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An 88,000-M_r *Giardia lamblia* Surface Protein Which Is Immunogenic in Humans

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Human anti-*Giardia lamblia* sera specifically immunoprecipitated an 88,000-M_r surface protein from radioiodinated trophozoites, establishing this protein as a potentially important immunogen in humans. A mouse monoclonal antibody (GL-1) was isolated which immunoprecipitated the same 88,000-M_r surface protein recognized by the human sera. GL-1 gave uniform fluorescent staining of the cell surface and flagella of *G. lamblia* trophozoites from the Portland 1 and WB strains as well as fresh clinical isolates, but not of *Giardia muris*, suggesting that the surface antigen is specific to *G. lamblia*. Other human parasites, including *Entamoeba histolytica*, *Trichomonas vaginalis*, and *Trichomonas hominis*, were not stained. A second mouse monoclonal antibody (GL-2) gave weaker immunofluorescent staining of living *G. lamblia* trophozoites but intense staining of fixed cells. None of the other parasites tested were stained, with the exception of *E. histolytica*, which may contain a cross-reactive antigen. No proteins were recognized in immunoprecipitation studies with iodinated trophozoites.

Giardia lamblia infection occurs world wide and is an important cause of diarrhea and malabsorption particularly in children and thus constitutes a significant public health problem (5). Although protective immunity to *G. lamblia* has not yet been demonstrated conclusively in humans, the development of such an immune response is suggested by the self-limiting nature of the infection (19), by the appearance of *G. lamblia*-specific antibodies in the sera of infected patients (18, 20, 23, 27), by the apparently increased susceptibility of hypogammaglobulinemic persons to this infection (1, 21) and the decreased susceptibility of long-term residents in endemic areas compared with new visitors, and by the results obtained from animal studies with *Giardia muris* (22, 24). Assessment of the importance of cellular and humoral immunity in controlling *G. lamblia* infection will ultimately involve identification of the relevant parasite components which elicit these responses. However, to date the surface antigens recognized by the *G. lamblia*-specific human sera have not been identified. Additionally, several studies directed toward the identification and characterization of the surface antigens of *G. lamblia*, the likely targets of the immune response in vivo, have reported somewhat conflicting results (4, 16, 17).

In the present study we demonstrate that human anti-*G. lamblia* sera recognize an 88,000-M_r protein present on the surface of axenically cultured trophozoites. In addition, we have prepared mouse monoclonal antibodies to trophozoite surface antigens and shown that one, GL-1, recognizes the same 88,000-M_r protein as the human sera.

MATERIALS AND METHODS

Parasites, cells, and media. The Portland 1 strain of *G. lamblia* (15) was obtained originally from L. Diamond of the Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, whereas the WB strain was from the American Type Culture Collection (ATCC 30957). Trophozoites were cultivated axenically at 37°C in modified TYI-S medium (2, 9) containing 10% heat-inactivated bovine serum (Biofluids Inc., Rockville, Md.) and 0.8 g of bovine bile (Sigma Chemical Co., St. Louis, Mo.) per ml. Stationary tube or roller bottle cultures were grown as described previously (7), and middle- to late-log-phase cultures were harvested by chilling on ice followed by agitation to dislodge attached cells. Trophozoites were collected by centrifugation at 500 × *g* for 20 min at 4°C and washed three times with phosphate-buffered saline (PBS). Fresh *G. lamblia* trophozoites were also obtained by duodenal aspiration from four patients with acute giardiasis and washed similarly before use in indirect immunofluorescent studies. *G. muris* was kindly provided by H. Ward Pillai, *Entamoeba histolytica* HM1 was provided by K. McGowan, and local isolates of *Trichomonas vaginalis* and *Trichomonas hominis* were provided by J. Ackers. P3x63Ag8.653 mouse myeloma cells (10) were grown in RPMI 1640 medium (Microbiological Associates Bioproducts, Walkersville, MD) supplemented with 10% fetal calf serum (FCS) (GIBCO Laboratories, Grand Island, N.Y.), 1 mM sodium pyruvate (Flow Laboratories, Inc., McLean, Va.) and 50 μM 2-mercaptoethanol (Bio-Rad Laboratories, Richmond, Calif.).

