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Experimental Human Infections with *Giardia lamblia*

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Fifteen healthy volunteers were inoculated enterally with trophozoites of two distinct human isolates of *Giardia lamblia*, GS/M and Isr. Each of two groups of five volunteers were inoculated with 50,000 (GS/M or Isr) trophozoites. All of the volunteers inoculated with GS/M and none of the volunteers inoculated with Isr became infected. Three of five volunteers infected with GS/M became ill, including two who had diarrhea and typical symptoms of giardiasis. In the second study, three patients who were previously infected with GS/M and treated were rechallenged 12 weeks after the first inoculation, together with five new control volunteers. All of the latter group became infected, and two developed loose stools; two rechallenged volunteers became reinfected but were asymptomatic, and a third was retrospectively found to be infected at the time of challenge. Serum IgM, IgG, and IgA antibody responses to *Giardia* and intestinal fluid IgA antibody responses to *Giardia* occurred in 100%, 70%, 60%, and 50%, respectively, of infected volunteers. These studies fulfill Koch's postulates and demonstrate strain variation in the pathogenicity of *Giardia* infections in humans.

Giardia lamblia is a flagellated protozoan parasite that infects the upper intestinal tract of humans and many animal species [1]. It is the most common gastrointestinal parasitic infection of humans in the United States [2], and infection is nearly universal early in life in underdeveloped areas [3]. In developed countries, infections occur most frequently in children (particularly in those attending day care facilities [4, 5]), homosexuals [6], institutionalized individuals, travelers [7], and backpackers [8]. Epidemics have occurred after contamination of municipal water supplies [2]. Although many persons are asymptomatic cyst excreters [9–11], other infected individuals may complain of diarrhea with or without malabsorption, nausea, vomiting, flatus, and abdominal cramping [7].

The pathogenesis of giardiasis and the host immune response to infection are still poorly understood. Improvement of illness after eradicating *Giardia* has been clearly documented in symptomatic patients. There is, however, a high rate of infection, but little associated symptomatology, in some populations, such as children attending day care centers [9]. In addition, in Rendtorff's classic studies [12], experimental infection of humans with *Giardia* cysts produced no clinical illness. Current knowledge about this parasite does not yet permit a unifying explanation for these disparate observations, although some progress in understanding has been made.

Recent studies demonstrate that *Giardia* isolates from humans differ biochemically and biologically, and these differences may explain some of the variability in the clinical features and chronicity noted in human infections. Restriction endonuclease analysis of the DNA from 15 isolates, followed by hybridization to nondefined *Giardia* probes, revealed marked differences between a majority of the isolates [13]. Analysis of the surface antigens and excretory-secretory (E-S) products of these isolates also revealed major differences [14]. Furthermore, when gerbils were infected with two well-characterized *Giardia* isolates, different patterns of infection and degrees of self-cure and varying abilities to induce resistance to homologous and heterologous

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challenge were observed [15]. In these studies the ability to maintain infection and induce resistance to reinfection was partly dependent on the isolate. Other investigators have shown different zymodemes in *Giardia* isolates [16, 17] and various surface antigens, as defined by agglutination with isolate-specific antisera to *Giardia* [17].

The most direct way to answer many basic biologic questions concerning *Giardia* infection in humans is by experimental infections of humans. Although four studies claim to have infected humans [1, 12, 18, 19], only Rendtorff's studies [12] are described in sufficient detail to allow a reasonable interpretation of the results. These studies used prison volunteers who ingested various doses of *Giardia* cysts obtained from humans. Infections were readily induced, but no symptoms could be attributed to the infection.

To determine if well-defined isolates of *G. lamblia* could cause infection and disease, we experimentally infected humans with trophozoites from two well-characterized, but unique, *Giardia* isolates, GS/M and Isr. Infection and clinical giardiasis occurred in 100% and 40% of GS/M-infected volunteers, respectively, whereas none of the volunteers inoculated with Isr became infected. For the first time, Koch's postulates have been fulfilled and the pathogenicity of *G. lamblia* formally established in humans. The inability of Isr to infect humans suggests that *Giardia* isolates differ in their inherent ability to cause infection.

Subjects and Methods

Giardia isolates. Two isolates with distinct DNA restriction endonuclease patterns, surface antigens, and E-S products were used [13, 14]. GS/M was isolated from a scientist from the National Institutes of Health who had typical symptoms of giardiasis. The patient had traveled to Japan and camped in the Alaskan wilderness about two weeks before the onset of illness. Purified cysts were inoculated into neonatal mice, and the trophozoites were recovered from the small intestines and axenized [13]. Cysts were obtained from a one-year-old child with diarrhea (isolate Isr) who lived in Bethesda, Maryland, and trophozoites were obtained as described above. Both GS/M and Isr were maintained in culture and passaged at least 10 times before sterility testing, expansion, and cryopreservation of inocula. After axenization, all culture materials and the trophozoites themselves were tested for the presence of bacteria, fungi, acid-fast bacilli (AFB), and mycoplasma. After these were found to be sterile, cultures were expanded in sterile medium and cryopreserved in individual vials. When needed, isolates were reestablished in culture from individual vials; retested for the presence of bacteria, fungi, and AFB; and used as the inoculum three days later. Inocula were always sterile.

