

Human Secretory and Serum Antibodies Recognize Environmentally Induced Antigens of *Giardia lamblia*

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Received 2 August 1991/Accepted 21 November 1991

The variability in duration and severity of infection with *Giardia lamblia* is likely to be due to trophozoite interactions with immune and nonimmune components of the small intestinal milieu. Despite its potential importance, nothing is known of the isotype or the specificity of the secretory antibody response to *G. lamblia*. In the present study, we show that serum and secretory antibodies recognize many *Giardia* antigens whose expression is induced by exposure to selected intestinal conditions. Isotype-specific immunoblots of antigens from trophozoites grown at pH 7.0 without bile or at the intestinal pH of 7.8 with bile were reacted with milk or serum antibodies from subjects with or without histories of giardiasis. While the results were complex, several key observations emerged. Serum and secretory immunoglobulin A (IgA), IgM, and IgG antibodies reacted with many regulated antigens. Antigen recognition patterns varied with isotype and between milk and serum antibodies of the same isotype. Antigen recognition also differed among subjects. Antibodies from virtually every patient recognized some *G. lamblia* antigens. Furthermore, milk and/or serum samples from putative controls without histories of giardiasis were positive more frequently than would be predicted from published prevalence studies, suggesting either that these antibodies may be cross-reactive or that undiagnosed infections with *G. lamblia* may be more common than previously thought. Thus, recognition of neoantigens induced by host conditions may be due to conserved or cross-reactive epitopes which could constitute a form of immune evasion by *G. lamblia*.

The spectrum of symptoms of giardiasis is extremely broad, ranging from asymptomatic infection to severe, prolonged diarrhea with malabsorption and failure of children to thrive (44). Despite its clinical importance, little is known of how *Giardia lamblia* causes disease, since no specific toxin or virulence factor has been identified (37). We have proposed that the variability in the manifestations of infection is due, in large part, to interactions of trophozoites with immune and nonimmune components of the small intestinal milieu which they colonize (13).

We and others have shown that *G. lamblia* can utilize specific nonimmune intestinal factors, such as bile (9, 15, 25) and mucus, to survive (46), attach (45), and grow (11). Moreover, although this is an extremely complex and dynamic environment, we have identified relatively defined factors which have profound effects on *G. lamblia*. For example, we found that exposure of cultured trophozoites to conditions which are unique to the small intestine, slightly alkaline pH (8) and bile (21), induces them to express a number of new or regulated antigens, which are likely to be expressed in the host small intestine but are not detected in trophozoites cultured at pH 7.0 without bile (33). These antigens were first detected in Western blots (immunoblots) by reaction with antibodies raised in rabbits against *G. lamblia* cysts purified from human feces (18). Immunocytochemical studies show that >80% of the parasites in the population express at least some of these antigens, while ~30% of the trophozoites differentiate into water-resistant cysts (10). Many studies have demonstrated serum (22) and secretory (29, 31) antibody responses to *G. lamblia*, but little is known about the identity and expression of the parasite antigens recognized or of the possible influence of these

antibodies on the course of infection (22). Since trophozoites are exposed to alkaline pH and bile in vivo, we have now asked whether any of the antigens induced by these factors are recognized by secretory and serum antibodies from patients.

MATERIALS AND METHODS

Organism and cultures. *G. lamblia* WB (ATCC 30957) was isolated from a patient with chronic symptomatic giardiasis (37) and belongs to the most common group of isolates from humans (30). Clone C6 of strain WB was isolated by limiting dilution in agarose (14) in 1983. Trophozoites were routinely subcultured twice weekly in filter-sterilized Diamond's TYI-S-33 medium (pH 7.0) with 10% adult bovine serum (Irvine Scientific) (5) and 500 µg of bovine bile ml⁻¹ (25) without added vitamins, antibiotics, or iron, at 37°C.

Reagents. Prestained rainbow molecular weight markers were obtained from Amersham (Arlington Heights, Ill.); nitrocellulose membrane (BA-83; pore size, 0.22 µm) was obtained from Schleicher & Schuell (Keene, N.H.) or Millipore (Bedford, Mass.). Unless otherwise specified, all other reagents were purchased from Sigma Chemical Co. (St. Louis, Mo.) and were of analytical grade.

