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# Isolation and Identification of a *Giardia lamblia*-Specific Stool Antigen (GSA 65) Useful in Coprodiagnosis of Giardiasis

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**A *Giardia lamblia*-specific antigen (GSA 65) was isolated from stools of *G. lamblia*-positive patients by crossed- and line-immunoelectrophoresis and counterimmunoelectrophoresis (CIE) in agarose by using rabbit antiserum prepared against *G. lamblia* cysts. CIE with rabbit anti-GSA 65 monospecific antiserum revealed that GSA 65 was present in aqueous stool eluates of giardiasis patients and in cysts and trophozoites of the parasite. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of immunoaffinity-purified antigen followed by Western blotting showed that the molecular weight of this molecule was about 65,000. GSA 65 was detectable by CIE in stool eluates of 36 of 40 giardiasis patients but not in eluates of 10 *G. lamblia*-negative asymptomatic controls. GSA 65 was detected in stool eluates of 2 of 18 individuals with chronic diarrhea who were negative for parasites by microscopic examination. Cross-specificity studies with other genera of parasitic protozoa performed by using CIE and immunofluorescence indicated that GSA 65 was present only in strains of *G. lamblia*. Based on these findings, GSA 65 may prove to have an important application in the design of sensitive diagnostic tests for giardiasis.**

Clinical diagnosis of giardiasis is currently made primarily by direct microscopic examination of stool or stool concentrates for the presence of cysts or trophozoites. The sensitivity of this method, even with multiple stool examinations, is only 50 to 70% (5, 6). With chronic giardiasis patients, the sensitivity of this method may be lower because cysts are excreted only intermittently and at low levels. Examination of fluid aspirates from the duodenum or jejunum and small-bowel biopsy are perhaps more sensitive methods of diagnosis (19), but are invasive and costly and for these reasons are rarely used. Serodiagnosis by indirect fluorescent antibody methods (17, 24, 25) or by enzyme-linked immunosorbent assay (ELISA) (13, 18, 25) may be useful but do not provide clear proof of active *Giardia* infection. Clearly, there is a need for more sensitive yet noninvasive methods for diagnosing giardiasis.

Coproimmunodiagnostic assays offer a possible solution to the problem of detecting *Giardia lamblia* infection in humans. Some efforts have already been made to design a practical method for detecting *Giardia* antigens in stool. Craft and Nelson (8) reported the use of counterimmunoelectrophoresis (CIE) with rabbit antiserum prepared against *G. lamblia* cysts for detection of *G. lamblia*-specific antigens in stool and reported >98% sensitivity with giardiasis patients. Vinayak et al. (23) used rabbit antiserum against trophozoites grown in culture in a CIE test for *Giardia* antigens in patients' stools and obtained 94% sensitivity. Ungar et al. (22) used rabbit and goat antisera prepared against trophozoites grown in culture in the development of an antigen-capture ELISA for detecting *Giardia* antigens in stool and reported 92% sensitivity. Green et al. (12) also devised an antigen-capture ELISA for use in *Giardia* coprodiagnosis; these authors used antisera prepared against trophozoites grown in culture as well as against cysts (no mention is made of which type of antisera was more effective). A sensitivity of >98% was reported for this method. Clearly, all of the above assays represent sensitive tests for *Giardia* antigens in stool; moreover, in all cases the specificity of the tests was reported to be high. However, these assays were based on the detection of a potentially broad array of undefined giardial antigens, some of which might prove to cross-react with antigens of other intestinal protozoa and of other intestinal organisms and fecal contents. For the purposes of standardizing such assays, determining their limits of sensitivity, and ensuring specificity for *Giardia*, it is important to identify and characterize the *G. lamblia*-specific stool antigens around which such assays are developed.

We describe here the isolation and identification of a *G. lamblia*-specific stool antigen (GSA 65) that may have an important application in the design of sensitive and specific coprodiagnostic tests for giardiasis.

## MATERIALS AND METHODS

**Parasite cultures.** Trophozoites of *G. lamblia* (WB strain, ATCC 30957), *Trichomonas vaginalis* (local isolate HMC-1, our designation), *Pentatrichomonas hominis* (Diamond strain, ATCC 30000), and *Entamoeba histolytica* (NIH 200 strain, ATCC 30458) were axenically cultured at 37°C as described by Einfeld and Stibbs (10), Torian et al. (20), and Diamond et al. (9). All cultures were grown in antibiotic-free medium. *Leishmania donovani* promastigotes were donated by Steven Reed, Issaquah Health Research Laboratory, Issaquah, Wash. *Candida albicans* was donated by Paula Sundstrom of our department and grown on Saboraud agar in vertical, loosely capped borosilicate tubes at 25°C.

