanta, GA) (PDB-M84) was pooled from 10 patients with amebic liver abscess. The immune sera blocked the binding of mAb to all six epitopes (Fig. 2), control sera inhibited binding by less than 10% (data not shown). A combination of

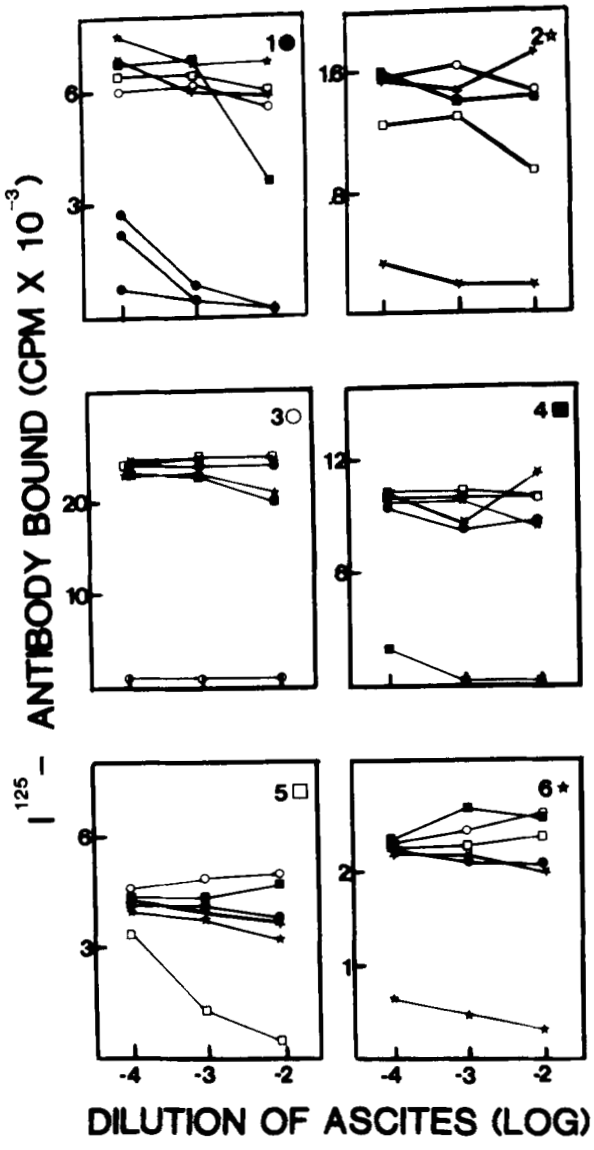


*Figure 1.* Cross-inhibition of the binding of $^{125}$I-labeled mAb to the adherence lectin. Tenfold dilutions of mAb ascites from each of the epitopes were used to inhibit binding of purified $^{125}$I-labeled mAb to adherence lectin-coated microtiter wells (*epitope 1*) or to lectin bound to 3F4-coated wells (*epitopes 2* to *6*). The $^{125}$I-labeled mAb used for each set of experiments were: *epitope 1*, 3F4 (●); *epitope 2*, 8A3 (★); *epitope 3*, 7F4 (○); *epitope 4*, 6H3 (■); *epitope 5*, 1G7 (□); and *epitope 6*, H85 (★).

