

## Role of the Galactose Lectin of *Entamoeba histolytica* in Adherence-Dependent Killing of Mammalian Cells

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***Entamoeba histolytica* extracellular killing of host cells is contact dependent. Adherence to human colonic epithelial cells and mucins is mediated by a galactose-specific lectin. The effect on cytotoxicity of a panel of monoclonal antibodies (MAb) directed against the galactose lectin was tested. As expected, those MAb which inhibited adherence also decreased cytotoxicity. However, one antilectin MAb blocked cytotoxicity after adherence had occurred, indicating that the lectin has a role in cell killing that is distinct from its adherence function.**

*Entamoeba histolytica* is a cytolytic protozoan parasite that is responsible for an estimated 100,000 deaths per year from amebic liver abscess and colitis. The initial steps in pathogenesis include adherence to colonic mucosa, subsequent lysis of the mucosal surface, and invasion through the colonic epithelium. The events leading to host cell destruction following adherence are unknown. The purpose of this study was to analyze the role of the galactose-specific lectin of *E. histolytica* in the killing of target cells.

Adherence is an absolute prerequisite for cytotoxicity (8) and is mediated in vitro by an amebic galactose-specific lectin which is a heterodimeric glycoprotein composed of 170- and 35-kDa subunits (3, 6, 9). This protein is a major cell surface antigen that is almost universally recognized by the immune sera of patients with amebic liver abscess (4, 10). Immunization with the galactose lectin prevents experimentally induced amebiasis in animal models (5). Six nonoverlapping epitopes have been mapped on the 170-kDa subunit with monoclonal antibodies (MAb) (7). MAb to epitopes 4 through 6 decreased adherence, MAb to epitope 3 had no effect, and MAb to epitopes 1 and 2 increased adherence (via a direct activation of the galactose-binding activity of the lectin) (7). We wished to continue this investigation by testing the effects of these MAb on cytotoxicity.

The model system used to test the antilectin MAb was the interaction of *E. histolytica* trophozoites with Chinese hamster ovary (CHO) cells. Amebic extracellular cytotoxicity has been shown to be entirely contact dependent in this system (8, 9). Adherence to CHO cells is mediated by the galactose-specific adhesin; inhibition of the adhesin with galactose or *N*-acetylgalactosamine almost completely blocks both adherence and CHO cell death. The effect of MAb to the 170-kDa adhesin subunit on CHO cell killing by *E. histolytica* trophozoites was measured by <sup>51</sup>Cr release from labeled CHO cells.

Axenic culture and harvesting of *E. histolytica* were performed as reported previously (3, 9). Adherence and killing of CHO cells were measured by the method of Ravdin and Guerrant (9), except that adherence was measured at 37°C and CHO cells were labeled with <sup>51</sup>Cr (0.25 mCi/5 ml) for 2 h in Eagle's minimal essential alpha medium (GIBCO, Grand Island, N.Y.). Labeled CHO cells were incubated for

30 min with unlabeled minimal essential alpha medium prior to their interaction with amebae. Antilectin MAb (protein A purified) were preincubated with amebae at a concentration of 10 µg/10<sup>4</sup> amebae for 45 min at 4°C. Amebae (10<sup>4</sup>) and CHO cells (2 × 10<sup>5</sup>) were pelleted and incubated together at 37°C in 1 ml of M199 medium (GIBCO) supplemented with 5.7 mM cysteine, 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), pH 6.9, and 10% heat-inactivated fetal bovine serum (GIBCO). After incubation, the supernatant was removed and assayed for <sup>51</sup>Cr release on a gamma counter (Micromedics, Horsham, Pa.). Because *E. histolytica* cytotoxic activity is altered by poorly defined variables such as age of culture and length of in vitro cultivation, experiments were reported only when <sup>51</sup>Cr release after 1 h of incubation with amebae was approximately 20% of the total CHO cell label. This occurred in more than 75% of experiments.