Preparing and administering inocula. Isolates were maintained in TYI-S-33 medium supplemented with bile salts and antibiotics [13, 20]. When used as inocula, only viable adhered *Giardia* were used. Tubes containing *Giardia* in late log-phase were decanted, the medium replaced, and the tubes cooled for 30 min on ice. The number of *Giardia* was determined by using a Coulter Counter (Coulter Electronics, Hialeah, Fla) [20]. Tubes were then kept on ice until ~1 hr before use. The *Giardia* were then washed three times in ice-cold, sterile PBS (pH 7.4), and 5×10^4 *Giardia* were added to 5 ml of ice-cold, sterile PBS held on ice. Visual assessment of the *Giardia* revealed that most were alive and showed flagellar movement. The *Giardia* were lavaged into the small intestine by using a polyvinyl tube, which was previously shown by pH testing (≥ 6) and distance from the mouth (130 cm) to be in the small intestine, and the tube was immediately rinsed with an additional 5 ml of ice-cold, sterile PBS.

Volunteers. Fifteen healthy men, 20–33 years of age, from the metropolitan Baltimore community were admitted to the Isolation Ward of the Center for Vaccine Development for 22 days. The study was explained in detail, and written consent was obtained and witnessed. To ensure the informed nature of the consent procedure, we required the volunteers to pass a written examination containing 25 multiple choice and true-false questions covering all aspects of the study.

The health of the participants was assessed before study entry by medical history, physical examination, electrocardiogram, psychological examination, and laboratory tests (including complete blood count; measurement of levels of electrolytes, creatinine, blood urea nitrogen, liver enzymes, and quantitative serum immunoglobulins; and serological tests for syphilis, hepatitis B surface antigen, and antibodies to the human immunodeficiency virus). Stool specimens were examined for bacterial enteropathogens, ova, and parasites. None of the volunteers were infected with *Giardia* at the time of inoculation. Ex-

amination of one to two stools, as well as a jejunal aspirate, for ova and parasites failed to reveal *Giardia*. In addition, five additional stool examinations after inoculation, but before patency, failed to reveal *Giardia*. Forty-eight hours after admission, after a period of acclimation on the ward, volunteers were enterally inoculated with *Giardia* as described below. All volunteers were treated with quinacrine, 100 mg orally every 8 hr for five days, beginning 15 days after inoculation and were assessed for cure on day 28.

Study design. We performed two separate studies. In the first study, two groups of five volunteers were inoculated at the same time with 5×10^4 *Giardia* trophozoites from isolates GS/M or Isr and were followed up serially. In the second study, two different groups were inoculated with isolate GS/M exactly as in the first study. One group consisted of five newly recruited volunteers and the other, a group of three volunteers (previously infected with GS/M) from the initial study who had been successfully infected 12 weeks earlier and then treated. Volunteers in the second study were followed up as outpatients for an additional five weeks after therapy.

At the same time the *Giardia* study was undertaken, another group of volunteers on the same ward and sharing the same facilities were inoculated with an attenuated *Salmonella typhi* live oral vaccine [23]. Both sets of volunteers were evaluated serially by daily stool examination or culture (as noted above) for the presence of *Giardia* and *S. typhi* to exclude cross transmission. Stools were examined for *Giardia* up to day 19 in the group inoculated with *Salmonella*.

Clinical surveillance. Volunteers were interviewed on a daily basis by using a standard questionnaire and were asked about the presence of the following specific symptoms: anorexia, malaise, abdominal gurgling and flatus, abdominal cramping, headache, fever, and vomiting. The duration and severity of symptoms were noted. Symptoms that were present for at least 1 hr and that were judged to be clearly abnormal by the volunteer were designated as present. The sum of all the symptoms from days 1 to 14 was the symptom score. Vital signs were taken every 8 hr. Volunteers were not told whether they became infected. Examiners were aware of whether volunteers were infected.

All stools were graded, weighed, and tested as described below; one portion was preserved in formalin, and another was frozen at −70 C. The consistency of all stools were graded as follows: grade 1, formed; grade 2, soft; grade 3, thick liquid; grade 4, opaque watery; grade 5, rice water. Diarrhea was defined as passage of two or more loose stools (grades 3–5) within 48 hr and of at least 200 ml in volume or as a single loose stool of 300 ml or greater [24]. Abnormal stools (grades 3–5) of insufficient volume or frequency to be considered diarrhea were defined as loose stools. The presence and degree of other signs, such as vomiting, were recorded by the nursing staff.

Parasitological and microbiological methods. All stools from day 2 to day 14, most stools during treatment, and all stools posttreatment on days 20 and 28 were examined, graded, and tested for the presence of *Giardia*. Initially, most stools were examined unconcentrated for the presence of trophozoites or cysts. If none were found, we examined the stools by using a modification of a formalin-ether method for concentrating cysts [21]. In some instances, particularly during the prepatent period or when there were questions regarding the presence or absence of infection, stools were examined by two, or even three, different laboratories. Stools previously frozen and stored at −20 to −70 C were retrospectively tested for the presence of *Giardia* antigen [22], and when results disagreed, preserved stools were examined again.

Daily stool samples were also cultured for the attenuated *S. typhi* vaccine strain by using methods previously described [23].

Jejunal fluid was collected from polyvinyl chloride intestinal tubes on days 0, 14, and 19 or from gelatin string-capsules (Enterotest®; HDC, Mountain View, Calif) on days 5 and 10 as previously described [24]. We immediately examined the jejunal fluid microscopically to determine the presence of *Giardia*. If no organisms were detected, jejunal fluid was repeatedly sampled and examined over 2–6 hr.

