

Clearance of *Giardia muris* Infection in Mice Deficient in Natural Killer Cells

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Immunocompetent C57BL/6J mice and beige mice (which are deficient in natural killer cells) were infected with *Giardia muris*. Both types of mice cleared *G. muris* infection at similar rates. This observation suggests that clearance of *G. muris* parasites from the mouse intestine is not mediated by natural killer cells.

Various studies have indicated that lymphocytes with natural killer (NK) cell activity are present in mouse intestinal mucosa (10, 15, 16). NK cells kill tumor cells in vitro and may prevent the development of malignant tumors in vivo (2). In addition, there is evidence that resistance to certain virus infections is mediated by NK cells (1). The role of NK cells in the intestinal mucosa is, however, unknown.

It is conceivable that intestinal NK cells contribute to the clearance of bacterial, fungal, and protozoan infections of the gastrointestinal tract. This possibility is supported by evidence that NK cells bind to fungal cells and inhibit the growth of fungi, and that intestinal lymphocytes kill bacteria in the absence of antibacterial antibody (5, 8, 9, 17). The aim of the present study was to test the hypothesis that NK cells are involved in the clearance of *Giardia muris* infection from the mouse intestine.

The time course of *G. muris* infection was compared in beige mice, which are deficient in NK cells (12), and in immunocompetent mice. The strain of *G. muris* used in this work was obtained from Frank W. Schaefer, U.S. Environmental Protection Agency, Cincinnati, Ohio, and was originally isolated from an infected golden hamster (13). C57BL/6J *bg⁺* (beige) mice and immunocompetent C57BL/6J mice were obtained from Jackson Laboratory, Bar Harbor, Maine. Female mice were used in the study, which was approved by the Animal Studies Subcommittee of the San Francisco Veterans Administration Medical Center. Mice aged 7 weeks were infected with *G. muris* by peroral intra-esophageal inoculation of 1,000 cysts via a blunt-ended feeding needle. At serial times during the following 6 weeks, the fecal output of the cysts was monitored, using a method described previously (11). Briefly, mouse fecal pellets were collected for 2-h periods and mixed with water to prepare suspensions, which were then centrifuged over an aqueous solution of 1 M sucrose. Cysts were aspirated from the interface above the sucrose solution and were counted by microscopic examination in a hemacytometer chamber.

To confirm that the beige mice had NK cell deficiency, splenic lymphocytes from both types of mice were tested for their ability to kill YAC-1 mouse lymphoma target cells (6, 7). At the age of 12 weeks, C57BL/6J mice and beige mice that did not have *G. muris* infection were sacrificed, and lymphocyte suspensions were prepared from their spleens. The lymphocytes (effectors) were then incubated with ⁵¹Cr-labeled YAC-1 cells (targets) at 37°C for 4 h in microtiter

plates with V-shaped wells, at various effector/target cell ratios (10⁴ YAC-1 cells per well). Incubation was carried out in RPMI 1640 medium containing 10% fetal calf serum, 3 mM L-glutamine, and antibiotics (penicillin and streptomycin). Killing of target cells was assessed by measuring release of radioactivity (⁵¹Cr) from the cells in counts per minute (cpm). This was done by gamma counting of supernatant fluid aspirated from microtiter plate wells, and specific

