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Protection of Mice Against *Giardia muris* Infection

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Strains of mice showing relatively rapid (BALB/c) and defective (C3H/He) spontaneous elimination of *Giardia muris* displayed marked differences in the degree of resistance to infection induced by prior injection of trophozoites in Freund complete adjuvant.

An understanding of human disease caused by the protozoan *Giardia lamblia* has been facilitated by the study of *Giardia muris* infection in mice (7). *G. muris*, like *G. lamblia*, resides as a trophozoite in the lumen of the upper small bowel and is transmitted by the oral ingestion of cysts, which are passed in the feces. The development of methods for quantitating *G. muris* infection led to studies of its natural history in several mouse strains (6). Whereas spontaneous resolution of infection was observed in most mouse strains, the infection was prolonged in C3H/He mice. Hypothymic nude (nu/nu) mice of a genotype showing relatively rapid spontaneous resolution of infection (BALB/c) were similar to intact C3H/He mice in that cyst output continued for several months. Resolution of infection in BALB/c nu/nu mice was achieved by injection of syngeneic thymus-derived (T) cells.

Since giardiasis is self-limiting both in humans (5) and in various strains of mice, it seems likely that infection is associated with an immune response and that this response is elicited by, and directed against, the intestinal trophozoite. Attractive but unproven mechanisms for anti-trophozoite immunity include inflammation of the intestinal mucosa mediated by T cell-dependent processes (6) and the action of specific immnoglobulin A antibodies inhibiting parasite attachment to epithelial cells. In this report, we describe the influence of systemic administration of trophozoites on the course of subsequent *G. muris* infection.

Studies were performed in mouse strains (3) showing relatively rapid (BALB/c) and defective (C3H/He) spontaneous elimination of *Giardia*. Mice were infected at 6 to 8 weeks of age and were derived from a specific pathogen-free facility. The protocol involved injection, into the peritoneal cavity and hind footpads, of 10^6 trophozoites in Freund complete adjuvant (FCA) (1:1 ratio), followed by a second injection of the same number of trophozoites without adjuvant after 4 weeks. Mice were challenged by giving 1,000 *G. muris* cysts into the esophagus 1 week after the second injection, and the subsequent course of infection was assessed by determining cyst excretion in feces (7) at intervals of 1 or 2 weeks. Trophozoites were obtained from infected nude mice by vibration (Vibromixer E1, Chemap, Zurich, Switzerland) of everted loops of upper small bowel in phosphate-buffered saline (pH 7.3) of appropriate tonicity for mice. Vibration in phosphate-buffered saline for 2 min resulted in the release of large numbers of trophozoites with few epithelial cells and little debris. Epithelial cell clumps were sedimented by centrifugation at 50 × *g* for 1 min, after which trophozoites were sedimented and washed by centrifugation (twice) at 300 × *g* for 5 min. Aerobic and anaerobic cultures of washed trophozoite preparations showed minimal bacterial contamination with a ratio of bacterial counts to trophozoite counts of less than 1:1,000.

There was marked resistance to infection in BALB/c mice given trophozoites in FCA (Fig. 1). However, cyst counts in feces from C3H/He mice, exposed to the same injection regime, did not differ significantly from those in uninjected controls or C3H/He mice given adjuvant alone. Additional studies showed that two injections of 10^6 trophozoites (without adjuvant) failed to influence the natural history of infection in both strains of mice.

A degree of protection was observed in BALB/c mice when soluble trophozoite products, prepared by sonication and dialysis against phosphate-buffered saline for 24 h at 4°C, were substituted for whole trophozoites. The method yielded about 1 mg of *G. muris* protein from 10^7 trophozoites. Injection of mice with 40 or 400 μg of protein in FCA, followed by injection of the same amount of protein without adjuvant after 4 weeks, resulted in cyst counts in the feces of injected mice that were significantly lower than