**Preparation of immune serum.** Rabbit antiserum was prepared against cysts recovered by the following procedure. Fresh, refrigerated stool specimens, previously verified to be *G. lamblia*-positive by microscopy, were suspended in 4 to 5 volumes of distilled water, filtered through two layers of cheesecloth, and centrifuged at 500 to 700 × *g* for 5 min. The supernatant was discarded, and the pellet was suspended in distilled water containing 0.5% Tween 80 for lipid removal.

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The suspended material was repeatedly washed in 0.5% Tween 80 until the supernatant remained clear after centrifugation. The pellet was then suspended in distilled water and layered upon an equal volume of a 0.85 M sucrose solution. Tubes were centrifuged for 20 min at 500 × *g*, and cysts from the water-sucrose interface were removed, washed repeatedly with distilled water, and then centrifuged at 900 × *g* for 2 min. Harvested cysts were stored in an aqueous solution of (per milliliter) 200 U of penicillin G, 200 μg of streptomycin, 50 μg of gentamicin, and 5 μg of amphotericin B in 1.5-ml polypropylene tubes at 4°C. Rabbits were immunized intramuscularly at several sites with 2 ml of intact cysts at a concentration of $5 \times 10^5$ cells per ml mixed with 2 ml of Freund incomplete adjuvant. After 3 weeks, the rabbits were boosted intravenously twice weekly with increasing doses of cysts (0.1-, 0.2-, 0.3-, and 0.4-ml doses at a concentration of $5 \times 10^5$ cysts per ml). One week after the last booster injection, the animals were anesthetized and bled by cardiac puncture, and serum was stored at −20°C.

Monospecific antiserum against *G. lamblia*-specific antigen present in stool eluates was prepared by a procedure previously described by Alexander and Kenny (1). Precipitin arcs from agarose line-immunoelectrophoresis plates (see below) were excised from 200 plates. For the initial injection, precipitin arcs in agarose were emulsified in Freund complete adjuvant and injected intradermally at multiple sites on the neck and upper back of two New Zealand White rabbits. After 3 weeks, a series of weekly intramuscular injections of precipitin arcs emulsified in Freund incomplete adjuvant was begun and was continued for about 3 months. Rabbits were exsanguinated as previously described, and sera were stored at −20°C. Immunoglobulin G (IgG) fractionation of antisera was performed by using a protein A-Sepharose column (Pharmacia Fine Chemicals, Piscataway, N.J.) as described by Goding (11).

**Preparation of antigens for electrophoretic techniques. (i) Stool eluates.** *G. lamblia* cyst-positive and control stools were collected from various clinical and private sources in Washington state. Approximately 1 g of non-Formalin-fixed (fresh) stool was added to 3 ml of distilled water, mixed well by stirring, and centrifuged at 900 × *g* for 5 min. Supernatants were stored at −20°C.

**(ii) *G. lamblia* cysts.** Previously purified *G. lamblia* cysts were suspended in 10 mM phosphate buffer (pH 6.8) containing 0.1% Triton X-100 at a concentration of $2 \times 10^6$ to $3 \times 10^6$ cysts per ml and were sonicated on ice with a minimum of eight 10-s pulses from a Branson cell disrupter. Complete disruption of the cyst preparation was verified by light microscopy. Sonicated preparations were centrifuged at 12,000 × *g* for 6 min, and the supernatant was assayed for protein by the method of Bradford (3). Supernatants were diluted to a final protein concentration of 1 to 2 mg/ml and stored at −20°C until needed.

**(iii) Trophozoites of *G. lamblia*, *T. vaginalis*, *P. hominis*, and *E. histolytica*.** After harvest in late log phase, trophozoites were chilled on ice for 20 min, pelleted by centrifugation at 800 × *g*, and washed four times in cold phosphate-buffered saline (PBS) or cold saline–1% glucose. After washing, cultures were suspended in 10 mM phosphate buffer (pH 6.8) containing 0.1% Triton X-100 and sonicated with six 10-s bursts on ice. Particulate matter from sonic extracts was removed by centrifugation at 12,000 × *g* for 6 min, and the supernatant was assayed for protein by the method of Bradford (3). Supernatants were diluted to a final protein concentration of 1 to 2 mg/ml and were stored at −20°C until needed.

