

**CLEARANCE OF *GIARDIA MURIS* INFECTION REQUIRES  
HELPER/INDUCER T LYMPHOCYTES**

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Mice infected with the intestinal protozoan parasite *Giardia muris* constitute an animal model of human giardiasis (1, 2). Whereas immunocompetent mice eliminate *G. muris* infection spontaneously, athymic (nude) mice are unable to clear the infection (2-5). This observation suggests that T (thymus-derived) lymphocytes are necessary for elimination of *G. muris* infection, but does not identify the T cell subset that is of major importance for clearance. Either helper/inducer (Th/i) or cytotoxic/suppressor (Tc/s) T lymphocytes might play a major part in clearance of the infection.

The availability of mAbs directed against mouse T lymphocyte surface antigens has enabled these antibodies to be used for depleting mouse T cell subsets in vivo (6, 7). Selective depletion of either Th/i (L3T4<sup>+</sup>) or Tc/s (Ly-2<sup>+</sup>) lymphocytes, by treatment of mice with mAbs directed against each of these T cell subsets, provides an opportunity to study the functions of the cells in vivo. In the present work, the objective was to determine which T cell subset is of major importance for immunological clearance of *G. muris* infection. Mice were depleted of L3T4<sup>+</sup> or Ly-2<sup>+</sup> lymphocytes by treatment with anti-L3T4 or anti-Ly-2 mAb, respectively, and were then infected with *G. muris*. The time-course of the infection was studied in each of these two groups of mice, and in control mice receiving PBS instead of mAb.

**Materials and Methods**

*Animals.* Female BALB/c mice aged 6-8 wk were obtained from Charles River Laboratories, Kingston, NY.

*Monoclonal Antibodies.* mAbs directed against mouse T cell surface antigens were used for treating BALB/c mice, and were produced by hybridoma cells grown as ascites-producing tumors in nude mice. The mAbs were (a) a rat IgG2b antibody directed against the T cell surface antigen L3T4, which is a marker of mouse Th/i lymphocytes (mAb GK1.5) (8), and (b) a rat IgG2a antibody directed against the cytotoxic/suppressor T cell antigen Ly-2 (mAb 53-6.7) (9). These mAbs were partially purified by incubating ascitic fluid with a 40% saturated solution of ammonium sulfate (10), and the resulting precipitate (containing mAb) was harvested and redissolved. Subsequently, each mAb solution was adjusted to a concentration of 5 mg/ml in PBS for administration to BALB/c mice.

Anti-L3T4 mAb for flow cytometry or immunohistochemistry (see below) was purified

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by affinity chromatography (10), and conjugated to fluorescein or biotin. Fluorescein-conjugated or biotin-conjugated anti-Ly-2 mAb (9), and fluorescein-conjugated mouse IgG2a mAb directed against rat immunoglobulin  $\kappa$  chains (MAR 18.5 mAb) (11) were obtained from the Becton Dickinson Monoclonal Center, Inc., Mountain View, CA.

*G. Muris Infection, and Treatment of Mice with mAbs.* BALB/c mice were injected intraperitoneally at weekly intervals with either 1 mg of anti-L3T4 mAb in 0.2 ml PBS, 1 mg of anti-Ly-2 mAb in 0.2 ml PBS, or 0.2 ml PBS alone (control group). Each animal received a total of seven such injections. After the second and before the third injection, all the mice were infected with *G. muris* by peroral intraesophageal inoculation of 1,000 *G. muris* cysts via a blunt-ended feeding needle (1). The time-course of *G. muris* infection was then studied by counting Giardia cysts in fecal specimens collected from the mice for 2-h periods at serial times (1, 2). Cysts were isolated by centrifuging fecal specimens over 1 M sucrose (1), and were then counted by microscopic examination in a hemocytometer chamber.

*Flow Cytometry and Immunohistochemistry.* At the end of the study, the mice were sacrificed by intraperitoneal injection of sodium pentobarbital (Nembutal), given in a dose of 15 mg per 100 g body weight. Their intestinal Peyer's patches were then examined to confirm that the relevant T cell subset had been depleted by mAb treatment. This examination was carried out by flow cytometry (for Peyer's patches from some mice) and by immunohistochemistry (for Peyer's patches from other mice), as described below.