Induction of regulated antigens. Preexperimental cultures were grown to late-log phase in TYI-S-33 medium with serum (pH 7.0 to 7.1), without bile, but containing the antibiotics piperacillin (500 µg ml⁻¹; Lederle Laboratories) and amikacin (125 µg ml⁻¹; Bristol Laboratories), which have not been observed to affect *G. lamblia* growth, differentiation, or expression of regulated antigens (18). Unless otherwise indicated, the spent medium and nonadherent trophozoites were removed and the adherent trophozoite monolayers were refed with TYI-S-33 medium containing antibiotics, lacking bovine bile, containing the intestinal

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stimuli porcine bile (250 $\mu\text{g ml}^{-1}$) and lactic acid (LA) (5 mM) and adjusted to pH 7.8 with NaOH (12). Porcine bile was used because earlier studies showed that it resembled human bile in its ability to efficiently induce cyst antigen expression and encystation (12). LA, a product of bacterial metabolism in the lower gut, was included because it acts late in encystation to increase cyst viability (2), although it does not appear to influence antigen expression directly (12, 21).

Parasite harvest, electrophoresis, and Western blotting. After incubation at 37°C for 48 h, or as specified for each figure, parasites were harvested by chilling cultures in ice water for 30 min to release attached parasites. Parasites harvested at the time of refeeding served as zero time controls. They did not differ detectably in antigen expression from trophozoites incubated for 66 h in medium at pH 7.0 without bile or LA. Parasites were collected by centrifugation at $833 \times g$ for 10 min, washed, resuspended at $3 \times 10^8 \text{ ml}^{-1}$ in sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis sample buffer (27) containing 2% SDS and 50 mM dithiothreitol, boiled for 6 min, and stored at -70°C until use.

Aliquots (20 μl) of the solubilized parasite extract were separated by using a discontinuous gel system, with a 3% stacking gel and 10% separating gel (27). The separated antigens were electrotransferred to nitrocellulose membranes according to the method of Towbin et al. (40) for 18 h at 120 mA and then for 1 h at 350 mA. Membranes were blocked in phosphate-buffered saline (PBS) containing 0.3% gelatin and 0.05% Tween 20 for 1 h and washed thoroughly.

Milk (1:10) or serum (1:50) samples from patients with current or recent histories of giardiasis or controls were used as sources of antibodies. Patients were identified by the presence of cysts or trophozoites in stool specimens. Putative negative controls were normal subjects from the San Diego, Calif., area with no known histories of giardiasis or other unexplained diarrheal disease. Rabbit antiserum against *G. lamblia* cysts prepared in vitro (used at 1:200) and antibody against the recombinant cysteine-rich surface antigen TSA 417 (1:50) were prepared as described previously (16, 18). The blots were incubated for 2 h with antibody as specified for each figure. Replicate blots were washed and then reacted with appropriate peroxidase-conjugated secondary antibodies (1:2,000 in PBS) for 1 h: goat anti-human secretory immunoglobulin A (IgA) (α -chain specific) from Cappel (West Chester, Pa.), goat F(ab')₂ anti-human IgM (μ -chain specific) from Tago (Burlingame, Calif.), or protein A (enzyme immunoassay grade) from Zymed (San Francisco, Calif.). Under our conditions, protein A appears highly specific for IgG. Other blots were reacted with peroxidase-conjugated wheat germ agglutinin (WGA) at 2 $\mu\text{g ml}^{-1}$ for 1 h. The strips were then developed with 4-chloro-1-naphthol (40).

