Cryptosporidium parvum Infection in T-Cell Receptor (TCR)- α - and TCR- δ -Deficient Mice

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Mice deficient in either $\alpha\beta$ or $\gamma\delta$ T cells were more susceptible to infection with the protozoan parasite *Cryptosporidium parvum* than were control mice. Both neonatal and adult $\alpha\beta$ -T-cell-deficient mice developed chronic infection. $\gamma\delta$ -T-cell-deficient neonatal mice were more susceptible than control mice but were able to clear the infection. Adult $\gamma\delta$ -T-cell-deficient mice were not susceptible to infection. These data indicate that $\alpha\beta$ T cells are important for resistance to *C. parvum* while $\gamma\delta$ T cells have a less critical role.

Cryptosporidium parvum is an intracellular protozoan parasite that causes diarrheal disease in humans and various other mammals (8). In immunocompetent hosts, the disease is mild and self-limiting. In immunosuppressed hosts, such as AIDS patients, the disease is often chronic and life threatening (25). Management of the disease in chronically infected individuals is hindered by the lack of a drug effective against the parasite (8). A better understanding of host responses to the parasite could lead to a more effective therapy for individuals infected with *C. parvum*.

Previous experiments have shown that T lymphocytes are necessary for resistance to and clearance of *C. parvum* infection in mice (13, 26). These studies demonstrated that *C. parvum*-challenged athymic mice (which lack T cells) develop chronic illness characterized by intermittent diarrhea and continuous shedding of oocysts. Other experiments have examined the roles of various T-cell subsets and cytokines (1, 6, 27). These studies demonstrated that CD4⁺ (T-helper) cells and gamma interferon are required to limit the duration and severity of infection, respectively. However, no studies have examined the roles of T-cell subsets which are defined by T-cell receptor (TCR) chains expressed on the surface of lymphocytes (i.e., $\alpha\beta$ T cells and $\gamma\delta$ T cells).

Recent developments in molecular genetics have made it possible to create mutant mice with a distinct genetic defect (5). These mice are useful models for the study of immune mechanisms underlying host resistance to many infectious agents (7, 15, 16). Disruption of the TCR-constant- α gene causes the complete loss of T cells bearing TCR $\alpha\beta$ chains (20, 22). Similarly, disruption of the TCR-constant- δ gene causes the complete loss of T cells bearing TCR $\gamma\delta$ chains (14). TCR-deficient mice have been utilized in studies of various protozoal, bacterial, and viral infections (7, 16). Studies have shown distinct roles for $\alpha\beta$ T cells and more subtle roles for $\gamma\delta$ T cells in several infections involving intracellular pathogens, i.e., *Listeria* spp., *Plasmodium* spp., and *Mycobacterium* spp. (16, 18, 19, 24).

In the present study, we examined the roles of $\alpha\beta$ and $\gamma\delta$ T cells in *C. parvum* infection of mice. We found that neonatal $\alpha\beta$ -T-cell-deficient mice challenged with *C. parvum* became persistently infected with the parasite. In contrast, neonatal $\gamma\delta$ -T-cell-deficient mice and C57BL/6 control mice challenged

with *C. parvum* did not become persistently infected with the parasite. However, neonatal $\gamma\delta$ -T-cell-deficient mice did shed oocysts for a longer period than did C57BL/6 control mice. Adult $\alpha\beta$ -T-cell-deficient mice challenged with *C. parvum* became persistently infected, while C57BL/6 and $\gamma\delta$ -T-cell-deficient mice challenged as adults with *C. parvum* did not become infected.

Breeding pairs of TCR- α -deficient, TCR- δ -deficient, and C57BL/6 control mice (the background strain for TCR-deficient mice) were purchased from Jackson Laboratories (Bar Harbor, Maine). A breeding colony was established and maintained at the National Animal Disease Center, U.S. Department of Agriculture, Agricultural Research Service (Ames, Iowa) for the generation of both neonates and adult mice for the experiments. Breeding stocks of TCR-deficient mice were housed on a laminar flow rack in HEPA-filtered hooded cages. The breeding stock received filtered distilled water and autoclaved rodent chow (Harlan Teklad, Madison, Wis.). Mice utilized in the experiments received tap water and autoclaved feed.

Purified oocysts were isolated from feces collected from calves experimentally inoculated with *C. parvum* oocysts by a method described previously (9). Oral challenge of mice consisted of 10⁴ oocysts (neonatal study) or 5 $\times$ 10⁵ oocysts (adult study) in 50 μ l of 0.15 M phosphate-buffered saline solution. Mice were orally challenged with *C. parvum* oocysts at 1 week of age (neonatal study) or 8 weeks of age (adult study) by gavage with a 26-gauge needle with tygon tubing fitted over the end of the needle (neonatal challenge) or a 24-gauge animal feeding tube (adult challenge).

