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Antibody Response to *Giardia muris* Trophozoites in Mouse Intestine

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The protozoan parasite *Giardia muris* colonizes the mouse small intestinal lumen. This parasite is cleared immunologically from the intestine of normal mice. In contrast, T-lymphocyte-deficient (nude) mice have an impaired immunological response to *G. muris* and become chronically infected. In the present study, trophozoites were harvested from the intestinal lumen of immunocompetent BALB/c mice and nude mice and examined for surface-bound mouse immunoglobulins by immunofluorescence microscopy. Immunoglobulin A (IgA) and IgG, but not IgM, were detected on trophozoites obtained from BALB/c mice, from day 10 of the infection onwards. Trophozoites from nude mice showed very little evidence of surface-bound mouse immunoglobulin at any time during the 5-week period immediately following infection of these animals with *G. muris* cysts. Intestinal *G. muris* infection was cleared by the BALB/c mice but not by the nude animals. The data suggest that parasite-specific IgA and IgG bind to *G. muris* trophozoites in the intestinal lumen of immunocompetent BALB/c mice. Intestinal antibodies that bind to trophozoite surfaces are likely to play an important part in the clearance of *G. muris* infection by immunocompetent mice. The inability of nude mice to clear this infection at a normal rate is likely to be due to impairment of *Giardia*-specific intestinal antibody production.

Giardia muris is a protozoan parasite that colonizes the mouse small intestinal lumen (14). Immunocompetent mice are able to clear *G. muris* infection spontaneously, whereas T-cell-deficient nude mice have an impaired capacity to eliminate this infection (2, 13, 18). It is likely that T lymphocytes play an important part in the immunological clearance of *G. muris* infection, but their actual role in this process is uncertain. Previous work has shown that intestinal Peyer's patches of nude mice have a profound deficiency of lymphocytes bearing the helper/inducer T cell marker L3T4 (J. R. Carlson, M. F. Heyworth, and R. L. Owen, submitted for publication). Several recent studies indicate that clearance of intestinal *Giardia* infections is likely to be antibody dependent (9, 10, 16). Impaired clearance of *G. muris* organisms by nude mice may therefore be a result of defective production of *Giardia*-specific intestinal antibody, due in turn to helper/inducer T cell deficiency. The previous literature contains very little information about the possible occurrence of antibodies on *G. muris* trophozoites harvested from the intestine of immunocompetent mice. In addition, the question of whether nude mice have an impaired intestinal antibody response to *G. muris* does not appear to have been addressed.

The aims of the present study were (i) to compare the ability of immunocompetent BALB/c mice and nude mice to generate an intestinal antibody response to *G. muris* trophozoites and (ii) to identify the immunoglobulin class(es) of anti-trophozoite antibody produced in the intestine of *G. muris*-infected mice. Antibody production was monitored by immunofluorescent staining of trophozoites for surface-bound mouse immunoglobulins at serial times during the course of *G. muris* infection.

MATERIALS AND METHODS

Mice. Female BALB/c mice and nude mice were obtained from Charles River Laboratories, Kingston, N.Y. Mice were screened for absence of *G. muris* infection by examination of fecal suspensions for cysts (14) and were housed in the Animal Care Facility, San Francisco Veterans Administration Medical Center. They were fed laboratory chow (Purina Co.) and water ad libitum. The study was approved by the Animal Studies Subcommittee of the Medical Center.

Infection with *G. muris* and harvesting of intestinal trophozoites. Mice were infected at the age of 8 weeks by esophageal inoculation of 1,000 *G. muris* cysts through a blunt-ended feeding needle. At serial times over the next 5 weeks, mice were fasted overnight with access to water and then anesthetized intraperitoneally with sodium pentobarbital, and the small intestine was removed. *G. muris* trophozoites were harvested from the intestinal lumen by a technique described previously (8). Briefly, the intestinal lumen was lavaged with 20 ml of Eagle minimum essential medium supplemented with nonessential amino acids and 10% fetal calf serum or with 20 ml of phosphate-buffered saline (PBS) containing 0.1% sodium azide. The resulting trophozoite suspensions were then centrifuged at 450 × *g* for 5 min, and the organisms were suspended in 0.5 ml of supplemented Eagle medium or PBS–0.1% sodium azide. Trophozoites were counted by microscopic examination in a hemacytometer.