RESULTS

Immunoblots showed that *G. lamblia* antigens that are induced by exposure to selected intestinal conditions (porcine bile and LA at pH 7.8) are recognized by serum IgG antibodies from giardiasis patients (Fig. 1). In each of four replicate panels, the first lane contains antigen from trophozoites grown under control conditions (pH 7.1, no bile), and the second lane contains antigen from parasites grown at pH 7.8, with porcine bile and LA. The recognition patterns of antibody from each of the four patients tested differed (Fig. 1). In each case, however, IgG antibodies reacted with

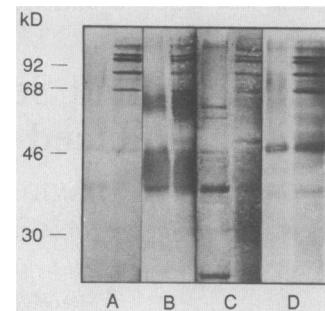


FIG. 1. Reaction of serum IgG antibodies from four different patients with symptomatic giardiasis with *G. lamblia* antigens. In each replicate panel, the first lane (closest to the molecular size markers on the left) contains antigen from trophozoites grown under control conditions (pH 7.1 and no bile); the second lane contains antigen from parasites grown at an intestinal pH of 7.8, with porcine bile and LA. Antigens which are present in only one lane of a panel are taken to be regulated by pH and/or bile. Results are shown for the following patients: a San Diego woman who was infected while pregnant and, although symptomatic, declined treatment (A); a San Diego man who returned from India with severe diarrhea and donated serum immediately after diagnosis (B); a Laotian woman whose giardiasis was diagnosed when she was hospitalized for childbirth (C); and a California woman who developed symptomatic giardiasis two days after giving birth, was treated at 17 days, and donated serum (and milk) 1 month later (D).

multiple antigens which were expressed only by parasites exposed to the intestinal factors. Prominent regulated antigens recognized by these sera were of approximately 66, 78, 94, 103, and 120 kDa. In addition to the regulated antigens, patients' sera recognized various numbers of constitutively expressed antigens present in both "intestinally" grown and control trophozoites (Fig. 1). Constitutive antigens included broad bands at ~ 66 and 40 kDa (Fig. 1B) and a discrete band at ~ 49 kDa (Fig. 1D), which we know from other studies (4, 16) to be trophozoite surface antigens (see below). The sharp induced band at 66 kDa (Fig. 1A to D) is not the same protein as the broad surface antigen band of approximately the same molecular size (both can be seen in panel B).

To begin to examine the relationship between infection and anti-giardial antibodies, we compared the reactivities of sera from patients (Fig. 2A and D) with those of controls with no known histories of giardiasis (Fig. 2B, C, E, and F). Antibodies from most control serum samples reacted with some or all of the regulated antigens, although the magnitude of the reactions tended to be less. We also compared the reactivity of serum from a patient infected in Leningrad, USSR, with that of his pretravel serum sample (Fig. 3). Reactivity with a number of the regulated antigens, including bands at ~ 66 and 78 kDa, was apparent only after infection.

We next examined the kinetics of expression of regulated and constitutive antigens, as well as the isotypes of the serum antibodies from a patient with chronic symptomatic giardiasis (Fig. 4). While most of the regulated antigens, including those at 66, 78, and 94 kDa, were first expressed after 24 h of exposure to intestinal conditions, the highest-molecular-mass antigen (~ 120 kDa) appeared at ~ 5 h and tended to decrease in intensity at 48 h. Although there was considerable overlap in antigen recognition by serum IgA, IgG, and IgM antibodies from this representative patient, there were also prominent differences. For example, the major constitutive, heterodisperse bands at ~ 40 and 66 kDa were mainly recognized by IgG antibodies. These prominent

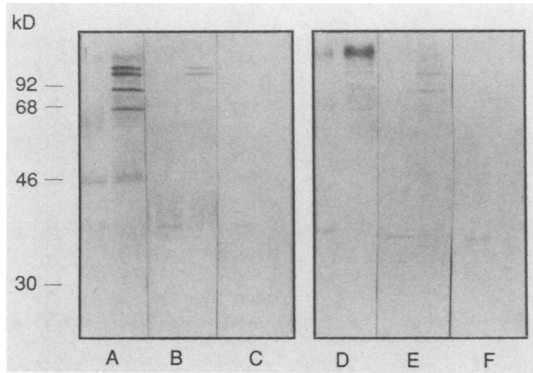


FIG. 2. Reaction of serum IgG antibodies from patients (A and D) and controls (B, C, E, and F) with antigen from trophozoites grown under control (first lane of each panel) and intestinal (second lane) conditions as described for Fig. 1. Results are shown for the same patient as described for Fig. 1A (A) and a male Vietnamese psychiatric patient (D).

