CONCISE COMMUNICATION

The Role of Normal Flora in *Giardia lamblia* Infections in Mice

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The presence of normal bacterial flora in the intestinal tract is thought to protect against colonization by pathogens. Only a few specific examples of this protection have been demonstrated for bacterial pathogens and protozoan infections. Mice from one commercial breeding farm were found to be less susceptible to infection with *Giardia lamblia* than were isogenic mice from another facility. When mice were housed together, resistance to infection was readily transferred to normally susceptible mice. After resistant mice were treated with neomycin, differences in susceptibility to infection were shown to be due to differences in the resident flora present in these mice. These results suggest the possible use of probiotic therapy for prevention of *G. lamblia* infections and may help explain some of the variability of outcomes seen in *G. lamblia* infections in humans.

A large number of parasites, both helminths and protozoa, normally inhabit the gastrointestinal tract. In model infections, bacterial flora decrease susceptibility to infection by *Cryptosporidium parvum* [1], and there is substantial literature indicating that certain bacteria increase the virulence of *Entamoeba histolytica.* The presence of normal bacterial flora in the gastrointestinal tract is often cited as a basic defense mechanism of the body against infections by pathogens [2, 3]. Several examples exist in which gnotobiotic mice or rats are much more susceptible to infections than are their conventionally reared counterparts or those specifically contaminated (e.g., *Salmonella enteritidis, Listeria monocytogenes, Clostridium difficile,* and *Helicobacter pylori*). Whether differences in the specific composition of the normal flora in these animals affect their ability to provide such protection has not been demonstrated.

Giardia lamblia is one of the most common pathogenic gastrointestinal parasites of humans and other animals [4]. Replicating trophozoites reside in the small intestine and are responsible for disease manifestations. *Giardia* infections are most common in young children, and newborn animals are usually more susceptible to infection than adults. Specifically, neonatal mice can be infected with a majority of clinical isolates, whereas adult mice are usually resistant to infection [5]. We recently developed an adult mouse model of *G. lamblia* and noted marked variability in our ability to infect adult mice [6, 7]. Although mice from one commercial supplier were susceptible to infection, mice with similar genetic backgrounds from a second firm were resistant to infection. The present study investigated the reason for this discrepancy.

Materials and Methods

Mice. We obtained B10.A mice, B10.A-H2[a] H2-T18/SgSnJ and B10.A/SgSnAi, from Jackson Laboratories (Bar Harbor, ME) and Taconic Farms (Germantown, PA), respectively (hereafter referred to as Jackson and Taconic). Immunodeficient mice were also obtained from Jackson (C57BL/6J RAG1$^{tm1\,Mom}$) and Taconic (C57BL/10SgSnAi-[KO]RAG2 N13, N2). Mice were housed in sterile microisolator cages. Some animals were given drugs in drinking water: neomycin (1.4 mg/mL; Phoenix Pharmaceuticals, St. Louis) or Bactrim (0.64 mg/mL sulfamethoxazole, 0.13 mg/mL trimethoprim; Teva Pharmaceuticals, Sellersville, PA).

Parasites and infections. *G. lamblia* clone GS/M H7 was cultured in TYI-S-33 medium, and 500,000 trophozoites were inoculated into mice by gavage, as described elsewhere [6, 7]. Trophozoites in the small intestine were counted by hemocytometer, as described elsewhere [7].

Results

We initially observed differences in mice from different suppliers when we attempted to infect mice with targeted mutations in a number of different genes important for immune responses. Mice from Jackson were all readily infected, whereas those from Taconic were always difficult to infect, despite the identical genotypes of the mice. To directly compare mice from these suppliers, we obtained immunocompetent B10.A mice from both firms. Table 1 shows that the mice from Taconic were not as easily infected as those from Jackson. Of interest, when mice from the two suppliers were housed in the same cage for 2

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Table 1. *Giardia lamblia* infections in B10.A mice.

Mouse source	No. infected	Intensity[a]
Taconic	2/4	12.5 ± 0
Jackson	3/4	104 ± 80
Taconic (+Jackson)	0/4	ND
Jackson (+Taconic)	1/4	37.5

NOTE. Female mice (6–8 weeks old) were inoculated with 5×10^5 trophozoites of *G. lamblia* GS(M)/H7 by gavage [6]. Mice were killed 2 weeks later, and the no. of parasites in the small intestine was determined [7]. Taconic, Taconic Farms (Germantown, PA); Jackson, Jackson Laboratories (Bar Harbor, ME); ND, none detected.

[a] Trophozoites per mouse $\times 10^3$ ± SD. Only infected mice were used to calculate the intensity of infection.

weeks prior to infection, resistance to infection was transferred to the previously susceptible Jackson mice. This suggested that a biologic agent may be responsible and that the prevention of infection was somehow dominant. Although both sets of mice are designated B10.A, it is possible that minor genetic differences had accumulated in these substrains. To exclude such a possibility, skin grafts were done between mice from Jackson and Taconic, and results indicated no incompatibilities in graft-rejection antigens between these mouse substrains (C. M. Collazo, C. Anderson, and A. Sher, personal communication).