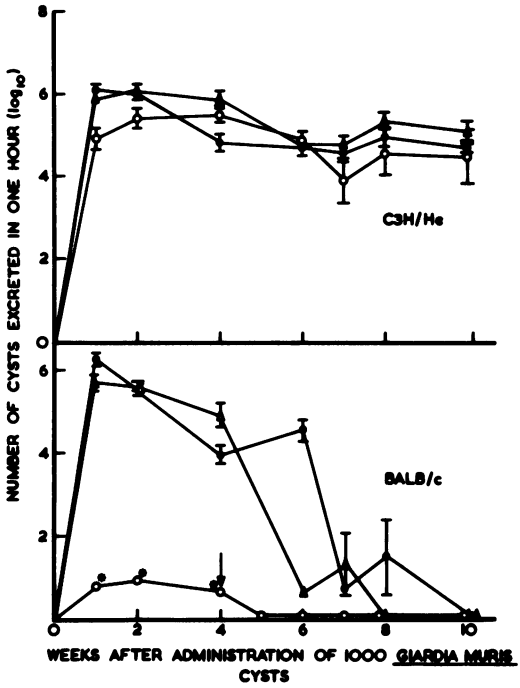

FIG. 1. *Geometric mean numbers (± standard error of the mean) of Giardia cysts in stools after oral administration of 1,000 G. muris cysts to groups of five male C3H/He and BALB/c mice. Mice were injected with trophozoites in FCA* (○), *were injected with FCA alone* (△), *or remained as uninjected controls* (●). *Only one* (*) *of five BALB/c mice injected with trophozoites in FCA had cysts in stools, and a further challenge with Giardia cysts at week 4 (arrow) failed to induce infection.*

those in uninjected control mice at 2 ($P < 0.01$), 3 ($P < 0.05$), and 5 ($P < 0.01$) weeks after challenge with 1,000 *G. muris* cysts (Student's *t* test). Both doses of antigen produced a similar degree of resistance to infection with *G. muris*.

Protection of mice against *G. muris* was further explored by the administration to CBA/H mice of trophozoites with various adjuvants. CBA/H mice show spontaneous resolution of infection, although at a slower rate than that observed in BALB/c mice (6). Injection of 10^6 trophozoites in FCA, followed by injection of the same number of trophozoites without adjuvant after 4 weeks, resulted in significantly lower cyst counts in feces at weeks 1 ($P < 0.05$) and 3 ($P < 0.02$) than those in mice given FCA alone (Student's *t* test). However, cyst counts in the feces of those mice receiving trophozoites with either Freund incomplete adjuvant (Difco Laboratories, Detroit, Mich.) or 10^9 *Bordetella pertussis* organisms (Commonwealth Serum Laboratories, Melbourne, Australia) were similar to

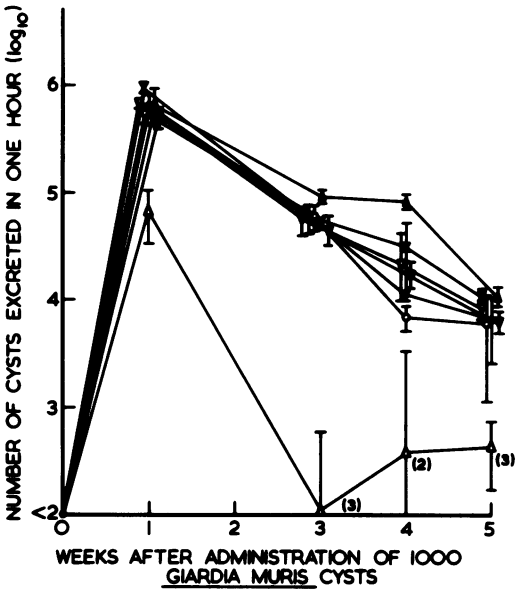

FIG. 2. *Geometric mean numbers (± standard error of the mean) of Giardia cysts in stools after oral administration of 1,000 G. muris cysts to groups of five female CBA/H mice injected with trophozoites in FCA* (△), *Freund incomplete adjuvant* (▲), *or B. pertussis vaccine* (×). *Control groups consisted of uninjected mice* (●) *and mice given FCA* (○), *Freund incomplete adjuvant* (▽), *and trophozoites* (▼) *alone.*

cyst counts in uninjected controls and mice receiving adjuvant or trophozoites alone (Fig. 2).

In this study the protective effect of injection of trophozoites in FCA was only observed in strains of mice showing spontaneous resolution of infection. The reasons for failure to induce resistance in C3H/He mice remain unclear, but one possibility is that this mouse strain, like the BALB/c nu/nu mouse, lacks adequate numbers of T cells reactive to the "host-protective" antigens of *G. muris*. In all probability the vaccination strategy in C3H/He mice, and by analogy in those patients with chronic rather than transient giardiasis, needs to be different from that adopted in individuals capable of spontaneous resolution of infection.

The potency of FCA, and the inadequacy of other readily available adjuvants, is a recurring theme in experimental protozoal vaccination studies (1, 2, 4, 8). One important research endeavor in all such studies will be to determine whether the influence of FCA can be explained in terms of quantitative or qualitative aspects of the immune response.

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