Materials. Sodium dodecyl sulfate (SDS) was purchased from Sigma, and Triton X-100 and all other reagents for SDS-polyacrylamide gel electrophoresis (SDS-PAGE) were from Bio-Rad. Radioisotopes were obtained from New England Nuclear Corp. (Boston, Mass.) and include carrier-free $Na^{125}I$ and ^{14}C-methylated proteins used as molecular weight markers for SDS-PAGE. Bovine milk lactoperoxidase was purchased from Calbiochem-Behring (La Jolla, Calif.). Formalin-fixed *Staphylococcus aureus* (IgSorb) was from the New England Enzyme Center (Malden, Mass.).

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Cell surface iodination. Trophozoites (10^8) were washed three times with PBS and suspended in 1 ml of PBS, and the surface membrane proteins were radioiodinated by using a lactoperoxidase procedure and 0.5 mCi of $Na^{125}I$ (14). Cells were washed four times with PBS, lysed by the addition of 1 ml of 10 mM Tris hydrochloride (pH 7.4)–1 mM $MgCl_2$–0.25 mM dithiothreitol–0.25 mM phenylmethylsulfonyl fluoride–0.5% Triton X-100, and incubated at 4°C for 30 min with periodic Vortex mixing. The lysate was centrifuged at 74,000 × *g* for 30 min and used immediately or stored frozen at −70°C.

Antisera and immunoprecipitation. The control human sera used in this work were from persons with no history of giardiasis, and they did not stain trophozoites by indirect immunofluorescence (described below). The two anti-*G. lamblia* human sera used in this work did stain trophozoites by indirect immunofluorescence and were obtained from a convalescent giardiasis patient and a person with no established history of infection. The mouse anti-*G. lamblia* serum was obtained from BALB/c mice immunized with whole trophozoites as described below. Immunoprecipitation with these polyclonal antibodies and the monoclonal antibodies described below was carried out with Formalin-fixed *S. aureus* cells as described originally by Kessler (11), with minor modifications (25, 26). In each experiment, the samples were precleared with 5 μl of ascites fluid of an irrelevant monoclonal antibody and 200 μl of *S. aureus*.

Electrophoresis. SDS-PAGE was carried out by the method of Laemmli (13) on 1.5-mm-thick slab gels at the percent acrylamide indicated in each figure legend. After electrophoresis, gels which contained ^{125}I were dried and exposed at −70°C to Kodak XAR-5 X-Omat film with an intensifying screen.

Hybridoma preparation. Mice were injected subcutaneously with 2 × 10^7 intact trophozoites and were boosted subcutaneously 2 months later with 2 × 10^7 trophozoites. Two months later, the mice were boosted intravenously with 2 × 10^7 trophozoites, and 4 days later they were sacrificed. Fusion to P3x63Ag8.653 myeloma cells in a ratio of 2:1 followed the procedure of Kohler and Milstein (12). Culture supernatants were analyzed for *G. lamblia*-specific antibodies by indirect immunofluorescence, strongly positive cultures were expanded and cloned twice by limiting dilution, and ascites fluid was prepared from the stable hybridoma cell lines. The hybridoma giving the brightest membrane fluorescence, 4.CE.3D2, was termed GL-1, whereas that giving the brightest ghost fluorescence, 4.CE.5D4, was termed GL-2. Immunoglobulin subclass was determined by Ouchterlony immunodiffusion in agarose using anti-mouse isotype-specific sera (Gateway Immunosera Co., St. Louis, Mo.).

Immunofluorescence. *G. lamblia* trophozoites were washed three times with PBS and deposited as smears on glass slides, air dried for 2 h at room temperature, and fixed in methanol at −20°C for 5 min. Methanol-fixed *G. lamblia* trophozoites were incubated for 40 min at room temperature with antibody, washed with PBS, incubated with a 1:20 dilution of fluorescein isothiocyanate-labeled rabbit anti-mouse immunoglobulin G (IgG; Cappel Laboratories, Cochranville, Pa.) for 40 min at room temperature in the dark, washed with PBS, and examined with a Nikon fluorescence microscope. Immunofluorescence studies with living trophozoites were carried out similarly, except the incubations were at 4°C and cells were washed by centrifugation at 1,000 × *g*. The control antibodies used in these studies were a series of isotype-matched monoclonal antibodies to varicella-zoster virus (3).