Jejunal fluid was also evaluated for the presence of enteric bacteria and yeast before and 14 days after *Giardia* inoculation. Jejunal fluid was diluted tenfold serially in PBS (pH 7.2), and 0.1 ml was inoculated onto plates containing MacConkey's *Salmonella-Shigella* or Saboraud agar. Quantitative counts of bacteria or yeast were obtained by counting colonies.

Measuring humoral responses. Specific IgM, IgG, and IgA antibody responses were measured by using an ELISA. Wells of a microtiter plate (Immulon® 2; Dynatech Laboratories, Alexandria, Va) were coated with 10^5 Isr or GS/M *Giardia*

trophozoites (frozen and thawed three times) in 100 μl of PBS (pH 7.4) for 1 hr at 37 C and overnight at 4 C. Plates were used immediately or stored at −20 C. A sample (100 μl) of a 1:20 dilution of serum in PBS–Tween 20 was added and incubated for 1 hr at 37 C. After washing, 100 μl of the appropriate dilution of horseradish peroxidase–conjugated, heavy-chain goat antibody to IgM, IgG, or IgA (Cooper Biomedical, Malvern, Pa) was added in PBS-Tween for 1 hr, and 2,2′-azino-di-[3-ethyl-benzthiazoline sulfonate] (ABTS; Kirkegaard & Perry Laboratories, Gaithersburg, Md) substrate in PBS–Tween 20 was then added. Wells were washed five times with PBS-Tween. The OD was then read automatically at 405 nm by using a Titertek Multiskan MC® (Flow Laboratories, McLean, Va). Serial sera from individual patients were tested on the same plate in triplicate, and positive and negative controls were also included. Antibody levels are expressed as the change in the OD above or below the preinoculation value. Values of ≥0.1 OD were considered positive. None of the noninfected volunteers had values >.08 OD, as detected by using homologous or heterologous isolates as antigen, and all positive values were significantly above preinoculation values ($P <$ .05). Rheumatoid factor was not detected in any of the five infected patients in the first group with increased IgM antibodies to *Giardia*.

Intestinal IgA responses to isolates GS/M and Isr were measured in jejunal fluid. The intestinal samples were heated at 56 C for 30 min, and the total amount of IgA was determined [25]. Briefly, polyvinyl chloride plates were coated overnight at 4 C with α-chain–specific goat antibody to human IgA. After washing with PBS, plates were blocked with PBS/5% fetal bovine serum (FBS). Secretory IgA purified from milk by column chromatography used at the standard and a 1:4 dilution of jejunal fluid specimens were allowed to incubate overnight at 4 C. After a washing step, alkaline phosphatase–labeled goat antibody to human IgA was incubated in the wells for 1 hr at 37 C. After washing, *p*-nitrophenylphosphate was added, and color change was recorded at 405 nm. Jejunal fluids were then standardized to 20 mg/100 ml for the *Giardia*-specific IgA assay.

To determine the *Giardia*-specific IgA response in jejunal fluids, we sensitized microtiter plates (Dynatech Laboratories) overnight at 4 C with 100 μl of 6×10^6 previously frozen and thawed *Giardia* organisms/ml. After a washing step with PBS, plates were blocked with PBS–Tween 20 with 5% FBS for 1 hr at 37 C. Twofold serial dilutions of jejunal specimens were then incubated overnight. Plates were washed, and alkaline phosphatase–conjugated goat antibody to human IgA (Kirkegaard & Perry Laboratories) was added for 1 hr at 37 C. After washing, *p*-nitrophenylphosphate was added and color change recorded at 405 nm. On the basis of the ODs generated in samples obtained from the subjects before oral inoculation with *Giardia*, a cut-off level of 0.2 was used to determine the *Giardia*-specific antibody titer.

Giardia antigen in stool was detected by using a previously described ELISA [22, 26]. Stools from the same patient were tested in duplicate on the same plate along with a panel of positive and negative controls. A stool sample was considered positive if the mean OD of the sample was >3 SD above the mean of the negative controls.

Axenization of *Giardia* and DNA restriction analysis were performed as previously described [13].

Values of antibodies to *Giardia* were compared by using Student's *t* test.

Results

Infectivity. In the first study, five of five volunteers inoculated with GS/M (volunteers 1–5) became infected, as judged by detecting *Giardia* cysts in multiple stool examinations, humoral responses to *Giardia*, and *Giardia* antigen in the stool [26]. In contrast, none of five persons inoculated with Isr became infected, as judged by failure to detect *Giardia* cysts or antigen in the stool and lack of detectable humoral responses ($P <$ .004, two-tailed Fisher's exact test). In the second experiment, five of five previously uninfected volunteers who were inoculated with GS/M (volunteers 6–10) became infected (infection was determined by using the above criteria). Therefore, 100% of 10 persons inoculated with GS/M became infected, compared with 0% of five persons inoculated with Isr ($P <$ .0005). These experiments establish that axenically grown *Giardia* are infectious for humans and demonstrate a difference in the ability of two unique human isolates of *Giardia* to initiate infection.