constitutively expressed antigen species (seen more clearly in Fig. 1B) appeared to correspond to TSA 417, the major cysteine-rich surface variant antigen of strain WB clone C6, whose gene we have isolated and expressed in *Escherichia coli* (16). To test this possibility, we reacted Western blots containing antigen from a *Giardia* clone which expresses TSA 417 and five subclones which do not express TSA 417 (Fig. 5) with antiserum from the same patient and with rabbit antirecombinant TSA 417. Both the rabbit antiserum sample and the patient's serum reacted with broad bands at ~85, 66, and 40 kDa only in the TSA 417-positive *Giardia* clone. However, the patient's serum also reacted with a number of other antigens which were present in most or all clones. The 85- and 66-kDa species are products of the same gene, and the 40-kDa species appears to be a proteolytic fragment (1, 16).

Since *G. lamblia* colonizes the small intestinal lumen, the secretory antibody response may quite be important. Therefore, we compared the antibody responses of paired milk (Fig. 6, subpanels A) and serum (Fig. 6, subpanels B) samples obtained from the same patients and controls.

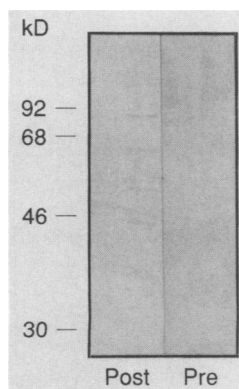


FIG. 3. Comparison of serum samples from a patient (male physician) prior to (Pre) and after (Post) travel to Leningrad, USSR, and acute symptomatic infection with *G. lamblia*. In each panel, the first lane contains antigen from trophozoites grown under control conditions (pH 7.1 and no bile); the second lane contains antigen from parasites grown under intestinal conditions (pH 7.8 with porcine bile and LA).

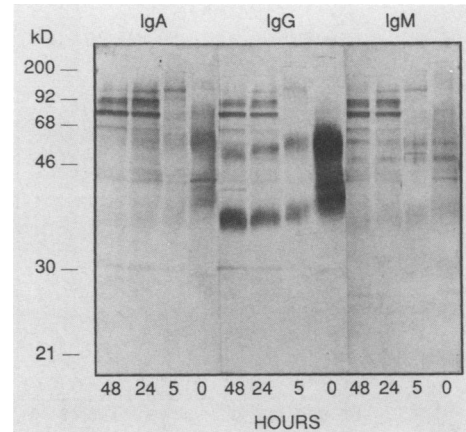


FIG. 4. Kinetics of expression of antigens recognized by IgA, IgG, and IgM antibodies from serum of a patient with chronic symptomatic giardiasis. The patient was a male scientist who was presumably infected by a child in day care. Parasite antigen was harvested after 0, 5, 24, or 48 h of exposure to bile and LA at pH 7.8.

Representative isotype-specific Western blots are shown in Fig. 6A to C. As was seen with serum antibodies (Fig. 2), many antigens recognized by antibodies in milk from both the patient and the control were expressed only after exposure to intestinal stimuli. Moreover, milk IgA, IgM, and IgG antibodies (Fig. 6, subpanels A) differed in the antigens they recognized. Finally, for each donor, milk (Fig. 6, subpanels A) and serum (Fig. 6, subpanels B) antibodies of a single isotype recognized different antigens. Although antibodies from controls did recognize most antigens, the reactions