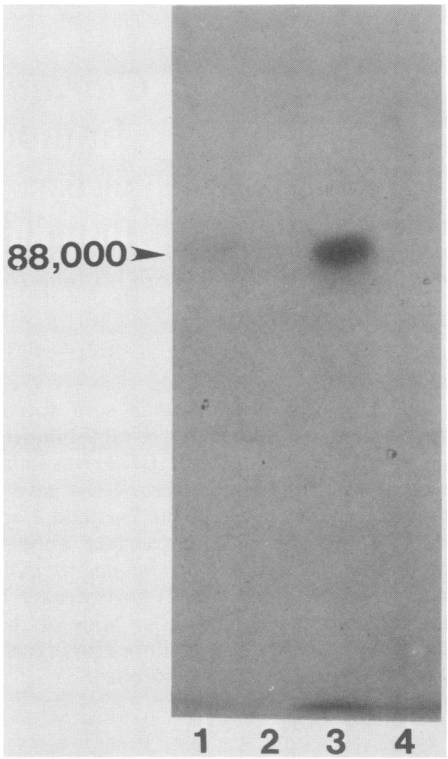

FIG. 1. ^{125}I-labeled *G. lamblia* proteins recognized by human anti-*G. lamblia* sera. Extracts containing ^{125}I-labeled proteins were immunoprecipitated with control human sera containing no *G. lamblia*-specific antibodies (lanes 2 and 4) or with human anti-*G. lamblia* sera (lanes 1 and 3). The serum in lane 1 was from a convalescent giardiasis patient. The samples were analyzed by 10% SDS-PAGE.

RESULTS

Protein specificity of human anti-*G. lamblia* sera. Several laboratories have reported the presence of *G. lamblia*-specific serum antibodies in humans (18, 20, 23, 27). To examine the protein specificities of such antisera, immunoprecipitation studies were performed with several human anti-*G. lamblia* sera which immunofluorescently stain trophozoites and with several negative control human sera. Trophozoites of the Portland 1 isolate were radioiodinated by the lactoperoxidase procedure, and a Triton X-100 extract was prepared and subjected to immunoprecipitation. Electrophoretic analysis of the immunoprecipitates (Fig. 1) revealed that the anti-*G. lamblia* human sera recognized an 88,000-M_r surface protein (lanes 1 and 3), whereas negative human sera did not (lanes 2 and 4).

***G. lamblia*-specific monoclonal antibodies.** To develop antibody reagents useful for the further characterization of the 88,000-M_r *G. lamblia* surface protein as well as other surface antigens, a series of *G. lamblia* surface antigen-specific monoclonal antibodies was isolated. Eleven stable cell lines secreting *G. lamblia* surface antigen-specific antibodies were prepared from BALB/c mice immunized with axenic trophozoites of the Portland 1 isolate. When ascitic fluids were assayed by indirect immunofluorescence, three reacted with surface membrane components, giving uniform fluorescent staining of the cell surface and flagella on both living and methanol-fixed cells (membrane pattern) (Fig. 2a and b). The remaining eight also reacted with living cells, giving a