The prepatent period in the first group was 7.2 ± 1.3 days (mean ± SD; range, 6–9 days) compared with a mean of 7.8 ± .45 days (range, 7–8 days) in the second group (figure 1). The mean prepatent period of all persons infected with GS/M was 7.5 ± .97 days. Each day, the presence of cysts was also

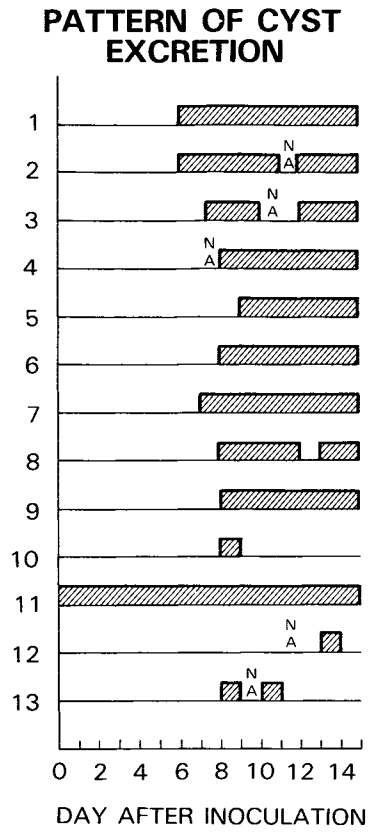

Figure 1. Cyst excretion by volunteers infected with GS/M (▭). Numbers 11, 12, and 13 are the cyst excretion patterns of reinfected volunteers 1, 3, and 4, respectively. The prepatent period for all initially infected persons was 7.5 ± .97 days. *NA* denotes stools were not available.

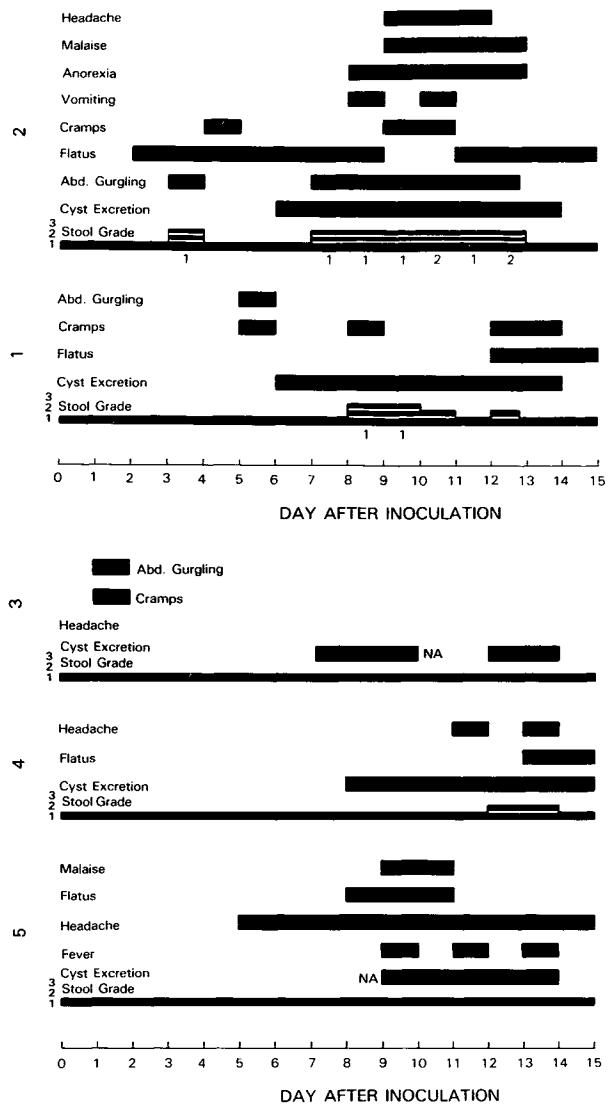

Figure 2. Chart of the presence (■) of a particular symptom, sign, or finding. *Top*, volunteers 1 and 2 developed loose stools or diarrhea along with other symptoms usually associated with giardiasis as noted. *Bottom*, volunteer 5 developed headache, fever, malaise, and flatus but did not experience diarrhea. Numbers under the horizontal bar denoting stool grade represent the number of grade 3 stools that occurred on that day.

variable (figure 1). Of the 10 infected persons, two had no detectable cysts on one day, even though excretion was documented both before and after these days. The number of cysts excreted varied markedly among individuals. Some excreted enormous numbers of cysts and others only an occasional cyst.

In the first study, because none of the volunteers inoculated with Isr became infected, they can reasonably serve as a control group. Three of five volunteers infected with GS/M became ill, two of whom had symptoms and signs typical of giardiasis (figure 2 top; table 1). Volunteer 2 complained of flatus, abdominal pain, anorexia, vomiting, cramps, headache, malaise, abdominal gurgling, and diarrhea for six days. Volunteer 1, although not as ill as volunteer 2, also developed diarrhea. One volunteer, no. 5, (figure 2 bottom) developed fever and headache but did not have diarrhea or loose stools. Although he was evaluated extensively, no other definite diagnosis could be established, and his symptoms and signs abated during therapy for *Giardia*. In contrast, one of the persons inoculated with Isr had a symptom score of +23 but had no diarrhea (table 1). A single isolated episode of loose stools occurred in

Table 1. Symptoms and signs of *G. lamblia* infection in volunteers.

