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## ANTIBODIES TO THE VENTRAL DISC PROTEIN $\delta$ -GIARDIN PREVENT IN VITRO BINDING OF *GIARDIA LAMBLIA* TROPHOZOITES

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**ABSTRACT:** A cDNA coding for  $\delta$ -giardin was cloned from *Giardia lamblia* trophozoites to localize the protein and to study its function in mediating surface attachment. Recombinant  $\delta$ -giardin antigen was expressed in *Escherichia coli* as a poly-histidine fusion protein and was purified by affinity chromatography for production of antisera to  $\delta$ -giardin. By immunoblotting analysis, antisera to recombinant  $\delta$ -giardin antigen recognized a 31-kDa protein on *G. lamblia* trophozoites. Anti-recombinant  $\delta$ -giardin was used to localize the native protein to the trophozoite ventral disk in both immunofluorescence and immunoelectron microscopy assays. Pre-treatment of *G. lamblia* trophozoites with anti- $\delta$ -giardin sera caused morphological changes in the parasite and inhibited trophozoite binding to the surface of cell culture slides. Binding of antibodies to  $\delta$ -giardin may provide a means of inhibiting attachment of *G. lamblia* trophozoites to the intestinal epithelium and thereby prevent clinical giardiasis.

*Giardia lamblia*, the causative agent of giardiasis, is a protozoan that infects the upper intestinal tract of many mammals, including humans. In general, giardiasis begins by the ingestion of water contaminated with *G. lamblia* cysts, which excyst in the small intestine, releasing 4 trophozoites that attach to epithelial cells lining the gut (for review, see Adam, 2001). Attachment is mediated by the ventral disk, an organelle that is composed of 3 distinct structures, i.e., microtubules that are coiled around a bare area; microribbons that protrude into the cytoplasm; and cross-bridges that connect adjacent microtubules, thereby providing structural support to the ventral disk (Elmendorf et al., 2003; de Souza et al., 2004; Sant'Anna et al., 2005). A ventro-lateral flange and lateral crest at the disk perimeter may play a role in attachment of the parasite to surfaces (Feely, 1982; Peattie et al., 1989; Tůmová et al., 2007). It has been proposed that adherence of *G. lamblia* trophozoites is mediated by a suction force between the ventral disk and epithelial cell surface (Holberton, 1974). At present, 3 major classes of proteins, termed  $\alpha$ -,  $\beta$ -, and  $\gamma$ -giardins, have been identified as components of the ventral disk (Peattie et al., 1989; Peattie, 1990; Nohria et al., 1992).  $\alpha$ -Giardins are a large class of annexin-like molecules that are localized to the outer edges of microribbons (Holberton et al., 1988; Bauer et al., 1999; Wenman et al., 1993; Weiland et al., 2003, 2005), whereas  $\beta$ -giardins seem to be closely associated with microtubules (Heyworth et al., 1999).  $\gamma$ -Giardin is a 38-kDa protein that may also be a component of microribbons (Nohria et al., 1992). A fourth giardin, named  $\delta$ -giardin, has been studied at the molecular level, but no information is available on its location in *G. lamblia* (Elmendorf et al., 2001).

Maintenance of the ventral disk architecture seems to be critical for trophozoite binding. For example, disruption of microtubule function and binding of microribbons by benzimidazoles prevented attachment of trophozoites to host cells (Chavez et al., 1992; Sousa et al., 2001). In a related study, polyclonal antibodies against *G. lamblia* surface proteins prevented attachment of trophozoites to host cells and glass tissue culture surfaces (Inge et al., 1988; Samra et al., 1991). Although the antigen target of this serum was unknown, cytoskeletal proteins, such as  $\alpha$ - and  $\beta$ -giardins, are known to be highly immunogenic

molecules (Roxstromlindquist, 2006). The purpose of the present study was to express recombinant *G. lamblia*  $\delta$ -giardin protein, produce antisera specific for recombinant  $\delta$ -giardin, use this antisera to localize the protein in *G. lamblia* trophozoites, and test its effect on in vitro attachment of trophozoites.

### MATERIALS AND METHODS

#### *Giardia lamblia*

*Giardia lamblia* (WB strain) trophozoites (assemblage A) were obtained from the American Type Culture Collection (ATCC 30957, Manassas, Virginia) and cultured in modified TYI-S-33 media (Miller et al., 1988) in 15-ml sterile polypropylene tubes at 37 C. *Giardia lamblia* were grown in continuous culture by inoculating 12 ml of new media every 2–3 days, with approximately  $5 \times 10^8$  trophozoites from a viable culture.

