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Tubulin and High-Molecular-Weight Polypeptides as *Giardia lamblia* Antigens

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We determined the antigenic specificities of nine murine monoclonal antibodies to *Giardia lamblia*. Four distinct antibody reaction patterns were detected by immunofluorescence with *G. lamblia* trophozoites. Four monoclonal antibodies which reacted with the body but not the flagella of the whole trophozoites recognized two polypeptides of 170,000 and 155,000 molecular weights by immunoblotting. Two antibodies reacting with both the flagella and body also reacted with 170,000- and 155,000-molecular-weight antigens. An antibody specific for the *G. lamblia* attachment disk and two specific for flagella by immunofluorescence were found to react with 53,000- and 55,000-molecular-weight polypeptides by immunoblotting. These antigens comigrated with purified bovine brain tubulin, and their respective antibodies reacted in immunoblots with purified bovine brain tubulin. The antigens identified in this report were found to be present in *Giardia* cytoskeletal preparations. All antigens were pronase labile and heat stable. Four strains of *G. lamblia* reacted similarly in immunofluorescence assays as well as in immunoblotting assays.

Giardia lamblia is a flagellated, parasitic protozoan which adheres to the human upper intestinal epithelium by a ventral attachment disk (7). Infection may produce a variety of clinical manifestations including diarrhea, flatulence, anorexia, and malabsorption (37). *G. lamblia* is the parasite most frequently found in fecal specimens submitted to U.S. State Health Department laboratories (1) and is a frequent cause of illness worldwide. Parasitized persons may shed infectious cysts in stool intermittently (5), and colonization may be asymptomatic (2). Transmission of the organism is thought to occur through ingestion of fecally contaminated food or water (15, 26, 28) or by fecal-oral contact as has been suggested in studies of child day care centers (2) and homosexual males (25).

Diagnosis of giardiasis is laborious and insensitive and is usually performed by direct microscopic examination of stool specimens for cysts or live trophozoites (5, 7). Alternate methods of laboratory diagnosis include microscopic examination of duodenal aspirates for trophozoites or counterimmunoelectrophoresis for the detection of trophozoite or cyst antigens in feces (4).

G. lamblia antigens and their roles in pathogenesis of disease have not been extensively investigated. Smith et al. (30) obtained *G. lamblia* isolates from widely divergent geographic locations and analyzed these specimens by the enzyme-linked immunosorbent assay (ELISA), gradient sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and crossed immunoelectrophoresis. Gradient SDS-PAGE demonstrated overall similarities in trophozoite proteins. We prepared nine independently derived hybrid cell lines that produce monoclonal antibodies against *G. lamblia*. Immunological evaluation by indirect immunofluorescence and immunoblotting techniques showed that these antibodies react with multiple strains of *G. lamblia* trophozoites.

MATERIALS AND METHODS

Organisms and culture. Four *G. lamblia* strains (Portland 1 [24], WB [31], RS [30], and LT [30]) were cultured in screw-cap borosilicate glass tubes (16 by 125 mm) at 37°C in Diamond's modified TYI-S-33 medium (6) supplemented with 10% newborn calf serum, or Meyer's HSP-1 medium supplemented with 15% horse serum (24). The WB strain was obtained from Louis S. Diamond, National Institute of Allergy and Infectious Disease, Bethesda, Md. The RS and LT strains were obtained from Murray Wittner, Department of Pathology, Albert Einstein College of Medicine, Yeshiva University, Bronx, N.Y. Portland 1 strain was obtained from Govinda Visvesvara, Centers for Disease Control, Atlanta, Ga. Cultures of *G. lamblia* were shown to be free of mycoplasmic contamination by culture methods and DAPI (4′-6-diamidino-2-phenylindole) staining (14, 29). Organisms for immunization were harvested by chilling culture tubes in an ice bath for 10 min to detach trophozoites. Organisms were centrifuged at 250 × *g* for 10 min and washed three times in phosphate-buffered saline (PBS; pH 7.0, 0.01 M), and the final pellet was resuspended in PBS at a concentration of 3.2×10^7 cells per ml and, for immunization, immediately inoculated into mice. More than 90% of the parasites excluded trypan blue at the time of inoculation. For indirect immunofluorescence, the final pellet was resuspended in PBS containing 1.0% Formalin. This suspension was left overnight at 4°C, centrifuged at 250 × *g* for 10 min, resuspended in PBS at an appropriate dilution, and used for immunofluorescence assay the same day. For immunoblotting and for ELISA, the washed, pelleted organisms were used immediately or stored at −20°C. Protein estimations of antigen preparations were determined by the method of Bradford (3).