Fecal pellets were collected by placing individual mice into beakers until they defecated. Fresh pellets were then smeared onto glass slides, stained with carbol fuchsin, and examined for the presence of *C. parvum* oocysts. Fecal smears were checked weekly. At the end of each experiment, mice were euthanized and intestinal sections from the distal ileum-cecum and proximal colon were fixed in 10% formalin and embedded in paraffin. Histologic sections were cut at a thickness of 4 μ m, stained with hematoxylin and eosin, and examined microscopically for *C. parvum*.

We prepared two-by-two contingency tables containing data representing the number of mice infected with *C. parvum* as determined by either oocyst shedding or the presence of the parasite on histologic examination. Comparisons were then made by using either the χ^2 or Fisher's exact test. Infectivity scores from histologic sections were analyzed by one-way anal-

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FIG. 1. Percentages of mice shedding *C. parvum* oocysts after oral challenge with the parasite at 1 week of age. $\square$, data from $\gamma\delta$ -T-cell-deficient mice; $\blacktriangle$, data from $\alpha\beta$ -T-cell-deficient mice; $\bullet$, data from C57BL/6 control mice.

ysis of variance and Tukey-Kramer multiple-comparison tests. Differences between groups were considered significant if probability values of <0.05 were obtained.

Prior to the experiments, flow cytometric analyses of spleen cells from mice in the breeding colony confirmed deficiency of $\alpha\beta$ T cells in TCR- α -deficient mice, deficiency of $\gamma\delta$ T cells in TCR- δ -deficient mice, and normal numbers of $\alpha\beta$ and $\gamma\delta$ T cells in C57BL/6 mice (data not shown).

The neonatal study consisted of two independent experiments. The oocyst shedding data are combined and shown in Fig. 1. Mice were euthanized at 4 weeks of age in the first experiment and 8 weeks of age in the second experiment. At 2 weeks of age (1 week postchallenge), all mice (C57BL/6, TCR- δ -deficient, and TCR- α -deficient mice) were shedding oocysts. At 3 weeks of age, significantly ($P < 0.05$) greater numbers of TCR- δ - and TCR- α -deficient mice were shedding oocysts than were C57BL/6 control mice. Significantly ($P < 0.05$) more TCR- α -deficient mice were shedding oocysts than were either of the other two groups from 5 weeks of age until the end of the second experiment (8 weeks of age). At 8 weeks of age, 100% of TCR- α -deficient mice were shedding oocysts. A decreasing number of TCR- δ -deficient mice shed oocysts from 3 weeks of age until 7 weeks of age, at which time none of the

mice in this group were shedding oocysts. At 6 weeks of age, the difference in levels of oocyst shedding between TCR- δ -deficient mice and C57BL/6 control mice was no longer significant. No oocysts were detected from C57BL/6 control mice after 3 weeks of age.

Histologic examination of intestinal sections from mice challenged as neonates and euthanized at 4 weeks of age showed that significantly ($P < 0.05$) greater numbers of TCR- δ -deficient and TCR- α -deficient mice were infected with *C. parvum* than were C57BL/6 control mice (Table 1). Both TCR- δ - and TCR- α -deficient mice showed a significantly ($P < 0.05$) greater severity of infection than did C57BL/6 control mice in both ileum-cecum and proximal colon sections. In addition, ileum-cecum sections from TCR- α -deficient mice showed a significantly ($P < 0.05$) greater severity of infection than that seen in ileum-cecum sections from TCR- δ -deficient mice (Table 1). Intestinal sections of mice challenged as neonates and euthanized at 8 weeks of age showed that significantly ($P < 0.05$) greater numbers of TCR- α -deficient mice were infected with *C. parvum* than were either C57BL/6 control mice or TCR- δ -deficient mice (Table 1). TCR- α -deficient mice had a greater severity of infection than did either C57BL/6 or TCR- δ -deficient mice, as evidenced by significantly ($P < 0.05$) greater infectivity scores from ileum-cecum and proximal colon sections (Table 1).

When challenged with *C. parvum* as adults, few C57BL/6 control mice or TCR- δ -deficient mice shed oocysts (Fig. 2). Initially, few TCR- α -deficient mice shed oocysts. However, by 7 weeks postchallenge (15 weeks of age) until the end of the experiment (20 weeks of age), approximately 60 to 70% of TCR- α -deficient mice shed oocysts.

Histologic examination of intestinal sections from mice challenged with *C. parvum* as adults showed that significantly ($P < 0.05$) greater numbers of TCR- α -deficient mice were infected with *C. parvum* than were either C57BL/6 control mice or TCR- δ -deficient mice (Table 2). TCR- α -deficient mice showed a significantly ($P < 0.05$) greater severity of infection in both ileum-cecum and proximal colon sections than did either C57BL/6 or TCR- δ -deficient mice (Table 2).