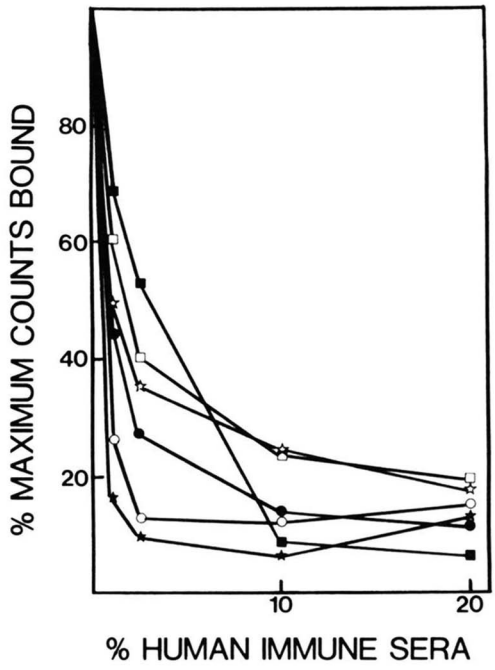


*Figure 2.* Human immune sera inhibition of the binding of $^{125}$I-labeled mAb to the adherence lectin. Serial dilutions of pooled human immune sera from patients with amebic liver abscess were used to inhibit binding of purified $^{125}$I-labeled mAb to the adherence lectin. The lectin was bound to microtiter wells coated with mAb 7F4 (for $^{125}$I-3F4 binding) or 3F4 (for binding of $^{125}$I mAb from *epitopes* 2 to 6). The symbols for the mAb are the same as in Figure 1.

TABLE I

*Effect of mAb to the amebic lectin on adherence of E. histolytica to CHO cells and human colonic mucins*

| Epitope | mAb | IgG Isotype | Adherence to *E. histolytica*[a] | |
|---|---|---|---|---|
| | | | CHO Cells (% Control) | Colonic Mucins (% Control) |
| 1 | 3F4 | 1 | 338 ± 8 | 481 ± 8 |
| 2 | 8A3 | 1 | 209 ± 4 | 259 ± 12 |
| 3 | 7F4 | 2B | 96 ± 5 | 90 ± 5 |
| 4 | 8C12 | 1 | 42 ± 8 | 49 ± 3 |
| 5 | 1G7 | 2B | 42 ± 12 | 25 ± 1 |
| 6 | H85 | 2B | 51 ± 4 | 97 ± 2 |

[a] Values expressed are percent of adherence in absence of any mAb and represent the mean ± SE ($n$ = 6 for CHO cell experiments), $n$ = 3 for mucin experiments). CHO cell adherence was measured by rosetting (7), mucin adherence by binding of $^{125}$I labeled purified colonic mucins (13).