Polyvalent rabbit anti-*G. lamblia* Portland 1 serum was the kind gift of David Einfeld, Department of Pathobiology, University of Washington, and was prepared by a previously described immunization procedure (13). A Braun homogenate of *Candida albicans* was obtained from Paula Sundstrom of the Department of Pathobiology, University of Washington, after culture in glucose-salts-biotin medium followed by culture in Medium 199 (GIBCO Laboratories, Grand Island, N.Y.) (22, 32). Threefold-purified bovine brain tubulin was the kind gift of R. L. Margolis of the Fred Hutchinson Cancer Research Center, Seattle, Wash.

Preparation of monoclonal antibodies. BALB/c mice were immunized intraperitoneally with 8×10^5 live *G. lamblia*

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(Portland 1 strain) trophozoites suspended in 0.25 ml PBS, pH 7.0. After 41 days, the mice were reinoculated intraperitoneally with 8×10^5 trophozoites, "rested" 21 days, and subsequently boosted intravenously with 8×10^5 trophozoites 3 days before sacrifice. Preparation of monoclonal antibodies was carried out as previously described (17, 21, 33). Monoclonal immunoglobulin subclasses were determined with an enzyme immunoassay kit (MONOAB-ID EIA; Zymed Laboratories, San Francisco, Calif.).

Antibody assays. Anti-*G. lamblia* antibodies were detected in culture supernatants by ELISA (35). Briefly, 96-well round-bottom microtiter plates were seeded with 50 μl of a suspension containing 8.6×10^5 *G. lamblia* trophozoites (Formalin-fixed or live organisms) per ml in PBS (pH 7.6) and incubated at 37°C overnight, leaving organisms adhering to the microtiter plate wells. The PBS was then discarded, and 100 μl of 5% bovine serum albumin in PBS (pH 7.6) was added to each well. The plates were incubated for 3 h at 37°C, and the PBS was discarded. Culture supernatant (50 μl) was added to each well, and the balance of the procedure was performed as previously described (33).

Immunoblotting procedure. Antigens were separated by SDS-PAGE (18) and then electrophoretically transferred to nitrocellulose sheets for immunoblotting (34). The procedures were carried out as previously described (33) with slight modifications. Blocking the nitrocellulose sheets with 5% bovine serum albumin was omitted, and incubations with antibody or conjugate were carried out in PBS (pH 7.6) with 0.3% Tween 20. Bound murine monoclonal antibodies were detected with anti-mouse peroxidase-conjugated goat immunoglobulin (Cappel Labs, West Chester, Pa.). The nitrocellulose sheets were stained with a solution of 3 ml of 4-chloro-1-naphthol (3.3 mg/ml in absolute methanol) in 17 ml of TES-saline [5 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid, 0.85% sodium chloride] with 0.06% hydrogen peroxide. India ink staining of nitrocellulose sheets for protein was performed according to the method of Hancock and Tsang (10).

Isolation of *Giardia* cytoskeletons. Isolation of *G. lamblia* cytoskeletons was performed by the method of Holberton and Ward (11) in TEDEMP (10 mM Tris-hydrochloride buffer [pH 8.2], 2 mM EDTA, 2 mM dithiothreitol, 1 mM ATP, 2 mM $MgSO_4$, 150 mM KCl, 0.5% Triton X-100). Cells were harvested as described above and were suspended in TEDEMP at a final density of 10^8 cells per ml. Cells were subjected to vortex agitation for 2 min. Membrane-free cytoskeletons were pelleted by centrifugation at 15,000 × *g* at 4°C for 10 min and washed three times by suspension in Triton-free TEDEMP, and the final pellet was suspended in PBS (pH 7.6) at an appropriate protein concentration for SDS-PAGE and immunoblotting procedures.