Reagents for immunofluorescent staining of trophozoites. Polyclonal rabbit antibodies directed against mouse immunoglobulin α, γ, and μ chains were obtained from Litton Bionetics, Charleston, S.C. These antibodies had been affinity purified by the supplier and tested for specificity against the relevant class of mouse immunoglobulin by Ouchterlony immunodiffusion and immunoelectrophoresis. The affinity-purified antibodies were conjugated to fluorescein by the supplier.

Rat immunoglobulin G (IgG) monoclonal antibody directed against the α chain of mouse IgA was produced by a hybridoma cell line (designated 11.44.2-23-5) kindly provided by John F. Kearney, University of Alabama, Birming-

TABLE 1. Fluorescence assay of *G. muris* trophozoites from intestinal lumen of mice[a]

No. of days postinfection	% Immunoglobulin-positive trophozoites (no. of mice)[b]					
	BALB/c mice			Nude mice		
	IgA	IgG	IgM	IgA	IgG	IgM
<10	2 ± 2 (16)	2 ± 2 (17)	0 (6)	1 ± 1 (6)	0 (6)	0 (6)
10–27 (BALB/c) or 11–35 (nude)	15 ± 2[c] (41)	10 ± 2[d] (35)	1 (13)	3 ± 1[e] (36)	2[e] (36)	0 (18)

[a] Fluorescent polyclonal antisera against mouse IgA, IgG, and IgM were used at a dilution of 1:10.
[b] Data are means ± standard error.
[c] Significantly different from BALB/c mice before day 10 ($P < 0.001$).
[d] Significantly different from BALB/c mice before day 10 ($P < 0.005$).
[e] Significantly different from BALB/c mice ($P < 0.001$).

ham (17). This hybridoma was cultured in vitro, and the resulting monoclonal antibody was purified on an affinity column of Sepharose beads conjugated to goat anti-rat IgG antibody. The purified monoclonal antibody was then conjugated to biotin.

Rat IgG monoclonal antibody against the Fd portion of mouse IgG (11) was obtained from Accurate Chemical and Scientific Corporation, Westbury, N.Y. This antibody is known to react with mouse IgG1, IgG2a, and IgG2b (5). A biotinylated form of the monoclonal antibody was a generous gift from Vector Laboratories, Burlingame, Calif.

Fluorescein-conjugated avidin DCS (cell sorter grade) and biotinylated polyclonal (goat) antibody to avidin were obtained from Vector Laboratories.

ELISA to check specificity of monoclonal antibodies. Wells of flat-bottomed, 96-well rigid plastic microtiter plates (Dynatech Laboratories, Alexandria, Va.) were coated with mouse IgA, IgG, or IgM (Miles Laboratories, Naperville, Ill.) by incubation with each of these immunoglobulins at 10 μg/ml. The wells were then washed, and 2% bovine serum albumin was added and the plates were incubated to block sites on the plastic that were unoccupied by mouse immunoglobulin. Nonbiotinylated monoclonal antibodies against mouse IgA and IgG were added in serial dilutions, starting at a maximum concentration of 40 μg/ml. After incubation for 1 h, the plates were washed to remove unbound antibodies and then incubated with peroxidase-conjugated goat anti-rat IgG at a dilution of 1:400 (Cooper Biomedical, Malvern, Pa.). This antiserum did not cross-react with mouse IgG. After further washing of the plates, *o*-phenylenediamine (Zymed Laboratories, South San Francisco, Calif.) and 0.03% hydrogen peroxide were added. The enzymatic reaction was stopped after 10 min by adding 2 N H_2SO_4, and the plates were scanned with an enzyme-linked immunosorbent assay (ELISA) reader (Bio-Tek Instruments, Burlington, Vt.) at a wavelength of 490 nm, to record the optical density in each well. This assay showed that the monoclonal antibodies to mouse IgA and IgG reacted with the appropriate class of mouse immunoglobulin at concentrations of monoclonal antibody down to approximately 1 μg/ml. The monoclonal antibodies did not bind to inappropriate classes of mouse immunoglobulin.

