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COMPARISON OF SERUM ANTIBODY RESPONSES TO *GIARDIA LAMBLIA* OF SYMPTOMATIC AND ASYMPTOMATIC PATIENTS

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Abstract. The circulating anti-parasite antibody response against *Giardia lamblia* in symptomatic and asymptomatic Egyptian children with confirmed giardiasis was examined. Symptomatic patients were identified using the following criteria: presence of only *G. lamblia* cysts in the feces, and one or more of the following symptoms, diarrhea, abdominal pain, loss of weight, vomiting and/or nausea, and abdominal distention. The anti-parasite humoral response was measured using indirect immunofluorescence (IFA), ELISA, and immunoblotting. There was a significant difference in the anti-parasite antibody response measured by IFA of asymptomatic and symptomatic patients, in which more than 34% of the asymptomatic patients had a titer equal to or less than 1:500, and more that 29% of the symptomatic patients had a titer of 1:8,000 or higher. The circulating anti-parasite total IgM and IgA but not IgG, measured by ELISA, was significantly higher in symptomatic than in asymptomatic patients, and were related to higher cyst output observed in symptomatic individuals. Although total anti-parasite IgG response was similar in symptomatic and asymptomatic patients, the analysis of the IgG isotype responses revealed that both IgG1 and IgG3 were significantly higher in symptomatic patients. The antigen recognition by anti-parasite IgM, IgA, IgG1, and IgG3 of symptomatic and asymptomatic individuals, determined by immunoblotting, was heterogeneous and revealed only minor differences in the response of the two groups.

Epidemiologic, clinical, and experimental observations indicate that *Giardia lamblia* elicits a host immune response.[1–4] Infections with *G. lamblia* are chronic among hypogammaglobulinemic patients, also suggesting a role for humoral immunity in the elimination of the parasite from the host.[5–7] A number of studies reported the presence of anti-parasite antibodies in infected individuals. Ridley and Ridley[8] were the first to report the presence of anti-*G. lamblia* antibodies in the serum of infected humans. Circulating antibodies to *G. lamblia* were first detected using an indirect immunofluorescence assay (IFA)[9, 10] and ELISA.[11–14] Although humans infected with *G. lamblia* produce specific circulating antibodies against the parasite, the role of these antibodies in protective immunity has not been completely elucidated, and the mechanisms of induction of specific anti-parasite antibody production remain to be identified.[3] These antibodies may play a role in resistance to infection by mediating complement fixation and/or antibody-dependent cellular cytotoxicity.[15–20]

Anti-*Giardia* antibodies have been used to detect parasite antigens in secretions and/or excretions.[21, 22] Vinayak and others[23] used sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting to distinguish symptomatic cases of giardiasis from asymptomatic cyst carriers, and showed that the 65-kD *G. lamblia*-specific antigen was detectable in stool eluates of gerbils infected with the parasite obtained from a asymptomatic patient while the 70-kD *G. lamblia*-specific antigen was detected in stool eluates of gerbils infected with the parasite obtained from an asymptomatic individual. In a number of studies, Nash and others[4, 22] reported significant heterogenicity among *Giardia* strains isolated from humans and demonstrated that the parasite undergoes antigenic variation both in vitro[24] and in vivo.[25]

Antigen recognition in patients infected with *G. lamblia* has also been examined in several studies.[26–29] Taylor and Wenman[26] identified a major 31-kD protein recognized by sera from patients during the acute phase of giardiasis. The 31-kD immunodominant *G. lamblia* protein was found it to be related to the alpha giardin antigen.[28] Other major antigens recognized by sera from infected humans had molecular weights of 27, 28, and 56 kD.[26, 28] In a different study, Einfeld and Stibbs[29] identified and characterized a major surface 82-kD glycoprotein of trophozoites common to four isolates of *G. lamblia*. This glycoprotein was similar to the molecule identified in other parasitic protozoa, including *Plasmodium knowlesi* and *Trypanosoma cruzi*,[30] and was shown to function as receptor for interaction with host cells, and as an antigen that elicits protective immune responses in Chagas' disease and malaria.[31, 32]

In the present study, the humoral immune responses of symptomatic and asymptomatic patients infected with *G. lamblia* were assessed. We report that symptomatic patients exhibited a stronger circulating anti-parasite IgM, IgG (IgG1 and IgG3), and IgA responses than asymptomatic individuals, and that the magnitude of the humoral response of the patients was related to the number of cysts released in the feces. However, there were no major differences in the antigen recognition of symptomatic and asymptomatic patients.

