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T-Cell-Dependent Control of Acute *Giardia lamblia* Infections in Mice

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We have studied immune mechanisms responsible for control of acute *Giardia lamblia* and *Giardia muris* infections in adult mice. Association of chronic *G. lamblia* infection with hypogammaglobulinemia and experimental infections of mice with *G. muris* have led to the hypothesis that antibodies are required to control these infections. We directly tested this hypothesis by infecting B-cell-deficient mice with either *G. lamblia* or *G. muris*. Both wild-type mice and B-cell-deficient mice eliminated the vast majority of parasites between 1 and 2 weeks postinfection with *G. lamblia*. *G. muris* was also eliminated in both wild-type and B-cell-deficient mice. In contrast, T-cell-deficient and *scid* mice failed to control *G. lamblia* infections, as has been shown previously for *G. muris*. Treatment of wild-type or B-cell-deficient mice with antibodies to CD4 also prevented elimination of *G. lamblia*, confirming a role for T cells in controlling infections. By infecting mice deficient in either αβ- or γδ-T-cell receptor (TCR)-expressing T cells, we show that the αβ-TCR-expressing T cells are required to control parasites but that the γδ-TCR-expressing T cells are not. Finally, infections in mice deficient in production of gamma interferon or interleukin 4 (IL-4) and mice deficient in responding to IL-4 and IL-13 revealed that neither the Th1 nor the Th2 subset is absolutely required for protection from *G. lamblia*. We conclude that a T-cell-dependent mechanism is essential for controlling acute *Giardia* infections and that this mechanism is independent of antibody and B cells.

Giardia lamblia is a common cause of both acute and chronic diarrheal disease in humans (reviewed in reference 1). In many regions of the world, giardiasis is endemic and infection is practically universal by 2 years of age (1). In developed countries, infections are more sporadic but nevertheless common whenever fecal contamination occurs, such as with contamination of water supplies or direct person-to-person spread in day care centers. The courses of infections are highly variable among individuals; some infections resolve quickly, whereas others can continue for years. This variability may be due to differences in pathogenicity among parasite isolates as well as to differences in host responses (3).

Several lines of evidence suggested that antibodies and T cells are required to control *Giardia* infections. Many studies have focused on immunoglobulin A (IgA) since it is found predominantly on mucosal surfaces. Infections of humans and rodents with *G. lamblia* and *Giardia muris* lead to the production of parasite-specific antibodies, including antibodies of the IgA isotype (5, 18, 28). Parasites recovered from infected animals were shown to be coated with IgA (19), and IgM and IgG antibodies have been shown to be cytotoxic in vitro by complement-dependent and complement-independent mechanisms (34). Furthermore, hypogammaglobulinemia is often associated with chronic giardiasis in humans (reviewed in references 1 and 40). Together, these data have led to the hypothesis that antibodies, particularly of the IgA isotype, are required to control *G. lamblia* infections. This idea has been further supported by experimental infections in mice with the related parasite *G. muris*. Mice depleted of B cells by treatment with anti-IgM antibodies were unable to control *G. muris* replication (44) as were *xid* mutant mice, which have reduced numbers of B cells (45). Finally, the discovery of antigenic variation of the surface proteins of *G. lamblia* was also consistent with a role for antibody in controlling the parasite, since antigenic variation is typically thought to be a mechanism used by microorganisms to evade host antibody responses (2, 9, 35). T cells are also important in controlling *Giardia* infections. Nude mice and anti-CD4 antibody-injected mice were unable to control *G. muris* replication (20, 47). Similarly, neonatal nude and SCID mice were unable to control infections with *G. lamblia* (13). However, it was unclear from these studies whether T cells were directly involved in eliminating the parasites or whether they were needed merely to augment production of antibodies.

Because *Giardia* replicates only in the lumen of the small intestine, we were interested in directly addressing the role of antibodies in controlling *G. lamblia* infections as well as defining any other components of the mucosal immune system responsible for controlling parasite infections. We therefore took advantage of a model of acute *G. lamblia* infection in adult mice (7). In this model parasites are introduced by gavage and replicate in the small intestines of the mice until parasite numbers drop dramatically between 1 and 2 weeks postinfection. However, small numbers of parasites continue to be detectable by culturing the intestinal contents for several months, although they cannot be detected by visual inspection of intestinal contents. We have used both *G. lamblia* and *G. muris* to infect B-cell-deficient mice and show that there is little difference in the levels of parasite clearance between these mice and wild-type mice for either parasite. In contrast, we show that αβ-T-cell-receptor (αβ-TCR)-bearing, $CD4^+$ T cells are required for control of acute *G. lamblia* infections in mice and that this requirement persists in the absence of B cells. Finally, infections in several lines of cytokine-deficient mice demonstrate that neither the Th1 nor the Th2 subset is absolutely required for control of acute *G. lamblia* infections.