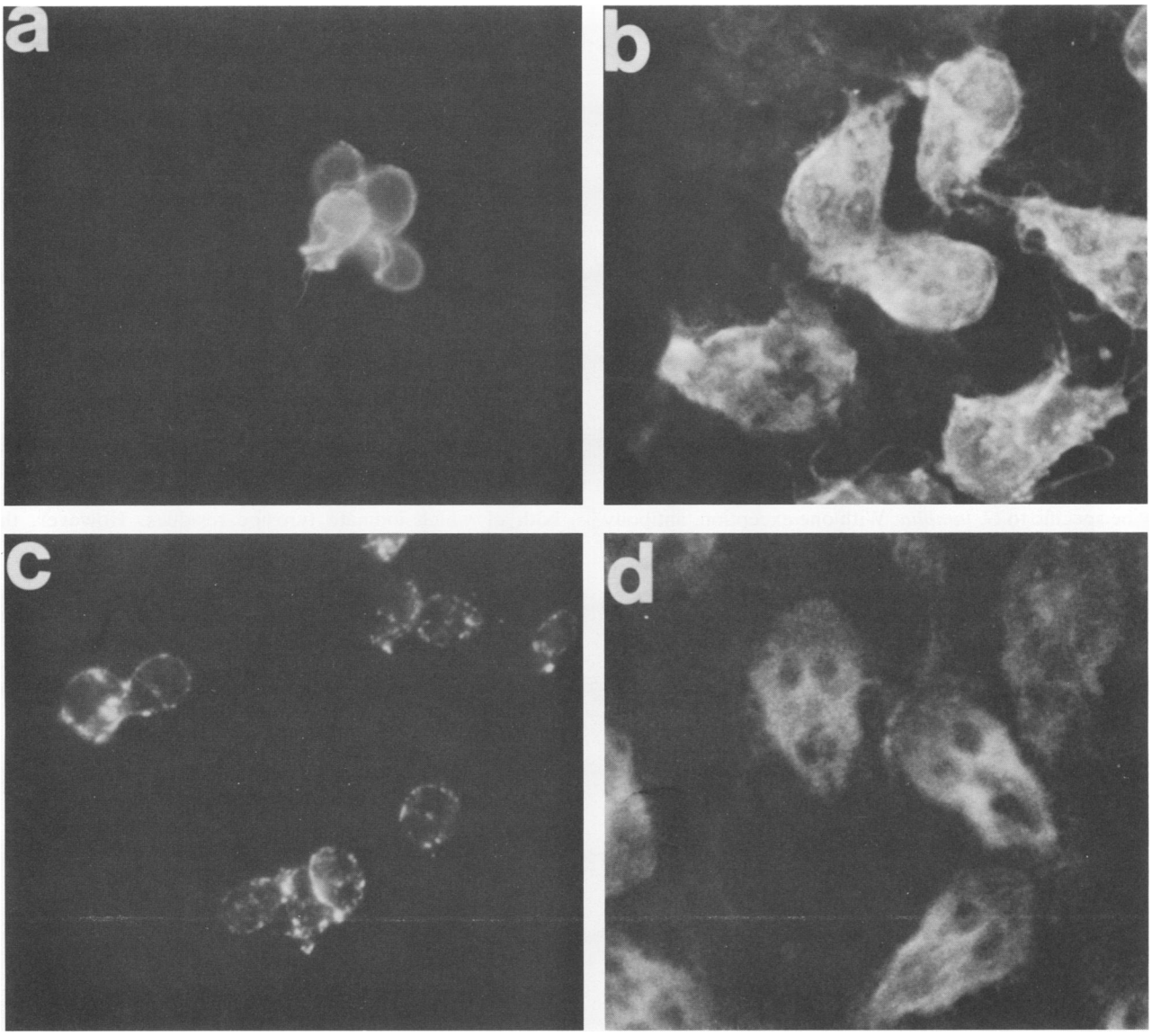

FIG. 2. Immunofluorescent staining of *G. lamblia* trophozoites with monoclonal antibodies GL-1 (a and b) and GL-2 (c and d). Living cells are shown in a and c, and methanol-fixed cells are shown in b and d.

punctate fluorescent staining of the cell body but not the flagella (Fig. 2c). With fixed cells, these eight antibodies gave a uniform staining except for the two nuclei and median bodies (ghost pattern) (Fig. 2d). The intensity of the immunofluorescent staining was unchanged by the presence of complete growth medium or its components, indicating that the antibodies were specific for trophozoite antigens. The hybridomas giving the brightest membrane (GL-1) and ghost (GL-2) fluorescence were chosen for further study, and some of their properties are summarized in Table 1. Identical fluorescent staining was observed with the WB isolate of *G. lamblia*, demonstrating that GL-1 and GL-2 do not recognize antigens restricted to the Portland 1 isolate.

TABLE 1. Properties of two *G. lamblia*-specific monoclonal antibodies

Antibody	Isotype	Structures recognized	Trophozoite aggregation	M_r of proteins recognized
GL-1	IgG1	Cell surface, flagella	+ +	88,000
GL-2	IgG2a	Cell surface, fixed cells except nuclei and median structures	+	?