Physicochemical characterization. The physicochemical characteristics of *G. lamblia* antigens were investigated by subjecting live trophozoites or isolated cytoskeletons to pronase or heat treatment before ELISA or immunoblot assay. For the ELISA procedure live trophozoites were subjected to heat treatment in a water bath for 20 min at 100, 80, or 65°C. Heat-treated organisms were then adsorbed to the surface of a 96-well microtiter plate overnight (25 μg per well) at 37°C for ELISA analysis. When pronase was used as a treatment, live organisms at a protein concentration of 25 μg per well were first adsorbed to the surface of the microtiter plates, and then 50 μl of pronase (100 μg/ml; Calbiochem-Behring, La Jolla, Calif.) in PBS (pH 7.0) was added to each well. The plates were then incubated at 37°C for 2 h and washed three times with PBS (pH 7.6) before the antibody binding assay. For immunoblotting isolated cytoskeletons, preparations were treated with pronase or heat and then subjected to SDS-PAGE analysis and immunoblotting. The same protein and pronase concentrations were used for this procedure as were used in the ELISA.

Indirect immunofluorescence. Indirect immunofluorescence assays with Formalin-fixed parasites were performed by the method of Wang and Grayston (36) with slight modifications as previously described (33). Formalin-fixed parasites were also used in the procedure outlined below for live organisms, to determine if the two different procedures yielded similar results with Formalin-fixed parasites.

To determine whether the antigens detected by immunofluorescence were located on the surface of the flagellates, we performed an immunofluorescence assay on live parasites. Briefly, 50 μl of heat-inactivated ascites fluid containing monoclonal antibody to *G. lamblia* was added to 100 μl of a suspension of freshly harvested, washed *G. lamblia* (10^8 cells per ml in PBS, pH 7.6). The mixture was incubated at 37°C for 45 min. The cells were washed three times in PBS (pH 7.6) and resuspended in 20 μl of PBS. Fluorescein-conjugated goat anti-mouse immunoglobulin (50 μl; 1:50 dilution in PBS) was then added to the cells. The cells were incubated for 30 min at 37°C, centrifuged at 1,000 × *g* for 10 min, washed three times in PBS, and resuspended in PBS. The parasites remained motile throughout this procedure. Portions (10 μl) of the suspension were air dried on glass slides. The slides were fixed in acetone for 10 min and air dried, and cover slips were mounted as described previously (12, 33). Polyvalent rabbit anti-*G. lamblia* antibody detected with fluorescein-conjugated goat anti-rabbit serum served as a positive control and was consistently positive. Negative controls included antigen to which monoclonal antibody specific for *Chlamydia trachomatis* was added or antigen to which no antibody, but fluorescein-conjugated anti-mouse immunoglobulin serum, was added. Both were consistently negative.

TABLE 1. Immunofluorescence reaction patterns, physicochemical sensitivities, and antibody subclasses

Antibody	Immunofluorescence staining pattern	Antigen sensitivity[a]		Antibody type[b]
		Heat	Pronase	
3-4	Body-flagella	−	+	ND[c]
3-17	Body-flagella	−	+	IgM
3-7	Body	−	+	IgG1
3-11	Body	−	+	ND
3-15	Body	−	+	IgG1
3-16	Body	−	+	ND
3-5	Flagella	−	+	IgM
3-14	Flagella	−	+	IgM
3-12	Attachment disk	−	+	IgM

[a] Sensitivities were tested in immunoblot assays.
[b] All antibodies contained k light chains. IgM, Immunoglobulin M.
[c] ND, Not done.