**(iv) *G. lamblia*-specific antigen from stool eluates.** *G. lamblia*-specific antigen present in eluates of stools from *G. lamblia*-positive patients was partially purified by the following procedure before affinity chromatography. Stool (10 g) was suspended in 500 ml of distilled water and centrifuged at 10,000 × *g* for 20 min. The pellet was discarded, and the supernatant was subjected to 50% ammonium sulfate precipitation followed by centrifugation at 10,000 × *g* for 20 min. The supernatant was discarded, and the pellet was suspended in distilled water to the original volume and subjected to a second 50% ammonium sulfate precipitation, followed by centrifugation at 10,000 × *g* for 20 min. The pellet was suspended in distilled water, extensively dialyzed at 4°C against distilled water containing 0.1% ammonium hydroxide, and lyophilized. The dried material was suspended in 5 ml of 50 mM Tris buffer (pH 8.2) containing 1 mM phenylmethylsulfonyl fluoride and 5 mM EDTA and stored at −20°C until needed for affinity chromatography.

**Electrophoretic techniques.** CIE with polyclonal anti-cyst antisera was used to verify the presence of *G. lamblia*-specific antigen in *G. lamblia* cyst-positive stool samples (14). By using glass slides (43 by 43 mm or 1 by 3 in [2.54 by 7.62 cm]) covered with 2.5 ml of 0.9% agarose in electrophoresis buffer (20 mM Ches, 16 mM Bicine, 0.05% Triton X-100 [pH 8.86]), two 3-mm-diameter wells separated by 5 mm were punched in the agarose, oriented parallel to the electric field. The well closest to the anode was filled with 10 μl of either rabbit anti-cyst antiserum or rabbit monospecific antiserum prepared against the isolated *G. lamblia*-specific stool antigen. The well closest to the cathode was filled with 10 μl of aqueous eluate (one part stool to three parts distilled water) of stool of a giardiasis patient or of a control patient. Electrophoresis with a field strength of 5 V/cm was applied for 90 min. Before being dried and stained with Coomassie blue R-250, plates were washed for 24 h in Tris-buffered saline (10 mM Tris, 150 mM NaCl [pH 7.2]), followed by a 24-h rinse in distilled water.

Antigen for monospecific antibody production was isolated by using line immunoelectrophoresis (2). Glass slides (43 by 43 mm) were covered with 2.5 ml of 0.9% agarose in electrophoresis buffer. The upper two-thirds of the agarose was excised and replaced with 1.6 ml of 0.9% agarose containing 2.5% of the IgG fraction of rabbit anti-cyst antiserum. A trough (3 by 35 mm) was cut in the lower one-third of the agarose and was subsequently filled with 50 to 70 μl of stool eluate from patients previously shown to be *G. lamblia*-positive by microscopy and *Giardia* antigen-positive by CIE, as described above, by using anti-cyst antiserum. Electrophoresis with a field strength of 1 V/cm was applied for 15 h. Slides were washed, and precipitin lines were excised as described by the methods of Norrild et al. (16).

To obtain profiles of the precipitable antigens found in *G. lamblia*-positive stool eluates, crossed-immunoelectrophoresis techniques were employed. These techniques were used to characterize the electrophoretic mobility of the stool antigen, to check for cross-reactivity of the monospecific antiserum with antigens from the trophozoite and cyst preparation, and to determine whether anti-trophozoite antiserum could precipitate similar *Giardia* antigens from stool eluate. Crossed immunoelectrophoresis, tandem crossed immunoelectrophoresis, and intermediate gel crossed immunoelectrophoresis (2) were performed on glass slides (43 by 43 mm) covered with 2.5 ml of 0.9% agarose in electrophoresis buffer. For first-dimensional electrophoresis, a field strength of 5 V/cm was applied for 60 min. Bovine serum albumin