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TABLE 1. *G. lamblia* infections in immunodeficient mice

Expt	Mice	Day 7		Day 14		Day 28	
		Infected[a]	Intensity[b]	Infected	Intensity	Infected	Intensity
1	C57BL/6J	3/3	7.1 ± 4.4	1/3	0.3 ± 0.6	0/3	ND[g]
	B6 Igh-6	3/3	22.1 ± 6.3[c,d]	1/3	0.3 ± 0.6[d]	0/3	ND
	B6 SCID	3/3	164.2 ± 62.1[c]	3/3	197.1 ± 100.3[c]	3/3[e]	150.8 ± 15.8
	B6 TCRβ	3/3	45.0 ± 8.2[c,d]	3/3	51.7 ± 12.1[c,d]	3/3[e]	53.8 ± 17.7[d]
2	C57BL/6J	3/3	13.3 ± 11.1	2/3	1.3 ± 1.2	1/3	0.3 ± 0.6
	B6 SCID	3/3	79.2 ± 37.4[c]	3/3	185.0 ± 73.7[c]	3/3	140.0 ± 47.7[c]
	B6 TCRβ	3/3	185.0 ± 50.7[c,d]	3/3	91.7 ± 36.2[c,d,f]	3/3	50.0 ± 10.0[c,d,f]

[a] The number of mice with detectable parasites in the gut/total number of mice analyzed on the day indicated postinfection.
[b] The average ± standard deviation of the number of parasites/mouse × 10^4.
[c] $P < 0.01$ versus the value for C57BL/6J mice.
[d] $P < 0.01$ versus the value for B6 SCID mice.
[e] $P < 0.08$ versus the value for C57BL/6J mice.
[f] $P < 0.01$ versus the value on day 7.
[g] ND, none detected.

MATERIALS AND METHODS

Mice. C57BL/6J (wild-type), BALB/cJ (wild-type), C57BL/6J Igh-6 tm1 Cgn (B-cell-deficient, B6 Igh-6 [26]), C57BL/6J Tcrb tm1 Mom (αβ-T-cell-deficient, B6 TCRβ [32]), C57BL/6J Tcrd tm1 Mom (γδ-T-cell-deficient, B6 TCRδ [23]), C57BL/6J *scid* (T- and B-cell-deficient, B6 SCID), BALB/cJ STAT-6 tm1 Gru (signal transducer and activator of transcription 6 [STAT-6]-deficient, BA STAT-6 [25]), C57BL/6J IFN tm1 Ste (gamma interferon [IFN-γ]-deficient, B6 IFN-γ [8]), and C57BL/6J IL-4 tm1 Nnt (interleukin-4 [IL-4]-deficient, B6 IL-4 [37]) mice were obtained from the Jackson Laboratories, Bar Harbor, Maine. IL-4 and IL-4 receptor gamma chain (IL-4R)-deficient mice on an inbred BALB/c background (BA IL-4 and BA IL-4R) were kindly provided by Nancy Noben-Trauth (National Institute of Allergy and Infectious Diseases, Rockville, Md.) (37, 38). All mouse experiments were performed in accordance with institutional guidelines.

Parasites and infections. The clone of *G. lamblia* GS/M-H7 has been previously described (3). Infections were initiated with 500,000 in vitro-cultivated trophozoites suspended in 0.1 ml of TYI-S-33 medium given by gavage (7). Groups of three mice were euthanized at various time points, and the proximal 10-cm sections of their small intestines were removed and placed in 5 ml of TYI-S-33 medium on ice. The small intestines were minced, and after 15 min on ice, parasites were counted with a hemocytometer. At least four separate grids on the hemocytometer were counted for each mouse, and mice with no detectable parasites were considered to have cleared the infections. Since a single parasite on one grid corresponds to 10^4 trophozoites/ml, the limits of detection were roughly 10^4 trophozoites/mouse. *G. muris* cysts were obtained from Frank Schaefer (U.S. Environmental Protection Agency, Cincinnati, Ohio), and an inoculum of 1,000 cysts/mouse was used to initiate infections by gavage.

VSP analysis. Expression of the H7 variant-specific surface protein (VSP) was analyzed essentially as described previously (3). After quantitation, parasites were cultured in 15 ml of TYI-S-33 medium in glass tubes at 37°C for 15 min to allow attachment. Nonadherent material was decanted, and tubes were filled with phosphate-buffered saline and chilled on ice to detach parasites. After concentration by centrifugation, parasites were then adhered to glass slides at 37°C, fixed in methanol-acetone (1:1), air dried, and stained with monoclonal antibody G10/4, which reacts with the VSP expressed by the H7 clone of the parasite. Staining was visualized with goat-anti-mouse IgG, IgA, IgM, and fluorescein isothiocyanate (Cappel) and viewed on a Zeiss axiophot microscope. Negative controls were incubated without primary antibody and showed no staining.

Anti-CD4 antibody treatment. Mice were depleted of $CD4^+$ T cells by administration of 2 mg of the monoclonal antibody GK1.5 given intraperitoneally in phosphate-buffered saline on days −4, 0, +6, and +9 pre- and postinfection (10). Control animals similarly received 2 mg of an isotype-matched monoclonal antibody, GL113. Both antibodies were provided by A. Sher (National Institute of Allergy and Infectious Diseases) as ammonium sulfate-purified ascites fluid. $CD4^+$-T-cell depletion was >95% effective as determined by fluorescence-activated cell sorter analysis of splenic lymphocytes with anti-CD3 phycoerythrin and anti-CD8 fluorescein isothiocyanate (Pharmingen, San Diego, Calif.) at the termination of the experiment (data not shown).

Statistics. Comparisons between the numbers of infected animals per group were done by chi-square analyses. Comparisons between the numbers of parasites observed in different groups were done by a *z* test. Mice with undetectable parasites ($<10^4$) were considered to have a statistical value of 0 for statistical comparisons. Both tests were done with Excel software (Microsoft Corp., Redmond, Wash.).