Volunteers	Symptoms and signs of volunteers inoculated with	
	GS/M	Isr
Group I		
Symptoms	66 (31, 21, 8, 4, 2)	29 (23, 3, 2, 1, 0)
Loose stools (grade 3)	11 (9, 2, 0, 0, 0)	1 (0, 0, 0, 1, 0)
Group II		
Symptoms	10 (5, 4, 1, 0, 0)	. . .
Loose stools (grade 3)	6 (3, 3, 0, 0, 0)	. . .

NOTE. Symptom score is the sum of the symptoms that occurred from day 1 to day 14 (Subjects and Methods). The numbers in parentheses are the values for each volunteer.

another volunteer inoculated with Isr who had a symptom score of +1. Altogether, there were 66 separate symptoms and 11 episodes of loose stools or diarrhea in the infected group compared with 29 symptoms and one episode of loose stools in the noninfected group. Although there were more symptoms in the infected group, the number of symptoms did not significantly differ from the control group ($P > .05$). In the second study, loose stools developed in two of five infected persons (three episodes each), accompanied by mild symptoms usually associated with giardiasis. Therefore, 50% of individuals infected with GS/M became ill, and 40% developed an illness compatible with *Giardia* infection. The presence of diarrhea or loose stools was significantly more frequent in the 10 volunteers inoculated with GS/M compared with five inoculated with Isr ($P \approx .035$, Yates' mean score).

Diarrhea or loose stools developed 7.25 ± 2.99 days (range, 3–10 days) after inoculation. Three patients developed diarrhea or loose stools one to two days after cyst excretion, and one patient had a single loose stool on day 3 before cyst excretion followed by multiple episodes of diarrhea on days 7–12.

There were no consistent changes or abnormal values in the white blood cell count or in the levels of amylase, serum aspartate aminotransferase (SGOT), and serum alanine aminotransferase (SGPT) before infection compared with 14 days after inoculation. The one individual who developed headaches and fever also developed slight elevations of the SGOT and SGPT. Another symptomatic volunteer also showed a slight transient rise in the SGOT with a normal SGPT.

Quantification of aerobic bacteria and fungi in the jejunal fluid was performed just before inoculation and on day 14 in all volunteers in the second study and on day 14 for fungi only in the first study. There was no correlation with the presence and number of fungi or aerobic bacteria in the jejunum with the development of infection or symptoms (data not shown).

Immune responses to initial infection. Humoral responses to *Giardia* occurred in all GS/M-infected patients (figure 3) and none of the volunteers inoculated with Isr (data not shown). Responses were qualitatively and quantitatively similar whether GS/M or Isr was used as antigen (data not shown). IgM responses were detected in all infected patients (figure 3 top): seven had responded significantly by day 14 (before treatment) and the rest by day 21. Levels tended to fall after therapy. IgG responses occurred in seven of 10 (figure 3 middle): four responded by day 14, one by day 21, and the rest showed delayed responses. IgA responses were seen in six volunteers (figure 3 bottom), five of whom responded significantly by day 14 and another by day 21.

Five of 10 volunteers demonstrated significant rises in intestinal IgA antibodies to *Giardia* (table 2); these five also showed significant, specific IgA responses to *Giardia* in the sera. One person showed a delayed serum response, but no jejunal fluid was available for analysis at the same time.

Rechallenge study. Three persons infected in the first study (volunteers 1, 3, and 4) were rechallenged 12 weeks after inoculation to see if resistance to infection had developed (figure 1). Volunteer 1 (no. 11 in figure 1) was retrospectively found to be infected at the time of challenge, and the results are therefore uninterpretable. Volunteer 3 (no. 12 in figure 1) showed a rare cyst on one occasion and did not have *Giardia* antigen in his feces. In comparison, he had easily demonstrable cysts in the first study. The third person, volunteer 4 (no. 13 in figure 1) had cysts demonstrable on days 8 and 10 but had *Giardia* antigen in the feces until he was successfully treated on day 14, a result suggesting continuing infection.

Immunological response to rechallenge. Humoral responses and intestinal responses are shown in table 2 and figures 4–6. Although volunteer 3 was only transiently infected, he had no detectable intestinal IgA antibodies to *Giardia*. In contrast, volunteer 4 had a titer of 1:128 intraintestinally, but con-