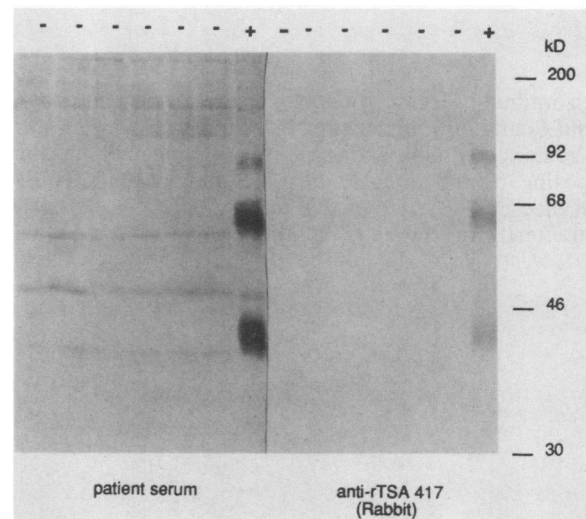


FIG. 5. Demonstration of reaction of IgG antibodies with TSA 417, the major cysteine-rich trophozoite surface antigen of strain WB clone C6. Duplicate blots contain antigen of conventionally grown trophozoites of strain WB clone C6 which express (+) the major TSA 417 cysteine-rich surface antigen and six sister subclones which do not express it (-). The left panel was reacted with serum from the same patient as described for Fig. 4. The right panel was reacted with rabbit antiserum against recombinant TSA 417 expressed in *E. coli* (16). It reacts only with the three species of TSA 417 in the expressing subclone. The patient serum recognizes TSA 417, as well as approximately five or six antigens which are expressed by the TSA 417-negative subclones.

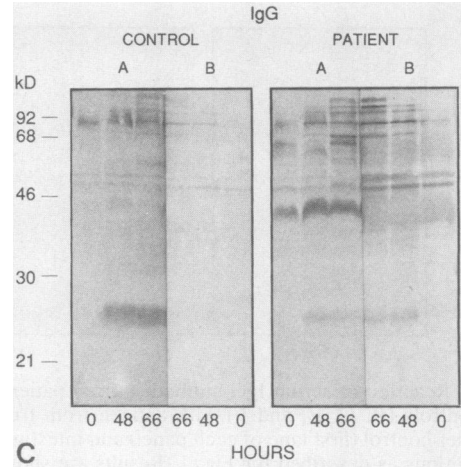
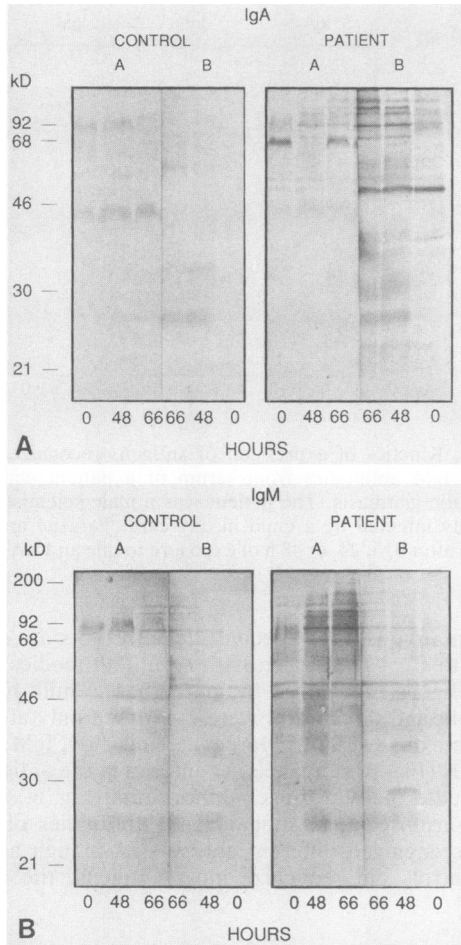


FIG. 6. Isotype-specific recognition of induced antigens by secretory and serum antibodies from a patient and a control. The patient was a San Diego woman with severe diarrhea, weight loss, and vomiting during her fifth month of pregnancy. She was treated during the fifth and seventh months and donated milk and serum 12 days postdelivery. She presumably was infected by a child in day care. Antigens were prepared from parasites exposed to bile and LA at pH 7.8 for 0, 48, or 66 h. Subpanels A were reacted with milk (1:10) and subpanels B were reacted with serum (1:50) from a single representative control and a single patient. Replicate sets of panels were reacted with peroxidase-conjugated rabbit anti-human IgA (A), IgM (B), or protein A (C).

with antibodies from patients tended to be more intense (Fig. 2 and 6) and of a higher titer (data not shown).