Immunofluorescent staining of *G. muris* trophozoites for surface-bound mouse immunoglobulins. Trophozoites were incubated with fluorescein-conjugated polyclonal antisera against mouse IgA, IgM, and IgG in microtiter plates with U-shaped wells for 1 h in the dark at 4°C. The fluorescent antisera were used at a dilution of 1:10. After this incubation, the trophozoites were washed twice in PBS containing 0.1% sodium azide, placed on microscope slides, and examined with a Zeiss fluorescence microscope. Fluorescent and nonfluorescent trophozoites were counted, and the percentage of fluorescent organisms was calculated.

Incubation of trophozoites with biotinylated monoclonal antibodies was also carried out in U-well microtiter plates. Trophozoites were incubated with 2 μg of biotinylated monoclonal antibody for 1 h at 4°C. Incubations were carried out in the presence of 1% heat-inactivated rat serum to minimize nonspecific binding of monoclonal antibodies to trophozoite surfaces. After being washed with PBS–0.1% sodium azide, the organisms were incubated sequentially with fluorescein-conjugated avidin DCS (2 μg, for 15 min), biotinylated polyclonal antiavidin (1 μg, for 30 min), and again with fluorescein-conjugated avidin DCS (2 μg, for 15 min). The trophozoites were then washed twice with PBS–0.1% sodium azide, and the percentage of fluorescent trophozoites was determined by fluorescence microscopy. Negative control experiments for immunofluorescent staining involved (i) omission of biotinylated monoclonal antibodies from the incubation sequence and (ii) substitution of biotinylated anti-mouse T cell monoclonal antibody (2 μg of rat IgG anti-Thy-1.2; Becton Dickinson, Mountain View, Calif.) for biotinylated anti-IgA or anti-IgG.

The mean number of total trophozoites counted per microscope slide was >140 for parasites from BALB/c mice and >220 for parasites from nude mice.

Statistics. Data were analyzed by Student's unpaired *t* test.

RESULTS

Time course of *G. muris* infection. Immunocompetent BALB/c mice cleared *G. muris* infection 5 weeks after cyst inoculation, whereas nude mice showed no evidence of ability to clear this infection from the gastrointestinal tract.

Detection of mouse immunoglobulins on trophozoite surfaces. Trophozoites from BALB/c mice showed surface fluorescence after incubation with fluorescent polyclonal antibodies against mouse IgA or IgG. Significantly higher percentages of trophozoites showed fluorescent staining for IgA or IgG from day 10 of the infection onwards (Table 1). Virtually no trophozoites from BALB/c mice showed fluorescent staining after incubation with fluorescein-conjugated polyclonal anti-IgM. Only small percentages of trophozoites from nude mice showed evidence of surface-bound mouse immunoglobulin at any time during the 5-week period immediately following inoculation of these mice with *G. muris* cysts (Table 1).

The use of monoclonal antibodies to detect mouse immunoglobulins on trophozoite surfaces gave results similar to those obtained with fluorescent polyclonal antibodies. From approximately day 10 of the infection onwards, trophozoites from BALB/c mice showed evidence of surface-bound IgA and IgG (Fig. 1 and 2). The percentage of trophozoites with

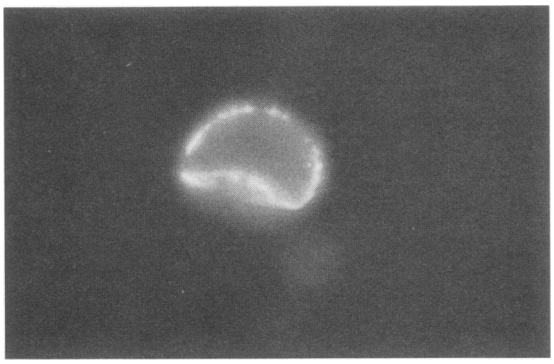

FIG. 1. Fluorescence photomicrograph of *G. muris* trophozoite from a BALB/c mouse, showing parasite-bound mouse IgA. The trophozoite was incubated with monoclonal anti-mouse IgA (see text for details of staining procedure).

surface fluorescence was higher for organisms incubated with anti-IgA than for organisms incubated with anti-IgG monoclonal antibody (Fig. 2). Although some trophozoites from BALB/c mice showed surface fluorescence after incubation with anti-Thy-1.2 monoclonal antibody, the percentage of such fluorescent organisms was lower than for parasites incubated with anti-IgG monoclonal antibody at corresponding times during the period that parasites were being cleared by the infected mice (Table 2).