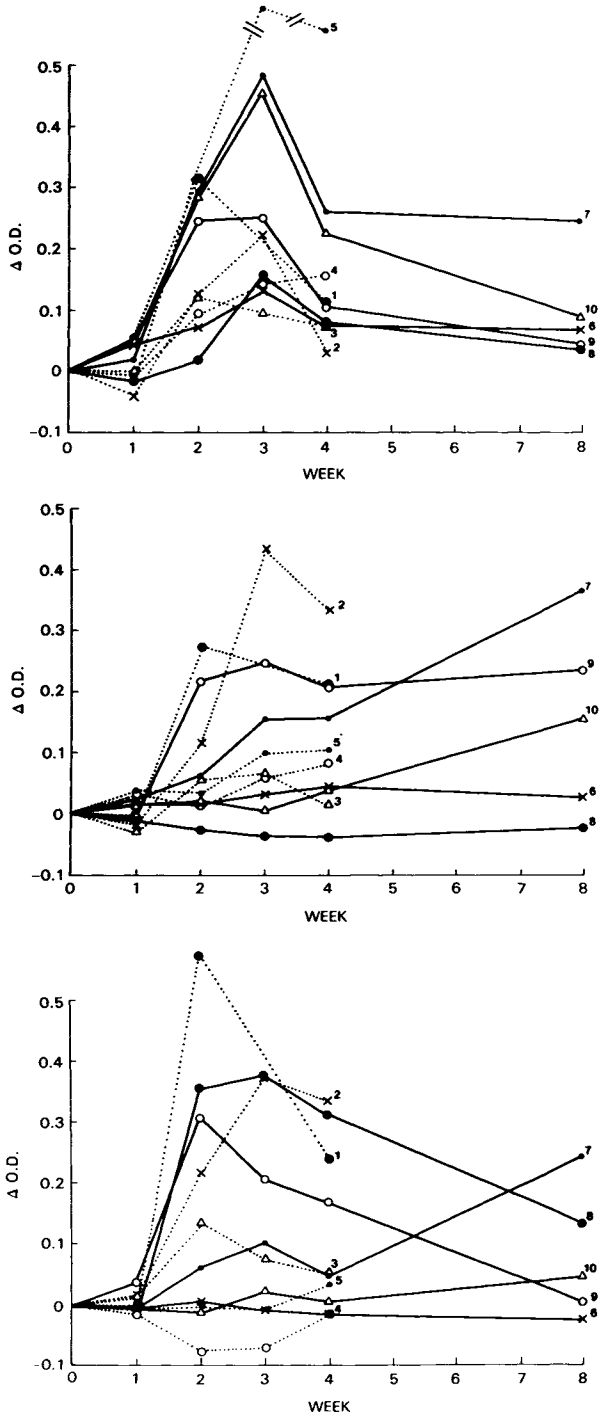

Figure 3. *Top*, IgM antibody responses (Group I) to *Giardia* using GS/M isolate as antigen. All volunteers had detectable increases in antibody levels that were significantly ($P < .05$) increased over preinoculation levels. Group I (. . .); Group II (———). Numbers to the right of each line represent volunteer number. *Middle*, IgG antibody responses to *Giardia* using the GS/M isolate as antigen. Increases of ≥0.10 OD were seen in six of 10 volunteers; volunteer 4 responded by week 12 (see figure 7). For explanation of symbols see top panel. *Bottom*, IgA antibody responses to *Giardia* using the GS/M isolate as antigen. Increases ≥0.1 OD were seen in six of 10 volunteers. For explanation of symbols see top panel.

Table 2. Reciprocal IgA antibody titer in jejunal fluid.

Volunteers	IgA antibody titer on Day 0	Days 11-14	Days 20-21
Group I			
1	<4	512	
2	16	128	128
3	<4	16	8
4	<4	<4	<4
5	<4	-	<4
Group II			
6	-	<4	<4
7	<4	<4	<4
8	<4	-	256
9	<4	-	-
10	<4	-	<4
Reinoculated			
1	2,048	2,048	2,048
3	<4	<4	<4
4	128	256	16

NOTE. IgA titers were determined by using GS/M as antigen. None of the volunteers inoculated with Isr showed any response to the homologous Isr isolate.

sistently shed cysts. In addition, volunteer 1 was found to be infected at the time of challenge despite a very high titer (1:2,048) of intestinal IgA antibody to *Giardia*.

All patients were intubated on day 14 to collect jejunal fluid and to reisolate *Giardia*. Only one of the 10 infected persons had *Giardia* trophozoites despite immediate microscopic evaluation, repeated sampling over many hours, and repositioning of the tube. We also failed to detect trophozoites by using gelatin string-capsules to examine duodenal fluid on days 5 and 10.

Characterizing recovered Giardia isolates. *Giardia* were reisolated and axenized from the stools of five of the 10 infected patients. Restriction endonuclease analysis revealed that these isolates were identical or nearly identical to the inoculating isolate (figure 7).

Cross-transmission analysis. Transmission of *Giardia* from the infected volunteers to the recipients of the oral *S. typhi* vaccine did not occur, as documented by the negative parasitological exami-

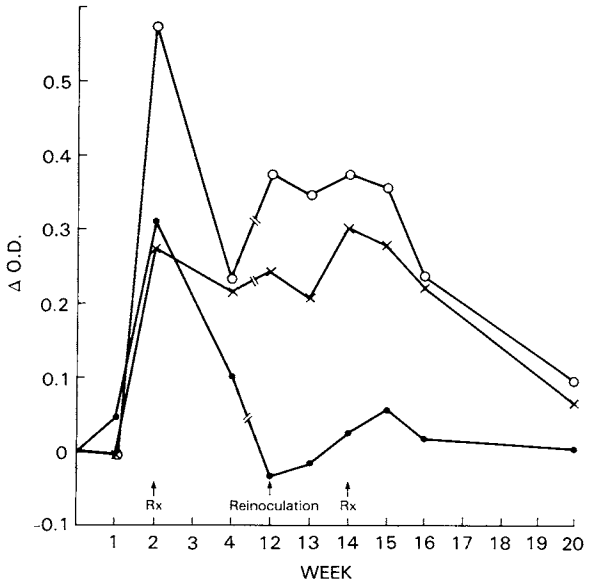

Figure 4. IgM (●), IgG (X), and IgA (O) antibody responses to GS/M isolate antigen in volunteer 1. At time 0, the volunteer was inoculated with GS/M; he was treated on day 14. He was infected and was retreated on day 30. A subsequent stool examination was negative, and he was therefore challenged. He was found retrospectively to be lightly infected at the time of challenge.