We next examined the regulation of antigen expression by exposing trophozoites to alkaline and neutral pH in the presence and absence of bile and LA (Fig. 7). To begin to characterize the antigens, parallel blots were reacted with a

patient's serum (Fig. 7A) and rabbit antiserum prepared against in vitro derived cysts (Fig. 7C). To assess whether the regulated antigens may be glycosylated, we reacted a third replicate blot (Fig. 7B) with WGA, the major lectin which reacts with *G. lamblia* trophozoites (19, 42), encysting cells (32, 33), and cysts (32, 33, 41).

The data in Fig. 7 show that simple exposure of trophozoites for 48 h to slightly alkaline pH, which is typical of the small intestine (lanes labeled 7.8-), induces expression of many regulated antigens. Many of the same antigens are also induced by exposure to bile and LA at neutral pH (lane labeled 7.0+). Interestingly, exposure of trophozoites to the combination of intestinal pH and bile and LA (lanes labeled

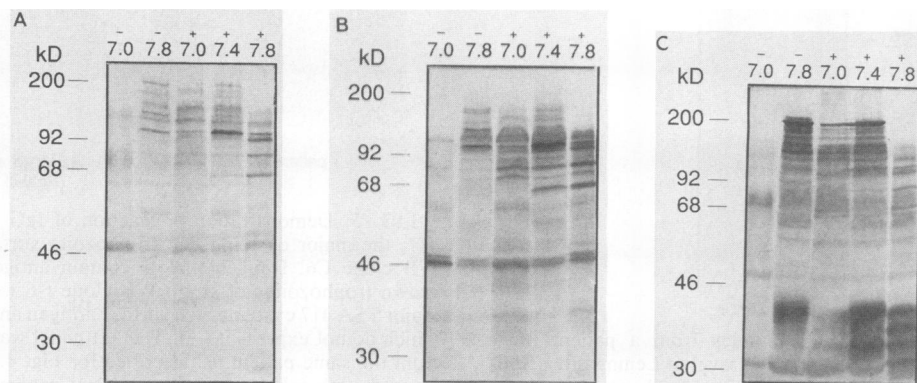


FIG. 7. Roles of alkaline pH and bile in inducing regulated antigen synthesis. Parasites were incubated at pH 7.0, 7.4, or 7.8 in the absence (-) or presence (+) of porcine bile and LA. Replicate blots were incubated with either serum from a patient (Fig. 2A) with giardiasis (A), peroxidase-conjugated WGA (B), or rabbit antiserum raised against cysts prepared in vitro (C). Antibodies in panels A and C were localized by reaction with protein A-peroxidase.

7.4+ and 7.8+) did not appear to induce synthesis of increased amounts or numbers of antigens. Indeed, some of the higher M_r antigens expressed at pH 7.8 without or at 7.0 and 7.4 with bile and LA were not observed at 48 h with parasites exposed to bile and LA at pH 7.8 (Fig. 7), although they were present at 24 h (data not shown), indicating earlier expression and possible turnover or processing under these conditions.

Virtually all of the antigens recognized by patients' sera were also recognized by rabbit anticyst serum (Fig. 7C) and WGA (Fig. 7B). Identity of these antigens was confirmed by affinity purification of antigens with WGA-agarose and reaction in Western blots with patients' sera or rabbit anticyst serum (data not shown). A minority of the antigens recognized by each serum and by WGA were present in control trophozoites incubated at pH 7.0 without bile and LA. We conclude that their expression is not regulated. One example is the glycoprotein at ~49 kDa (4), which is often seen as a doublet. Conversely, many antigens, particularly in the lower-molecular-mass range (<45 kDa), which reacted with the rabbit anticyst serum (Fig. 7C) were not recognized by human serum and milk samples (Fig. 1 to 7).

To examine whether reactivity of patients' antibodies with the induced antigens may be restricted to carbohydrate epitopes, we reacted replicate nitrocellulose transfers, containing control and induced antigens, with 50 mM NaIO₄ overnight. Periodate oxidizes adjacent hydroxyl groups of sugars and tends to destroy antigenicity of carbohydrate epitopes. This treatment ablated all reactivity with WGA but did not affect the TSA 417 surface protein antigen. NaIO₄ treatment did not detectably decrease reactivity with antibodies from patients or controls (data not shown).