RESULTS

We began by investigating whether antibodies are required for control of acute infections with *G. lamblia*. Wild-type or B-cell-deficient mice which lack all antibody due to disruption of the Ig heavy-chain (IgH) locus (26) were infected with *G. lamblia* trophozoites, and parasite loads were determined at various times after infection. Table 1 shows that while all the mice were infected 1 week after infection, B-cell-deficient mice, αβ-T-cell-deficient mice, and SCID mice all had significantly more parasites than did wild-type controls, suggesting that both B-cell and T-cell functions can affect parasite load. However, while both B-cell-deficient and wild-type mice eliminated the majority of parasites by 2 weeks postinfection and had no detectable parasites ($<10^4$/mouse) 4 weeks postinfection, αβ-T-cell-deficient mice and *scid* mice were completely unable to control the parasites, suggesting a crucial role for T cells in control of this parasite (Table 1) (7). There was some indication that SCID mice might harbor more parasites than the B-cell or TCRβ-deficient mice 7 days postinfection (Table 1, experiment 1), suggesting that the role of T cells and B cells in controlling parasite loads might be additive.

G. lamblia undergoes antigenic variation during infections of immunocompetent hosts (2, 35). The VSPs expressed by parasites in the inoculum are replaced by antigenically distinct VSPs, presumably due to elimination of the parasites expressing the original VSP and outgrowth of parasites which have spontaneously switched to expression of new VSPs. Gottstein and Nash showed that neonatal nude mice were capable of selecting for antigenic variants but that neonatal SCID mice were not (13), and Stäger and Müller recently showed that selection of antigenic variants failed to occur in B-cell-deficient mice, showing that B cells are required for selecting antigenic variants of *G. lamblia* (46). Our analysis of the VSPs expressed by the parasites recovered from all of these strains confirmed that B cells were required for the selection of antigenic variants (data not shown). Interestingly, antigenic variants were selected for in the TCRβ mutant mice even though the parasites could not be eliminated. It is possible that IgM is able to select for antigenic variants or that the γδ T cells are able to provide help for isotype switching in the TCRβ-deficient mice. Thus, while antibodies are essential for selection of VSPs, they are not required for control of parasite numbers in the gut.

G. muris naturally infects mice and other rodents. Studies of *G. muris* infections in anti-IgM-treated mice and *xid* mutant

TABLE 2. *G. muris* infections in B-cell-deficient mice

Mice	Day 7		Day 21		Day 35	
	Infected[a]	Intensity[b]	Infected	Intensity	Infected	Intensity
C57BL/6J	3/3	43.8 ± 26.5	1/3	0.3 ± 0.6	0/3	ND[c]
B6 Igh-6	3/3	31.2 ± 24.6	2/3	1.0 ± 1.0	1/3	0.7 ± 1.2

[a] The number of mice with detectable parasites in the gut/total number of mice analyzed on the day indicated postinfection.
[b] The average ± standard deviation of the number of parasites/mouse × 10^4.
[c] ND, none detected.

mice have suggested that antibodies are required for parasite clearance (44, 45). We wanted to see if our results with *G. lamblia* in B-cell-deficient mice differed from the results of these earlier studies due to differences between *G. lamblia* and *G. muris*. We therefore infected wild-type and B-cell-deficient mice with *G. muris* cysts. While the clearance of *G. muris* in mice is slower than for *G. lamblia*, both wild-type and B-cell-deficient mice eliminated the majority of parasites by 3 weeks postinfection (Table 2). Thus, the data indicate that antibodies are not required for control of either *G. lamblia* or *G. muris* infections. Importantly, our results are not unique to the adult-mouse model of *G. lamblia* infections, as *G. muris* is also eliminated in B-cell-deficient mice. Furthermore, our results can be directly compared to those of earlier experiments. The differences between our results and those previously published for *G. muris* are likely due to conditions in B-cell-deficient mice resulting from disruption of the IgH locus (26) that are different from conditions in mice with the *xid* mutation or following treatment with anti-IgM antibodies (44, 45).

Since antibodies were not required for control of acute *Giardia* infections in mice, we examined the role of T cells in these infections. $CD4^+$ T cells were shown to be required for control of *G. muris* infections (20). In order to determine whether these T cells were required to augment antibody production or if they acted through a distinct pathway, 4 days prior to infection with *G. lamblia* we treated both wild-type and B-cell-deficient mice with anti-CD4 antibody. As had been seen for *G. muris*, depletion of the $CD4^+$ T cells prevented *G. lamblia* clearance in wild-type mice (Table 3). In addition, depletion of $CD4^+$ T cells in B-cell-deficient mice prevented the elimination of the parasites, suggesting that the role of these T cells is not merely to provide help for antibody production and that there is a distinct antibody-independent, but T-cell-dependent, pathway leading to elimination of the parasite.

The T-cell compartment in the small intestine contains both αβ-TCR- and γδ-TCR-bearing T cells. While the former are known to recognize peptide antigens bound to molecules encoded in the major histocompatibility complex, it is still unclear which ligands are recognized by the latter, although recent data suggest that they recognize invariant molecules expressed by intestinal epithelial cells under certain conditions (15). We therefore infected mice deficient in either T-cell subset due to disruption of either the TCRβ or -δ locus (23, 32). As seen in Table 4, the response to *G. lamblia* is clearly dependent on the αβ-TCR-bearing T cells and not the γδ-TCR-bearing T cells. Most of the mice were infected with *Giardia* at day 7 postinfection. The low numbers of parasites seen in the wild-type and TCRδ-deficient mice in this experiment at day 7 suggest that immunity-mediated clearance may have already begun by day 7. While wild-type and TCRδ-deficient mice eliminated the majority of parasites by 2 weeks postinfection, the TCRβ-deficient mice could not control this acute phase of the infection. The reduction in the number of parasites from week 1 to week 4 postinfection seen in the TCRβ-deficient mice (Table 1, experiment 2) was not reproducible (Table 1, experiment 1).