DISCUSSION

The findings in the present study provide strong evidence that immunocompetent BALB/c mice generate an intestinal IgA response to infection with *G. muris* trophozoites. Previous work has shown that free anti-trophozoite IgA is present in the intestinal lumen of *Giardia*-infected mice (1, 16). However, there have been very few documented attempts to look for IgA or other immunoglobulins on trophozoites harvested from the intestine of infected hosts. In one study, human IgA was demonstrated on *G. lamblia*

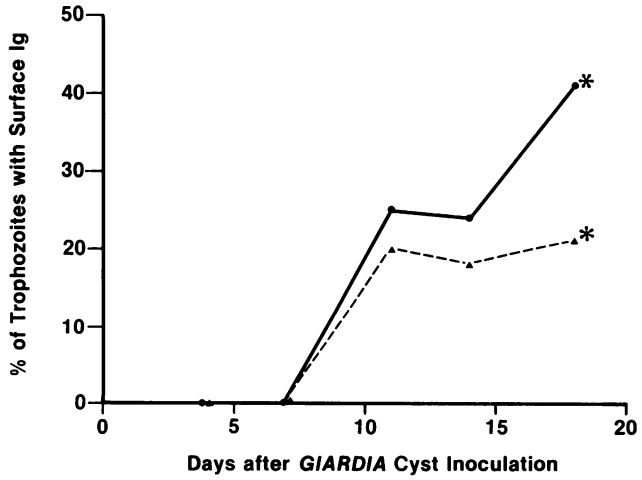

FIG. 2. Percentage of *G. muris* trophozoites from infected BALB/c mice showing surface fluorescence after incubation with monoclonal anti-mouse IgA (——) or IgG (– – – –). Details of the staining technique are given in the text. Trophozoites were obtained from BALB/c mice at serial times during *G. muris* infection. Each point shows the mean result of data for three mice, except for points marked *, which show mean results for two animals.

TABLE 2. Surface fluorescence of *G. muris* trophozoites after incubation with various monoclonal antibodies[a]

Day post-inoculation	% Fluorescent trophozoites (no. of mice)			
	With monoclonal antibody			Without monoclonal antibody
	Anti-IgA	Anti-IgG	Anti-Thy-1.2	
5	<1 (3)	<1 (3)	2 (3)	1 (3)
13	5 (3)	2 (3)	<1 (3)	0 (3)
15	21 (3)	9 (3)	1 (3)	1 (3)
17	17 (3)	6 (3)	1 (3)	<1 (3)
18	31 (2)	18 (3)	9 (3)	4 (3)

[a] Trophozoites from the intestinal lumen of immunocompetent BALB/c mice were sequentially incubated with biotinylated monoclonal antibody to mouse IgA, IgG, Thy-1.2, or no biotinylated monoclonal antibody, with fluorescein-conjugated avidin DCS, with biotinylated antiavidin, and again with fluorescein-conjugated avidin DCS. Means are shown for various time points during the course of *G. muris* infection.

trophozoites obtained from patients with giardiasis (M. Briaud, M. Morichau-Beauchant, C. Matuchansky, G. Touchard, and P. Babin, Letter, Lancet ii:358, 1981). Because the trophozoites in that study were obtained from each patient at a single time point, it is not known whether the IgA was parasite specific (Briaud et al., Letter, Lancet ii:358, 1981). In the present work, increased staining of trophozoites for IgA from day 10 of *G. muris* infection onwards suggests that IgA on trophozoites was specifically directed against these organisms.