SUBJECTS, MATERIALS, AND METHODS

Subjects. A sample of 150 giardiasis patients from Egypt was studied: attendants of the Mansura University Hospital (58 patients), primary and preparatory schools (40 patients), and rural health units (52 patients). The Human Experimentation Ethics Committee of the Faculty of Mansoura University reviewed and approved this research project. Patients were informed about the study and were required to provide consent for the procedures used and participation in the study. Patients that had only *G. lamblia* in their feces were included in the study. This population consisted of 49 asymptomatic and 81 symptomatic giardiasis patients. A sample of 20 noninfected individuals with no recent history of diarrheal illness and no parasites in the stool upon three

TABLE 1
Distribution of symptoms in giardiasis patients from Egypt

Symptom	Percent of patients
Diarrhea	78
Abdominal pain	75
Weight loss	60
Flatulence	58
Loss of appetite	45
Vomiting	26
Fatigue	22
Headache	18
Fever	12

consecutive fecal examinations was selected as the control negative population. The mean ± SD age of the subjects was 7.56 ± 3.23 and 9.87 ± 6.68 years for asymptomatic and symptomatic patients, respectively. The sample consisted of 81 males and 69 females.

The distribution of symptoms of giardiasis patients included in this study is shown in Table 1. The most prevalent symptoms, reported by 50% or more of the patients, were diarrhea, abdominal pain, weight loss, and flatulence. A number of minor symptoms were also reported by the patients: they included loss of appetite, vomiting, fatigue, headache, and fever.

Collection of stool and examination for parasites. Stool samples were collected for three consecutive days in plastic containers. Each stool sample was examined for the presence of parasites using three different methods: direct saline smear, merthiolate-iodine-formalin concentration (MIFC), and zinc sulfate flotation.

For direct saline smear examination, a drop of saline was placed in the center of the slide and a small amount of feces (approximately 2 mg) was applied using an applicator stick. The sample was stirred gently until completely mixed with saline and a 22 × 22-mm coverslip was placed on top of the mixture. The sample was examined using bright field microscope with low (10×) and high (40×) power objectives.

For the MIFC, 1 g of feces from each collection was suspended in a vial containing 9 ml of MIF and stirred until suspended and strained through two layers of surgical gauze into a 15-ml centrifuge tube. Three milliliters of ether were added and the tube was shaken vigorously. The stopper was removed, the tubes was incubated at room temperature for 2 min, and centrifuged for 2 min at 1,000 × *g*. The plug of the fecal detritus was loosened by ringing with an applicator stick, and the supernatant was decanted off. A drop of the sediment was placed on a slide, a coverslip was placed on top of the slide, and the slide was examined using a bright field microscope with low (10×) and high (40×) power objectives.

For zinc sulfate flotation, 1 g of the fecal sample was suspended in 5 ml of MIF and emulsified. The sample was strained through two layers of surgical gauze into 15-ml centrifuge tubes. The sample was centrifuged at 1,200 × *g* for 1 min and the supernatant was decanted, leaving approximately 0.5–1 ml at the bottom of the tube. The sediment was resuspended in a saturated zinc sulfate solution and centrifuged at 1,000 × *g* for 2 min. The surface of each tube was skimmed off and placed on a slide, covered with a coverslip, and examined using a bright field microscope with low (10×) and high (40×) power objectives.

Collection of serum. Peripheral blood samples (10 ml) were obtained from all subjects. Hyperimmune rabbit serum was obtained from rabbit immunized with sonicated protein extract of *G. lamblia* trophozoites (WB strain; American Type Culture Collection, Rockville, MD). Specific, pathogen-free rabbit serum was a kind gift of Dr. David Neil (University of Alberta Animal Care Facilities). Human cord blood was a kind gift of Dr. Dennis Kunimoto (Department of Medical Microbiology and Immunology, University of Alberta). All serum samples were stored at −70°C.

Enumeration of cyst output. The cyst output of individual patients was estimated using the technique described by Danciger and Lopez.[33] Briefly, 10 g of stool was placed in a container, weighed, and emulsified in 5 ml of phosphate-buffered saline (PBS). Three 50-μl aliquots of the fecal suspension were placed on slides and mounted with a 22 × 22-mm coverslips. The preparation was examined using a bright field microscope at 40x in the following manner: starting at the upper left corner of the coverslip and moving in a straight line to the right edge of the coverslip, then moving down the width of one field and going back to the left edge in a straight line. Total number of cysts for each sample (coverslip) was determined and an average number of cysts for the three aliquots calculated and expressed per gram of feces.