Galactose (100 mM) blocked adherence via the galactose adhesin and inhibited cytotoxicity, as previously described (9) (Fig. 1). As expected from their effects on adherence at 4°C (7), MAb to epitopes 4 through 6 inhibited adherence and cytotoxicity at 37°C, while MAb to epitope 3 had no effect on adherence or cytotoxicity (Fig. 1).

Unexpectedly, MAb 3F4 (directed against epitope 1) decreased cytotoxicity by 30% at the same time that it increased adherence twofold, resulting in an overall inhibition of chromium release from adherent cells of 60% at 45 min (Fig. 1). The inhibition of cytotoxicity by MAb 3F4 was observed during incubations of 1 to 3 h (Fig. 2) and at concentrations of 10 to 100 µg/ml (data not shown), the concentration range at which maximal effects on adherence at 4°C were seen (7). The effect of the MAb was independent of its ability to cross-link the lectin on the cell surface, as Fab fragments decreased cytotoxicity to 85 ± 6% (4 µg/10<sup>4</sup> amebae) and 71 ± 4% (80 µg/10<sup>4</sup> amebae) of the control value. Reductions in cytotoxicity were not due to an effect of MAb 3F4 on amebic viability; 95% of trophozoites incubated with the MAb and 96% of control amebae were viable after a 1-h incubation at 37°C.

The identification of an antilectin MAb that blocked cytotoxicity without inhibiting adherence implicated the galactose lectin in either signaling the initiation of or directly participating in the cytolytic event. We next tested whether the affinity-purified lectin was cytotoxic. Earlier studies had

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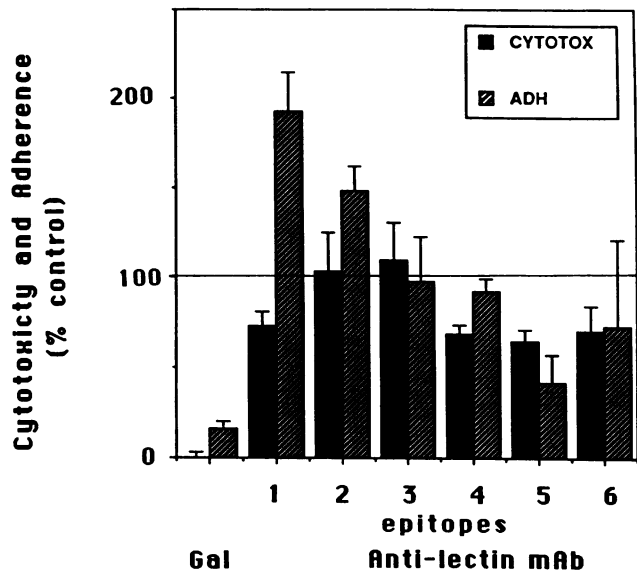


FIG. 1. Effect of antilectin antibodies on *Entamoeba histolytica* adherence and killing of CHO cells. Amebae were preincubated at 4°C for 1 h in the presence of protein A-purified MAb to six epitopes of the lectin.  $^{51}\text{Cr}$  release and adherence were measured after 45 min of incubation of amebae with CHO cells at 37°C with a CHO cell-to-ameba ratio of 20 to 1. Data are expressed as percentages of the control values (no MAb present). Each point is an average of three or more determinations. Bars indicate standard errors.

shown that the purified lectin retained its ability to bind to CHO cells in a galactose-specific manner and that its binding caused elevations in the intracellular calcium levels of the cells (11, 13). Because elevation in CHO cell intracellular calcium is an early event in amebic killing of CHO cells but is not by itself sufficient for cell death (11), we tested the cytotoxic effects of the purified lectin by the more direct measurement of chromium release.