Positive staining of trophozoites for IgG was the most unexpected finding in the current work; this observation warrants further study. It might, for example, be feasible to quantify free anti-trophozoite IgG in the intestinal lumen of *Giardia*-infected mice by an ELISA technique. Previous work has shown that trophozoite-specific IgG occurs in the serum of *Giardia*-infected mice and humans (15, 16), but the present study appears to be the first in which IgG has been demonstrated on *Giardia* trophozoites harvested from the intestinal lumen of any mammalian species. A clinical observation which suggests that IgG plays a part in the clearance of human *G. lamblia* infection is that patients with common variable hypogammaglobulinemia or X-linked immunoglobulin deficiency (who are IgG- and IgA-deficient) appear to be more susceptible to chronic giardiasis than patients with selective IgA deficiency (3, 6, 10). It is conceivable that IgG is important for clearing various intestinal infections, besides giardiasis, in humans and other mammals.

In the present work, the percentages of IgA^+ or IgG^+ trophozoites from nude mice were significantly lower than those from immunocompetent animals. As in earlier studies, nude mice were found to have an impaired ability to clear *G. muris* infection. Inadequate production of trophozoite-specific IgA or IgG or both is the likely reason why these animals fail to eliminate *Giardia* parasites at a normal rate. This impairment of anti-*Giardia* antibody production may result from deficiency of helper/inducer T lymphocytes in Peyer's patches and other lymphoid tissues of nude mice (Carlson et al., submitted for publication). I recently found that experimental depletion of helper/inducer T lymphocytes in previously immunocompetent BALB/c mice by injection with anti-L3T4 monoclonal antibody (4) inhibits the clearance of *G. muris* infection (unpublished data). This recent finding supports the view that helper/inducer T lymphocytes play a major part in the immune response of mice to *G. muris*.

Although clearance of *G. muris* infection is likely to

depend on intestinal anti-trophozoite antibody, the mechanism by which this antibody exerts its effect is not known. It has been suggested that antibody may prevent trophozoite adherence to intestinal epithelial cells (by antibody coating of trophozoite adhesive discs) and that parasite motility may be impaired by antibody coating of flagella (9). It is unlikely that ingestion of antibody-coated (or uncoated) trophozoites by phagocytes in the intestinal lumen plays a significant part in the elimination of *Giardia* infections. Only small numbers of phagocytes are present in the intestinal lumen of *Giardia*-infected mice, and there is no difference between the number of intraluminal phagocytes in immunocompetent BALB/c mice and nude mice (7). Two recent observations suggest that the clearance of *G. muris* infection by immunocompetent mice is not mediated by cytotoxic lymphocytes. First, identical numbers of lymphocytes bearing the cytotoxic/suppressor T cell antigen Lyt-2 are present in the intestinal lumen of immunocompetent mice and nude mice infected with *G. muris* (7). Second, mice with natural killer cell deficiency (beige mice) clear this infection at a normal rate (M. F. Heyworth, J. E. Kung, and E. C. Eriksson, Gastroenterology **88**:1418, 1985).

In the present study, not all the *G. muris* trophozoites from the intestinal lumen of any BALB/c mouse had detectable immunoglobulin on their surface. Previous work has shown that cultured *G. lamblia* trophozoites shed surface antigens into the culture medium (12). Shedding of surface antigens may be a mechanism by which *Giardia* trophozoites achieve some degree of protection against parasite-specific antibodies in the intestinal lumen of infected hosts. It is possible that *Giardia* trophozoites can shed surface antigens after immunoglobulin molecules have become bound to these antigens. Loss of surface antigens may explain why not all of the *G. muris* trophozoites from any BALB/c mouse were immunoglobulin positive in the present study. An important goal of future work will be to identify the trophozoite antigens which are targets for intestinal antibodies in mice infected with *G. muris*.