Cultivation of *G. lamblia*. *Giardia lamblia* (WB strain) was cultured axenically in filter-sterilized Diamond's TYI-S-33 medium using the procedures described previously.[34] The trophozoites were grown at 37°C in 5% CO_2 in sterile polystyrene tissue culture flasks (Corning Inc., Corning, New York) for 48 hr. The parasites were harvested by chilling on ice for 10 min and washing by centrifugation at 200 × *g* for 10 min. The trophozoites were suspended in PBS (pH 7.2) prior to use in the assays.

Preparation of crude extract of trophozoites. Trophozoites were washed two times in PBS by centrifugation at 400 × *g* for 10 min at 4°C. The supernatant was removed and the pellet was suspended in 1 ml of PBS. The solution was sonicated at maximum power four times for 30 sec using a microultrasonic disrupter (Kontes, Vineland, NJ) and each sonicate was examined microscopically to ensure that trophozoites were completely disrupted.[35] The sonicate was then centrifuged at 2,000 × *g* for 10 min and the supernatant was removed and used in the assays as crude *G. lamblia* extract. The crude *G. lamblia* protein concentration was estimated using the micro protein assay reagent kit (Pierce, Inc., Rockford, IL) and the sample was aliquoted into 100-μg quantities and stored at −20°C until used.

Indirect immunofluorescence assay. The trophozoites suspended in PBS were enumerated using a hemocytometer and their number was adjusted to a concentration of 1×10^6/ml. Each well of 10-well slide (Cell-Line Associates Inc., Newfield, CA) was seeded with 1×10^4 trophozoites. Slides were dried at 37°C for 30 min and the parasites were fixed in acetone for 10 min and stored at 20°C prior to use in the assays. Prior to each IFA, slides were brought to room temperature, and 10 μl of diluted serum in PBS was placed in each well starting from a dilution of 1:10 up to 1:8,000. Sera that were positive at a 1:8,000 dilution were reanalyzed

up to a dilution of 1:32,000. A 1:50 dilution of control negative human serum was applied to one well of each slide. The slides were incubated in a humidified incubator (100% humidity) at room temperature for 60 min. After incubation, the slides were washed with PBS for 5 min and air-dried. Goat anti-human polyvalent immunoglobulin conjugated with fluorescein isothiocyanate (Sigma, St. Louis, MO), diluted with PBS (1:1,000), was placed in each well and the slides were reincubated for 60 min in a humidified incubator. The slides were air-dried, and a drop of buffered glycerin and a glass coverslip were placed on top of each slide. The slides were examined under epifluorescence using the procedure described previously.[10]

Enzyme-linked immunosorbent assay. The assay was performed using the procedure of Turunen and others[36] with the following modifications. Flat-bottom microtiter plates were used (Immulon 2; Dynatech Laboratories Inc., Chantilly, VA). Each well received 1 5 g of trophozoite soluble protein in 100 μl of PBS (pH 7.2). The plates were covered with parafilm M (American National, Inc., Greenwich, CT) and incubated at 4°C overnight. The plates were washed three times with washing buffer (1× PBS containing 0.1% Tween 20) to minimize nonspecific reactivity. The plates were air-dried and 200 μl of blocking buffer (1x PBS containing 0.1% Tween 20 and 1% bovine serum albumin) was added in each well, and incubated at 4°C overnight.

To measure total anti-parasite immunoglobulins, serum samples and a human cord blood sample (negative control) were diluted 1:50–1:500. Hyperimmune rabbit serum served as a positive control, and was diluted 1:16,000. Anti-parasite serum IgM, IgA, IgG, and IgG isotypes were measured by adding 100 μl of a 1:500 dilution of human serum (symptomatic or asymptomatic) in PBS. Peroxidase-labeled goat anti-human polyvalent immunoglobulins (Sigma) and peroxidase-labeled goat anti-rabbit IgG (heavy plus light chain) (Bio-Rad Laboratories, Hercules, CA) were diluted 1:1,000 in PBS. Peroxidase-labeled goat anti-human IgM (Gibco, Burlington, Ontario, Canada) and peroxidase-conjugated goat anti-human IgA (Pierce, Inc.) were diluted 1:16,000 and 1:4,000, respectively. All assays were done in triplicate on each microtiter plate and the measurements of anti-parasite antibody responses were done in three separate experiments.