The specificity of the GL-1 antibody was examined by indirect immunofluorescent staining of several other human parasites (Table 2). GL-1 did not react with any of the organisms tested. However, since the Portland 1 and WB isolates have been in culture for some time, it was important to examine freshly isolated *G. lamblia* which had never been grown axenically. Washed trophozoites, prepared from four patients with active giardiasis, were methanol fixed and found to be stained by the GL-1 antibody as well. To determine whether GL-1 recognized other species of *Giardia*, trophozoites of *G. muris*, which is morphologically distinct from *G. lamblia* and considered to be a different species, were examined. Neither living nor fixed trophozoites were stained, suggesting that the surface component

TABLE 2. Specificities of two *G. lamblia*-specific monoclonal antibodies

Parasite	Immunofluorescent staining			
	GL-1		GL-2	
	Fixed	Live	Fixed	Live
G. lamblia trophozoites				
Portland 1 strain	+	+	+	+
WB strain	+	+	+	+
Clinical isolates[a]	+	NT[b]	+	NT
G. muris trophozoites	−	−	−	NT
E. histolytica HM1	−	−	+	−
T. vaginalis	−	NT	−	NT
T. hominis	−	NT	−	NT

[a] Four clinical isolates were examined.
[b] NT, Not tested.

may be specific to *G. lamblia*. With one exception, antibody GL-2 reacted similarly. The single exception was methanol-fixed *E. histolytica*, which may contain an antigen cross-reactive with *G. lamblia*.

Protein specificity of the monoclonal antibodies. To examine the surface protein specificities of the monoclonal antibodies, immunoprecipitation experiments were performed on Triton X-100 extracts of radioiodinated trophozoites. Serum from the mouse used to obtain GL-1 and GL-2 immunoprecipitated a major iodinated protein of 88,000 M_r (Fig. 3, lane 1). Similar Triton X-100 extracts were subjected to immunoprecipitation with GL-1 and GL-2. Electrophoretic analysis of the immunoprecipitates revealed that GL-1 recognized an 88,000-M_r protein (Fig. 3, lane 2), whereas GL-2 immunoprecipitated no iodinated protein (Fig. 3, lane 3). A control immunoprecipitate with an irrelevant monoclonal antibody isotype-matched to GL-1 is shown in Fig. 3, lane 4. The mobility of the 88,000-M_r protein on SDS-PAGE was the same under reducing or nonreducing conditions, indicating that it is not disulfide linked to any other protein.

Since GL-1 and the human anti-*G. lamblia* sera both immunoprecipitated 88,000-M_r proteins, it was of interest to determine whether the sera recognized the same protein. To determine this, a Triton X-100 extract of radioiodinated trophozoites was subjected to preclearing with GL-1. Electrophoretic analysis of the material cleared by GL-1 is shown in Fig. 3, lane 5. Preclearing was considered complete when additional immunoprecipitation gave no detectable 88,000-M_r band on SDS-PAGE (Fig. 3, lane 6). The precleared extract was then subjected to immunoprecipitation with the two human anti-*G. lamblia* sera. Electrophoretic analysis of the immunoprecipitates (Fig. 3, lanes 7 and 8) established that GL-1 and the human sera recognized the same 88,000-M_r protein.

DISCUSSION

In the present study, we found that human anti-*G. lamblia* sera contain antibodies to an 88,000-M_r protein present on the surface of trophozoites, a result which establishes this protein as a potentially important *G. lamblia*-specific immunogen in humans. In addition, we have isolated parasite-specific mouse monoclonal antibodies and used them to analyze the surface antigens of trophozoites in more detail. The monoclonal antibodies yielded two immunofluorescence patterns, membrane (GL-1) and ghost (GL-2). Immunoprecipitation studies on detergent extracts of radioiodinated trophozoites revealed that monoclonal antibody GL-1 (membrane) recognized the same 88,000-M_r surface protein as the human anti-*G. lamblia* sera.

While this work was in progress, workers in two other laboratories described studies using surface radioiodination to identify the major trophozoite surface antigens. In the first, Nash et al. (16, 17) found a major polydisperse band of material from 94,000 to 200,000 M_r, present sometimes as a smear and sometimes as a discrete ladder of bands. On occasion, we have observed a similar ladder of bands, but since this material is not present reproducibly in our preparations, we have been unable to study it further. In the second report, Einfeld and Stibbs (4) observed a major iodinated surface protein of 82,000 M_r present on the four *G. lamblia* isolates examined. From their description, it is likely that this protein is the same as the 88,000-M_r protein we report here. Interestingly, this group and our own have observed only one major iodinated trophozoite surface protein by either the lactoperoxidase or Iodogen procedures, both of which iodinate tyrosine residues. However, this should not be taken as evidence that *G. lamblia*, like several other parasitic protozoans, has only one major surface membrane protein, since at least 20 *G. lamblia* surface proteins are radioiolabeled after incubation of trophozoites with the Bolton-Hunter reagent, which iodinates amino groups (8). Rather, these results indicate that the 82,000- or 88,000-M_r protein is the major surface protein with appropriately exposed tyrosine residues.