In many other parasitic infections the immune response becomes biased towards either the Th1 or the Th2 subset (42). To determine if either subset was required for protection in this model of *G. lamblia* infection, we infected mice deficient in the Th1 subset due to disruption of the IFN-γ gene (8) or in the Th2 subset due to disruptions of the gene for IL-4, IL-4R, or STAT-6 (25, 37, 38). Table 5 shows that all of these mice were able to control the acute phase of the infection in a manner similar to the way wild-type mice could, although mice lacking IFN-γ showed a slight delay in eliminating the parasites in one experiment. Interestingly, the STAT-6 knockouts lacked parasites even on day 7, suggesting that they might in fact be hyperimmune to this parasite or that they were refractory to infection initially.

DISCUSSION

In this study we clearly demonstrate that a T-cell-dependent mechanism is required for control of acute *G. lamblia* infections in adult mice. However, since our assay for parasites cannot discriminate between low parasite burdens and complete elimination of infections, no role for T cells in controlling chronic *G. lamblia* infections can be assigned at this time. TCRβ-deficient mice maintain high parasite burdens in the small intestine for over 4 weeks postinfection. In addition, depletion of $CD4^+$ T cells from either wild-type or B-cell-deficient mice significantly enhances infections, suggesting that the role of these T cells is distinct from their ability to augment antibody production. We also show that T cells expressing αβ TCRs but not those expressing γδ TCRs are able to mediate clearance of *G. lamblia*. Finally, we show that neither IFN-γ-

TABLE 3. *G. lamblia* infections in anti-CD4-antibody-treated mice

Mice	Treatment	Day 7		Day 14	
		Infected[a]	Intensity[b]	Infected	Intensity
C57BL/6J	Anti-CD4 antibody	3/3	30.8 ± 30.7	3/3[c]	39.6 ± 22.2
	Control IgG	3/3	11.7 ± 14.4	0/3	ND[e]
B6 Igh-6	Anti-CD4 antibody	3/3	41.4 ± 25.9	3/3	82.1 ± 23.9[d]
	Control IgG	3/3	4.3 ± 4.2	1/3	0.3 ± 0.6

[a] The number of mice with detectable parasites in the gut/total number of mice analyzed on the day indicated postinfection.
[b] The average ± standard deviation of the number of parasites/mouse × 10^4.
[c] $P = 0.8$ versus the value for the control mice treated with IgG.
[d] $P < 0.01$ versus the value for the control mice treated with IgG.
[e] ND, none detected.

TABLE 4. *G. lamblia* infections in T-cell-deficient mice

Mice	Day 7		Day 14		Day 28	
	Infected[a]	Intensity[b]	Infected	Intensity	Infected	Intensity
C57BL/6J	2/3	0.7 ± 0.6	0/3	ND[e]	0/3	ND
B6 TCRδ	3/3	14.1 ± 21.0[c]	1/3	0.4 ± 0.7	0/3	ND
B6 TCRβ	3/3	78.8 ± 47.5[c]	3/3[d]	62.5 ± 19.2	3/3[d]	35.8 ± 30.8

[a] The number of mice with detectable parasites in the gut/total number of mice analyzed on the day indicated postinfection.
[b] The average ± standard deviation of the number of parasites/mouse × 10^4.
[c] $P < 0.01$ versus the value for C57BL/6J mice.
[d] $P = 0.08$ versus the value for C57BL/6J mice.
[e] ND, none detected.

producing Th1 cells nor IL-4- or IL-13-dependent Th2 cells are absolutely required for control of this parasite.

In contrast to T cells, B cells and antibodies are not required for control of acute *G. lamblia* or *G. muris* infections in mice (Tables 1 to 3). Antibodies can be cytotoxic to *Giardia* in vitro (33), however, and may be able to reduce the parasite load during the acute phase of infection (Tables 1 and 3 but not Table 2). It is also possible that antibodies may be essential for controlling parasites during the chronic phase of infection. Indeed, Stäger and Müller found that *G. lamblia* infections persisted longer in B-cell-deficient mice than in wild-type mice, consistent with a role for antibody during the chronic phase of infection (46). Antibodies are also required for selection of VSPs during infections. Importantly, however, our results demonstrate that an antibody-independent mechanism exists and is essential for control of the acute phase of *Giardia* infections.

How can our experiments demonstrating that T cells are required to control *G. lamblia* infections be reconciled with the results of previous studies indicating an essential role for B cells in controlling *G. muris* infections (44, 45)? Since in our experiments both *G. muris* and *G. lamblia* infections are controlled in B-cell-deficient mice, it seems likely that the differences observed are due to differences between the mice we have used and those used in earlier studies. While Snider et al. used either wild-type mice treated with anti-IgM or *xid* mutant mice, we have used mice with a disruption of the IgH locus (44). Disruption of the IgH locus produces mice completely devoid of both antibodies and B cells (26). It is also now known that anti-IgM treatment can have effects on the immune system in addition to the depletion of B cells and antibody. For example, Fc receptor ligation on macrophages can inhibit the production of IL-12 in response to lipopolysaccharide (48, 49). Thus, anti-IgM treatment might have affected cells other than B cells in ways which prevented control of the parasite. Similarly, while *xid* mutant mice, which have a defect in the *btk* gene, exhibit abnormal B-cell development, they also have abnormal mast cells that are deficient in cytokine production following Fcε receptor cross-linking (17). Thus, the interpretation of earlier experiments should be recast in light of recent findings. The earlier results seen with *G. muris* do not necessarily imply a requirement for B cells in controlling infections. The ability of IgH-targeted mice to control *Giardia* infections is therefore most consistent with antibody not being essential during the acute phase of infection. Importantly, the fact that depleting $CD4^+$ T cells in these B-cell-deficient mice prevented the infection from being controlled argues that this antibody-independent pathway for control of the parasite is indeed T cell dependent (Table 3).