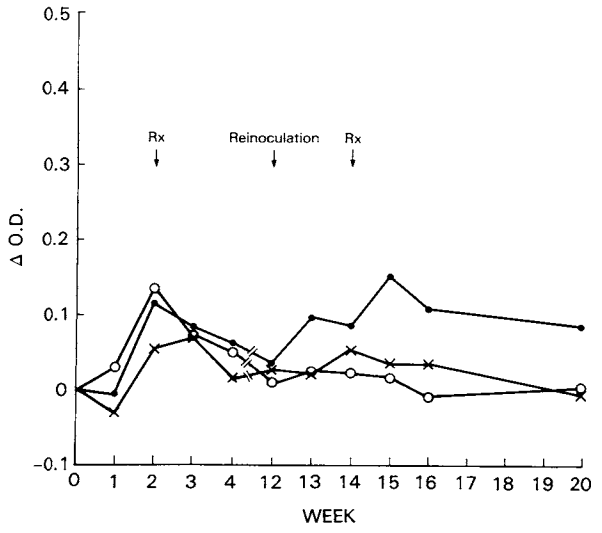

Figure 5. IgM (●), IgG (X), and IgA (O) antibody responses to GS/M isolate antigen in volunteer 3. At time 0, the volunteer was inoculated with GS/M, and the subsequent infection was treated on day 14; he was challenged 10 weeks later and treated 14 days later.

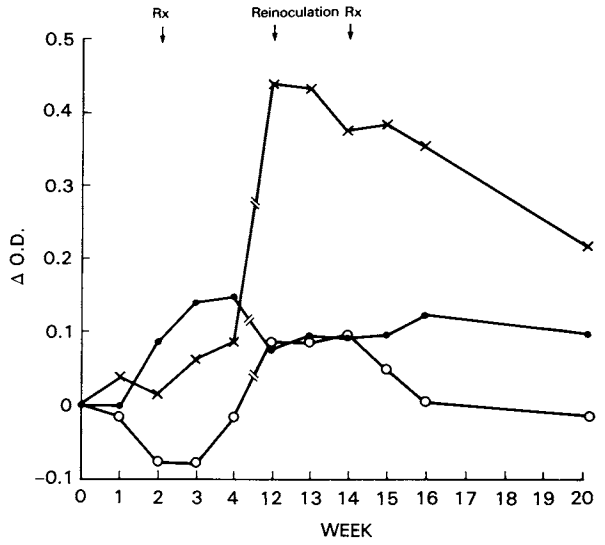

Figure 6. IgM (●), IgG (X), and IgA (O) antibody responses to GS/M isolate antigen in volunteer 4. At time 0, the volunteer was inoculated with GS/M, and the subsequent infection was treated on day 14; he was challenged 10 weeks later and treated 14 days later.

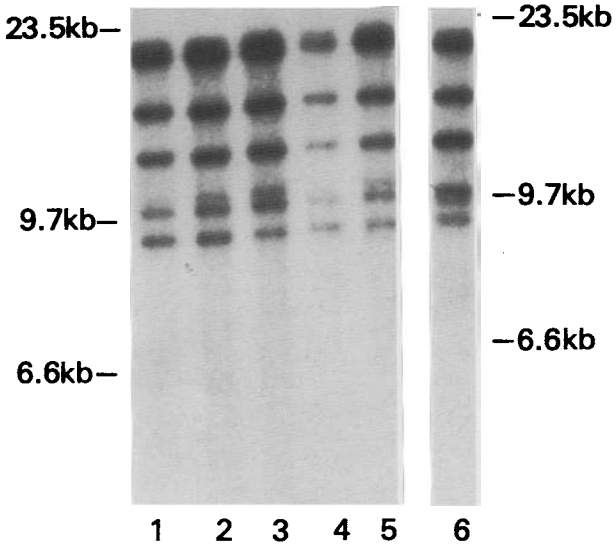

Figure 7. Autoradiograph of a Southern blot of DNA from *Giardia* reisolated from five volunteers infected with GS/M. The DNA was cleaved with *Hin*dIII and hybridized to probe G6A10. *Lane 1*, patient 2; *lane 2*, patient 1; *lane 3*, patient 6; *lane 4*, patient 4; *lane 5*, patient 7; and *lane 6*, GS/M inoculating isolate. Banding patterns are nearly identical to the original GS/M isolate. Failure to observe the upper band of the 9.7-kilobase (*kb*) doublet in lane 4 is most likely due to a decreased amount of DNA. All the other isolates are identical, although the doublet is not very apparent in lane 6 because of decreased migration. Lane 6 was run at a different time.

nation of stools from these individuals throughout the 22-day inpatient study.

Discussion

These studies formally establish the pathogenicity of *G. lamblia* for humans and fulfill Koch's postulates. GS/M was isolated from a symptomatic patient, established in culture, and used to infect 10 volunteers, 40% of whom developed diarrhea or loose stools associated with the appearance of *Giardia* in the feces and symptoms usually associated with giardiasis. The *Giardia* in the feces was reaxenized and shown to be the same or similar to the initial infecting isolate by using restriction endonuclease analysis. Although the association of *Giardia* infection and disease is indicated by a number of lines of evidence, previously published experimental studies were either not sufficiently detailed to be interpretable or demonstrated infection without disease [1, 12, 18, 19]. Rendtorff [12] inoculated prison volunteers with known numbers of cysts collected from the feces of infected humans. Although infection was easily induced, no disease or diarrhea could be attributed to the infection. There are a number of possible reasons for this, including differences in virulence among isolates of *Giardia* or the prior development of resistance to those as yet undefined factors responsible for the development of diarrhea in giardiasis. Although Rendtorff's studies were well done and informative, the choice of the inoculating isolate of *Giardia* was necessarily a random selection.