Unconjugated mouse anti-human IgG1, IgG2, IgG3, and IgG4 (Pierce, Inc.) diluted 1:1,000, followed by a 1:250 dilution of peroxidase-labeled goat anti-mouse IgG whole molecule (Sigma), were used to detect IgG isotypes. Each well received 100 μl of appropriate conjugate or the second antibody unconjugated (IgG isotypes). The plates were incubated and washed as described above. Peroxidase substrate (Bio-Rad Laboratories) was added (100 μl to each well) and the plates were gently shaken for 2 min at the time of color development. The reaction was read using a microplate reader at 405 nm (Bio-Tek Instruments Inc., Highland Park, VT). All data are presented as the mean optical density of triplicate samples, and the measurements of anti-parasite IgG isotype response were done in three separate experiments.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis and immunoblotting. The SDS-PAGE was performed as described by Laemmli.[37] *Giardia lamblia* trophozoite soluble antigen and biotinylated high and low molecular weight protein markers (Bio-Rad Laboratories) were solubilized in sample buffer containing 2% SDS and 2% β-mercaptoethanol, then boiled for 5 min using a multi-block heater (Lab-Line Instruments, Inc., Melrose Park, IL). Electrophoresis was performed using a gradient 8–15% polyacrylamide minigel, with a 4% stacking gel. Ten micrograms of *G. lamblia* soluble antigen was loaded in each well. The running buffer was 0.025 M Tris, 0.19 M glycine, and 1% SDS, pH 8.3. Electrophoresis was done at 100 volts/gel at room temperature until the tracking dye reached the bottom of the gel. The gels were stained with Coomassie blue or a silver staining kit (Bio-Rad Laboratories).

Separated proteins were transferred from gel to nitrocellulose paper using a Trans Blot Cell (Bio-Rad Laboratories) containing transfer buffer (0.192 M glycine, 0.025 M Tris, 20% [v/v] methanol, pH 8.3) at 100 V for 1 hr at room temperature. The blots were blocked with 1% skim milk in Tris-buffered saline (TBS) at 4°C overnight. The blots were cut in strips, and each strip was incubated at 4°C overnight in TBS containing 0.2% Tween 20 (TBST) with either a 1:100 dilution of hyperimmune rabbit serum (positive control), a 1:10 dilution of sera from infected patients, or a 1:10 dilution of control negative sera. The strips were rinsed once in TBST at room temperature and washed three times in the same solution at room temperature for 20 min. The strips were then incubated for 1 hr at room temperature with either 1:1,000 dilutions of peroxidase-conjugated goat anti-human IgM or peroxidase-conjugated goat anti-human IgA in TBST. To detect proteins reacting with IgG1 or IgG3 isotypes, strips were incubated with unconjugated mouse anti-human IgG1 or IgG3 at dilutions of 1:1,000, followed by incubation with a 1:250 dilution of peroxidase-labeled goat anti-mouse IgG at room temperature for 1 hr. The blots were rinsed once and washed three times in TBST at room temperature for 20 min. The color was developed using 0.5 mg/ml of 3,3′-diaminobenzidine in PBS (pH 7.4) containing 0.1% hydrogen peroxide. The reaction was stopped by extensive washing of strips in double-distilled, deionized water. The strips were either photographed immediately or stored in dark until photographed.

Statistical analysis. The data were analyzed using one-way analysis of variance (ANOVA) and frequency distribution programs of a statistical software package, Statview 4.0 (Abacus Concepts, Inc., Berkeley, CA) for the Apple Power Macintosh computer (Apple Canada, Inc., Markham, Ontario, Canada). Probability values of $P < 0.05$ were considered significant.

RESULTS

Symptomatology and cyst output of giardiasis patients. The distribution of symptoms of giardiasis patients is shown in Table 1. The mean ± SEM cyst output per gram of feces was significantly higher in symptomatic individuals: $131 \pm 22 \times 10^3$ versus $575 \pm 35 \times 10^3$ for asymptomatic and symptomatic patients, respectively. There were no significant differences in the mean cyst output of patients of different age groups ($P > 0.05$, by one-way ANOVA).