RESULTS

Isolation of hybrid cell lines producing anti-*G. lamblia* antibodies. Of 768 wells seeded, 47 culture supernatants demonstrated antibody against *G. lamblia*, and hybrids from these wells were serially passaged at low density (5 to 10 cells per well), reassayed, cloned by limiting dilution twice, and assayed for antibody after each cloning. This process resulted in 17 phenotypically stable, cloned cell lines that produced monoclonal antibody against *G. lamblia*. Nine of the monoclonal antibodies were chosen for further analysis

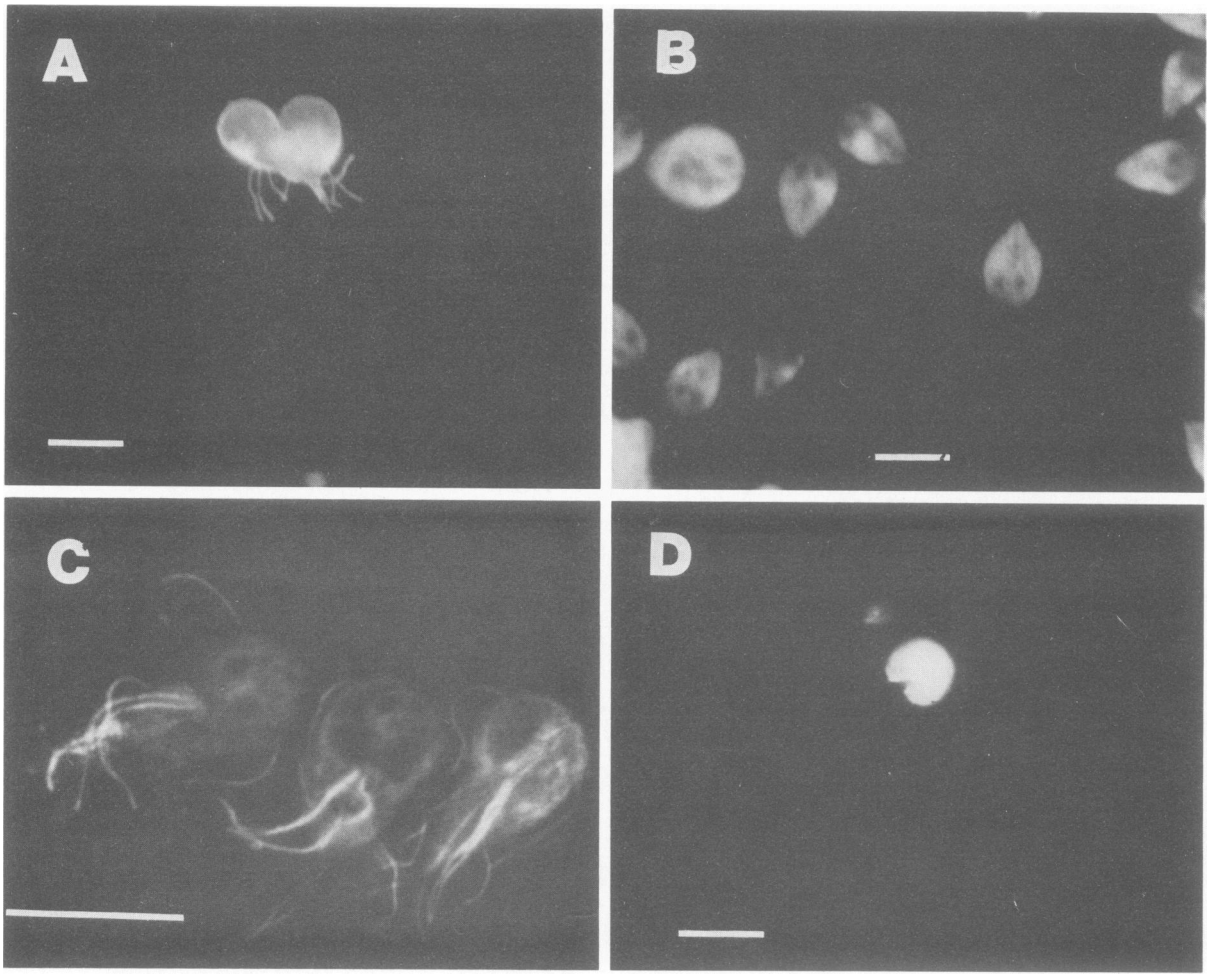

FIG. 1. Indirect immunofluorescence of Formalin-fixed *G. lamblia* WB trophozoites with monoclonal antibodies. (A) Antibody 3-17 reacting with both the body and flagella of the trophozoites. (B) Antibody 3-16 reacting with the body of the trophozoites. Note that the nuclei do not stain. (C) Antibody 3-14 reacting with flagella. (D) Antibody 3-12 was specific for the attachment disk. Bars, 10 μm.