#### Identification and real-time reverse transcription-polymerase chain reaction (RT-PCR) analysis of *G. lamblia* $\delta$ -giardin cDNA cloning and expression of *G. lamblia* $\delta$ -giardin cDNA

A cDNA clone was identified in subtracted *G. lamblia* trophozoite libraries by searching the nr database using BLAST-X and found to be nearly identical (98–99%) to *G. lamblia*  $\delta$ -giardin DNA sequences in GenBank (accessions AAK32143.1, XP001707448.1, XP001707449.1). Oligonucleotide primers (Gl $\delta$ G-forward (F), 5'-AGGAGTCCTTTGGCGCCTTATTG-3' and Gl $\delta$ G-reverse (R), 5'-ATTCATGTGCGGTGG-CATGCTTGGAG-3') directed to the  $\delta$ -giardin cDNA sequence were used in real-time RT-PCR with a 3000XP real-time PCR machine (Stratagene, La Jolla, California) to compare the relative abundance of  $\delta$ -giardin mRNA between *G. lamblia* cysts and trophozoites. In brief, 1 ng of total RNA was subjected to real-time RT-PCR using 1 pmol of Gl $\delta$ G-forward and -reverse primers, and the Superscript III one-step RT-PCR system (Invitrogen, Carlsbad, California) in a 25- $\mu$ l reaction volume. RT-PCR consisted of reverse transcription at 47 C for 1 hr, denaturation at 94 C for 1 min, followed by 35 cycles of 94 C for 30 sec, 59 C for 30 sec, 72 C for 1 min, and a final extension at 72 C for 5 min. In addition, RT-PCR reactions were performed using primers directed to *G. lamblia* glyceraldehyde-3-phosphate dehydrogenase (GAPDH) to control for slight differences in RNA levels between cysts and trophozoites. Reaction conditions were identical to those described for  $\delta$ -giardin RT-PCR described above, except using GIGAPDH-F (5'-AGCTTACCGGTATGGCCTTTCGT-3') and GIGAPDH-R (5'-TTGT-CGTACCAGGCAACCAGCTTA-3') primers. All reactions were run in triplicate and were further analyzed by polyacrylamide gel electrophoresis to ensure that the real-time RT-PCR signal was generated from the expected size amplification product ( $\delta$ -giardin, 118 base pairs [bp]; GAPDH, 267 bp). Relative expression of  $\delta$ -giardin mRNA in trophozoites and cysts were calculated after normalizing the data to the *G. lamblia* GAPDH signal using Q-gene software (Mueller et al., 2002).

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### Cloning and expression of *G. lamblia* $\delta$ -giardin cDNA

Plasmid DNA containing  $\delta$ -giardin cDNA sequence was prepared and digested with EcoRI to release the  $\delta$ -giardin coding region, which was inserted into EcoRI-digested pET28c (Novagen, Madison, Wisconsin) using DNA ligase and reaction conditions recommended by the manufacturer (New England Biolabs, Ipswich, Massachusetts). The ligation mixtures were used to transform *Escherichia coli* DH5 cells according to standard procedures (Hanahan, 1983). Recombinant  $\delta$ -giardin clones were identified by restriction digestion of plasmids derived from the *E. coli* transformation. Maintenance of the reading frame between the cloning vector and  $\delta$ -giardin cDNA was confirmed by DNA sequencing. Recombinant clones were used to transform *E. coli* Rosetta 2 cells (Novagen) for high-level protein expression. Cultures of *E. coli* Rosetta 2 cells harboring pET28- $\delta$ -giardin were grown at 30 C in Luria-Bertani broth containing 50  $\mu$ g/ml kanamycin and 50  $\mu$ g/ml chloramphenicol until an optical density<sub>600</sub> = 1.0. Induction of recombinant  $\delta$ -giardin was accomplished by growth of cultures at 30 C for 4 hr in the presence of 1 mM isopropylthiocyanate (Sigma, St. Louis, Missouri).