Anti-parasite serum antibody response measured by IFA. The sera from all symptomatic and asymptomatic patients were positive in the IFA. The circulating anti-parasite antibodies recognized the surface, flagella, and the internal

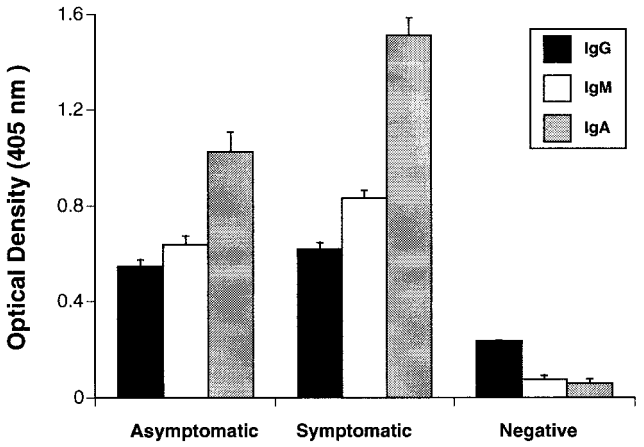

FIGURE 1. Circulating total IgM, IgG, and IgA anti-parasite antibody response measured by ELISA in 81 symptomatic and 49 asymptomatic patients infected with *Giardia lamblia.* Bars show the mean ± SEM.

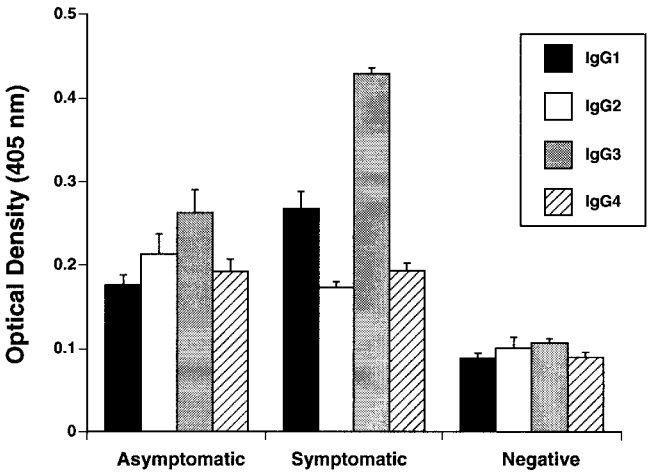

FIGURE 2. Circulating IgG1, IgG2, IgG3, and IgG4 anti-parasite response measured by ELISA in symptomatic and asymptomatic patients infected with *Giardia lamblia.* Bars show the mean ± SEM.

structures of the trophozoites. Serum titers for asymptomatic patients ranged between 1:250 and 1:4,000 and those of symptomatic patients ranged between 1:500 and 1:16,000. The frequency distribution of 49 asymptomatic patients was as follows: titer 1:500 or lower, 17 patients (34.7%); 1:1,000, nine patients (18.4%); 1:2,000, 10 patients (20.4%); 1:4,000, 11 patients (22.45%); and 1:8,000 or higher, two patients (4.1%). The frequency distribution of 81 symptomatic patients was as follows: titer 1:500 or lower, one patient (1.2%); 1:1,000, eight patients (9.9%); 1:2,000, 19 patients (23.5%); (1:4,000, 29 patients (35.8%); and 1:8,000 or higher, 24 patients (29.6%). The difference between IFA titers of between asymptomatic and symptomatic patients was significant ($P < 0.05$, by one-way ANOVA).

Anti-parasite IgG, IgM, and IgA response measured by ELISA. The anti-parasite IgG, IgM, and IgA of asymptomatic and symptomatic patients were measured using the ELISA (Figure 1). There were significant differences in the anti-*G. lamblia* IgM ($P < 0.02$) and IgA ($P < 0.01$) responses between asymptomatic and symptomatic patients. However, no significant differences ($P > 0.05$) were observed in the total anti-*G. lamblia* IgG response of asymptomatic and symptomatic patients.

With the exception of a significantly lower anti-parasite IgA response in the oldest symptomatic patient group ($P < 0.05$, by one-way ANOVA), there were no significant differences in the other circulating anti-parasite antibody responses of patients in different age groups of both asymptomatic and symptomatic patients (Table 2).

Anti-parasite IgG isotype response. The IgG1, IgG2, IgG3, and IgG4 isotype responses of 49 asymptomatic patients were examined using the ELISA (Figure 2). There were significant differences ($P < 0.05$, by one-way ANOVA) between the anti-*G. lamblia* IgG1 and IgG3 responses of asymptomatic and symptomatic patients, but not between the IgG2 and IgG4 isotype responses.

Recognition of parasite antigens by anti-parasite immunoglobulins. Antigen recognition by anti-parasite IgA and IgM from sera of 24 asymptomatic and 24 symptomatic patients was examined using SDS-PAGE and immunoblotting. The IgA and IgM from sera of asymptomatic and symptomatic patients recognized several distinct parasite molecules (Figures 3 and 4). There appeared to be no clear differences in the recognition profiles of anti-parasite IgA or IgM from sera of the asymptomatic and symptomatic patients: similar antigens varying in molecular weight from 18 kD to 113 kD were recognized by anti-parasite IgA (Figure 3) and antigens varying in molecular weight from 25 kD to 225 kD by anti-parasite IgM (Figure 4).