(Table 1). To aid in identifying antibodies against medium components, yeast extract (Difco Laboratories, Detroit, Mich.), Trypticase-peptone (BBL Microbiology Systems, Cockeysville, Md.), and Phytone (BBL) components were assayed by ELISA. Antibodies 3-5, 3-14, and 3-12 were reactive to yeast extract but to no other medium components. All other antibodies assayed were unreactive toward medium components.

Specificity of monoclonal anti-*G. lamblia* antibodies by microimmunofluorescence. Serial dilutions of each of the nine antibodies tested were reacted against four strains of *G. lamblia*. The antibody titers of the ascites fluids ranged from 1:1,000 to 1:10,000 by immunofluorescence. All monoclonal antibodies reacted with the four strains of *G. lamblia* trophozoites with similar intensity at equivalent titration endpoints. The antibodies varied, however, in which locations in the cells were stained. Four monoclonal antibodies reacted only with the body of the trophozoites, whereas two monoclonal antibodies reacted with both the flagella and body of the trophozoites with similar intensity (body-flagella) (Table 1; Fig. 1). Two of the antibodies reacted primarily with flagella, although some weak staining of the body was evident at low dilutions of antibody. We have termed this reaction flagella specific. One antibody was specifically reactive with the attachment disk of the trophozoites. The antibody stained the anterior portion of the ventral side of the parasite, outlining the classic bilobed disk profile. Only about half of the trophozoites were stained in this pattern; the others did not appear to be stained at all, probably due to their orientation on the slide. Four general reactivity patterns were thus demonstrated: body only, body-flagella, flagella, and attachment disk (Fig. 1). The body-specific antibodies (3-7, 3-11, 3-15, and 3-16) reacted evenly over most of the body of the trophozoites; however, the nuclei or areas over the nuclei were unstained. The body-flagella class (3-4 and 3-17) of antibody reacted evenly with the body and flagella of the trophozoites. As a control, monoclonal antibody specific for *C. trachomatis* failed to react with *G. lamblia*. The four antibody reaction patterns mentioned above (body, flagella, body-flagella, and attachment disk) were the same for Formalin-fixed trophozoites when the assay was performed by the microimmunofluorescence method or by using Formalin-fixed trophozoites in the protocol described for live organisms. In the immunofluorescence assays with live organisms, none of the monoclonal antibodies reacted to *G. lamblia*, probably because the monoclonal antibodies do not

react with antigenic determinants which are located on the external surface of the plasma membrane. The polyvalent anti-*G. lamblia* serum control was consistently positive.

Physicochemical nature of *Giardia* antigens. The sensitivity of *Giardia* antigens to heat treatment and pronase digestion was examined by immunoblotting and ELISA. By immunoblotting, all antigens were sensitive to pronase and resistant to heat treatment (Table 1). In contrast, by ELISA the antibody-antigen reactions of antibodies 3-5, 3-14, and 3-12 were actually enhanced by pronase treatment only (data not shown).

Identification and characterization of *Giardia* antigens by immunoblotting. Monoclonal antibodies reacting with the trophozoite body (3-7, 3-11, 3-15, and 3-16) and with the body and flagella (3-4 and 3-17) in immunofluorescence assays reacted with two closely migrating components of approximate molecular weights 170,000 and 155,000 in all four *G. lamblia* strains (Fig. 2). This was consistent irrespective of whether the antigen used was whole solubilized trophozoites or cytoskeletons. On 7.5% gels, the separation of the two components is clear (Fig. 2). Rabbit polyvalent *G. lamblia* antiserum stained the 170,000- and 155,000-molecular-weight components as intensely as any other components (Fig. 2). Flagellum- (3-5 and 3-14) and disk-specific (3-12) antibodies reacted with 53,000- and 55,000-molecular-weight polypeptides. At lower antibody dilutions, five other peptides were clearly seen on the immunoblots stained with antibody 3-5 (data not shown) or 3-14. Approximate molecular weights for these polypeptides in cytoskeletal preparations were 160,000, 116,000, 110,000, 38,000, and 31,000 (Fig. 3). The polypeptides reactive with all three of these antibodies (3-5, 3-14, and 3-12) also comigrated with a 53,000- to 56,000-molecular-weight band of a purified bovine brain tubulin preparation (Fig. 4). All monoclonal antibodies