(BSA) (3 μg) was added to the antigen to be used as a migration standard. Before electrophoresis in the second dimension, a gel containing 2.5% rabbit antiserum and 0.25% rabbit anti-BSA antiserum was poured above the 1-cm strip containing the separated antigen preparation, and electrophoresis was performed for 15 h at 1 V/cm. Washing and staining procedures were the same as those used in CIE.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used for characterization of the *G. lamblia*-specific antigen in the immunoadsorbent column eluates and in *G. lamblia* trophozoite and cyst sonic extracts. SDS-PAGE was performed basically as described by Laemmli (15). A 5% stacking gel and an 8% separating gel were used. Immunoadsorbent column fractions, trophozoite sonic extracts, and cyst sonic extracts were mixed with an equal volume of sample buffer consisting of 4% sodium dodecyl sulfate, 20% glycerol, 10% 2-mercaptoethanol, 0.004% bromophenol blue, and 0.2 M Tris hydrochloride buffer (pH 6.8) and boiled for 2 min before addition to the sample slots. After electrophoresis, the unstained slabs were used for Western blotting.

**Western blotting.** Antigens separated by SDS-PAGE were transferred to nitrocellulose paper and analyzed by the method of Towbin et al. (21) as modified by Burnette (7). After transfer, nitrocellulose sheets were incubated for 1 h at room temperature in blocking buffer (160 mM NaCl, 5 mM 4-sodium EDTA, 0.25% gelatin, 0.1% Tween 20 in 10 mM TES [*N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid] buffer [pH 7.3]). Sheets were transferred to fresh blocking buffer containing 0.32% rabbit antiserum, incubated with slow shaking for 1 h, and then washed repeatedly in 0.02 M PBS (pH 7.6) containing 0.5% Tween 20 (PBS-T). The sheets were placed in fresh blocking buffer containing 0.1% peroxidase-conjugated goat anti-rabbit IgG (Antibodies Inc., Davis, Calif.) for 1 h at 25°C and then washed six times in PBS. Nitrocellulose sheets were developed by the addition of freshly prepared 0.06% (wt/vol) 4-chloro-1-naphthol (Sigma Chemical Co., St. Louis, Mo.) and 0.01% hydrogen peroxide in PBS.

**Immunoadsorbent chromatography.** A total of 60 mg of the IgG fraction of the monospecific rabbit antiserum was bound to 2 g (dry weight) of CNBr-Sepharose 4B (Pharmacia) in 20 ml of coupling buffer (0.1 M $NaHCO_3$ [pH 8.5] containing 0.5 M NaCl), as recommended by the manufacturer. Excess active groups on the gel were blocked by incubation of the slurry overnight at 4°C with 40 ml of 0.1 M glycine in coupling buffer. The gel was transferred to a 10-ml column and washed alternately with coupling buffer and 0.1 M acetate buffer (pH 4.0) containing 0.5 M NaCl. The gel was prepared for use by washing in PBS-T. Partially purified *G. lamblia*-specific antigen from stool eluates and *G. lamblia* cyst and trophozoite sonic extracts, prepared as described above, were applied to separate immunoadsorbent columns and allowed to react for 2 h at 4°C. Unadsorbed antigens were removed by washing the column with 100 ml of PBS-T. Nonspecifically adsorbed antigens were eluted by using 50 ml of PBS-T containing 1 M NaI. The more avidly bound *Giardia* antigen was then eluted with 25 ml of PBS-T containing 3 M NaI, exhaustively dialyzed against distilled water at 4°C, and lyophilized or concentrated by ultrafiltration. Concentrates were analyzed by crossed immunoelectrophoresis, SDS-PAGE, and Western blotting.

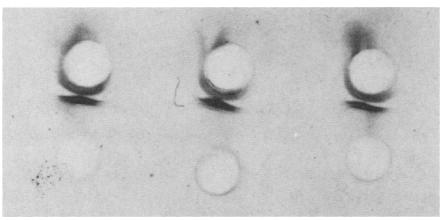

FIG. 1. CIE analysis of three *G. lamblia*-positive stool eluates by using anti-cyst antiserum. Note the characteristic anodal precipitin lines, each of which existed as a doublet.