In addition to the results of *G. muris* infections in mice, the association between chronic *Giardia* infection and hypogammaglobulinemia in humans has also been used to argue for a requirement for B cells in the immune response to *Giardia*. Hypogammaglobulinemia, however, can have multiple etiologies (51). These include various T-cell defects (43) as well as mutations of the *btk* gene, which, as noted above for *xid* in mice, is expressed by cells in addition to B cells. Thus, the association of chronic giardiasis with hypogammaglobulinemia cannot exclude the presence of a T-cell-dependent, but antibody-independent, mechanism for controlling this parasite during the acute phase of infections. Alternatively, antibody may play an important role in controlling parasites during the chronic phase of infection, when low numbers of parasites persist in the host for several months. This persistence may be particularly important in human disease, since infections can last a long time and recur. Our study did not address the role of antibody in chronic infections.

Antigenic variation is a common way for pathogenic microorganisms to evade the host antibody response. The experiments with B- and T-cell-deficient mice reported here show that while B cells are necessary to select for antigenic variants, T cells are not. This finding is in contrast to the requirement for T cells, but not B cells, to eliminate the majority of parasites during the acute phase of the infection. The discovery of the antigenic variation of the major surface protein of *G. lamblia* augured poorly for the development of a vaccine against *G. lamblia*, since the common belief was that antibodies reactive with this protein are the major protective mechanism against this parasite (34, 46a). This suggests that antigenic variation may be important in the chronic phase of infection or perhaps in the transmission of the parasite. The discovery of antibody-independent pathways for eliminating the parasite, however, once again makes the idea of a protective vaccine feasible. Indeed, it has previously been shown that infection of gerbils with *G. lamblia* isolates with different surface coats can induce some cross-protective immunity (3). A study of gerbils has also shown that administration of steroids to suppress cellular immunity can lead to reactivation of chronic infections (27), consistent with an important role for T cells in controlling the parasite. The existence of these T-cell-dependent pathways may also allow development of novel immunotherapies for giardiasis.

Our study demonstrates that, instead of B cells, $CD4^+$, αβ-TCR-bearing T cells are essential and that they function in an antibody-independent pathway to promote parasite clearance

TABLE 5. *G. lamblia* infections in cytokine-deficient mice

Expt	Mice	Day 7		Day 14		Day 28	
		Infected[a]	Intensity[b]	Infected	Intensity	Infected	Intensity
1	C57BL/6J	2/3	4.2 ± 3.8	0/3	ND[e]	0/3	ND
	B6 IL-4	3/3	1.7 ± 1.2	0/3	ND	0/3	ND
	B6 IFN-γ	3/3	11.7 ± 7.3	2/3[c]	2.0 ± 2.6	0/3	ND
2	C57BL/6J	3/3	3.5 ± 1.0	0/3	ND	0/3	ND
	B6 IL-4	3/3	3.5 ± 1.8	0/3	ND	0/3	ND
	B6 IFN-γ	2/3	4.2 ± 4.2	0/3	ND	0/3	ND
3	BALB/cJ	4/6	3.5 ± 1.0	0/6	ND	0/6	ND
	BA IL-4	2/3	3.5 ± 1.0	0/3	ND	0/2	ND
	BA IL-4R	3/4	3.5 ± 1.0	0/3	ND	0/2	ND
	BA STAT-6	0/3[d]	ND	0/3	ND	0/3	ND

[a] The number of mice with detectable parasites in the gut/total number of mice analyzed on the day indicated postinfection.
[b] The average ± standard deviation of the number of parasites/mouse × 10^4.
[c] $P = 0.16$ versus the value for C57BL/6J mice.
[d] $P = 0.15$ versus the value for BALB/c mice.
[e] ND, none detected.

(Tables 1, 3, and 4). The requirement for $CD4^+$ T cells was not unexpected since anti-CD4 treatment was previously shown to prolong *G. muris* infections (20). Similarly, because the parasite is noninvasive, it would be unlikely for $CD8^+$, class I major histocompatibility complex-restricted T cells to be important in their control. Indeed, class I-deficient mice (β_2 microglobulin knockouts) controlled parasites as well as wild-type controls (data not shown). Finally, data from human studies may also suggest a role for T cells in controlling *Giardia* infections. *Giardia* infections are associated with nodular lymphoid hyperplasia in humans, a disease associated with decreased B- and T-cell functions (29, 36, 54).

A prominent feature of the mucosal immune system is the presence of T cells bearing γδ TCRs (24). These, however, are not required for protection against *Giardia* (Table 4). Instead, the more conventional $CD4^+$ αβ-TCR-bearing T cells are required for control of parasite replication. This situation is similar to that in intracellular intestinal infections by other parasitic protozoa, e.g., *Eimeria vermiformis* and *Cryptosporidium parvum*, in which γδ-TCR-bearing T cells are also thought not to be important in immunity, although they can regulate pathology (39, 41, 55). Thus, no essential role for these γδ-TCR-bearing T cells in protection against intestinal infections can yet be assigned.