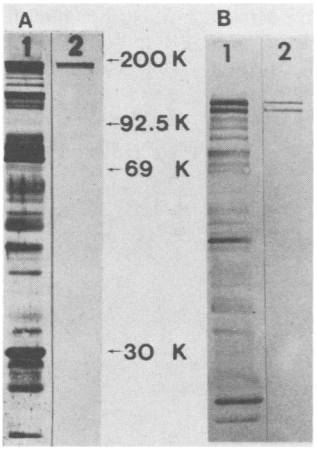

FIG. 2. Antigens of *G. lamblia* WB whole solubilized trophozoites (25 μg of protein per lane) reacted with rabbit polyvalent or murine monoclonal antibodies in immunoblots. (A) Polypeptides separated on a 10% SDS-PAGE gel. (B) Polypeptides separated on a 7.5% SDS-PAGE gel. (A and B) Lane 1, Polyvalent anti-*G. lamblia* Portland 1 antiserum at a 1:1,000 dilution reacting with numerous antigenic components; lane 2, monoclonal antibody 3-4 reacting with 170,000- and 155,000-molecular-weight components. Numbers show ^{14}C-methylated protein standards visualized after autoradiography (not shown), including myosin (molecular weight, 200,000), phosphorylase *b* (92,500), bovine serum albumin (69,000), carbonic anhydrase (30,000), and cytochrome *c* (22,300) (New England Nuclear Corp., Boston, Mass.). K, 10^3.

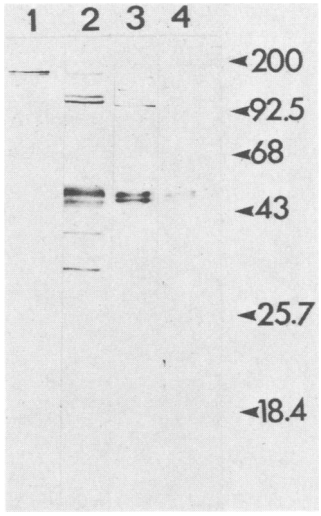

FIG. 3. Antigens of *G. lamblia* WB cytoskeletal preparations (25 μg of protein per lane) reacted with monoclonal antibodies in immunoblots. Lane 1, Antibody 3-7 (body specific by immunofluorescence) reacting with 170,000- and 155,000-molecular-weight components. Lane 2, Flagellum-specific antibody 3-14 (1:400 dilution) reacting with 53,000- and 55,000-molecular-weight components, as well as with 160,000-, 116,000-, 110,000-, 38,000-, and 31,000-molecular-weight components. Lane 3, Antibody 3-14 (1:1,000 dilution). Lane 4, Antibody 3-12 (disk specific, 1:1,000 dilution) reacting with 53,000- and 55,000-molecular-weight components. Numbers on right show molecular weights of standards ($\times 10^3$).

failed to react with cytoskeletal preparations that were treated with pronase and then subjected to SDS-PAGE followed by immunoblotting (data not shown). Antibodies 3-5, 3-14, and 3-12 reacted strongly with a purified bovine brain tubulin preparation, and antibody 3-14 was tested and found to react with a peptide of *C. albicans* comigrating with bovine brain tubulin (Fig. 5). No positive reactions were demonstrated with any of the monoclonal antibodies when serum (horse or calf), yeast extract, Trypticase-peptone, or Phytone medium components were tested by immunoblotting at protein concentrations equivalent to those used for the organisms or at protein concentrations 10 times those used for the organisms. In contrast, flagellum-specific antibodies reacted with a 54,000-molecular-weight polypeptide of *C. albicans* (as stated above) as well as another, faster-migrating polypeptide. Disk-specific antibody was not tested against *C. albicans*.