Since *G. lamblia* is normally grown with 0.5 mg of bovine bile ml⁻¹ (at neutral pH [25]), an important question is why the regulated antigens are not observed under these conditions. In an earlier study, we found that porcine bile resembled human bile in its ability to induce cyst antigen expression and encystation (12). In contrast, much higher concentrations (10 mg ml⁻¹) of bovine bile are required to induce encystation (12, 35) and cyst antigen expression (43).

DISCUSSION

Since little is known of the effect of serum and secretory antibodies on the course of giardiasis, these studies were undertaken to identify the parasite antigens which are recognized by local and systemic antibodies. We have asked whether exposure of cultured trophozoites to specific small intestinal conditions induces expression of antigens which are recognized by antibodies from infected patients. We found that a subset of induced antigens is frequently recognized by IgA, IgM, and IgG antibodies in both serum and milk. These same specific intestinal factors, slightly alkaline pH (8) and bile (21), induce some cultured trophozoites to differentiate into cysts (12, 17). We found earlier (33) that exposure of cultured trophozoites to these intestinal conditions induced expression of a number of new antigens recognized by rabbit antibodies raised against cysts either purified from human feces (6) or prepared *in vitro* (Fig. 7). Trophozoites begin to express these antigens early in encystation. One group of low-molecular-mass polydisperse protein antigens (~22 to 39 kDa) was detected after only approximately 4 h of exposure to intestinal conditions (pH 7.8 and bile), while a second group of glycoprotein antigens (>60 kDa) was expressed at ~24 h (33).

It is likely that trophozoites express these antigens *in vivo*,

as they are exposed to both alkaline pH and high concentrations of bile in the small intestinal lumen. In addition, we have observed vesicular patterns of immunofluorescence with the anticyst serum in trophozoites aspirated from the upper jejunum of patients with giardiasis (unpublished data). *In vitro*, >80% of trophozoites exposed to bile at pH 7.8 express regulated antigens in vesicles (10, 33). Since only ~30% of these parasites go on to form water-resistant cysts, expression of the regulated antigens is not restricted to encystation (10). It is also likely that only a minority of trophozoites encyst *in vivo* (18). Indeed, many antigens are induced by exposure either to pH 7.8 or to bile (Fig. 7), conditions which do not lead to efficient encystation (10, 33). Some of these antigens are not observed after 48 h of exposure to both pH 7.8 and bile (Fig. 7), although they are expressed earlier (data not shown), suggesting that they may be transiently expressed or processed in encystation. Although some, if not all, of the regulated antigens are destined for the cyst wall (33, 34), they may have other nonstructural roles in the cell.

Interestingly, it is mainly the regulated antigens with molecular masses of >60 kDa which appear after 24 h that tend to be recognized by human serum and secretory antibodies. Most of these antigens may contain *N*-acetylglucosamine, because they react with WGA (Fig. 7) (32, 33) and are substrates for exogenous galactosyltransferase, an enzyme specific for terminal GlcNAc residues (32). One group (32) has found GlcNAc in encysting *G. lamblia*-solubilized membranes by gas chromatography-mass spectrometry, while another has found mainly GalN or GalNAc in whole cysts or cyst walls (23). In either case, some of the regulated antigens recognized by human antibodies may contain carbohydrate, although the antigenicity was not determined by periodate-sensitive epitopes.

We also observed that certain nonregulated antigens are recognized by antibodies in patients' sera and secretions. Two of the nonregulated antigens correspond to surface-iodinated antigens, which we have identified and characterized in other studies (4, 16). The broad bands of 85, 66, and 40 kDa are all products of the TSA 417 gene which we have cloned (1, 16) and are species of a single cysteine-rich trophozoite variable surface antigen (Fig. 5). We have detected no glycosylation of TSA 417 by lectin binding or metabolic labeling (4, 33). In contrast, the gp49 surface antigen is metabolically labeled with fatty acids, glucosamine, and inositol and has an unusual glycosylphosphatidylinositol anchor. We do not know whether its reactivity with WGA is due to GlcNAc residues outside the anchor moiety (4).