Infections with many protozoan and helminth parasites result in T-cell responses strongly biased toward either the Th1 or the Th2 subset (42). In particular, nematodes in the small intestine often lead to very strong Th2 responses, and these responses are, in some cases, absolutely required for elimination of these parasites (14, 50). On the other hand, mice deficient in IFN-γ, STAT-6, IL-4, and IL-4R all control *Giardia* infections (Table 5), indicating that Th1 and Th2 cells are not required together. This finding may reflect the fact that in the absence of IFN-γ-producing Th1 cells, Th2 cells are sufficient for control of the parasite or that in the absence of Th2 cells, Th1 cells are sufficient. Alternately, the important cytokines for controlling *Giardia* may be produced by both the Th1 and the Th2 subset or neither subset. For example IL-3, which can augment mast cell activation in vitro (31), is produced by both Th1 and Th2 cells (33), and transforming growth factor β is typically produced by mucosal T cells sometimes labeled Th3 cells (22, 56). There was some delay in parasite clearance observed in the IFN-γ-deficient mice (Table 5), suggesting that the Th1 response might have a role in controlling *Giardia* infections. However, these mice were able to completely eliminate the parasites by 4 weeks postinfection, indicating that this response is not essential. This result is consistent with earlier reports showing IFN-γ production by human intraepithelial and blood lymphocytes in response to *G. lamblia* parasites (11) and enhanced *G. muris* infections in mice treated with anti-IFN-γ antibody (52). We are currently examining the production of cytokines in the intestine during infection of wild-type mice with *G. lamblia*.

The ability of antibody-deficient mice to eliminate most of the parasites in an acute infection, however, suggests an alternate (and T-cell-dependent) effector mechanism for controlling *Giardia*. Several groups have previously implicated mast cells in the control of *G. muris* infections (12, 16, 53). And while *c-kit*$^{w/wv}$ mutant mice that are deficient in mast cells are also unable to control infections with *G. lamblia* (our unpublished observations), it remains to be shown that reconstitution of these mice with mast cells restores immunity to *Giardia* and that other aspects of the *c-kit* mutant phenotype are not responsible. And while several IgE-independent pathways have been shown to exist for the activation of mast cells in vitro and in vivo (reviewed in reference 31 and see references 6, 21, and 30), it is difficult to reconcile a role for mast cells with the kinetics of the anti-*Giardia* response and the lack of effect of eliminating IL-4 from the system since it is a mast cell growth factor (31). *G. lamblia* has also been shown to be susceptible to defensins, antimicrobial peptides whose production might be influenced by T-cell products (4). The T-cell-dependent pathways responsible for controlling *Giardia* replication remain to be elucidated.

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REFERENCES

1. **Adam, R. D.** 1991. The biology of *Giardia* spp. Microbiol. Rev. **55:**706–732.
2. **Aggarwal, A., and T. E. Nash.** 1988. Antigenic variation of *Giardia lamblia* in vivo. Infect. Immun. **56:**1420–1423.
3. **Aggarwal, A., and T. E. Nash.** 1987. Comparison of two antigenically distinct *Giardia lamblia* isolates in gerbils. Am. J. Trop. Med. Hyg. **36:**325–332.
4. **Aley, S. B., M. Zimmerman, M. Hetsko, M. E. Selsted, and F. D. Gillin.** 1994. Killing of *Giardia lamblia* by cryptdins and cationic neutrophil peptides. Infect. Immun. **62:**5397–5403.
5. **Anders, R. F., I. C. Roberts-Thomson, and G. F. Mitchell.** 1982. Giardiasis in mice: analysis of humoral and cellular immune responses to *Giardia muris*. Parasite Immunol. **4:**47–57.
6. **Bhattacharyya, S. P., I. Drucker, T. Reshef, A. S. Kirshenbaum, D. D. Metcalfe, and Y. A. Mekori.** 1998. Activated T lymphocytes induce degranulation and cytokine production by human mast cells following cell-to-cell contact. J. Leukoc. Biol. **63:**337–341.
7. **Byrd, L. G., J. T. Conrad, and T. E. Nash.** 1994. *Giardia lamblia* infections in adult mice. Infect. Immun. **62:**3583–3585.
8. **Dalton, D. K., S. Pitts-Meek, S. Keshav, I. S. Figari, A. Bradley, and T. A. Stewart.** 1993. Multiple defects of immune cell function in mice with disrupted interferon-gamma genes. Science **259:**1739–1742.
9. **Deitsch, K. W., E. R. Moxon, and T. E. Wellems.** 1997. Shared themes of antigenic variation and virulence in bacterial, protozoal, and fungal infections. Microbiol. Mol. Biol. Rev. **61:**281–293.
10. **Dialynas, D. P., Z. S. Quan, K. A. Wall, A. Pierres, J. Quintans, M. R. Loken, M. Pierres, and F. W. Fitch.** 1983. Characterization of the murine T cell surface molecule, designated L3T4, identified by monoclonal antibody GK1.5: similarity of L3T4 to the human Leu-3/T4 molecule. J. Immunol. **131:**2445–2451.
11. **Ebert, E. C.** 1999. *Giardia* induces proliferation and interferon gamma production by intestinal lymphocytes. Gut **44:**342–346.
12. **Erlich, J. H., R. F. Anders, I. C. Roberts-Thomson, J. W. Schrader, and G. F. Mitchell.** 1983. An examination of differences in serum antibody specificities and hypersensitivity reactions as contributing factors to chronic infection with the intestinal protozoan parasite, *Giardia muris*, in mice. Aust. J. Exp. Biol. Med. Sci. **61:**599–615.
13. **Gottstein, B., and T. E. Nash.** 1991. Antigenic variation in *Giardia lamblia*: infection of congenitally athymic nude and scid mice. Parasite Immunol. **13:**649–659.
14. **Grencis, R. K.** 1997. Th2-mediated host protective immunity to intestinal nematode infections. Philos. Trans. R. Soc. Lond. Ser. B **352:**1377–1384.
15. **Groh, V., A. Steinle, S. Bauer, and T. Spies.** 1998. Recognition of stress-induced MHC molecules by intestinal epithelial γδ T cells. Science **279:** 1737–1740.
16. **Hardin, J. A., A. G. Buret, M. E. Olson, M. H. Kimm, and D. G. Gall.** 1997. Mast cell hyperplasia and increased macromolecular uptake in an animal model of giardiasis. J. Parasitol. **83:**908–912.
17. **Hata, D., Y. Kawakami, N. Inagaki, C. S. Lantz, T. Kitamura, W. N. Khan, M. Maeda-Yamamoto, T. Miura, W. Han, S. E. Hartman, L. Yao, H. Nagai, A. E. Goldfeld, F. W. Alt, S. J. Galli, O. N. Witte, and T. Kawakami.** 1998. Involvement of Bruton's tyrosine kinase in FcεRI-dependent mast cell degranulation and cytokine production. J. Exp. Med. **187:**1235–1247.
18. **Hautus, M. A., H. Abdillahi, and J. J. Laarman.** 1987. Circulating IgG and IgA anti-*Giardia lamblia* antibodies in sera of symptomatic giardiasis patients. Acta Leiden **56:**47–55.
19. **Heyworth, M. F.** 1986. Antibody response to *Giardia muris* trophozoites in mouse intestine. Infect. Immun. **52:**568–571.
20. **Heyworth, M. F., J. R. Carlson, and T. H. Ermak.** 1987. Clearance of *Giardia muris* infection requires helper/inducer T lymphocytes. J. Exp. Med. **165:** 1743–1748.
21. **Inamura, N., Y. A. Mekori, S. P. Bhattacharyya, P. J. Bianchine, and D. D. Metcalfe.** 1998. Induction and enhancement of FcεRI-dependent mast cell