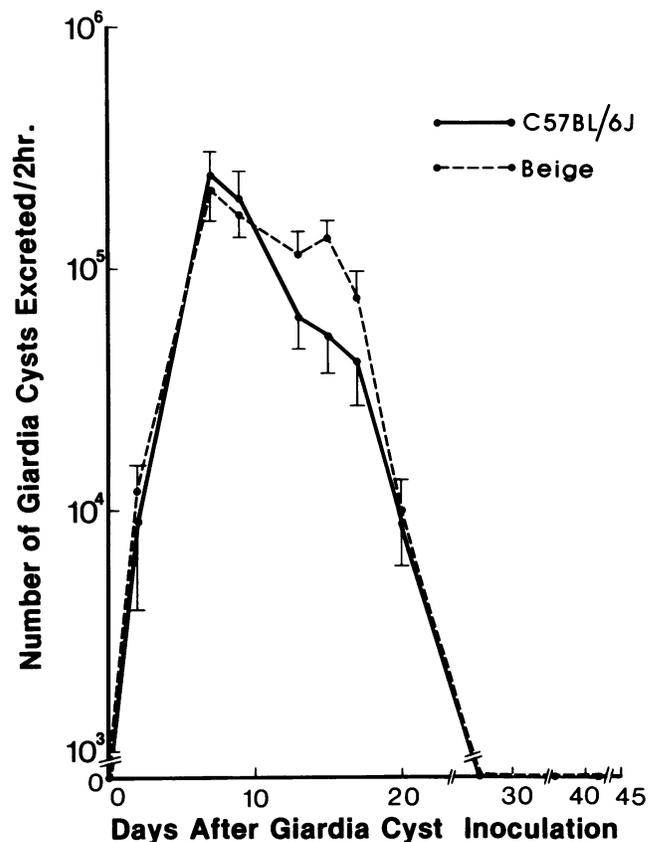


FIG. 1. Time course of *G. muris* infection in C57BL/6J mice and beige mice. The graphs show numbers of *G. muris* cysts in mouse fecal specimens collected for 2-h periods at various times during the infection. Mean values \pm standard errors are shown, for *G. muris* cyst output from 10 C57BL/6J mice and 10 beige mice. Mice were infected by peroral inoculation of *G. muris* cysts on day 0.

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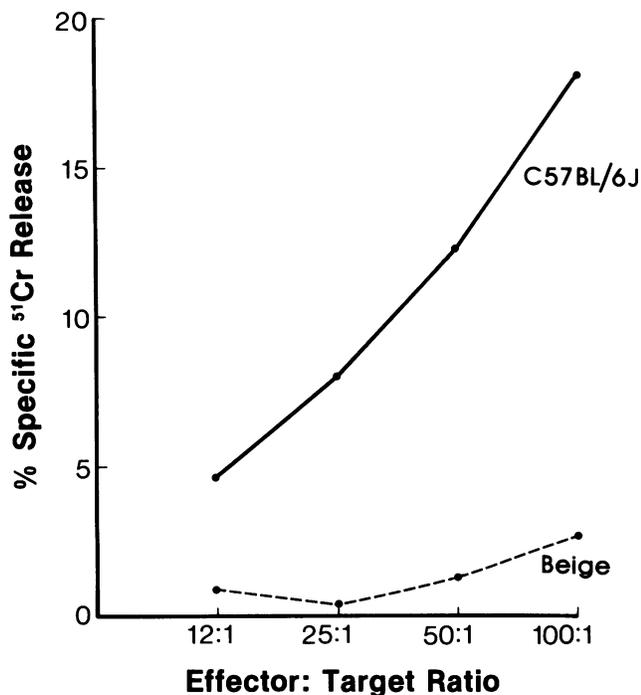


FIG. 2. NK cell activity of splenic lymphocytes from C57BL/6J mice and beige mice without *G. muris* infection. Specific release of ⁵¹Cr from ⁵¹Cr-labeled YAC-1 cells is shown, at various effector (spleen cell)/target (YAC-1 cell) ratios. Each point shows the mean result of data obtained by using spleen cells from three mice. All assays with spleen cells from each individual mouse were set up in triplicate.

release of ⁵¹Cr (at various effector/target ratios) was calculated by using the following formula: percent specific release = [observed release (cpm) - spontaneous release (cpm)]/[maximum release (cpm) - spontaneous release (cpm)] × 100.

In this formula, observed release is the release of ⁵¹Cr from target cells incubated with effector cells, and spontaneous release is the leakage of ⁵¹Cr from target cells incubated with medium but without effector cells. Maximum release of ⁵¹Cr was determined by saponin lysis of ⁵¹Cr-labeled target cells, and gamma counting of supernatant fluid. In the assay of NK cell cytotoxicity, microtiter wells containing effector cells and ⁵¹Cr-labeled target cells were set up in triplicate at each effector/target ratio, and the mean release of ⁵¹Cr (cpm) was determined for each triad of wells.