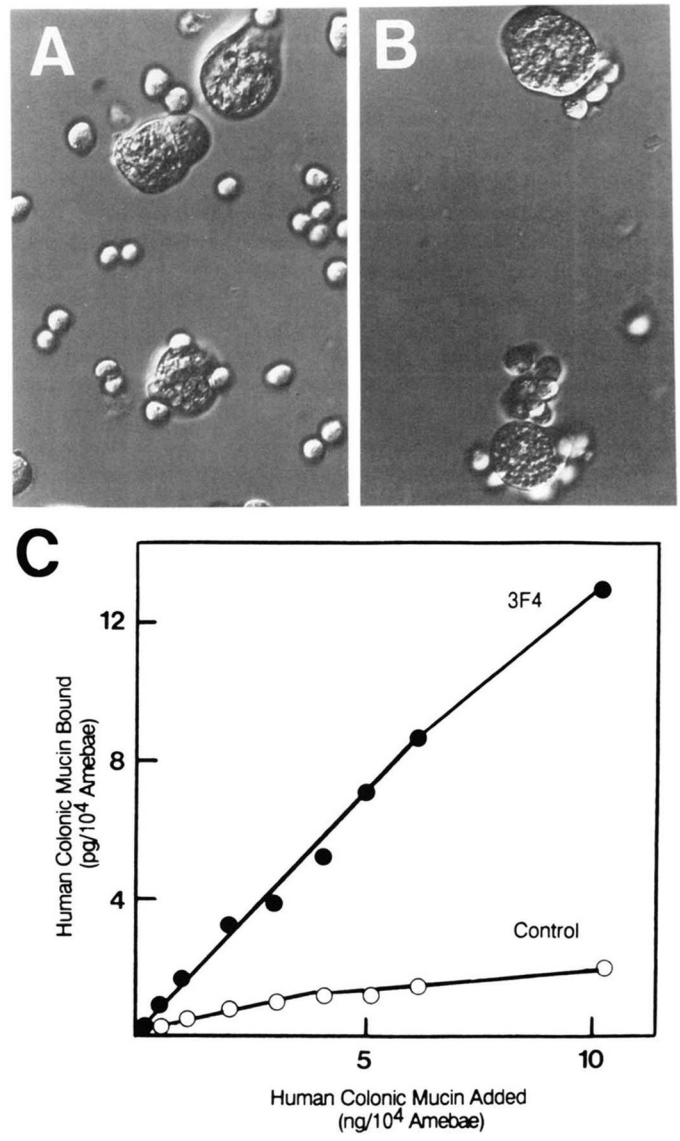


*Figure 3.* Enhancement of *E. histolytica* adherence to CHO cells and human colonic mucins by anti-lectin monoclonal antibody 3F4. *A*, Adherence of *E. histolytica* trophozoites to CHO cells at 4°C in the absence or (*B*) presence of anti-lectin mAb 3F4. Note lack of free CHO cells in *B*. *C*, Binding of $^{125}$I-labeled colonic mucins to trophozoites in the presence and absence of mAb 3F4.

mAb to epitopes 2 to 6 of the H chain decreased human antibody binding to the lectin (immobilized in microtiter wells by epitope 1 mAb) by 40% indicating that there are additional human antibody epitopes in addition to the six mapped on the heavy subunit of the adherence lectin (data not shown).

*Enhancement of amebic adherence in presence of mAb.* The ability of lectin H chain-specific antisera to completely inhibit adherence suggested a predominant role for the heavy subunit in mediating adherence (9). mAb to the six epitopes defined on the heavy subunit were tested for their effects on adherence at 4°C to CHO cells and human colonic mucins. mAb to epitope 3 bound to the lectin only upon its solubilization from the membrane (data not shown), had no effect on the adherence of intact amebae, and served as useful controls for these experiments. Enhanced adherence of *E. histolytica* trophozoites to both CHO cells and human colonic mucins at 4°C was seen in the presence of mAb to epitopes 1 and 2 (Table I; Fig. 3). The enhancement of adherence was most dramatic for the epitope 1 mAb, which increased the number of amebae having at least three adherent CHO cells from 26 ± 9 to 88 ± 2% (338% of control) and increased the binding of colonic mucins almost fivefold from 34 ± 1 to 164 ± 3 pg/$10^5$ amebae (481% of control). Adherence remained 90 to 100% galactose inhibitable in the presence of epitope 1 and 2 mAb (data not shown). mAb to epitopes 4 and 5 inhibited adherence to both CHO cells and colonic mucins. Epitope 6 antibodies inhibited CHO cell but not mucin adherence, indicating subtle differences in the binding of whole cells vs soluble mucin glycoproteins to the lectin (Table I). Inhibitory mAb were unable to completely overcome the enhancement of amebic adherence by antibody 3F4. The combination of mAb from all three inhibitory epitopes (8C12, 1G7, and H85, each at 10 $\mu$g/$10^4$ amebae) decreased the percent of amebic rosettes from control values of 40 ± 6 to 12 ± 2%, but only decreased amebic rosettes in the presence of 3F4 antibody from 99 ± 1 to 57 ± 4%.

The mechanism of the antibody-mediated increase in adherence was investigated. Univalent Fab fragments of 3F4 at a concentration of 10 $\mu g/10^4$ amebae augmented mucin binding to 238% of control levels (Fig. 4), indicating that neither the antibody Fc portion nor antibody cross-linking of the lectin was necessary for enhancement. Amebae-CHO cell rosette formation was also increased by 10 $\mu g$ Fab/$10^4$ amebae from a control of 26 ± 19 to 62 ± 11%. The lower potency of Fab fragments than the intact 3F4 antibody may have been due to a lower affinity of univalent than bivalent antibody for antigen or partial denaturation of the Fab fragments during preparation and purification.

*Effect of 3F4 mAb on binding of lectin to CHO cells.* The purified lectin binds to CHO cells in a galactose-inhibitable manner and competitively inhibits amebic adherence (8, 12). We tested whether mAb that increased amebic adherence would directly increase binding of lectin to Lec 2 CHO cells. Lec 2 CHO cells express a greater number of galactose-terminal glycoproteins than parental CHO cells and are a more suitable cell line for measuring lectin binding (12). The binding of purified lectin to target cells was measured in the presence or absence of epitope 1 mAb 3F4. In the absence of 3F4 antibody the CHO cells bound 1.1 ± 0.1 ng lectin/$10^3$ CHO cells and the binding was 73% inhibited by galactose. mAb 3F4 at 50 $\mu g$/ml increased lectin binding by 240%; the enhanced binding was 84% galactose inhibitable (Fig. 5).