For flow cytometry, Peyer's patches were removed from the small intestine, and cell suspensions were prepared from the patches, using a mechanical technique described previously (12). For each individual mouse, cells obtained from all the Peyer's patches were pooled. The cells were then incubated with fluorescein-conjugated anti-L3T4 mAb, or with fluorescein-conjugated anti-Ly-2 mAb, and were analyzed in a Becton Dickinson FACS II fluorescence-activated cell sorter, to determine the percentage of leukocytes bearing the L3T4 or Ly-2 surface antigen (12). Peyer's patch leukocytes from each group of mice were also incubated with fluorescein-conjugated MAR 18.5 mAb (see above), and were analyzed in the fluorescence-activated cell sorter. This was done to check that lymphocytes in mice that were treated with anti-L3T4 or anti-Ly-2 mAb (rat IgG mAbs) did not become coated with rat IgG and remain in the patches, rather than being depleted by mAb treatment.

For immunohistochemistry, mouse Peyer's patches were removed from the small intestine, embedded in O.C.T. embedding compound (Tissue-Tek; Miles Laboratories, Inc., Naperville, IL) on pieces of cork, and frozen in Freon 22 cooled to its freezing point by liquid nitrogen in a surrounding chamber (13). Frozen sections of the patches were then cut with a cryostat microtome (Hacker Instruments, Inc., Fairfield, NJ), and were incubated with biotinylated anti-L3T4 or anti-Ly-2 mAb, followed by avidin conjugated to biotinylated horseradish peroxidase (ABC reagent) (Vector Laboratories, Inc., Burlingame, CA). The sections were then incubated with 0.05% diaminobenzidine tetrahydrochloride (DAB) (Litton Bionetics, Charleston, SC), 0.03%  $\text{CoCl}_2$ , and 0.03%  $\text{H}_2\text{O}_2$  in 0.1 M Tris-HCl buffer at pH 7.3, and were counterstained with methyl green. Finally, the Peyer's patch sections were examined by light microscopy.

## Results

*Time-course of G. Muris Infection.* Depletion of Th/i but not Tc/s lymphocytes markedly impaired the clearance of *G. muris* infection (Fig. 1). BALB/c mice that were treated with PBS or with anti-Ly-2 mAb cleared *G. muris* infection within 6 wk of cyst inoculation. In contrast, mice that were given anti-L3T4 mAb did not clear the infection (Fig. 1). These data indicate that Th/i lymphocytes play a major part in elimination of *G. muris* from the mouse intestine, and suggest that Tc/s lymphocytes are not required for this process.

*Flow Cytometry and Immunohistochemical Staining.* Flow cytometry of Peyer's patch cell suspensions showed that the appropriate T cell subset was depleted by

mAb treatment (Fig. 2). Flow cytometry of cells incubated with fluorescein-conjugated MAR 18.5 mAb showed that lymphocytes in the Peyer's patches of mAb-treated mice were not coated with rat IgG at the time when the mice were sacrificed.

Peyer's patch frozen sections that were stained immunohistochemically for L3T4<sup>+</sup> cells are shown in Fig. 3. In sections of Peyer's patches from mice that had been treated with anti-L3T4 mAb, follicles and interfollicular areas were devoid of L3T4<sup>+</sup> cells, whereas in control mice, these regions contained many such cells. A similar depletion of Ly-2<sup>+</sup> cells, primarily in interfollicular areas, was seen in mice that had been treated with anti-Ly-2 mAb.