TABLE 1. Cross-reactivity studies of anti-GSA 65 antiserum as assessed by CIE

| Stool eluate | No. of positive reactions/ total no. of reactions |
|---|---|
| *G. lamblia* only; patient symptomatic | 36/40 |
| Intestinal parasites other than G. *lamblia* | 4/31 |
| Gastrointestinal symptoms but negative by O & P exam[a] for parasites | 2/18 |
| No *G. lamblia*; asymptomatic, nonexposed controls | 0/10 |

[a] O & P exam, Ovum and parasite stool examination.

**Immunofluorescence.** Purified *G. lamblia* cysts and all cultured protozoan trophozoites were washed three times in cold sterile saline–1% glucose before use in the immunofluorescence assay. The concentration of cells was adjusted to $10^6$ per ml in sterile glucose, and 10 μl of each was applied to multispot, Teflon (E. I. Du Pont de Nemours & Co., Inc., Wilmington, Del.)-coated glass microscope slides, air dried, and then fixed for 5 min in acetone. Assay by immunofluorescence was also performed with smears of feces containing *Blastocystis hominis* or cysts of *E. histolytica*, with oocysts of *Cryptosporidium* purified from cattle, and with cysts of *Chilomastix mesnili* prepared from human stools by ethyl acetate-Formalin concentration (4). Assay by indirect immunofluorescence was performed by standard methods in humid chambers at 37°C using 1-h incubations with both the primary and secondary antibodies. The secondary, fluorescein-conjugated antibody used was fluorescein-labeled goat anti-rabbit IgG (IgG fraction) (Cappel Division, Cooper Biomedical, Inc., West Chester, Pa.) diluted 1:40 in PBS containing rhodamine-albumin counterstain (Difco Laboratories, Detroit, Mich.) diluted 1:80. Slides were mounted with fluorescence mounting medium (Difco) and viewed by using epifluorescence. Photographs were taken at ×190 magnification on Kodak Ektachrome 160 Tungsten film (Eastman Kodak Co., Rochester, N.Y.).

## RESULTS

**Isolation of GSA 65.** To determine whether anti-cyst antiserum was capable of detecting antigens in *Giardia* cyst-positive stools, CIE was used. A well-defined double precipitin line (doublet) (Fig. 1) was visible for stool eluates from 36 of 40 *Giardia* cyst-positive stool eluates, but was not visible in 10 cyst-negative controls derived from patients without clinical symptoms. With anti-cyst antiserum, precipitin lines were seldom visible before washing and staining. Use of the IgG fraction of the anti-cyst antiserum changed the doublet appearance of the precipitin to that of a single line. All of the 36 stool eluates shown to be positive by CIE with anti-cyst antiserum were also shown to be positive

TABLE 2. Reactivity of cultured organisms for anti-GSA 65 antiserum as assessed by CIE

| Cultured organism (cell type) | Reactivity[a] |
|---|---|
| *G. lamblia* (trophozoites) | + |
| *P. hominis* (trophozoites) | − |
| *T. vaginalis* (trophozoites) | − |
| *E. histolytica* (trophozoites) | − |
| *L. donovani* (promastigotes) | − |
| *C. albicans* (yeasts and germ tubes) | − |

[a] +, Positive reaction; −, negative reaction.

by CIE with monospecific antiserum (Table 1) and showed the same characteristic anodal precipitin arcs.

The electrophoretic mobility and purity of the antigen precipitated in CIE were assessed by crossed immunoelectrophoresis. When 10 stool eluates from patients previously verified as *G. lamblia*-positive by microscopy and CIE were used in crossed immunoelectrophoresis against the monospecific antiserum or against the IgG fraction of the anti-cyst antiserum, a single peak with a mobility of 1.26 (migration distance in relation to BSA, assigned a value of 1.0) was observed (Fig. 2). The peak height of this precipitin arc varied from sample to sample, presumably reflecting variations in the quantity of antigen in the stools. Using the same 10 stool eluates, no precipitin peaks were observed when rabbit anti-trophozoite antiserum or preimmune rabbit serum was used in crossed immunoelectrophoresis. Comparative analysis of these stool eluates by tandem crossed immunoelectrophoresis using anti-cyst antiserum revealed that the precipitable antigens from each stool specimen were immunologically identical; i.e., immunoprecipitates from different stools were fused in a single uneven line and no spurs were seen (data not shown). Intermediate gel crossed immunoelectrophoresis of *G. lamblia*-positive stool eluates using anti-cyst antiserum with monospecific antiserum in the intermediate gel produced a single precipitin peak, suggesting that the monospecific and the anti-cyst antisera were precipitating the same antigen (data not shown).