### Analysis of recombinant and native *G. lamblia* $\delta$ -giardin protein

*Escherichia coli* expressing *G. lamblia*  $\delta$ -giardin protein were harvested by centrifugation at 3,000 g for 10 min. The cell pellets were extracted with native binding buffer (Invitrogen) containing phenylmethylsulfonyl fluoride (PMSF) protease inhibitor (Sigma), frozen-thawed 2 times between a dry ice-ethanol bath and a 37 C water bath, and sonicated twice for 15 sec each, with incubation on wet ice for 1 min between sonications. The protein extracts were treated with 1 U/ml RNase and DNase for 30 min at room temperature and pelleted by centrifugation at 5,000 g for 30 min. The insoluble pellet was extracted by resuspension in denaturing binding buffer (Invitrogen) for 30 min at room temperature on a rocker. The extracts were pelleted by centrifugation at 5,000 g for 30 min, and the supernatant was subjected to nickel-nitrilotriacetic acid (Ni-NTA) affinity chromatography to purify recombinant  $\delta$ -giardin protein using procedures recommended by the manufacturer (QIAGEN, Valencia, California).

*Giardia lamblia* trophozoites were obtained by placing growing cultures on ice for 5 min followed by centrifugation for 5 min at 2,000 g. The pelleted trophozoites were washed once with incomplete media, suspended in protein extraction buffer (10 mM Tris-HCl, pH 7.3, and 1 mM MgCl<sub>2</sub>) containing PMSF, and extracted by freeze-thawing, sonication, and treatment with RNase and DNase as described above.

Recombinant and native *G. lamblia* protein were treated with sample buffer (Laemmli, 1970) with and without the reducing agent 2-mercaptoethanol, heated for 1 min at 95 C, and fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, followed by transblotting to Immobilon membrane (Millipore, Billerica, Massachusetts) in a semi-dry transblotter apparatus (Bio-Rad, Hercules, California). After transfer, the membranes were treated with phosphate-buffered saline (PBS) containing 2% nonfat dry milk (PBS-NFDM) to block non-specific immunoglobulin binding in subsequent steps. After blocking, the membranes were incubated with anti-recombinant  $\delta$ -giardin sera or preimmune sera for 2–4 hr at room temperature on a laboratory shaker, followed by 2-hr incubation with biotinylated goat-anti-rabbit immunoglobulin (Ig) G (1:1,000 dilution; Sigma), and 1 hr with avidin-peroxidase (1:5,000 dilution; Sigma). All antibodies were diluted in PBS containing 0.05% Tween 20 (PBS-TW) and removed after each step by 3 washes with PBS-TW. Binding of  $\delta$ -giardin antibodies was assessed by a final incubation with the peroxidase substrate 0.5 mg/ml 4-chloro-1-naphthol (Sigma) and 0.015% H<sub>2</sub>O<sub>2</sub> (Sigma) in PBS.

### Preparation of antisera against recombinant $\delta$ -giardin

Ni-NTA-purified recombinant  $\delta$ -giardin was mixed with ImmunoMax SR adjuvant (Repros Therapeutics, The Woodlands, Texas) and used to immunize 2 rabbits (New Zealand White, Covance, Denver, Pennsylvania) by subcutaneous injection. Pre-immunization sera were collected from both rabbits, and in immunofluorescence assay (below) they were found to be devoid of antibodies to *G. lamblia* trophozoites. Primary and booster immunizations consisted of 25  $\mu$ g of Ni-NTA-purified recombinant  $\delta$ -giardin in a total volume of 500  $\mu$ l, and they were administered 1 mo apart. Rabbits were killed by exsanguination, and blood was processed for serum following protocols approved by the BARC Animal Care and Use Committee. Preliminary studies showed that anti-

$\delta$ -giardin titers were similar in both rabbits; thus, sera were pooled for use in all assays described below.

### Immunofluorescence staining (IF) of *G. lamblia* trophozoites

*Giardia lamblia* trophozoites were harvested from cell culture as described above, suspended to 10<sup>6</sup> parasites/ml in PBS, pipetted onto individual wells of multi-well glass slides (10<sup>4</sup> trophozoites/well; Erie Scientific Co., Portsmouth, New Hampshire), and allowed to air dry. After drying, the wells were fixed for 5 min with methanol, then gently rinsed with PBS. After fixation, the wells were treated with PBS-NFDM for 30 min at room temperature in a humidified chamber, gently rinsed with PBS, air-dried, and then incubated for 2 hr at room temperature with a 1:1,000 dilution of rabbit anti-*G. lamblia*  $\delta$ -giardin sera or control sera (pre-immune sera or antisera to a non-*G. lamblia* polyHis recombinant protein). The wells were gently rinsed 3 times with PBS, allowed to air dry, and then incubated for 1 hr at room temperature with a 1:100 dilution of fluorescein isothiocyanate-anti-rabbit IgG (Sigma). Again, the wells were gently rinsed 3 times with PBS, allowed to air dry, overlaid with several drops of Vectashield mounting medium (Vector Laboratories, Burlingame, California) followed by a coverslip, and then examined using epifluorescence microscopy.