Beige mice and immunocompetent C57BL/6J mice cleared *G. muris* infection at similar rates (Fig. 1). The cytotoxicity assay confirmed that the beige mice had NK cell deficiency (Fig. 2).

It has previously been shown that NK cell activity of mouse intestinal epithelial lymphocytes parallels that of spleen cells (15). Thus, lymphocytes isolated from the small intestinal epithelium and spleen of beige mice have impaired NK cell activity (15). The fact that beige mice clear *G. muris* infection at essentially the same rate as immunocompetent mice suggests that NK cells are not involved in elimination of *G. muris* parasites from the mouse intestine. Evidence is accumulating that clearance of *G. muris* infection is dependent on antitrophozoite antibodies, and not on cytotoxic lymphocytes (3, 4, 14; M. F. Heyworth, J. R. Carlson,

J. E. Kung, and T. H. Ermak, *Gastroenterology* 90:1459, 1986).

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LITERATURE CITED

- Bukowski, J. F., B. A. Woda, S. Habu, K. Okumura, and R. M. Welsh. 1983. Natural killer cell depletion enhances virus synthesis and virus-induced hepatitis in vivo. *J. Immunol.* 131:1531-1538.
- Herberman, R. B., and J. R. Ortaldo. 1981. Natural killer cells: their role in defenses against disease. *Science* 214:24-30.
- Heyworth, M. F. 1986. Antibody response to *Giardia muris* trophozoites in mouse intestine. *Infect. Immun.* 52:568-571.
- 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.
- Jimenez, B. E., and J. W. Murphy. 1984. In vitro effects of natural killer cells against *Paracoccidioides brasiliensis* yeast phase. *Infect. Immun.* 46:552-558.
- Luini, W., D. Boraschi, S. Alberti, A. Aleotti, and A. Tagliabue. 1981. Morphological characterization of a cell population responsible for natural killer activity. *Immunology* 43:663-668.
- Mantovani, A., W. Luini, G. Peri, A. Vecchi, and F. Spreafico. 1978. Effect of chemotherapeutic agents on natural cell-mediated cytotoxicity in mice. *JNCI* 61:1255-1261.
- Nabavi, N., and J. W. Murphy. 1985. In vitro binding of natural killer cells to *Cryptococcus neoformans* targets. *Infect. Immun.* 50:50-57.
- Nencioni, L., L. Villa, D. Boraschi, B. Berti, and A. Tagliabue. 1983. Natural and antibody-dependent cell-mediated activity against *Salmonella typhimurium* by peripheral and intestinal lymphoid cells in mice. *J. Immunol.* 130:903-907.
- Petit, A., P. B. Ernst, A. D. Befus, D. A. Clark, K. L. Rosenthal, T. Ishizaka, and J. Bienenstock. 1985. Murine intestinal intraepithelial lymphocytes. I. Relationship of a novel Thy-1⁻, Lyt-1⁻, Lyt-2⁺, granulated subpopulation to natural killer cells and mast cells. *Eur. J. Immunol.* 15:211-215.
- 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.
- Roder, J. C. 1979. The beige mutation in the mouse. I. A stem cell predetermined impairment in natural killer cell function. *J. Immunol.* 123:2168-2173.
- Schaefer, F. W., III, E. W. Rice, and J. C. Hoff. 1984. Factors promoting in vitro excystation of *Giardia muris* cysts. *Trans. R. Soc. Trop. Med. Hyg.* 78:795-800.
- 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.
- Tagliabue, A., A. D. Befus, D. A. Clark, and J. Bienenstock. 1982. Characteristics of natural killer cells in the murine intestinal epithelium and lamina propria. *J. Exp. Med.* 155:1785-1796.
- Tagliabue, A., W. Luini, D. Soldateschi, and D. Boraschi. 1981. Natural killer activity of gut mucosal lymphoid cells in mice. *Eur. J. Immunol.* 11:919-922.
- Tagliabue, A., L. Nencioni, L. Villa, D. F. Keren, G. H. Lowell, and D. Boraschi. 1983. Antibody-dependent cell-mediated antibacterial activity of intestinal lymphocytes with secretory IgA. *Nature (London)* 306:184-186.