degranulation following coculture with activated T cells: dependency on ICAM-1- and leukocyte function-associated antigen (LFA)-1-mediated heterotypic aggregation. J. Immunol. **160:**4026–4033.
22. **Inobe, J., A. J. Slavin, Y. Komagata, Y. Chen, L. Liu, and H. L. Weiner.** 1998. IL-4 is a differentiation factor for transforming growth factor-beta secreting Th3 cells and oral administration of IL-4 enhances oral tolerance in experimental allergic encephalomyelitis. Eur. J. Immunol. **28:**2780–2790.
23. **Itohara, S., P. Mombaerts, J. Lafaille, J. Iacomini, A. Nelson, A. R. Clarke, M. L. Hooper, A. Farr, and S. Tonegawa.** 1993. T cell receptor δ gene mutant mice: independent generation of alpha beta T cells and programmed rearrangements of γδ TCR genes. Cell **72:**337–348.
24. **Kagnoff, M. F.** 1998. Current concepts in mucosal immunity. III. Ontogeny and function of γδ T cells in the intestine. Am. J. Physiol. **274:**G455–G458.
25. **Kaplan, M. H., U. Schindler, S. T. Smiley, and M. J. Grusby.** 1996. Stat6 is required for mediating responses to IL-4 and for development of Th2 cells. Immunity **4:**313–319.
26. **Kitamura, D., J. Roes, R. Kuhn, and K. Rajewsky.** 1991. A B cell-deficient mouse by targeted disruption of the membrane exon of the immunoglobulin μ chain gene. Nature **350:**423–426.
27. **Lewis, P. D., Jr., M. Belosevic, G. M. Faubert, L. Curthoys, and J. D. MacLean.** 1987. Cortisone-induced recrudescence of *Giardia lamblia* infections in gerbils. Am. J. Trop. Med. Hyg. **36:**33–40.
28. **Loftness, T. J., S. L. Erlandsen, I. D. Wilson, and E. A. Meyer.** 1984. Occurrence of specific secretory immunoglobulin A in bile after inoculation of *Giardia lamblia* trophozoites into rat duodenum. Gastroenterology **87:** 1022–1029.
29. **Matuchansky, C., G. Touchard, M. Lemaire, P. Babin, F. Demeocq, Y. Fonck, M. Meyer, and J. L. Preud'Homme.** 1985. Malignant lymphoma of the small bowel associated with diffuse nodular lymphoid hyperplasia. N. Engl. J. Med. **313:**166–171.
30. **Mekori, Y. A., N. Inamura, and D. D. Metcalfe.** 1996. Induction of mast cell degranulation by heterotypic aggregation with T cells. J. Allergy Clin. Immunol. **97:**263A.
31. **Metcalfe, D. D., D. Baram, and Y. A. Mekori.** 1997. Mast cells. Physiol. Rev. **77:**1033–1079.
32. **Mombaerts, P., A. R. Clarke, M. A. Rudnicki, J. Iacomini, S. Itohara, J. J. Lafaille, L. Wang, Y. Ichikawa, R. Jaenisch, M. L. Hooper, et al.** 1992. Mutations in T-cell antigen receptor genes α and β block thymocyte development at different stages. Nature **360:**225–231.
33. **Mosmann, T. R., and R. L. Coffman.** 1989. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu. Rev. Immunol. **7:**145–173.
34. **Nash, T. E., and A. Aggarwal.** 1986. Cytotoxicity of monoclonal antibodies to a subset of *Giardia* isolates. J. Immunol. **136:**2628–2632.
35. **Nash, T. E., A. Aggarwal, R. D. Adam, J. T. Conrad, and J. W. Merritt, Jr.** 1988. Antigenic variation in *Giardia lamblia*. J. Immunol. **141:**636–641.
36. **Nicolov, N. P., S. G. Stoinov, D. M. Todorov, M. A. Drumtscheva, and V. K. Matov.** 1983. Nodular lymphoid hyperplasia of the jejunal mucosa associated with hypogammaglobulinemia. Increased number of mast cells in the jejunal mucosa. Dtsch. Z. Verdau Stoffwechselkr. **43:**157–163.
37. **Noben-Trauth, N., P. Kropf, and I. Muller.** 1996. Susceptibility to *Leishmania major* infection in interleukin-4-deficient mice. Science **271:**987–990.
38. **Noben-Trauth, N., L. D. Shultz, F. Brombacher, J. F. Urban, Jr., H. Gu, and W. E. Paul.** 1997. An interleukin 4 (IL-4)-independent pathway for CD4+ T cell IL-4 production is revealed in IL-4 receptor-deficient mice. Proc. Natl. Acad. Sci. USA **94:**10838–10843.
