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Giardia lamblia Infections in Adult Mice

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An adult mouse-*Giardia lamblia* model was developed and used to study host-parasite interactions, including antigenic variation. The H7/1 clone of isolate GS infected mice consistently and produced infections in 14 mouse strains tested. Infection patterns were mouse strain and *Giardia* isolate dependent. Antigenic variation occurred in immunocompetent mice but not in mice with severe combined immunodeficiency.

Giardia lamblia is the most frequent cause of waterborne diarrhea in developed regions, and infections are common in developing countries. Many animal models have been utilized to describe the biology of and/or immunity to *Giardia* infections, including the *G. muris*-mouse model (3, 5, 6, 9, 10, 13, 22, 23), the neonatal mouse-*G. lamblia* model (11), the weanling mouse-*G. lamblia* model (1, 15, 25, 26), and the gerbil-*G. lamblia* model (4, 27). This report describes a novel adult mouse-*G. lamblia* model which overcomes many of the limitations of previous animal models (inability to study surface antigen change in *G. muris* trophozoites, development of resistance to *Giardia* organisms at the time of immune maturation, use of uncharacterized *Giardia* isolates, and lack of immunologic reagents) and allows the study of the dynamic interactions which occur between the host and the parasite.

Cultured *G. lamblia* trophozoites (maintained as previously described [14] and harvested during the log phase) from nine *G. lamblia* isolates (Be-2, 1182, Erin, E-4, E-9, the A6 clone of WB [A6], PM, N, and the H7 clone of GS [16, 19, 20]) were tested for infectivity in C3H/HeJ mice (4-week-old females). Only H7 was consistently detected 1 and 2 weeks postinoculation (p.i.). Infections were determined as follows. The proximal 4 in. (1 in. = 2.54 cm) of small intestine was removed and minced while immersed in TYI-S-33 medium with antibiotics (12). The mixture was removed to a glass tube, chilled on ice for 0.5 h to facilitate release of trophozoites from the intestine, and placed at 37°C for 1 h to allow trophozoites to attach to the tube. The pieces of intestine were transferred to a second tube, fresh medium was added to the original tube, and both tubes were maintained at 37°C for 1 h. Medium was replaced one additional time. Cultures were assessed microscopically for the presence of attached trophozoites on days 0, 1, 4, and 6. Mice were considered uninfected if no trophozoites were detected after 6 days in culture.

The dose dependence of H7 infection was determined after inoculation of 4-week-old C3H/HeJ mice with 500, 5,000, 50,000, or 500,000 trophozoites. Infections occurred with all doses; however, the high dose gave more consistent infections (data not shown). Therefore, the 500,000-trophozoite dose was chosen as the standard inoculum.

Three- and four-week-old mice of several strains (obtained from the Frederick Cancer Research Facility, National Cancer Institute, Frederick, Md., and Taconic Farms, Inc., as specific-pathogen-free animals and housed under specific-pathogen-free conditions) were evaluated for experimental infection. From 90 to 100% of the animals from the following mouse strains were infected by 6 days p.i.: C3H/HeN, NIH:Swiss, C57BL/6N, BALB/cAn, DBA/2N, BALB/c.*nu*/*nu*, BALB/cAn.*nu*/+, NIH:III (*nu*/*bg*/*xid*), CR:NIH(S).*nu* (all from the Frederick Cancer Research Facility), C.B-17, and C.B-17.SCID (both from Taconic). Mouse strains which showed inconsistent infections were C57BL/6N.*bg* (40%) and NIH:(*nu*/*bg*) (65%) and severe combined immunodeficiency (SCID) mice (all from the Frederick Cancer Research Facility) (20%). In all cases [except the CR:NIH(S).*nu* strain, which was tested only once], mice were assessed for infection in two or three separate experiments, with six mice per strain in each experiment.

The patterns of infection in 4-week-old mice differed among mouse strains. Immunocompetent C3H/HeJ and BALB/c mice showed similar patterns of infection. At 6 days p.i., numerous trophozoites (≈20 to 30 per microscopic field) were recovered. By 13 days, the numbers had markedly decreased but trophozoites were detected after 6 days of culture. On days 34 and 41, trophozoites were detected in most mice (Fig. 1). DBA/2N mice were easily infected and showed numerous intestinal trophozoites (≈10 to 15 per microscopic field); however, no trophozoites were detected on day 13, 34, or 41. SCID mice (Taconic) maintained a large number of intestinal trophozoites (≈35 to 50 per microscopic field) at all time periods p.i. (Fig. 1).

Six- and nine-week-old BALB/cAn female mice were also infected. In two experiments, between 50 and 100% of 6-week-old mice were infected (up to 20 days p.i.). Trophozoites were recovered from five of six and six of six 9-week-old mice on days 6 and 13, respectively.

No changes were observed in the overall general appearance and health of infected mice. Hematoxylin-and-eosin-stained slides of the small intestines of H7-infected BALB/cAn mice (6, 10, and 12 days p.i.) showed no changes in histologic appearance compared with uninfected mice.