To determine the approximate molecular weight(s) of the stool-derived antigen and also to determine whether this antigen could be identified in *G. lamblia* cysts and trophozoites, sonic extracts from trophozoites grown in culture, purified cyst sonic extracts, and partially purified antigen from cyst-positive stool eluates were individually passed over Sepharose affinity columns prepared with the IgG fraction of the monospecific antiserum. SDS-PAGE followed by Western blotting of reconcentrated affinity column eluates revealed that all of the eluates had identical banding patterns (Fig. 3). Each demonstrated a band (molecular weight, 65,000) with light smearing from 62,000 to 68,000 above and below the main band; hence, we referred to this antigen as GSA 65 (*Giardia* stool antigen 65). Crossed immunoelectrophoresis of affinity column eluates against monospecific antiserum revealed precipitin peaks of the same electrophoretic mobility (1.26) as those seen by using cyst-positive stool eluates. Crude sonic extracts of both trophozoites and cysts also were found to contain enough GSA 65 to be detected with the monospecific antiserum by CIE and by crossed immunoelectrophoresis.

**Specificity testing.** The species specificity of GSA 65 was assessed by CIE on stool eluates from patients with various protozoan and other parasitic diseases and on a variety of species of cultured protozoa and cultured *C. albicans*; specificity was assessed also by immunofluorescence, in all cases using the monospecific anti-GSA 65 antiserum.

A total of 49 *G. lamblia*-negative stool eluates (controls) were assayed for the presence of GSA 65 by CIE with the monospecific antiserum against this antigen (Table 1). Of 31 stool eluates from patients with other non-*Giardia* protozoan and helminth parasites, 4 were positive for the antigen. Of these positives, one stool contained *Entamoeba coli*, one contained *E. histolytica*, and two contained *Entamoeba nana*. However, four of five *E. histolytica*-positive stools were CIE negative, as were three of four *E. coli*- and four of six *E. nana*-containing stools. Of 18 stool eluates from patients with gastrointestinal symptoms who were negative for intestinal parasites by microscopic examination, 2 were positive for the presence of GSA 65. Sonic extracts of cultured *P. hominis*, *E. histolytica*, *T. vaginalis*, *C. albicans*, and *L. donovani* promastigotes run in CIE were negative for GSA 65. Sonic extracts of *G. lamblia* trophozoites run as a positive control yielded precipitin lines, as expected (Table 2).

In immunofluorescence studies, monospecific antiserum reacted strongly with purified *G. lamblia* cysts. Cyst walls fluoresced brightly, and this fluorescence precluded visualization of internal morphology (Fig. 4A). Fluorescence of the cyst wall was negligible when cysts were reacted with preimmune serum (Fig. 4B). *Giardia* trophozoites did not fluoresce when reacted with monospecific antiserum or preimmune serum, a surprising observation, given the fact that trophozoites were shown to contain GSA 65 by affinity chromatography and CIE. Monospecific antiserum failed to react with cultured trophozoites of *E. histolytica*, *P. hominis*, *T. vaginalis*, and *C. albicans*. Fecal smears containing cysts of both *E. histolytica* and *Entamoeba hartmanni* and preparations of *C. mesnili* cysts, *Cryptosporidium* oocysts, and *B. hominis* were also nonreactive.

## DISCUSSION

A *G. lamblia*-specific antigen that is excreted in the stool of giardiasis patients was isolated and partially character-

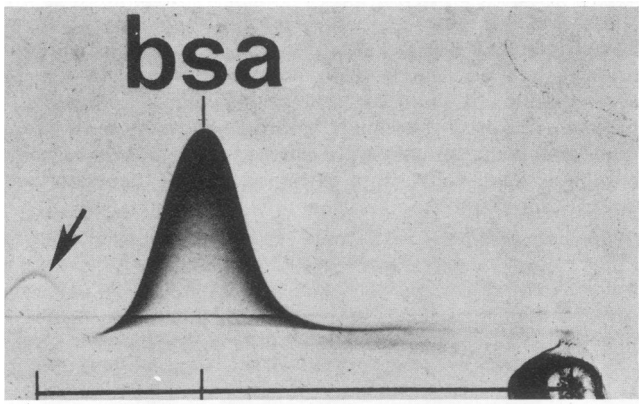


FIG. 2. Crossed-immunoelectrophoresis analysis of a *G. lamblia*-positive stool eluate. The antibody bed of 1.6 ml contained 2.5% polyspecific anti-cyst antiserum in addition to anti-BSA antiserum. The anode was on the left in the first dimension and at the top in the second dimension. The large precipitin peak is BSA. The small precipitin peak (arrow) is GSA 65.