TABLE 2

Serum anti–*Giardia lamblia* IgM, IgG, and IgA responses of different age groups of symptomatic and asymptomatic patients with confirmed giardiasis

Age group (years)	Patient status*	n	Optical density at 405 nm (mean ± SEM)		
			IgM	IgG	IgA
<6	S	26	0.84 ± 0.11	0.58 ± 0.09	1.68 ± 0.19
	A	12	0.76 ± 0.32	0.59 ± 0.08	1.57 ± 0.27
7–10	S	19	0.68 ± 0.09	0.67 ± 0.08	1.52 ± 0.21
	A	15	0.71 ± 0.34	0.48 ± 0.09	1.32 ± 0.35
11–18	S	25	0.79 ± 0.17	0.66 ± 0.13	1.73 ± 0.23
	A	12	0.73 ± 0.40	0.53 ± 0.14	1.54 ± 0.43
≥19	S	11	0.93 ± 0.12	0.62 ± 0.14	1.11 ± 0.24†
	A	10	0.82 ± 0.34	0.49 ± 0.12	1.38 ± 0.17

* S = symptomatic patients; A = asymptomatic patients.
† Significantly different from the IgA response of different asymptomatic age groups ($P < 0.05$).

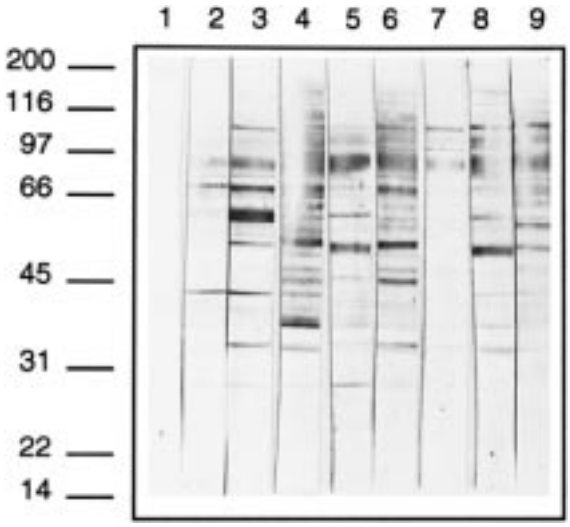

FIGURE 3. Representative immunoblot of anti-parasite IgA antigen recognition of symptomatic and asymptomatic patients infected with *Giardia lamblia*. Lane 1, serum from noninfected human; lanes 2–5, sera from four different symptomatic patients; lanes 6–9, sera from four different asymptomatic patients. Values on the left are in kilodaltons.

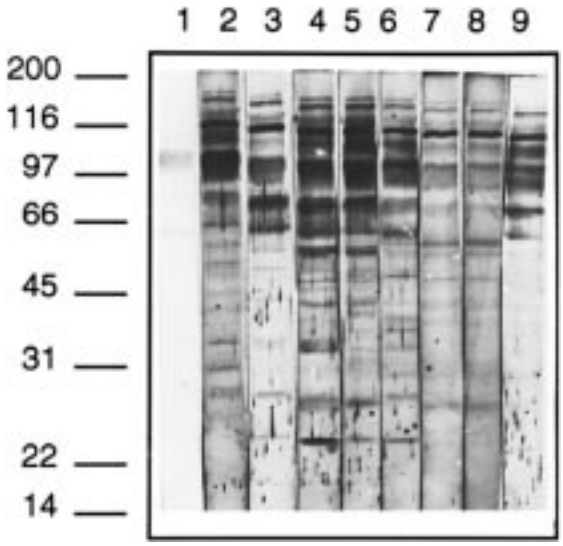

FIGURE 4. Representative immunoblot of anti-parasite IgM antigen recognition of symptomatic and asymptomatic patients infected with *Giardia lamblia*. Lane 1, serum from noninfected human; lanes 2–5, sera from four different symptomatic patients; lanes 6–9, sera from four different asymptomatic patients. Values on the left are in kilodaltons.

The response was heterogeneous, and there were no obvious differences in the antigen recognition profiles of anti-parasite IgA or IgM from asymptomatic and symptomatic patients or different patient age groups.