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LITERATURE CITED

1. **Anders, R. F., I. C. Roberts-Thomson, and G. F. Mitchell.** 1982. Giardiasis in mice: analysis of humoral and cellular immune responses to *Giardia muris*. Parasite Immunol. **4**:47–57.
2. **Brett, S. J., and F. E. G. Cox.** 1982. Immunological aspects of *Giardia muris* and *Spironucleus muris* infections in inbred and outbred strains of laboratory mice: a comparative study. Parasitology **85**:85–99.
3. **Brown, W. R., D. C. Savage, R. S. Dubois, M. H. Alp, A. Mallory, and F. Kern, Jr.** 1972. Intestinal microflora of immunoglobulin-deficient and normal human subjects. Gastroenterology **62**:1143–1152.
4. **Dialynas, D. P., Z. S. Quan, K. A. Wall, A. Pierres, J. Quintáns, M. R. Loken, M. Pierres, and F. W. Fitch.** 1983. Characterization of the murine T cell surface molecule, designated L3T4, identified by monoclonal antibody GK1.5: similarity of L3T4 to the human Leu-3/T4 molecule. J. Immunol. **131**:2445–2451.
5. **Galfrè, G., C. Milstein, and B. Wright.** 1979. Rat × rat hybrid myelomas and a monoclonal anti-Fd portion of mouse IgG. Nature (London) **277**:131–133.
6. **Hermans, P. E., J. A. Diaz-Buxo, and J. D. Stobo.** 1976. Idiopathic late-onset immunoglobulin deficiency: clinical observations in 50 patients. Am. J. Med. **61**:221–237.
7. **Heyworth, M. F., R. L. Owen, and A. L. Jones.** 1985. Comparison of leukocytes obtained from the intestinal lumen of *Giardia*-infected immunocompetent mice and nude mice. Gastroenterology **89**:1360–1365.
8. **Heyworth, M. F., R. L. Owen, W. E. Seaman, F. W. Schaefer III, and A. L. Jones.** 1985. Harvesting of leukocytes from intestinal lumen in murine giardiasis and preliminary characterization of these cells. Dig. Dis. Sci. **30**:149–153.
9. **Loftness, T. J., S. L. Erlandsen, I. D. Wilson, and E. A. Meyer.** 1984. Occurrence of specific secretory immunoglobulin A in bile after inoculation of *Giardia lamblia* trophozoites into rat duodenum. Gastroenterology **87**:1022–1029.
10. **LoGalbo, P. R., H. A. Sampson, and R. H. Buckley.** 1982. Symptomatic giardiasis in three patients with X-linked agammaglobulinemia. J. Pediatr. **101**:78–80.
11. **Milstein, C., and J. R. L. Pink.** 1970. Structure and evolution of immunoglobulins. Prog. Biophys. Mol. Biol. **21**:209–263.
12. **Nash, T. E., F. D. Gillin, and P. D. Smith.** 1983. Excretory-secretory products of *Giardia lamblia*. J. Immunol. **131**:2004–2010.
13. **Roberts-Thomson, I. C., and G. F. Mitchell.** 1978. Giardiasis in mice. I. Prolonged infections in certain mouse strains and hypothymic (nude) mice. Gastroenterology **75**:42–46.
14. **Roberts-Thomson, I. C., D. P. Stevens, A. A. F. Mahmoud, and K. S. Warren.** 1976. Giardiasis in the mouse: an animal model. Gastroenterology **71**:57–61.
15. **Smith, P. D., F. D. Gillin, W. R. Brown, and T. E. Nash.** 1981. IgG antibody to *Giardia lamblia* detected by enzyme-linked immunosorbent assay. Gastroenterology **80**:1476–1480.
16. **Snider, D. P., J. Gordon, M. R. McDermott, and B. J. Underdown.** 1985. Chronic *Giardia muris* infection in anti-IgM-treated mice. I. Analysis of immunoglobulin and parasite-specific antibody in normal and immunoglobulin-deficient animals. J. Immunol. **134**:4153–4162.
17. **Stohrer, R., and J. F. Kearney.** 1983. Fine idiotype analysis of B cell precursors in the T-dependent and T-independent responses to α1→3 dextran in BALB/c mice. J. Exp. Med. **158**:2081–2094.
18. **Underdown, B. J., I. C. Roberts-Thomson, R. F. Anders, and G. F. Mitchell.** 1981. Giardiasis in mice: studies on the characteristics of chronic infection in C3H/He mice. J. Immunol. **126**:669–672.