Binding of Cholera Toxin to *Giardia lamblia*

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Received 12 March 1987/Accepted 1 June 1987

Binding of cholera toxin to *Giardia lamblia* was demonstrated by two slightly different methods: an immunofluorescence technique using antibody to cholera toxin and anti-rabbit immunoglobulin G conjugated to fluorescein isothiocyanate, and a one-step fluorescence method in which *G. lamblia* was incubated with the B subunit of cholera toxin conjugated to fluorescein isothiocyanate.

*Giardia lamblia* is a flagellated, parasitic protozoan which adheres to the human upper-intestinal epithelium and produces symptoms that include diarrhea, abdominal cramps, anorexia, and malabsorption (3, 4, 10, 11). It is the parasite most frequently found in fecal specimens submitted to U.S. state health department laboratories (5) and has been implicated in a number of epidemics of food- or waterborne gastrointestinal illness (2, 8, 14, 16).

Binding of cholera toxin (CT) to the trophozoites of *Giardia muris* has been shown by Ljungström et al. (12). We demonstrate here the binding of CT to both the cysts and trophozoites of *G. lamblia* by two slightly different techniques: one is an immunofluorescence method which uses antibody to CT; the other, a fluorescence method, requires no antibody.

Trophozoites of *G. lamblia* (Portland-1, ATCC 30888; and WB, ATCC 30957; American Type Culture Collection, Rockville, Md.) were cultured at 37°C in a filter-sterilized liquid medium supplemented with 0.05 to 0.1% bile and 10% calf serum (9). Cultures were maintained by twice-weekly transfer (0.2 ml of a 4- to 5-day-old culture to 8 ml of the same medium in a screw-cap tube [13 by 100 mm]).

**Infection of mice.** Trophozoites cultured in vitro (WB or Portland-1) were centrifuged (125 x g for 5 min), washed with 5 ml of sterile 0.85% saline solution, counted by using a hemacytometer, and suspended to a concentration of approximately 5 x 10^6/ml. Adult (18 to 20 g) C3H/HEN mice (Harlan Sprague-Dawley, Indianapolis, Ind.) were inoculated intragastrically with 0.1 ml of culture by means of a 2-in. (5.08-cm), curved, ball-tipped, stainless steel, 18-gauge feeding needle (Popper and Sons, Hyde Park, N.Y.).

Fecal concentrates were prepared from a mixture of three pellets (per mouse) emulsified in 1 ml of sterile 0.85% saline solution, filtered through several layers of gauze, and centrifuged for 3 min at 300 x g (centrifuge model DPR-6000; International Equipment Co., Div. Damon Corp., Needham Heights, Mass.). After three 5-ml washes, 1 drop of sediment was mixed with 1 drop of 0.85% saline to detect motile trophozoites, and 1 drop of sediment was mixed with 1 drop of Lugol iodine (20%) to detect cysts. Fecal specimens, concentrates, or intestinal contents (removed from mice sacrificed by CO_2 asphyxiation) were examined on wet mounts for the presence of trophozoites or cysts. In some experiments, duplicate positive specimens and negative controls were placed in both thimerosal-iodine-Formalin solution or 10% Formalin (1) to determine whether either preserved interfered with the fluorescence methods.

Sixteen human fecal samples containing *G. lamblia* (obtained as part of an ongoing study at the Center for Vaccine Development, University of Maryland, Baltimore) and one negative control were concentrated and examined as described for mouse feces. However, these 17 fecal samples had been frozen, thawed when needed, and preserved in 10% Formalin.

Initially, during the development of the immunofluorescence method, a drop of fecal sample or concentrate emulsified in preservative was placed on a glass slide and allowed to air dry. In later experiments, however, fecal samples or concentrates were spotted onto nitrocellulose (0.45 µm pore size; Schleicher & Schuell, Inc., Keene, N.H.). Either the slide or nitrocellulose was immersed in a solution of 0.02 M Tris-buffered saline (TBS), pH 7.4, containing 1% bovine serum albumin; the slide or nitrocellulose was incubated either overnight at 4°C or for 1 h at 37°C. The nitrocellulose (or slide) was then incubated with CT (diluted in TBS to a concentration of 10 µg/ml) (Sigma Chemical Co., St. Louis, Mo.) for 1 h, washed three times, and incubated with a 1:500 dilution of antibody to CT. Antibody to CT was prepared by subcutaneous injection of New Zealand White rabbits with increasing amounts, 0.1 to 20 µg, of CT during several weeks (13). The nitrocellulose (or slide) was then washed again three times and incubated with anti-rabbit immunoglobulin G–fluorescein isothiocyanate (FITC) (1:1,000) (Sigma) for 1 h. TBS was used for all washes, and all washes and incubations were done at 37°C. The nitrocellulose was then cut into small pieces (each containing a fecal sample), cleared with xylene, and mounted on a slide with Pro-Text mounting medium (Lerner Laboratories, New Haven, Conn.). In experiments in which a slide was used, a cover slip was mounted with Pro-Text mounting medium. All slides were examined with a Leitz Ortholux 2 fluorescence microscope with a 100-W mercury bulb and 1.2 filter cube containing a narrow band blue filter (Leitz Inc., Rockleigh, N.J.).