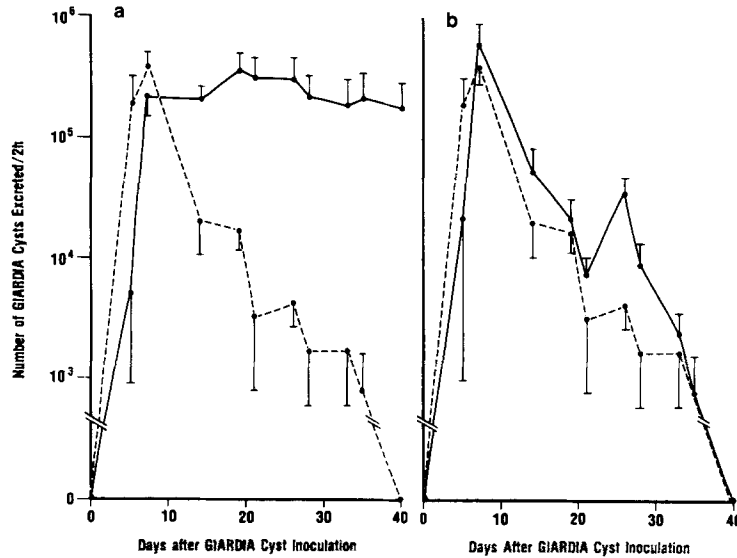


FIGURE 1. Numbers of *G. muris* cysts in mouse fecal specimens collected for 2-h periods, at serial times after the start of *G. muris* infection. Mice were infected with *G. muris* cysts on day 0. In a, fecal cyst counts are shown for mice treated with anti-L3T4 mAb (solid line) or with PBS alone (broken line). In b, fecal cyst counts are shown for mice treated with anti-Ly-2 mAb (solid line) or with PBS alone (broken line). Data are mean values  $\pm$  SE for cyst output from six mice in each group, at each time.

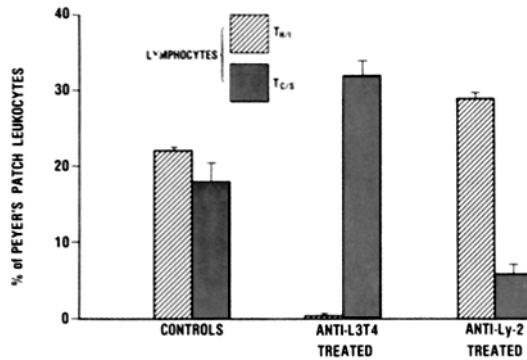


FIGURE 2. Percentages of helper/inducer (Th/i; L3T4<sup>+</sup>) and cytotoxic/suppressor (Tc/s; Ly-2<sup>+</sup>) T lymphocytes in mouse Peyer's patches. These percentages were determined by flow cytometry of Peyer's patch cell suspensions that had been incubated with fluorescein-conjugated anti-L3T4 or fluorescein-conjugated anti-Ly-2 mAb. Mean values  $\pm$  SE are shown, for percentages of L3T4<sup>+</sup> and Ly-2<sup>+</sup> Peyer's patch leukocytes from four mice in each treatment group.