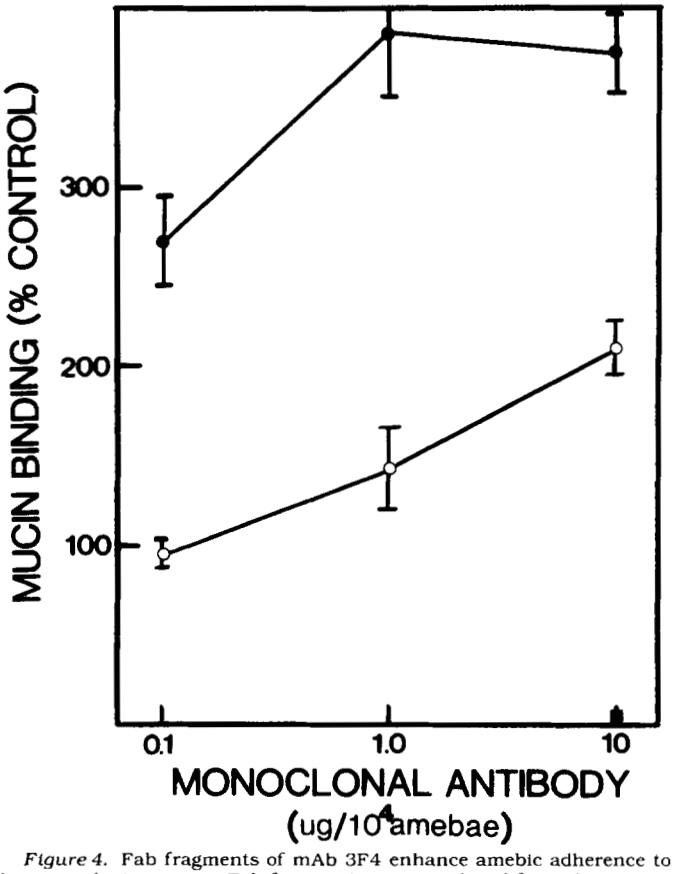


*Figure 4.* Fab fragments of mAb 3F4 enhance amebic adherence to human colonic mucins. Fab fragments were produced from the protein A-purified 3F4 mAb by digestion with immobilized papain (Pierce Chemical Co., Rockford, IL) followed by removal of the Fc fragments and undigested antibody by protein A chromatography. Mucin binding to amebae was measured in the presence of intact 3F4 (●), 3F4 Fab (○), and 3F4 plus 110 mM galactose (■).

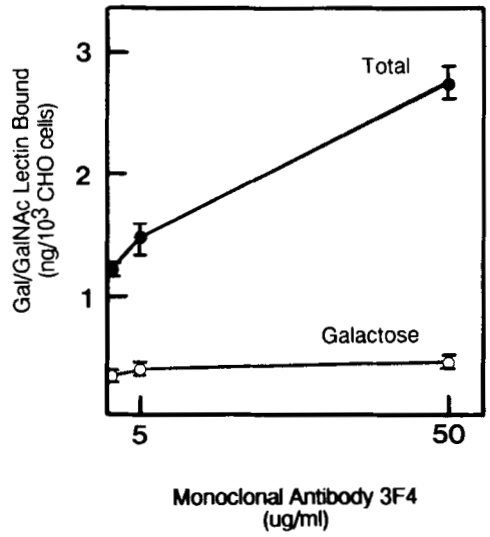


*Figure 5.* Binding of purified lectin to CHO cells is increased by antilectin mAb 3F4. The affinity-purified lectin (10 $\mu g$/ml) was incubated with $5 \times 10^5$ Lec 2 CHO cells for 60 min in ice-cold M199s medium. The lectin and CHO cells were incubated with increasing concentrations of 3F4 antibody with (○) or without (●) 110 mM galactose. At the end of the incubation the CHO cells were washed twice and bound lectin measured with $^{125}$I-labeled 7F4 antibody by RIA (9).