For the fluorescence method (without antibody), 20 µl of each fecal sample was spotted onto nitrocellulose and allowed to air dry at room temperature. The nitrocellulose was incubated either overnight at 4°C or at 37°C for 1 h with 1% bovine serum albumin in TBS. The nitrocellulose sheet was then incubated for 1 h at 37°C with B subunit-FITC conjugate (diluted 1:200 in TBS; List Biological Laboratories, Campbell, Calif.). After three washes with TBS, the nitrocellulose was cut into small sections, each containing one fecal sample. Each section was cleared with xylene.
Mouse fecal samples positive for *G. lamblia* cysts or trophozoites (or both) by direct microscopic examination were tested by the immunofluorescence technique by using CT and its antibody. Both trophozoites and cysts of either the WB or Portland-1 strain were easily detectable by their bright fluorescence (Fig. 1A). Feces from control mice were negative by both direct microscopic examination and immunofluorescence.

The immunofluorescence method was also tested on human fecal samples to determine whether the cysts and trophozoites detected by direct microscopic examination also bind CT. Binding of CT to *G. lamblia* cysts or trophozoites (or both) was demonstrated in all 16 human fecal samples and concentrates. Feces from a control that was negative by direct microscopic examination were also negative by this method. Although the *Giardia* trophozoites and cysts gave off a very bright fluorescence, there was some background fluorescence in these slides. Other objects gave off a faint green glow (Fig. 1A).

The 16 positive human fecal samples and one negative control were reexamined by using a one-step procedure that consisted of incubation with a CT B-FITC conjugate. The binding of CT to cysts was easily detectable by this one-step method (Fig. 1B). Trophozoites (not shown) also bound the B subunit-FITC conjugate. The negative control showed no fluorescence, except for the nitrocellulose itself, which glowed faintly.

Of the two methods used to show binding of CT to *G. lamblia*, the one-step B subunit-FITC procedure was easier to use, with less background fluorescence (Fig. 1). No other recognizable organism or debris in the fecal samples fluoresced when the B subunit-FITC method was used, although the nitrocellulose itself glowed faintly green (Fig. 1B). No fluorescence was detected when pure cultures of CT-producing *Vibrio cholerae* or toxigenic *Campylobacter jejuni* were spotted onto nitrocellulose and treated with B subunit-FITC conjugate (data not shown). Other protozoa have not yet been tested; however, if the binding of CT eventually proves to be specific for *Giardia* spp., the methods described here may be applicable for detection of *G. lamblia* in the clinical laboratory.

Although the phenomenon of CT binding is interesting, no role in pathogenesis for this interaction has yet been identified. It is possible, however, that *G. lamblia* interacts with toxigenic bacteria such as those of the genera *Vibrio*, *Campylobacter* (13), *Aeromonas* (6), *Salmonella* (15), and *Escherichia* (7), because all of these genera reportedly produce CT or CT-related toxins.

**LITERATURE CITED**


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**FIG. 1.** Trophozoites and cysts of *G. lamblia* bound to CT or its B subunit (magnification, ×1,000). (A) Mouse fecal sample (on glass slide) containing trophozoite (WB strain) emerging from cyst (C) (see arrow). The normally pear-shaped trophozoite has an ovoidround shape immediately after excystation. Flagella (F) (see arrow) are still emerging from the cyst. Internal structures (axostyle and a pair of nuclei) can be observed in the trophozoite. The slide was treated with CT, anti-CT, and anti-rabbit immunoglobulin G–FITC. (B) Human fecal sample (on nitrocellulose) containing cysts of *G. lamblia* treated with B subunit-FITC.