Sera from 24 asymptomatic and 24 symptomatic patients were assayed by immunoblotting to identify antigens reacting with IgG1 and IgG3 isotypes. Anti-parasite IgG1 and IgG3 recognized a number of major different parasite molecules, varying in molecular weight from 33 kD to 113 kD (anti-parasite IgG1) and from 25 kD to 113 kD (anti-parasite IgG3). The results summarized in Table 3 show the molecular weights of different antigens recognized by serum anti-parasite IgG1 and IgG3 of asymptomatic and symptomatic patients. For example, 46% of the asymptomatic patient sera IgG3 recognized 33-kD and 103-kD molecules, while none of the sera from symptomatic patient sera recognized these antigens. Similarly, 36% of the symptomatic patient sera IgG3 recognized the 25-kD molecule while none of the asymptomatic patient sera recognized this antigen. However, the recognition of these parasite antigens was not observed in the entire population of either symptomatic or asymptomatic patients. Thus, the responses were heterogeneous, and there were no obvious differences in the recognition profiles of these two anti-parasite IgG isotypes or patients of different age groups.

DISCUSSION

In the present study, we examined the serum antibody response of asymptomatic and symptomatic giardiasis patients from Egypt. All symptomatic and asymptomatic patients with confirmed giardiasis exhibited a specific antibody response to *G. lamblia*. In general, the anti-parasite antibody response of the symptomatic patients was higher than that of asymptomatic individuals, and was related to the magnitude of the cyst output. A significantly higher total anti-parasite IgM and IgA but not IgG response was observed in symptomatic individuals. In addition, symptomatic patients exhibited a significantly higher IgG1 and IgG3 isotype responses when compared with the asymptomatic individuals. Interestingly, there were no differences in the serologic response between different age groups. The antigen recognition of anti-parasite antibodies was heterogeneous, and there were no clear differences in the recognition of parasite molecules by anti-parasite IgM, IgA, IgG1, or IgG3 between the populations of asymptomatic and symptomatic patients.

A number of studies reported the presence of anti-parasite antibodies in infected individuals. Ridley and Ridley[8] were the first to report the presence of anti-*G. lamblia* antibodies in the serum of infected humans. Circulating antibodies to *G. lamblia* were first detected using an IFA[9, 10] and ELISA.[11–14] Chaudhuri and others[13] measured circulating IgM and IgG response of symptomatic and asymptomatic patients and found that a strong IgM response was related to the symptomatology of the infection, in which symptomatic patients exhibited a significantly higher IgM response than asymptomatic individuals. In this

TABLE 3

Summary of the molecular weights of major antigens recognized by anti-parasite IgG1 and IgG3 from the sera of asymptomatic and symptomatic giardiasis patients determined by immunoblotting*

	Percent of patients			
	Asymptomatic		Symptomatic	
Antigen (kD)	IgG1	IgG3	IgG1	IgG3
113	41	46	57	57
103	33	46	–	–
64	42	63	36	43
56	41	54	29	43
49	29	50	29	43
33	21	46	–	–
25	–	–	18	36

* – = not present.

study, we also observed a significantly higher circulating IgM level in the symptomatic patients. The IgM antibody is generally produced early during the course of the infection and in response to T cell–dependent antigens.[38] Due to its pentameric structure, IgM is also a major complement-fixing antibody and has been shown to participate in the complement-mediated lysis of bacteria and protozoan parasites.[18, 39] In contrast to the IgM response of giardiasis patients, the total anti-parasite IgG response was similar between symptomatic and asymptomatic patients, and could not be used to discriminate between the two groups. On the other hand, symptomatic individuals also had a significantly higher circulating IgA response. These findings are similar to those of Char and others,[27] who reported higher anti-parasite IgA response of Indian children infected with *G. lamblia.* Elevated circulating and secretory IgA levels have been reported during the elimination phase of the infection and following a challenge infection of mice with *G. muris,* particularly in animals that are genetically resistant to the infection.[16, 19, 40–43] These data suggest that elevated serum or secretory IgA after a challenge infection with *Giardia* may contribute to the resistance of the host to subsequent exposure to the parasite. It is tempting to speculate that elevated circulatory IgA in symptomatic patients may represent the response of the hosts to secondary exposure to the parasite. This prominent circulating IgA response may be an excellent tool for the early detection of the symptomatic cases of giardiasis.