ized. The antigen had an approximate molecular weight of 65,000; thus, we named it GSA 65. The presence of GSA 65 in cysts and trophozoites was established by agarose immunoelectrophoretic techniques and by SDS-PAGE followed by Western blotting. Immunofluorescence testing revealed that the antigen was present in or immediately adjacent to the cyst wall of *G. lamblia* cysts. The presence of the antigen in trophozoites was not confirmed by immunofluorescence, but was established both by immunoaffinity chromatographic isolation of the antigen from both cysts and trophozoites and by agarose immunoelectrophoretic precipitation of the antigen from sonicated trophozoites. Presumably, the antigen in trophozoites was either present in quantities too low to detect by immunofluorescence or present in a conformation or cellular location that did not allow for antibody binding.

A CIE assay with a monospecific anti-GSA 65 antiserum was tested and found to detect antigen in 90% of *Giardia* cyst-positive stool eluates, whereas no reactivity was observed in controls. These results compare well with those of Craft and Nelson (8) and those of Vinayak et al. (23), both of whom also claimed >90% sensitivity in CIE with *G. lamblia*-positive stool eluates. These authors also reported detecting *Giardia* antigens in eluates of stools from some patients not found to be excreting *G. lamblia* by microscopic examination. Although we were unable to follow up on the two patients in our study who were GSA 65 positive but negative in one microscopic stool exam for *G. lamblia*, it would seem likely, given the findings of the above authors and our own results revealing a general absence of cross-reactivity of this antigen with other intestinal protozoa and helminths, that what we detected was GSA 65 in the stools of patients with cryptic cases of giardiasis. Some CIE positives were obtained with stools that had been found to contain various amoebae, including *E. histolytica*; however, since the majority of amoebae-containing stools (e.g., 80% with *E. histolytica*) were CIE negative and since the amoebae themselves proved negative for GSA 65 by CIE and by immunofluorescence, it seems very probable that the CIE-positive patients with amoebae also had concurrent giardial infections. Such mixed infections are common. Cumulatively, these data furnish evidence that GSA 65 is unique to *G. lamblia*.

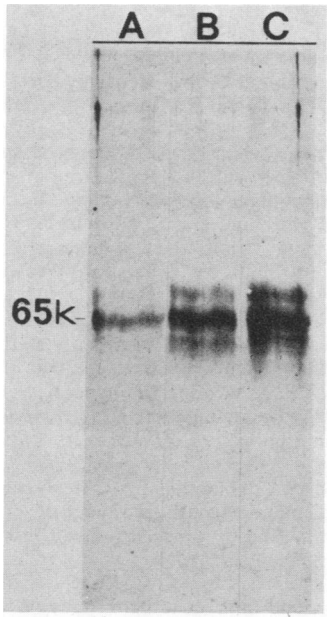


FIG. 3. Immunoblots of affinity-purified preparations of GSA 65 from trophozoite sonic extracts (lane A), cyst sonic extracts (lane B), and purified *G. lamblia*-positive stool eluate (lane C) reacted with monospecific anti-GSA 65 antiserum.