### Immunoelectron microscopic (IEM) staining of *G. lamblia* trophozoites

*Giardia lamblia* trophozoites were harvested from cell culture and pelleted by centrifugation for 2 min at 5,000 g. The trophozoite pellet was briefly mixed and then suspended in 100  $\mu$ l of fixative consisting of 3% paraformaldehyde in 0.1 M cacodylate buffer. After a 5-min fixation, the trophozoites were transferred to a 1.5-ml microcentrifuge tube, pelleted by centrifugation for 5 min at 5,000 g, gently washed twice with cacodylate buffer, and then briefly mixed to form a dispersed pellet in the bottom of the tube. The trophozoite mixture was dehydrated in a graded ethanol series, infiltrated overnight with LR White hard-grade acrylic resin (London Resin Company, London, U.K.), and cured at 55 C for 24 hr. Thin sections (90 nm thickness) were obtained using a Diatome diamond knife on a Reichert/AO Ultracut microtome and collected on 200-mesh Formvar-coated nickel grids. The grids were floated for 5 min with the tissue section facing down on drops of PBS containing 0.1 M glycine and 1% bovine serum albumin, followed by 5 min on drops containing PBS-TW-NFDM. Grids were incubated tissue-side down for 2 hr at room temperature on drops of PBS-TW containing a 1:1,000 dilution of rabbit anti-*G. lamblia*  $\delta$ -giardin sera or control sera (pre-immune sera or antisera to a non-*G. lamblia* polyHis recombinant protein). The grids were rinsed 3 times with PBS-TW, incubated for 1 hr at room temperature on PBS containing a 1:100 dilution of gold-labeled anti-rabbit IgG (Sigma); washed 2 times with PBS-TW, once with PBS, and once with H<sub>2</sub>O; air-dried; stained with 5% uranyl acetate for 30 min; and examined with a Hitachi H7000 electron microscope.

### In vitro testing of anti-*G. lamblia* $\delta$ -giardin sera against *G. lamblia* trophozoites

*Giardia lamblia* trophozoites were harvested from culture by transferring to 15-ml polypropylene tubes (Falcon 2059, Falcon; BD Biosciences Discovery Labware, Bedford, Massachusetts) and suspended to 5  $\times$  10<sup>5</sup> trophozoites/ml in complete culture medium containing 1:100 dilution of anti-*G. lamblia*  $\delta$ -giardin sera or control sera (antisera to a non-*G. lamblia* polyHis recombinant protein). The trophozoites were mixed on an orbital rocker for 1 hr at room temperature and then aliquoted to 4-well Lab-Tek Chamber Slides (Nalge Nunc International, Naperville, Illinois). The culture slides were placed in a stationary 37 C incubator to allow for attachment of *G. lamblia* trophozoites to the slide surface. A sample of trophozoites were also adhered to glass microscope slides using Cytopro 7620 cytocentrifuge (Wescor, Logan, Utah) by centrifugation for 1 min at 1,000 RPM. Adherent trophozoites were fixed for 5 min with methanol, and then stained for 10 min with 200  $\mu$ l of Ladd Multiple Stain (Ladd Research Industries, Burlington, Vermont). After staining, the slides were washed with PBS, and the adherent trophozoites were overlaid with a drop of mounting medium (50% glycerol in PBS) and a coverslip. After 4 hr, individual wells of the chamber slides were gently washed 3 times with PBS, and the attached trophozoites were treated with methanol and Ladd Multiple Stain as described above. After staining, the slides were overlaid with mount-