DISCUSSION

We have prepared monoclonal antibodies to *G. lamblia* which show four different staining patterns by immunofluorescence: body, flagella, body-flagella, and attachment disk.

Body-flagella- (3-4 and 3-17) and body-specific (3-7, 3-11, 3-15, and 3-16) antibodies reacted with 170,000- and 155,000-molecular-weight antigens which were pronase labile and heat stable. The lack of reactivity of these antibodies in immunofluorescence assays of live organisms suggests that the antigenic determinants are not located on the surface of the parasite membrane. Although both the body-flagella- and body-specific antibodies recognize the same molecular weight components in whole cell and cytoskeletal prepara-

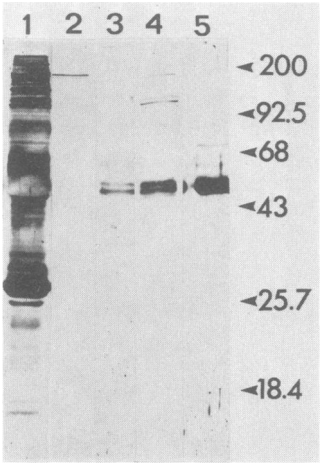

FIG. 4. Antigens of *G. lamblia* WB cytoskeletal preparations (25 μg of protein per lane) visualized after immunoblotting compared with an India ink protein stain of purified bovine brain tubulin (25 μg of protein per lane). Lane 1, Rabbit anti-*G. lamblia* Portland 1 antiserum at a 1:1,000 dilution reacting with numerous *G. lamblia* cytoskeletal antigens. Lane 2, Monoclonal antibody 3-7 (body specific) reacting with 170,000- and 155,000-molecular-weight components of *G. lamblia*. Lane 3, Monoclonal antibody 3-12 (disk specific, 1:750 dilution) reacting with 53,000- and 55,000-molecular-weight antigens of *G. lamblia*. Lane 4, Monoclonal antibody 3-14 (flagella specific, 1:1,000 dilution) reacting with 53,000- and 55,000-molecular-weight antigens of *G. lamblia* as well as three higher-molecular-weight components. Lane 5, India ink stain of purified bovine brain tubulin. Numbers on right ($\times 10^3$) are prestained molecular weight standards (not shown) (Bethesda Research Laboratories, Gaithersburg, Md.), including myosin (200,000), phosphorylase B (92,500), bovine serum albumin (68,000), ovalbumin (43,000), chymotrypsinogen (25,700), β-lactoglobulin (18,400), and cytochrome *c* (12,300).

tions by immunoblotting, the difference in immunofluorescence staining suggests that at least two forms of the antigen exist in different cellular locales.

In this study, we showed that three antibodies (two flagella specific and one disk specific) react not only with *G. lamblia* but also with purified bovine brain tubulin. Because these antibodies react to *Giardia* polypeptides which comigrate with tubulin, react with bovine brain tubulin, and stain microtubule-containing structures by immunofluorescence, we conclude that the polypeptides comigrating with bovine brain tubulin are *G. lamblia* tubulin. The higher-molecular-weight peptides visualized in cytoskeletal preparations by flagellum-specific antibody at lower antibody dilutions may be tubulin polymers which have not been completely solubilized or dissociated, although increasing the boiling time of samples for SDS-PAGE and increasing the 2-mercaptoethanol concentration did not alter the profiles. Thus, the higher-molecular-weight peptides may be unrelated proteins. The two lower-molecular-weight bands may be tubulin precursors, proteolytic fragments, or polypeptides sharing a determinant with tubulin. The phenomenon of monoclonal antibody cross-reactivity to unrelated proteins has been reviewed by several authors (19, 27) and has been previously observed with monoclonal antibodies developed against bovine brain tubulin (8).