*Amebic adherence in presence of human immune sera.* To test whether individual variations existed in the effect of immune sera on adherence, we tested sera from 44 patients with amebic liver abscess and 17 uninfected individuals, all from the Durban and Gazankula areas of South Africa. Sera were collected within 3 days of hospitalization from the liver abscess patients. All of the infected and none of the uninfected individuals' sera contained antibody to the galactose lectin as determined by Western blots and ELISA to purified lectin (20). Adherence was measured after preincubating amebae with a 1/100 dilution of sera in M199s. Enhancement of adherence (more than 3 SD from mean of adherence in absence of sera) was observed with sera from 16 liver abscess patients and no control individuals; inhibition of adherence occurred in the presence of sera from 25 liver abscess patients and 3 uninfected controls (Fig. 6). The inhibition of adherence in the 3 control sera was apparently nonspecific; the 17 control patients' sera lacked antibodies to whole *E. histolytica* trophozoites as well as to the lectin.

## DISCUSSION

We have demonstrated that antibodies directed against the *E. histolytica* adherence lectin can stimulate as well as inhibit adherence. Adherence plays a critical role in colonization of the large bowel and is required for amebic lysis of target cells (1–7). Recognition of the potential for an antilectin antibody response to either inhibit or stimulate adherence may prove important both in understanding the role of humoral immunity in *E. histolytica* infection and in the development of antiamebic vaccines. Antilectin antibody responses may not necessarily be protective and, indeed, if adherence-enhancing could facilitate *E. histolytica* colonization or tissue invasion. Murine mAb to epitopes 1 and 2 of the 170-kDa H subunit of the lectin enhanced adherence up to fivefold by an Fc-independent direct activation of its galactose-binding activity. Striking individual variations in the effect of im-

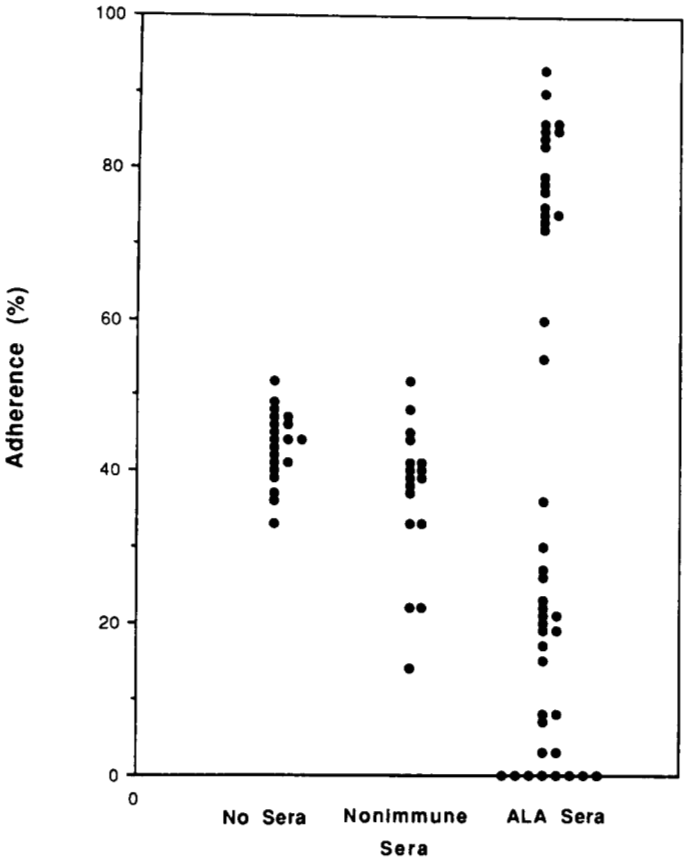


*Figure 6.* Sera from patients with amebic liver abscess enhance or inhibit adherence to CHO cells. Amebae were preincubated in the absence ($n$ = 20) or presence of sera (1/100 dilution) from patients with amebic liver abscess ($n$ = 44) or the sera of uninfected individuals ($n$ = 17) from South Africa. Adherence was then measured to CHO cells by rosette formation. Each *point* represents an individual serum sample. Adherence is expressed as the percent of amebae forming CHO cell rosettes.

mune sera from 44 amebic liver abscess patients were also seen. Although all 44 sera had high titer antilectin antibodies, 16 patients' sera increased and 25 sera inhibited adherence by more than 3 SD from control levels. A prospective study of patients with amebic liver abscess is required to determine if the severity of infection or likelihood for relapse is correlated with serum inhibitory or enhancing effects on adherence.