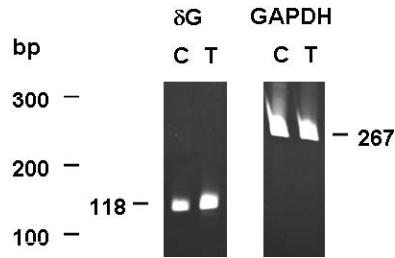


FIGURE 1. Analysis by polyacrylamide gel electrophoresis of amplification products from real-time reverse transcription-polymerase chain reaction of RNA from *Giardia lamblia* cysts (C) or trophozoites (T) using  $\delta$ -giardin-specific primers ( $\delta$ G) or glyceraldehyde-3-phosphate dehydrogenase-specific primers.

ing medium and a cover slip. Attached trophozoites were enumerated by counting total attached trophozoites in at least 5 random length-wise scans of each culture well. Average counts were compared between treatments for statistical differences using a two-way Student's *t* test in the GraphPad InStat software program (GraphPad Software Inc., San Diego, CA). Adherent and attached trophozoites were examined by phase contrast microscopy using a Zeiss Axioskop 2 microscope, and images were exposed using the AXIOVs v4.6.3.0 software (Zeiss, Göttingen, Germany).

## RESULTS

### Comparison of $\delta$ -giardin mRNA levels between *G. lamblia* cysts and trophozoites

Real-time RT-PCR analysis revealed a 16-fold greater level of  $\delta$ -giardin mRNA signal in trophozoites compared with cysts. GAPDH mRNA signals were nearly identical in real-time RT-PCR analysis of trophozoites. Analysis of  $\delta$ -giardin or GAPDH real-time RT-PCR products by acrylamide gel electrophoresis confirmed that the amplicons were of expected size (Fig. 1).

### Analysis of recombinant and native *G. lamblia* $\delta$ -giardin protein

Recombinant *G. lamblia*  $\delta$ -giardin protein was highly expressed in *E. coli*, representing nearly 50% of total denaturing

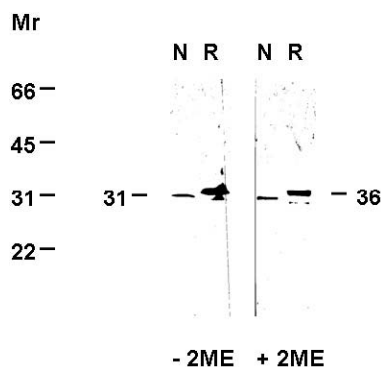


FIGURE 2. Immunoblotting analysis of native (N) *Giardia lamblia* protein or recombinant (R)- $\delta$  giardin protein fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis using antisera specific for recombinant- $\delta$  giardin protein. -2ME, native and recombinant proteins treated with sample buffer in the absence of 2-mercaptoethanol; +2ME, native and recombinant proteins treated with sample buffer in the presence of 2-mercaptoethanol;  $M_r$ , molecular size markers (kilodaltons).

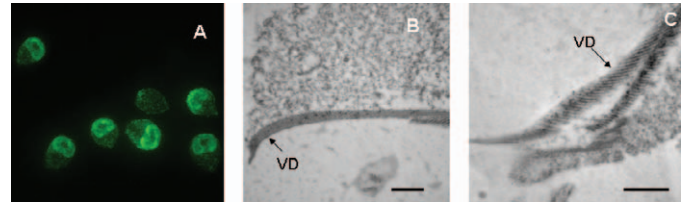


FIGURE 3. Immunofluorescence (A) and immunoelectron microscopic (B–C) staining of *Giardia lamblia* trophozoites using antisera specific for recombinant- $\delta$  giardin protein. VD, ventral disk. Bar = 500 nm.

soluble protein at concentration of approximately 5  $\mu$ g/ml culture medium (data not shown). Antisera specific for recombinant *G. lamblia*  $\delta$ -giardin protein recognized a 36-kDa recombinant protein and a 31-kDa native *G. lamblia* trophozoite protein by immunoblotting assay (Fig. 2). The  $M_r$  differences between recombinant and native  $\delta$ -giardin proteins are because of the short polyHis fusion peptide at the amino terminus of the  $\delta$ -giardin coding sequence. Inclusion of 2-mercaptoethanol had no effect on the apparent  $M_r$  of both recombinant and native *G. lamblia*  $\delta$ -giardin protein (Fig. 2).