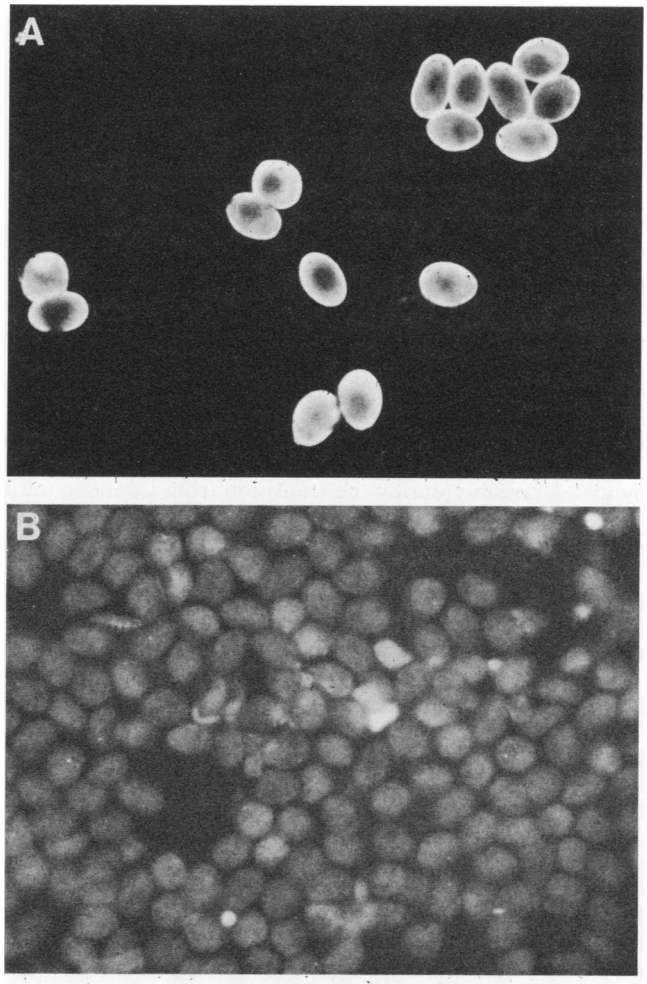


FIG. 4. Indirect immunofluorescence of *G. lamblia* cysts with monospecific anti-GSA 65 antiserum (A) and preimmune control serum (B).

GSA 65 was likely to have been among the antigens detected in the previously published studies on *Giardia* coprodiagnosis. Craft and Nelson (8) reported similar double immunoprecipitin lines in *Giardia*-positive stools assayed by CIE with anti-cyst antiserum. They also noted that rabbit anti-trophozoite antiserum did not precipitate *Giardia* stool antigens in CIE which used the same stool eluates that gave strong precipitins when run against anti-cyst antiserum. Our results support this finding, but conflict with the results of Vinayak et al. (23), who apparently obtained excellent results in CIE with antiserum against trophozoites. These findings suggest that the trophozoite does contain antigens that appear in the stool of infected patients, but at lower levels than found in cysts, so that only a very potent antiserum against trophozoites can detect such antigens by CIE or other agarose immunoelectrophoretic methods. In fact, anti-trophozoite antiserum is capable of detecting GSA

65 in Western blots of affinity-purified GSA 65 (unpublished data), suggesting that the anti-cyst and anti-trophozoite sera may be detecting at least one common antigen in stool eluates assayed by CIE or by antigen-capture ELISA. Green et al. (12) reported that their antigen-capture ELISA method was more sensitive in detecting cyst protein than trophozoite protein when sonic extracts of both were diluted in reference-negative stools, implying either that the cyst contains more potent immunogens or that it contains more of the relevant detectable stool antigens than does the trophozoite. However, Vinayak et al. (23) have stated that they believe that the stool antigens represent trophozoite antigen "elaborated by degenerating trophozoites" and that stool is unlikely to contain cyst antigen since cysts are unlikely to degenerate and release antigens during their transit down the gut. We feel that while some of the population of dividing trophozoites in the small intestine may release some antigens, perhaps as a result of degeneration, antigens released by cysts, possibly during the transition from trophozoite to the cyst stage, are the more abundant, the more stable, and the more readily detectable antigens in stool. The function of GSA 65 in the parasite, the mechanisms by which it is released into stool, and the possibility that it may be a molecule involved in the encystation process of the parasite are all questions that remain to be investigated.

Unlike the serological methods that have been applied to diagnosis of giardiasis, detection of GSA 65 in a patient's stool would be theoretically indicative of an active giardial infection and would represent a more meaningful clinical finding than the demonstration of serum antibody. The fact that this antigen is the only one consistently precipitated by anti-cyst antiserum in agarose gel immunoelectrophoresis suggests that GSA 65 is the predominant immunologically detectable *Giardia* antigen in the stool of giardiasis patients. Preliminary studies (submitted for publication) have shown that this molecule is stable under a variety of conditions, including 10% Formalin fixation and boiling. In conclusion, it would appear that GSA 65 represents an ideal antigen around which to design antigen detection methods useful in coprodiagnosis of giardiasis.

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