The galactose lectin was purified on an MAb affinity column (3) from *E. histolytica* trophozoites solubilized in the detergent  $\beta$ -D-octylglucoside. The residual detergent was removed from the purified lectin by dialysis to prevent any artifactual increases in CHO cell chromium release. Chromium release from CHO cells exposed to the lectin for 4 h at 37°C was measured at both pH 5.4 and 6.8 because of reports that cytotoxic activity of intact amebae is dependent upon the maintenance of acidic pH in intracellular vesicles (12). The purified lectin used at concentrations equivalent to 1,000 to 8,000 times the number of intact amebae used in the cytotoxicity assays had only a minimal effect on chromium release. At 10  $\mu\text{g}$  of purified lectin per ml, chromium release was  $8.3 \pm 0.9\%$  at pH 5.4 and  $8.8 \pm 9.3\%$  at pH 6.8. The small amount of chromium release that occurred was non-specific, as judged by its lack of inhibition in the presence of mouse antilectin polyclonal antibodies. These experiments could not rule out the possibility that the lectin is a cytotoxin, as the purification method may have removed essential cofactors required for cytotoxicity or denatured the cytotoxic properties of the lectin while preserving its cell-binding activity. Nevertheless, the cytotoxic activity of sonicated preparations of trophozoites was uninhibited by polyclonal or monoclonal antilectin antibodies (data not shown), making it less likely that the lectin had cytotoxic activities that were somehow destroyed by the purification process.

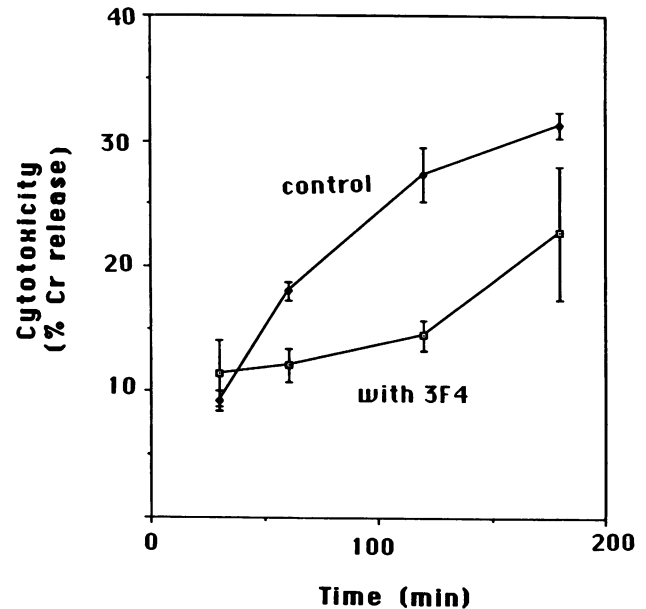


FIG. 2. Time course of *E. histolytica* cytotoxicity in the presence or absence of MAb 3F4. Amebae were preincubated with MAb 3F4 (50%  $\text{NH}_4\text{SO}_4$  precipitate of ascites) at a concentration of 0.3 mg/ml.  $^{51}\text{Cr}$  release was measured after incubation of amebae with CHO cells for the times indicated. Each point is an average of three or more determinations. Bars indicate standard errors.

Rather than inhibiting a direct cytotoxic activity of the lectin, MAb 3F4 could be interfering with the ability of the lectin to transduce the signal to initiate cytolysis. A cell-signaling role for the lectin had been suggested by experiments studying the interaction of synthetic liposomes containing galactose-terminal glycolipids with amebae. These liposomes triggered a rapid polymerization of cytoplasmic actin, presumably via their interaction with the galactose-specific lectin (1). Additional evidence that suggested that the lectin transmits signals across the plasma membrane was the presence of a region that shares sequence identity with the autophosphorylation site of the epidermal growth factor receptor tyrosine kinase on the 170-kDa subunit cytoplasmic domain (2, 14). We are investigating the potential role of the lectin in transducing activation signals across membranes in light of the data presented here, which demonstrate that the functions of the lectin are more complex than solely the mediation of adherence.

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