### Immunofluorescence (IF) and IEM staining of *G. lamblia* trophozoites with anti-recombinant $\delta$ -giardin sera

In the IF assay, antibodies to recombinant  $\delta$ -giardin protein recognized an antigen associated with the *G. lamblia* trophozoite ventral disk (Fig. 3A). Varying degrees of staining intensity were observed, with an occasional trophozoite exhibiting negligible staining of the ventral disk (Fig. 3A). IEM staining of *G. lamblia* trophozoites with anti-recombinant  $\delta$ -giardin sera localized the protein to microribbons of the ventral disk (Figs. 3B, C). Close observation of numerous IEM images revealed an even distribution across the entire ventral disk.

### Effect of anti-recombinant $\delta$ -giardin sera on in vitro attachment of *G. lamblia* trophozoites

Binding of anti-recombinant  $\delta$ -giardin sera had a noticeable effect on the morphology of *G. lamblia* trophozoites (Fig. 4B). Although a few control antisera-treated trophozoites displayed an irregular appearance (Fig. 4A), a much larger proportion of *G. lamblia* treated with anti-recombinant  $\delta$ -giardin exhibited a distorted morphology (Fig. 4B). Many trophozoites were misshapen, and all were noticeably less active than those treated with control sera. In addition, anti-recombinant  $\delta$ -giardin sera

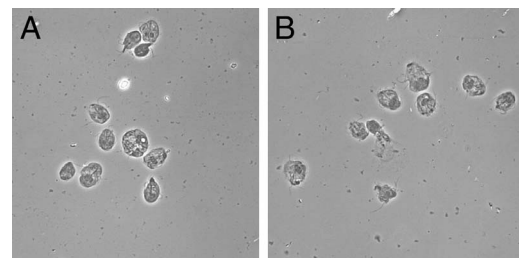


FIGURE 4. Morphology of *Giardia lamblia* trophozoites after treatment with control antisera (A) or with antisera specific for recombinant- $\delta$  giardin protein (B) as observed by phase contrast microscopy at  $\times 400$  magnification.



TABLE I. Comparison of numbers of *Giardia lamblia* trophozoites bound to surface of glass culture slides after treatment with either anti-recombinant  $\delta$ -giardin or control sera.

Study No.	Serum treatment	Average no. trophozoites/scan*	Percent inhibition**
1	Control	231 $\pm$ 58	
	Immune	127 $\pm$ 29	45.0
2	Control	79 $\pm$ 23	
	Immune	11 $\pm$ 3	86.1
3	Control	263 $\pm$ 41	
	Immune	104 $\pm$ 18	60.5

\* Values are an average of 5 random vertical scans of well surface.

\*\* No. trophozoites control wells – no. trophozoites immune wells/no. trophozoites control wells

affected the binding of trophozoites to the culture well surface. In all 3 studies, a significant decrease ( $P < 0.05$ ) was observed in the number of bound *G. lamblia* trophozoites that were treated with anti- $\delta$ -giardin sera compared to those treated with control sera (Table I). Although the absolute number of bound trophozoites varied between studies, trophozoites attachment after anti- $\delta$ -giardin sera treatment was consistently lower (45.0–81.1% inhibition) than attachment observed in control serum (Table I).

## DISCUSSION

The present study demonstrates that  $\delta$ -giardin is a component of the *G. lamblia* trophozoite ventral disk. Similar to other giardins, native  $\delta$ -giardin (31 kDa) is between 29 and 38 kDa and seems to be associated with microribbons that, together with microtubules and bridges, make up the ventral disk cytoskeleton. The  $\delta$ -giardin cDNA shares no sequence similarity to  $\alpha$ - or  $\gamma$ -giardins, which are different from one another. Although the overall sequence similarity is low (<20%),  $\delta$ -giardin shares conserved amino acid motifs with  $\beta$ -giardin, suggesting that they belong to the same protein family. Regardless, it remains unclear how the 4 classes of giardins interact to provide structure to the ventral disk. Antibodies to purified native ~32-kDa protein (Crossley and Holberton, 1983, 1985; Crossley et al., 1986) or to recombinant protein produced by molecular cloning (Holberton et al., 1988; Peattie et al., 1989; Alonso and Peattie, 1992) have shown that  $\alpha$ -giardins are a large class of proteins that are related to annexins (Morgan and Fernandez, 1995; Weiland et al., 2005).  $\alpha$ -Giardins are found in variety of *G. lamblia* cytoskeletal components, such as flagella, ventral disk, and the plasma membrane (Alonso and Peattie, 1992; Wenman et al., 1993; Bauer et al., 1999; Weiland et al., 2003, 2005; Vahrman et al., 2008). In particular,  $\alpha$ -1 giardin has been localized to the outer edges of microribbons of the ventral disk (Peattie et al., 1989). Other microribbon proteins, such as  $\beta$ -giardin (29 kDa) and  $\gamma$ -giardin (38 kDa), were identified in studies of 29–38-kDa proteins of *G. lamblia* trophozoites (Crossley and Holberton, 1983; Crossley et al., 1986) or by molecular cloning of the respective genes for these proteins (Baker et al., 1988; Holberton et al., 1988; Aggarwal and Nash, 1989; Nohria et al., 1992).