TABLE 1. Numbers of naive mice infected by cysts excreted from mice inoculated with 500,000 *G. lamblia* GS-H7/1 trophozoites

Naive strain housed with inoculated mice	Strain inoculated with trophozoites (no. of mice infected)	No. of days naive mice exposed to inoculated mice	No. of naive mice infected/total no. of naive mice (6-day culture)
BALB/c	BALB/c (5)	14	2/4
SCID	SCID (2)	42	1/4
SCID	SCID (2)	14	1/2
SCID	SCID (2)	21	1/2

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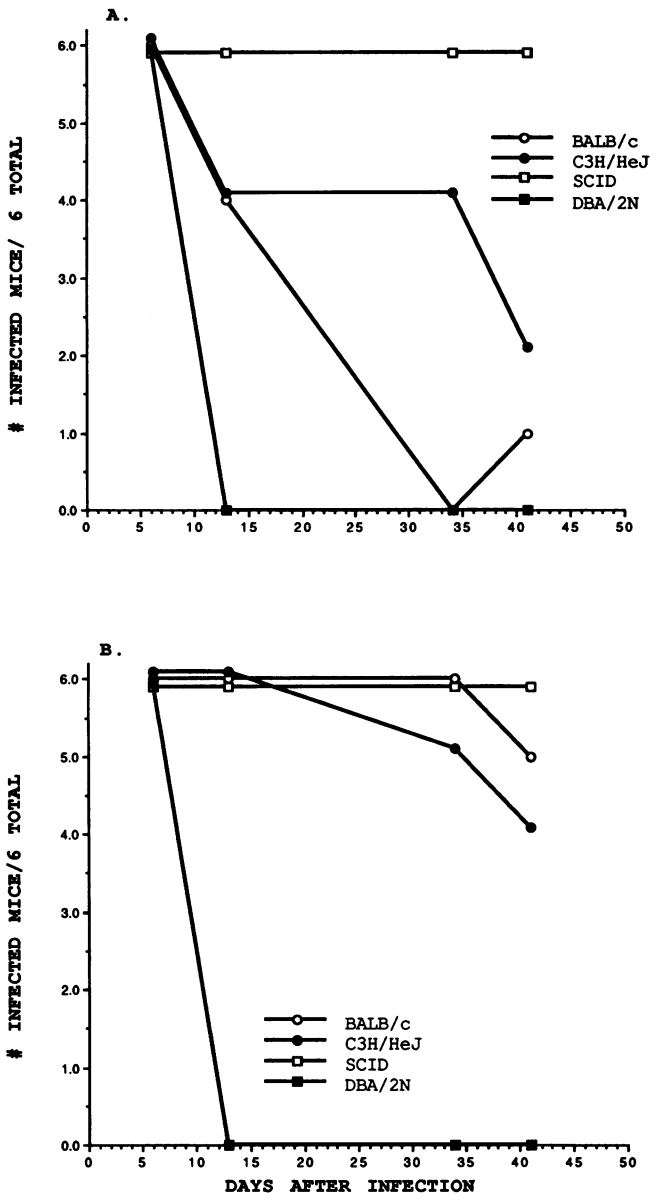

FIG. 1. Time course analysis of tubes containing *G. lamblia* trophozoites obtained from the intestinal contents of 4-week-old mice after 1 (A) or 6 (B) days of culture.

Approximately five cysts per mg of feces were detected in H7-inoculated SCID mice with fluorescein isothiocyanate-conjugated monoclonal antibody 5-3C (24). Natural transmission occurred; 25 to 50% of the naive mice housed with inoculated mice became infected (Table 1).

To determine if antigenic variation occurred (18), the percentage of trophozoites expressing the 57-kDa surface antigen (2, 21) recognized by the monoclonal antibody G10/4 (17) was determined as previously described (7, 8, 17). A minimum of 600 trophozoites were viewed. After 6 days in vivo, 90 to 100% of the trophozoites recovered were G10/4 positive, even when the initial inoculum was as low as 60% G10/4 positive (Table 2). However, by day 13 p.i., G10/4-positive trophozoites declined to below 20%; no G10/4-positive trophozoites were detected in many of the samples. In contrast, trophozoites recovered from SCID mice were consistently 90 to 100% G10/4 positive, even at 10 weeks p.i.

TABLE 2. Percentages of G10/4-positive *G. lamblia* trophozoites recovered from various mouse strains

Strain (age [wk])	No. of days p.i.	No. of days in vitro	% of culture G10/4 positive (no. of samples)	% of G10/4 positive in inoculating culture
BALB/c (3)	6	1	99–100 (5)	80
BALB/c (3)	13	4	0 (5)	80
BALB/c (4)	6	1	50–100 (5)	60
C3H/HeJ (3)	6	1	99–100 (4)	80
C3H/HeJ (3)	13	4	5–20 (3)	80
C3H/HeJ (4)	6	1	99–100 (5)	60
SCID (3)	6	1	95–100 (6)	80
SCID (3)	13	4	90–95 (6)	80
SCID (3)	34	1	99–100 (6)	80
SCID (4)	6	1	99 (1)	60

In the present report, we describe a reproducible *G. lamblia*-adult mouse model infection utilizing (i) a clone of *G. lamblia* which expresses predominately one surface antigen to infect adult mice and (ii) only 500,000 trophozoites to inoculate the mice. Three separate phases of the infection were noted. The initial phase involved host-parasite factors which allowed establishment of infections (e.g., natural or innate resistance). In established infections, large numbers of trophozoites were present initially but by day 13 (the second phase) a marked decline in the numbers of trophozoites was observed. Immunological factors were most likely responsible for this decline, because SCID mice showed no similar decline. Phase three was characterized by either cure or chronic infection. This model offers a way to understand those immunological responses which are characteristic of each infection pattern and allows analysis of the interplay between the trophozoite variant-specific surface protein and the immune system of the mammalian host.

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