G. lamblia infections in adult mice are much more robust in SCID mice than in immunocompetent mice [6, 7]. These animals maintain very high levels of infection throughout life. Because the results with B10.A mice were not statistically significant, we next infected B and T cell–deficient recombinant-activating gene (RAG)–knockout mice from both Jackson and Taconic with *G. lamblia.* Again, the mice from Taconic were naturally resistant to infection, whereas the mice from Jackson were susceptible, and resistance could be transferred by simply housing animals together for 4 weeks (table 2, experiment 1). To demonstrate a role for bacterial flora in this difference, we treated mice from Taconic with neomycin for 2 weeks (experiment 2) or 4 weeks (experiment 1) prior to and throughout the infection with *G. lamblia.* Treatment with neomycin completely reversed the inability to infect these mice, a result that is consistent with a protective role for the normal flora (table 2). Treatment with trimethoprim-sulfamethoxazole failed to induce susceptibility to infection in the mice from Taconic. Although high doses of neomycin can affect anaerobic bacteria, trimethoprim-sulfamethoxazole has no effect on anaerobic bacteria.

Discussion

An inherent assumption in the arguments above is that mice from Jackson and Taconic differ in their normal flora, and this is almost certainly the case. All mice distributed by Taconic are inoculated with a combination of 8 anaerobic microorganisms (collectively known as altered Schaedler flora) on introduction of the individual lines into their production facilities [8]. Jackson does not provide a similarly defined flora for its mice. In addition, both groups of mice will accumulate any number of unknown organisms during expansion of the production colonies. It is tempting to speculate that this defined flora is responsible, because these mice are housed under highly contained conditions. The altered Schaedler flora includes 2 lactobacilli species, 1 *Bacteroides* species, 1 spirochete, and 4 species of *Fusiform* bacteria. Of particular interest are the lactobacilli, which tolerate the acidic conditions of the duodenum and ileum, where *G. lamblia* replicates. *Lactobacillus salivarius,* a component of the altered Schaedler flora, reduces susceptibility to *H. pylori* infection [9]. Recent work with lactobacilli has also shown that these microorganisms have strong adjuvant effects on immune responses in vitro [10], although similar effects were not seen in vivo [11].

Normal flora could inhibit *G. lamblia* infections through several mechanisms, including competition for resources, direct toxicity, induction of cross-reactive adaptive immune responses, or differences in the innate mucosal immune system [2, 3]. Because these effects are seen in RAG-deficient mice, which lack adaptive immune responses, it is unlikely that induction of cross-reactive antibody or T cell responses are responsible. However, induction of innate immune responses can prevent *G. lamblia* infection, and its role must be considered.

The protective effects of normal flora are not unique to *G. lamblia. C. parvum* is another protozoan parasite that infects the small intestines of mammals. As with *G. lamblia,* infections of neonatal mice with *C. parvum* are much more common than infections in adult mice [12]. Also, gnotobiotic adult SCID mice can be infected with *C. parvum,* whereas conventional SCID mice from Taconic cannot be infected [1]. Although isogenic mice from Taconic and Jackson have not been examined, it will be of interest to see whether the same bacterial species and mechanisms are responsible for resistance to these 2 parasitic infections.

Table 2. *Giardia lamblia* infections in RAG-deficient mice.

	Experiment 1		Experiment 2	
Mouse source or source + treatment	No. infected	Intensity[a]	No. infected	Intensity
Taconic	0/4	ND	0/4	ND
Jackson	3/4	262 ± 381	4/4[b]	378 ± 77
Taconic (+Jackson)	0/4	ND	—	—
Jackson (+Taconic)	0/4	ND	—	—
Taconic (+neomycin)	4/4[b]	691 ± 139	4/4[b]	609 ± 411
Jackson (+Bactrim)	—	—	4/4[b]	406 ± 210
Taconic (+Bactrim)	—	—	0/4	ND

NOTE. Female mice (6–8 weeks old) were inoculated with 5×10^5 trophozoites of *G. lamblia* GS(M)/H7 by gavage [6]. The mice were killed 2 weeks later, and the no. of parasites in the small intestine was determined [7]. RAG, recombinant-activating gene; Taconic, Taconic Farms (Germantown, PA); Jackson, Jackson Laboratories (Bar Harbor, ME); ND, none detected. Bactrim, 0.64 mg/mL sulfamethoxazole and 0.13 mg/mL trimethoprim.

[a] Trophozoites per mouse $\times 10^3$ ± SD.

[b] $P < .05$ vs. untreated Taconic mice (by χ^2 test).

As noted, neonatal mice are much more susceptible than adult mice to infections with *G. lamblia* and *C. parvum.* These parasites also infect human infants much more readily than adults. Because the composition of normal flora in mice changes substantially on weaning [2], it is possible that this change in flora is directly responsible for the greater resistance to infection seen in adults, compared with that seen in infants. Alternatively, changes in the mucosal immune system that occur at weaning, in part as a result of the changes in flora, may be responsible [3, 13].

Infectivity of *Giardia* organisms and the course of infection and disease manifestations vary in giardiasis. *G. lamblia* infections produce highly variable outcomes in both humans and animals. Differences in the normal flora present in the hosts are likely contributors to this variability. To our knowledge this is the first suggestion that the composition of resident flora, rather than its presence or absence, blocks infection with a pathogen.

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