The IgG isotype response of human giardiasis patients is reported for the first time in this study. The results of the measurements of the IgG isotype responses indicated that symptomatic individuals had significantly higher anti-parasite IgG1 and IgG3 responses when compared with the asymptomatic patients. The IgG1 isotype is produced in humans in response to polypeptide antigens.[44] Different cytokines produced by T cell subsets have also been shown to affect IgG isotype production. For example, the Th2 T helper cell product interleukin-4 induces a strong IgG1 and IgG3 responses, while interferon-gamma regulates a strong IgG2 response.[45] The IgG1 isotype, along with IgG3, are the IgG isotypes that readily fix complement, and the in vitro complement-mediated lysis of the trophozoites of *Giardia* has been reported previously.[18, 40] The IgG3 isotype is produced in response to carbohydrate antigens, especially those of bacterial pathogens.[46] Anti-parasite IgG3 monoclonal antibodies have been shown to agglutinate *Giardia* trophozoites in vitro[40] and kill them in the presence of complement.[18, 40] This observation, plus the fact that IgG3 will cross the placental barrier six times more readily than other isotypes,[47] suggests that IgG3 may play an important role in intestinal immunity to protozoa and bacterial pathogens.

Circulating and secretory antibodies have been reported in murine giardiasis.[16–19, 41, 48, 49] Snider and others[16] detected anti-parasite serum IgG and IgA but not IgM in mice during the course of the infection. Of the three immunoglobulins, only anti-parasite IgA was present in gut secretions, particularly during the elimination phase of the infection. In contrast, Heyworth[43] detected IgA and IgG, but not IgM, bound to the surface of the trophozoites harvested from the intestinal lumen of immunocompetent mice on day 10 after infection.

An unexpected observation from this study was that the circulating anti-parasite antibody responses were similar between the children and adolescents of different age groups (broad group to the middle period of puberty). The exception was a significantly reduced ($P < 0.05$) anti-parasite IgA response of symptomatic patients more than 19 years of age. It is well established that anti-*Giardia* antibody titer increases with age.[9, 10, 27] A possible explanation for these findings is that in the areas of high endemicity, the continuous, high level exposure of humans to the parasite early in life induces a stronger circulating anti-parasite antibody response that transects different age groups of the population.

Antigen recognition in patients infected with *G. lamblia* has also been examined in this study. In general, the antigen recognition by IgM, IgA, and IgG isotypes was heterogeneous, and there were no obvious differences in antigen recognition of antibodies from symptomatic and asymptomatic patients. Three antigens with molecular weights of 56, 75, and 116 kD were recognized by IgM antibody and two antigens with molecular weights of 97 kD and 110 kD were recognized by IgA antibody from all symptomatic and asymptomatic patients. Using immunoblotting, Taylor and Wenman[26] identified a major 31-kD protein recognized by sera from patients during the acute phase of the infection. The 31-kD immunodominant *G. lamblia* protein was found it to be related to alpha giardin.[28] Other major antigens recognized by sera from infected humans had molecular weights of 27, 28, and 56 kD. In a different study, Einfeld and Stibbs[29] identified and characterized a major surface 82-kD glycoprotein of trophozoites common to four isolates of *G. lamblia.* This glycoprotein was similar to the molecule identified in other parasitic protozoa, and was shown to function as receptor for interaction with host cells,[30] and as an antigen that elicits protective immune responses.[31, 32]

Anti-parasite antibodies have been used to detect parasite antigens in secretions and/or excretions.[21, 22] In a number of studies, Nash and others[21, 22] reported significant heterogeneity among *G. lamblia* strains isolated from humans, and demonstrated that the parasite undergoes antigenic variation both in vitro[24] and in vivo.[25] Despite the fact that our studies were done using the trophozoites of the in vitro grown WB strain of *G. lamblia,* a number of major antigens were recognized by anti-parasite antibodies from sera of symptomatic and asymptomatic patients. However, further antigenic profile analyses must be conducted using different isolates of *G. lamblia,* particularly those prevalent in Egypt, to fully elucidate antigen recognition by antibodies from symptomatic and asymptomatic individuals in this endemic area.

Higher anti-parasite immunoglobulin responses to *G. lamblia* of symptomatic patients reported in this study, were similar to the responses of the genetically resistant mice to *G. muris,* in which IgM, IgA, IgG1, and IgG3 levels are significantly higher in resistant hosts compared with susceptible ones.[3, 16, 19] In this regard, symptomatic patients behave immunologically like resistant hosts, while asymptomatic individuals are similar to the susceptible hosts. It remains to be determined whether the duration of the course of the infection and cyst release, albeit at low levels, is of longer duration in asymptomatic compared with symptomatic giardiasis patients.

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