A related question is whether the antibodies which react with the regulated antigens are induced by infection with *G. lamblia*. A number of earlier studies (e.g., 36) detected antibodies to *G. lamblia* in >70% of patients and in ~9 to 25% of putative controls. The critical study by Nash et al. (29) demonstrated increases in anti-*Giardia* serum IgG, IgM, and IgA and intestinal fluid IgA antibodies after experimental infection of humans. In these studies, conventionally cultured trophozoites were used as the antigen.

In contrast, several early studies reported specific serum antibodies against cysts isolated from feces in naturally infected humans (22). However, in Finland, where there is no endemic giardiasis, Jokipii et al. (24) found IgG reactivity against purified fecal cysts in sera from both patients and controls, although antibody titers for the latter were lower. Our studies are consistent with the idea that cysts, or

encysting trophozoites, may express commonly recognized antigens.

Since the major goal of these studies was to identify stage-specific antigens which were recognized by serum and secretory antibodies, we did not use antibody concentrations designed to maximize distinctions between patients and controls. Although patient antibodies tended to react with greater intensity and higher titer than those of controls, the reactions of control milk and serum antibodies with *G. lamblia* antigens, especially the environmentally regulated antigens, were more frequent than would be expected from previous studies (e.g., reference 36) with conventionally grown trophozoites as the antigen.

Taken together, these results suggest either that exposure to *G. lamblia* may be more common than is currently accepted (22, 44) or that the reactivity of antibodies from uninfected people may be due to conserved or cross-reactive epitopes (24). If the cross-reactivity is to antigens in the intestinal environment, this phenomenon might provide a form of immune evasion for parasites to "hide" among an excess of the same antigen. Preexisting antibody titers may be boosted by infection, which may help explain the widespread antibody responses to this noninvasive parasite and may influence the variable severity of giardiasis.

Previous identification of *G. lamblia* antigens recognized by human antibodies has been limited to assays of the reactivity of serum antibodies with conventionally cultured trophozoites as antigen (39). The important study by Nash et al. (28) showed that a major immune response of experimentally infected people was directed against the predominant 72-kDa variable cysteine-rich protein species of the infecting strain GS/M. By 22 days of infection, the surface protein had changed so that the organism was no longer recognized by the patients' sera. Our observation that the TSA 417 variable trophozoite antigen of our strain WB clone C6 is recognized by some, but not all, serum and secretory antibodies is consonant with this finding. In other studies, recognition of 31- (39), 57- (3), 82- (26) and 88-kDa (7) trophozoite antigens by serum IgG antibodies from naturally infected patients has been reported.

Many pathogenic bacteria have proteins which are induced by environmental stimuli, such as temperature, osmolarity, iron, or hydrogen ion concentration. For example, *Aeromonas hydrophila*, an aquatic bacterium which can cause dysentery or choleralike disease, has a pilin which is regulated by temperature and iron concentration, as well as a second, constitutively expressed pilin (20).

While *Giardia* trophozoites do not naturally live outside the host, they occupy and must be able to adapt to several microenvironments within the small intestine. The dense mucus layer which lines the intestine provides a barrier which physically and chemically separates the luminal compartment and the epithelial compartment (38). Trophozoites which adhere to the small intestinal epithelium are exposed to a neutral or slightly acidic pH. In contrast, below the upper duodenum, the intestinal fluid is rather alkaline (pH 7.4 to 8 [8]). Moreover, bile salts are in high concentration in the intestinal lumen from the entrance of the common bile duct to the ileum, where they are reabsorbed (21). Thus, trophozoites on the luminal side of the mucus layer are exposed to higher pH and bile concentrations than they are on the epithelial side. Our earlier studies (33) demonstrated expression of a number of regulated antigens in response to these environmental stimuli. Our present studies show that certain of these regulated antigens are recognized by human serum and secretory antibodies. Further studies are needed

to elucidate the functions of the pH- and bile-regulated *G. lamblia* antigens and their relationship to infection.

ACKNOWLEDGMENTS

These studies were supported by grants AI 19863, AI 24285, and AM 35108 from the NIH.

We are grateful to Michael Hetsko for skilled technical assistance and to our colleagues in the Division of Infectious Diseases for critiques of the manuscript.

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