Antibodies 3-5, 3-14, and 3-12 reacted to yeast extract by ELISA. However, yeast extract failed to react in immunoblots, and furthermore, *G. lamblia* grown in HSP-1 (medium which contains no yeast extract) reacted with flagellum- and disk-specific antibodies (3-5, 3-14, and 3-12) by immunofluorescence, immunoblotting, and ELISA. This suggests that the antigen(s) of *G. lamblia* detected by these antibodies shares a determinant(s) with one or more of the many components of yeast extract (from *Saccharomyces* spp.). Since the yeast extract was an autolysate, the components reacting with antibodies 3-5, 3-14, and 3-12 may have been digested to the point at which they are too small to be retained by nitrocellulose paper, but can adsorb to ELISA plates. The reactivity with yeast extract is not surprising since antibodies 3-5 and 3-14 (flagella specific) also reacted with a 54,000-molecular-weight peptide of *C. albicans*. The peptide is probably a tubulin species.

Holberton and Ward (11) reported that the cytoskeleton of *G. lamblia* is composed largely of two components, tubulin and a 30,000-molecular-weight component which those authors refer to as "giardin." Tubulin is the principal component of microtubules, which are present in the cytoskeletons of all eucaryotic cells. *G. lamblia* possesses several types of microtubule-containing structures such as the flagellar axonemes and the attachment disk. Microtubules are composed largely of a protein dimer consisting of one α and one β tubulin subunit. Despite their universal distribution in eucaryotes, tubulins are not a homogeneous species. Each subunit (α or β) can be resolved into a set of isoforms by isoelectric focusing (16). *Giardia* cytoskeletons have been shown to possess five tubulin isoforms by isoelectric focusing (10). Other eucaryotic cells may express multiple forms of tubulin (9), and tubulin isoforms may be distributed in different cellular locales (23).

The antibodies specific for the flagella (3-5 and 3-14) may react with a population of flagellar microtubules which differ by at least one determinant from those microtubules found in the attachment disk (recognized by antibody 3-12). Alternatively, the attachment disk and flagellar tubulins may differ only in conformation, thus masking specific determinants and making them differentially accessible to the two classes of tubulin-specific antibodies. However, the former possibil-

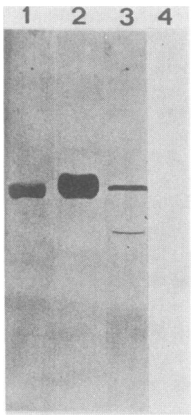

FIG. 5. Purified bovine brain tubulin (25 μg of protein per lane) or Braun homogenate of *C. albicans* (36 μg protein per lane) in immunoblots. Lane 1, Monoclonal antibody 3-12 reacting with purified bovine brain tubulin. Lane 2, Monoclonal antibody 3-14 reacting with purified bovine brain tubulin. Lane 3, Monoclonal antibody 3-14 reacting with *C. albicans*. Lane 4, Monoclonal antibody specific for *C. trachomatis* did not react with purified bovine brain tubulin.

ity of tubulin isoforms possessing different determinants recognized by the two antibody types has precedent. The existence of a flagellum-specific tubulin has been reported in *Chlamydomonas* sp. (20) and *Polytomella* sp. (23) and has been demonstrated to be the result of the presence of an additional α-tubulin isomer. Gallo and Anderton (8) recently reported the ability of two antitubulin monoclonal antibodies to distinguish between subpopulations of trypanosome microtubules. In that report, bovine brain tubulin was used as the immunogen to develop monoclonal antibodies; the antibodies were then tested for reactivity to trypanosomes. We obtained antibodies to tubulin by using a parasite as immunogen and then tested bovine brain tubulin for reactivity. As Gallo and Anderton (8) have done, we have suggested the likelihood of tubulin isoforms possessing different determinants and of the isoforms having different cellular locations.

In the present study, four strains of *G. lamblia* reacted in an equivalent manner by immunofluorescence, immunoblotting, and ELISA with all antibodies tested. Owing to their extensive cross-reactivity, the monoclonal antibodies to tubulin are not likely to be useful for a specific test to detect *G. lamblia* antigen in stool. In contrast, those antibodies which reacted with high-molecular-weight polypeptides may prove useful as a diagnostic probe. However, other isolates of *G. lamblia* must be tested before drawing conclusions about the diagnostic ability of these antibodies.

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