39. **Roberts, S. J., A. L. Smith, A. B. West, L. Wen, R. C. Findly, M. J. Owen, and A. C. Hayday.** 1996. T-cell αβ+ and γδ+ deficient mice display abnormal but distinct phenotypes toward a natural, widespread infection of the intestinal epithelium. Proc. Natl. Acad. Sci. USA **93:**11774–11779.
40. **Roberts-Thomson, I. C.** 1993. Genetic studies of human and murine giardiasis. Clin. Infect. Dis. **16**(Suppl. 2)**:**S98–S104.
41. **Rose, M. E., P. Hesketh, L. Rothwell, and R. A. Gramzinski.** 1996. T-cell receptor γδ lymphocytes and *Eimeria vermiformis* infection. Infect. Immun. **64:**4854–4858.
42. **Sher, A., and R. L. Coffman.** 1992. Regulation of immunity to parasites by T cells and T cell-derived cytokines. Annu. Rev. Immunol. **10:**385–409.
43. **Sneller, M. C., W. Strober, E. Eisenstein, J. S. Jaffe, and C. Cunningham-Rundles.** 1993. NIH conference. New insights into common variable immunodeficiency. Ann. Intern. Med. **118:**720–730.
44. **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.
45. **Snider, D. P., D. Skea, and B. J. Underdown.** 1988. Chronic giardiasis in B-cell-deficient mice expressing the *xid* gene. Infect. Immun. **56:**2838–2842.
46. **Stäger, S., and N. Müller.** 1997. *Giardia lamblia* infections in B-cell-deficient transgenic mice. Infect. Immun. **65:**3944–3946.
46a. **Stäger, S., and N. Müller.** 1997. Systemic and local antibody response in mice induced by a recombinant peptide fragment from *Giardia lamblia* variant surface protein (VSP) H7 produced by a *Salmonella typhimurium* vaccine strain. Int. J. Parasitol. **27:**965–971.
47. **Stevens, D. P., D. M. Frank, and A. A. Mahmoud.** 1978. Thymus dependency of host resistance to *Giardia muris* infection: studies in nude mice. J. Immunol. **120:**680–682.
48. **Sutterwala, F. S., G. J. Noel, R. Clynes, and D. M. Mosser.** 1997. Selective suppression of interleukin-12 induction after macrophage receptor ligation. J. Exp. Med. **185:**1977–1985.
49. **Sutterwala, F. S., G. J. Noel, P. Salgame, and D. M. Mosser.** 1998. Reversal of proinflammatory responses by ligating the macrophage Fcγ receptor type I. J. Exp. Med. **188:**217–222.
50. **Urban, J. F., Jr., K. B. Madden, A. Svetic, A. Cheever, P. P. Trotta, W. C. Gause, I. M. Katona, and F. D. Finkelman.** 1992. The importance of Th2 cytokines in protective immunity to nematodes. Immunol. Rev. **127:**205–220.
51. **van der Meer, J. W., and B. J. Zegers.** 1994. Agammaglobulinaemia. Neth. J. Med. **45:**250–256.
52. **Venkatesan, P., R. G. Finch, and D. Wakelin.** 1996. Comparison of antibody and cytokine responses to primary *Giardia muris* infection in H-2 congenic strains of mice. Infect. Immun. **64:**4525–4533.
53. **Venkatesan, P., R. G. Finch, and D. Wakelin.** 1997. A comparison of mucosal inflammatory responses to *Giardia muris* in resistant B10 and susceptible BALB/c mice. Parasite Immunol. **19:**137–143.
54. **Ward, H., K. N. Jalan, T. K. Maitra, S. K. Agarwal, and D. Mahalanabis.** 1983. Small intestinal nodular lymphoid hyperplasia in patients with giardiasis and normal serum immunoglobulins. Gut **24:**120–126.
55. **Waters, W. R., and J. A. Harp.** 1996. *Cryptosporidium parvum* infection in T-cell receptor (TCR)-alpha- and TCR-delta-deficient mice. Infect. Immun. **64:**1854–1857.
56. **Weiner, H. L., A. Friedman, A. Miller, S. J. Khoury, A. al-Sabbagh, L. Santos, M. Sayegh, R. B. Nussenblatt, D. E. Trentham, and D. A. Hafler.** 1994. Oral tolerance: immunologic mechanisms and treatment of animal and human organ-specific autoimmune diseases by oral administration of autoantigens. Annu. Rev. Immunol. **12:**809–837.

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