The ability of one isolate (GS/M) to infect all and the second isolate (Isr) to infect none of the volunteers indicates intrinsic differences between the isolates. Earlier studies showed that these isolates, as well as others, differed in their restriction endonuclease patterns [13], surface antigens [14, 20], E-S antigens [14, 20] and the pattern of infection in the Mongolian gerbil [15]. Our study proves that not only are there biochemical differences between isolates but that these or other differences may alter the biologic behavior of *Giardia* in humans.

Axenized trophozoites instead of cysts were used to initiate infection for a number of reasons. Cysts cannot be stored without changes in viability and infectivity over time; therefore, there would be batch-to-batch variation in cyst preparations and no way to control for the size of inoculum. This is particularly important in challenge experiments. Second, at the time of this study, cysts could only be obtained from feces, and there is always the possibility of administering adventitial agents along with cysts. Even though trophozoites were used, the prepatent period of the volunteers was similar to that found by Rendtorff when stool concentration techniques were used. The prepatent period was 7.5 ± .97 days (mean ± SD) in our study with a minimum of 6 days, compared with 8.3 ± 1.55 days with a minimum of 6 days in Rendtorff's study when fresh, unstored cysts were ingested. When stored cysts were used, the prepatent period was 13.1 days [27]. In the present study, diarrhea or loose stools began 7.25 ± 2.99 days after inoculation, a period somewhat shorter than that deduced from natural infections in humans [7, 28, 29]. In another study [30], the incubation period was eight days, but the median prepatent period was 14 days. Our studies suggest shedding can occur before developing symptoms.

Although total and IgG humoral responses to *Giardia* have been noted previously [31-35], the presence of IgM and IgA responses has not been appreciated. A!so, intestinal IgA responses have not been thoroughly studied. IgM antibody responses to *Giardia* were detected in all infected persons. Responses were documented by day 14 in 70%, and therefore could not have been initiated by treatment. This response was not widely accepted previously. The levels of IgM antibody to *Giardia* in the two patients who were reinfected did not greatly increase. The single patient with continuing infection also had relatively low levels at that time. Goka et al. [36] have recently reported the usefulness of IgM antibody responses to *Giardia* in infected humans. As noted above, responses in chronically infected and rechallenged individuals were low and could limit the usefulness in diagnosing chronic or repeated infections. Rises in IgG antibody to *Giardia* were detected in 7 (70%) infected volunteers. In four, responses were detected by day 14, in two by day 21, and in another by week 12. Humoral IgA and intestinal IgA responses were detected at the same time. Of the seven (70%) who responded, five had done so by day 14.

The importance of intestinal IgA antibody responses to *Giardia* is not clear. In the limited number of patients studied, the presence of IgA antibody in the intestine did not prevent the establishment of infection, nor was it associated with cure in the one volunteer with persistent infection.

Neither of the two persons challenged 10 weeks

after treatment for their initial infection were fully protected from reinfection; however, both persons had not self-cured at the time of initial therapy, and a longer duration of infection may be necessary for an optimal immune response. One patient was possibly transiently infected and therefore partially protected. The question of whether humans develop protective immunity cannot be answered by our study.

A number of studies have suggested that colonization of the small intestine with aerobic enteric bacteria [37, 38] or yeast [39] was in some way responsible for, or contributed to, infection and/or symptoms. In our study we showed no correlation between the presence or amount of bacteria or yeast and infection or the development of symptoms.

Generally, most studies have found jejunal aspiration more sensitive than routine stool examination in detecting *Giardia* infections [40]. The failure to detect *Giardia* trophozoites in the intestinal fluids in all but one of the 10 infected patients was surprising and casts doubt on the utility of this method in diagnosing some *Giardia* infections. Detecting parasites in stool, jejunal fluid, or other body fluids depends to a large degree on proper preparation of the sample, training of the observer, and the amount of time spent examining the specimen. In these studies, factors that could lead to failure to detect *Giardia* in small intestinal fluid were minimized. We immediately examined intestinal contents; when no *Giardia* were found, repeated attempts were made, over several hours, to detect parasites. *Giardia* may be positioned lower in the intestine early in the infection and only with time reside more proximally in the small intestine.

Transmission of *Giardia* between adults living in dormitory-like facilities for 22 days was not observed, consistent with the epidemiological observations that *Giardia* infections are seen more commonly in groups where fecal-oral contamination (children in day care, homosexuals) occurs. *Giardia* transmission is probably unusual among adults in developed areas who observe normal hygienic practices.

Although some of the major questions about *Giardia* have been answered, many remain unanswered. Does immunity develop in humans, and if it does is resistance to the infection isolate specific? What is the pathophysiology of diarrhea? How does the variability of *Giardia* occur? Human experimental infections with *Giardia* are safe and are a useful, direct approach to answer some of these questions.

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