Activation of the lectin by epitope 1 and 2 mAb was shown to be due to a direct effect of mAb on the lectin. Adherence in the presence of these mAb remained 90 to 100% galactose-inhibitable, indicating that the augmented adherence was still lectin mediated. Enhanced adherence was seen at 4°C, where mAb-induced recruitment of lectin to the cell surface could not occur. Fab fragments of mAb 3F4 increased adherence to colonic mucins and CHO cells, demonstrating that the Fc or bivalent $(Fab)_2$ portions of the antilectin mAb were not required. Increased binding of purified lectin to CHO cells in the presence of the 3F4 mAb was the strongest evidence for lectin activation by mAb to epitopes 1 and 2. The mechanism of mAb activation of the lectin could either be via changes in its conformation, which is the case for antibodies that activate enzymes such as $\beta$-galactosidase and phenylalanine hydrolase, or changes in the states of aggregation, as has been postulated to occur during activation of the insulin and epidermal growth factor receptors (21–23). Further experiments will be required to distinguish between these possibilities.

The enhancement of galactose-binding activity of the lectin by epitope 1 and 2 mAb raises the possibility that amebae may regulate their adherence to galactose-containing substrates, such as human colonic mucins, via similar changes in the lectin's activity. It is becoming evident that the activity of eukaryotic cell adherence proteins can be modulated (24–27). The human neutrophil adhesion molecule CR3 requires activation as well as surface expression to mediate adhesion (24), and the LFA-1 adhesion molecule of T lymphocytes is transiently activated by antigen binding to the TCR (27). Amebae may need to have a mechanism for deadhesion from epithelial cells and mucins as they invade the colon and move through the tissues of the infected host. We are currently pursuing the identification of biologic stimuli that may signal the amebae to activate or inactivate adherence via the lectin.

The 10 murine mAb reported here mapped to six distinct epitopes on the heavy subunit. Only one of the six epitopes (epitope 3) was not exposed on the surface of amebic trophozoites. The parallel effects of the mAb on adherence to whole cells and soluble human mucin glycoproteins, with the exception of epitope 6, indicated that both activities were not mediated by functionally distinct domains of the lectin. This supported previous observations that adherence to CHO cells and mucin could be inhibited by galactose or antibody to the lectin (8, 9, 13). The ability of epitope 6 mAb to inhibit CHO cell but not mucin adherence, however, demonstrated subtle differences in the adherence to cells and soluble mucin glycoproteins.

Recently we have shown that the lectin from pathogenic and nonpathogenic strains of *E. histolytica* is antigenically different (28). mAb to epitopes 1 through 6 reacted on RIA with all 16 pathogenic strains tested. In contrast, only mAb to the adherence-enhancing epitopes 1 and 2 bound to the lectin from the 34 nonpathogenic strains tested. These antilectin mAb make possible the rapid differentiation of pathogenic from nonpathogenic *E. histolytica*, which may be very important clinically. In addition, the antigenic conservation of only epitopes 1 and 2 is interesting in light of the enhancement of adherence that occurs when antibody binds to them.

Understanding the role of the lectin light subunit in adherence and contact-dependent cytolysis is hindered by the current inability to produce antibodies against it. Immunization of BALB/c mice with native lectin in CFA resulted in an antibody response that on Western blots was solely directed to the heavy subunit (9). The use of reduced and alkylated lectin or purified light subunit for immunizations also did not generate an antilight subunit antibody response. We are currently testing other adjuvants, including IL-2, in an attempt to develop these antibodies. The adherence-enhancing and inhibitory domains on the heavy subunit defined by the mAb described here indicate an important role for the heavy subunit in mediating and regulating adherence, with the contribition of the light subunit yet to be defined.

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