Results in the present study indicate that  $\delta$ -giardin (Elmendorf et al., 2001) is also a microribbon protein that may be involved in attachment of *G. lamblia* trophozoites. The cyto-

skeletal locale of  $\delta$ -giardin is consistent with its predicted structure. For example, motif searching using PFAM (<http://pfam.sanger.ac.uk> or <http://ebi.ac.uk/interpro>) and Conserved Domains (<http://www.ncbi.nlm.nih.gov/structure/cdd>) programs revealed that  $\delta$ -giardin shares homology with the striated fiber-assemblin (SFA)/ $\beta$ -giardin family (E value =  $2.00e^{-03}$ ). SFAs are acidic 33-kDa proteins that represent a major component of striated microtubule-associated fibers.

In this study, binding of the ventral disc using antibodies to  $\delta$ -giardin appeared to have an effect on morphology and motility of trophozoites. Examination of *G. lamblia* trophozoites immediately after the 1 hr treatment with control or immune serum revealed a high percentage of distorted trophozoites in those incubated with anti- $\delta$ -giardin antibodies (Fig. 4B). In addition, *G. lamblia* trophozoites treated with anti- $\delta$ -giardin sera were noticeably less mobile than trophozoites treated with control sera. While control trophozoites exhibited a classical rapid, tumbling movement, virtually all of those parasites bound with anti- $\delta$ -giardin antibodies were immobile. Subsequent binding assays revealed an effect of anti- $\delta$ -giardin serum on trophozoite attachment to glass surface. Although it is clear that in our assay most trophozoites in both control and immune treatments did not bind to glass culture surface (the maximum number observable in a single scan would equal 2,000 trophozoites), there was a consistent and highly significant inhibition of *G. lamblia* attachment in the presence of anti- $\delta$ -giardin sera. Our data indicates that binding of  $\delta$ -giardin antibodies to the ventral disc affects the ability of trophozoites to bind/or remain attached to inanimate surfaces. This phenomenon is not without precedent. Antibodies to whole *G. lamblia* blocked in vitro binding of trophozoites to enterocytes (Inge et al., 1988) and glass culture surfaces (Samra et al., 1991). How binding of a cytoskeletal protein by antibodies prevents attachment of *G. lamblia* trophozoites is unknown. It is possible that binding of cytoskeletal elements either directly interferes with trophozoite binding or hinders the flexibility of the ventral disk, thereby preventing parasite attachment to gut epithelial cells. The morphological change in trophozoites that had been treated with anti-recombinant  $\delta$ -giardin sera may indicate that *G. lamblia* attachment is at least partly dependent on morphology. Binding to cultured cells or glass surfaces is probably multi-factorial because a role for lectins in attachment of trophozoites has also been observed (Inge et al., 1988; Sousa et al., 2001). Whether post-translational moieties on giardins play a role in *G. lamblia* binding to surfaces or host cells remains unknown. Many giardins, such as  $\alpha$ 2-,  $\gamma$ -, and  $\delta$ -giardin seem to have sites for potential *N*-glycosylation and *O*-glycosylation, but it is unknown whether lectins that have been shown to interfere with trophozoite attachment (Inge et al., 1988; Sousa et al., 2001) are binding to giardins or to other surface proteins. The ability of antibodies that react with whole *G. lamblia* trophozoites or with specific giardin molecules to inhibit attachment of the parasite in vitro may indicate a possible therapy against giardiasis. Indeed, it seems that a humoral immune response, in particular secretory IgA, is necessary for development of immunity against *G. lamblia* (Faubert, 2000; Gillin and Eckmann, 2002; Langford et al., 2002; Eckman 2003) and that a strong response to cytoskeletal proteins is observed in natural infections (Roxström-Lindquist et al., 2006).

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