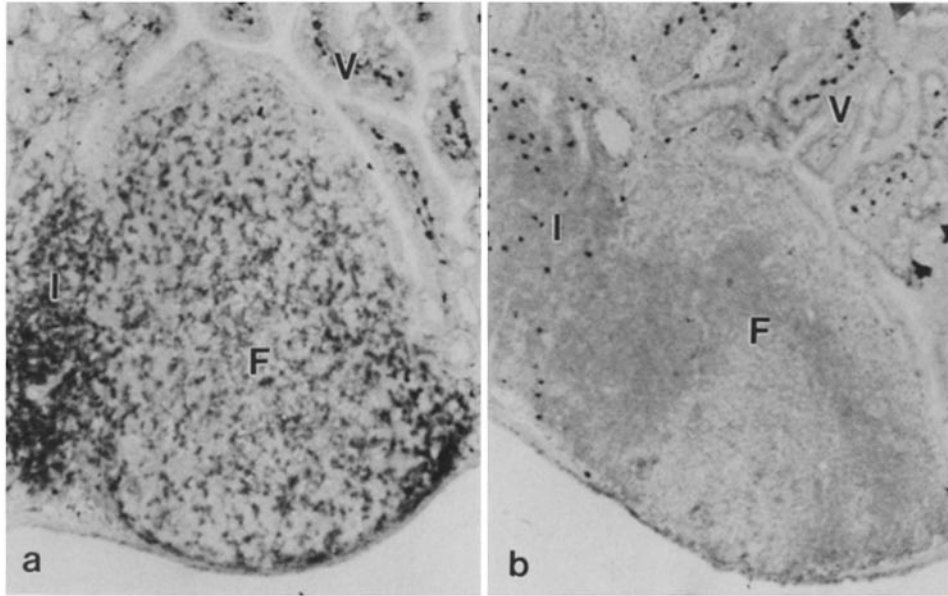


FIGURE 3. Peyer's patch sections from *G. muris*-infected mice that were given either PBS alone (a), or anti-L3T4 mAb (b). These sections were stained immunohistochemically for L3T4<sup>+</sup> cells. In a Peyer's patch from a control mouse (a), L3T4<sup>+</sup> cells (dense precipitate) are abundant in interfollicular areas (I) and are scattered throughout a follicle (F). In a patch from a Th/i-depleted mouse (b), L3T4<sup>+</sup> cells are absent, and the only labeled cells are granuloctes containing endogenous peroxidase, which are located in interfollicular areas (I) and intestinal villi (V).

### Discussion

The results of this work suggest that helper/inducer T cells are required for physiological clearance of *G. muris* infection, and that cytotoxic T cells do not play a significant part in this process.

A number of previous studies have examined the immunological response of mice to *G. muris*, although the actual mechanism by which immunocompetent mice clear *Giardia* infection remains uncertain. Available evidence, however, suggests that clearance of *G. muris* infection is mediated by antitrophozoite antibody rather than by cytotoxic lymphocytes. Thus, previous work has shown that immunocompetent mice are able to mount an intestinal antibody response to *G. muris* trophozoites (14–17). Furthermore, nude mice, and mice treated from birth with an antiserum directed against mouse IgM, have impairment of intestinal antibody production against *G. muris* trophozoites (15, 16); in both these types of mice, *G. muris* infection is persistent (rather than transient as in immunocompetent mice). Nude mice have a profound deficiency of L3T4<sup>+</sup> lymphocytes (18, 19), which may be the reason for the impairment of anti-*Giardia* antibody production in these animals. Mice that are deficient in natural killer cells (beige mice) eliminate *G. muris* infection at a normal rate (20). This observation suggests that natural killer cells are not involved in clearance of *G. muris* from the mouse intestine.

In vitro studies with cultured Peyer's patch cells indicate that Th/i lymphocytes

play an important role in the initiation of antibody responses against foreign antigens that have been introduced into the gastrointestinal lumen (21, 22).

Two potentially fruitful areas of further study are suggested by the present work, and by previous studies of murine giardiasis: (a) to determine whether experimental depletion of Th/i lymphocytes impairs production of intestinal antibody against *G. muris* trophozoites, and (b) to investigate whether *G. muris* trophozoites are killed by mouse intestinal antitrophozoite antibody. Recently, it has been shown that *G. lamblia* trophozoites are killed by mAbs that are directed against a trophozoite surface antigen (23).

### Summary

The aim of this work was to identify the T cell subset (helper/inducer or cytotoxic/suppressor) that plays a major part in the clearance of *Giardia muris* infection. BALB/c mice were selectively depleted of helper/inducer (Th/i) or cytotoxic/suppressor (Tc/s) T cells, by treatment with rat IgG monoclonal antibodies directed against the Th/i cell surface antigen L3T4, or against the Tc/s cell surface antigen Ly-2, and were infected with *G. muris* cysts. While mice depleted of Tc/s cells cleared *G. muris* infection at a normal rate, mice depleted of Th/i cells did not clear the infection. Depletion of the relevant T cell subset was confirmed by flow cytometry and by immunohistochemistry. The data indicate that helper/inducer T cells, but not cytotoxic T cells, are of major importance for elimination of *G. muris* from the mouse intestine.

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