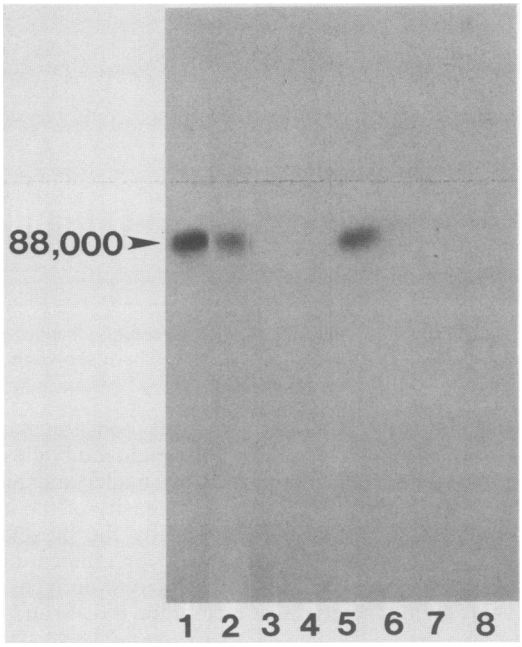

FIG. 3. *G. lamblia* proteins recognized by monoclonal antibodies GL-1 and GL-2. ^{125}I-labeled trophozoite proteins were solubilized in Triton X-100 and immunoprecipitated with a polyclonal mouse anti-*G. lamblia* serum (lane 1), with GL-1 (lanes 2 and 5), with GL-2 (lane 3), or with a control monoclonal antibody 1.CE.5C3 which recognizes a varicella-zoster virus glycoprotein (3) (lane 4). Samples of the ^{125}I-labeled trophozoite proteins were precleared of the 88,000-M_r protein and then immunoprecipitated with GL-1 (lane 6) and the human anti-*G. lamblia* sera (lanes 7 and 8). The serum in lane 7 was from a convalescent giardiasis patient. The samples were analyzed by 10% SDS–PAGE.

Examination of additional isolates and of other parasites showed that the epitope recognized by GL-1 was specific for *G. lamblia* and was present on fresh clinical isolates. Further, we have found that the antigen recognized by GL-1 is shed into the culture supernatant (M. J. G. Farthing, C. M. Edson, J. Prokopec, P. M. G. Inge, and A. J. K. Goka, Gastroenterology **88**:1379, 1985). These observations raise the possibility that GL-1 may prove useful in the development of a specific stool enzyme-linked immunosorbent assay for *G. lamblia*.

It is interesting that GL-1 does not recognize *G. muris*; although further analyses are clearly required, the results suggest that the antigen might be species specific. Since sera from BALB/c mice immune to *G. muris* immunoprecipitate a major immunogenic *G. muris* protein of 82,000 M_r (6), it is tempting to speculate, therefore, that major surface proteins, analogous to the 88,000-M_r protein reported here for *G. lamblia*, are present on trophozoites of each *Giardia* species.

Although we found that human anti-*G. lamblia* sera contain antibodies to the 88,000-M_r surface protein, no clear correlation has been established between the appearance of specific serum antibodies to *G. lamblia* and protective immunity to the parasite. This may reflect the fact that the site of replication, the small intestine, is accessible only to secretory antibody, predominantly IgA. Consequently, it will be important to compare the protein specificity of the IgA antibodies in duodenal fluid from controls and persons with active giardiasis.

Finally, it would be of great interest to know whether the protein identified in our studies plays any role in the pathogenesis of the parasite. Results from experiments investigating attachment of trophozoites to intestinal epithelial cells indicate that monoclonal antibody GL-1 blocks parasite adherence (M. Farthing and C. Edson, submitted for publication), suggesting that the 88,000-M_r protein may be involved in this critical event.

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