The mechanisms of immunity by which *C. parvum* infection is controlled and resolved are not clearly defined. Considering the intracellular location of the parasite in the host, it is not likely that antibodies play a major role (11, 21, 23). It is more likely that cellular effector mechanisms are of major importance in the host response to *C. parvum*. Previous studies have shown a requirement for the presence of CD4⁺ T cells and gamma interferon in limiting the duration and severity, respec-

TABLE 1. Histology results of mice challenged with 10^4 *C. parvum* oocysts at 1 week of age

Mouse group (<i>n</i>)	% Positive ^a	Mean infectivity score ^b ± SEM of section type:	
		Ileum-cecum	Proximal colon
Mice euthanized at 4 wk of age			
C57BL/6 (22)	5	0.0 ± 0.0	0.05 ± 0.05
TCR-δ deficient (27)	67 ^c	0.67 ± 0.11 ^c	0.41 ± 0.10 ^c
TCR-α deficient (31)	81 ^c	1.10 ± 0.13 ^d	0.52 ± 0.09 ^c
Mice euthanized at 8 wk of age			
C57BL/6 (17)	0	0.0 ± 0.0	0.0 ± 0.0
TCR-δ deficient (28)	4	0.04 ± 0.04	0.0 ± 0.0
TCR-α deficient (24)	100 ^d	1.67 ± 0.10 ^d	1.33 ± 0.10 ^d

^a Mice were considered positive if *C. parvum* was seen in either ileum-cecum or proximal colon histologic sections.

^b Infectivity scores for histologic sections were determined as follows: 0, no *C. parvum* seen; 1, few *C. parvum* seen; 2, many *C. parvum* seen.

^c $P < 0.05$ (compared with C57BL/6 control mice euthanized at the same age).

^d $P < 0.05$ (compared with C57BL/6 control mice and TCR- δ -deficient mice euthanized at the same age).

FIG. 2. Percentages of mice shedding *C. parvum* oocysts after oral challenge with the parasite at 8 weeks of age. □, data from $\gamma\delta$ -T-cell-deficient mice; ▲, data from $\alpha\beta$ -T-cell-deficient mice; ●, data from C57BL/6 control mice. Data presented are the means $\pm$ standard errors of the mean of two independent experiments.

tively, of *C. parvum* infection in mice (6, 27). Also, CD4-enriched splenocytes of mice primed in vivo with *C. parvum* proliferate and produce gamma interferon in response to *C. parvum* antigen in vitro (10). The relative importance of T lymphocytes expressing the $\alpha\beta$ or $\gamma\delta$ form of the TCR in resistance to *C. parvum* infection has not been previously addressed. Studies with mice deficient in either the $\alpha\beta$ or $\gamma\delta$ TCR have demonstrated the importance of $\alpha\beta$ -TCR⁺ cells in clearance of other intracellular pathogens such as *Listeria* spp., *Plasmodium* spp., and *Mycobacterium* spp. (16, 18, 19, 24). In contrast, $\gamma\delta$ -TCR⁺ cells appear to have a less critical role.

In the present study, mice deficient in $\alpha\beta$ T cells were susceptible to *C. parvum* infection both as neonates and as adults and were unable to clear the parasite. Since most CD4⁺ cells express the $\alpha\beta$ TCR and can be a rich source of gamma interferon, the increased susceptibility and inability of $\alpha\beta$ -T-cell-deficient mice to clear infection could be due to insufficient numbers of CD4⁺ cells and the concomitant lack of gamma interferon production. One possible pathway requiring both CD4⁺ cells and gamma interferon for resistance to *C. parvum* might begin with the uptake of luminal parasite antigens by M cells, followed by the presentation of antigens to CD4⁺ T cells located within Peyer's patches or lymphoid fol-

icles of the intestinal tract. The T cells may then become activated and migrate to the lamina propria or intestinal epithelium and elaborate cytokines such as gamma interferon. Gamma interferon could then have direct effects on infected or potentially infected enterocytes. In addition, gamma interferon could activate lymphocytes or macrophages within the intestinal epithelium or lamina propria. These cells could then provide effector functions in the clearance of the parasite.

Delayed clearance of the parasite by $\gamma\delta$ -T-cell-deficient mice suggests that $\gamma\delta$ T cells also play a role in *C. parvum* infection. A possible role for $\gamma\delta$ T cells in *C. parvum* infection could be their ability to secrete epithelial growth factors. For instance, activated $\gamma\delta$ T cells within the intestinal epithelium can produce the epithelial mitogen keratinocyte growth factor (2), which may be necessary for the proficient replacement of damaged enterocytes. It has been shown that mice deficient in $\gamma\delta$ T cells have a decreased epithelial cell turnover (17). In addition, $\gamma\delta$ TCRs are able to bind products of damaged cells, such as heat shock proteins (3, 4, 12). Thus, $\gamma\delta$ T cells may mediate nonspecific immunity through recognition of heat shock proteins expressed on the surface of *C. parvum*-infected enterocytes. The delayed clearance of *C. parvum* in neonatally challenged $\gamma\delta$ -T-cell-deficient mice compared with that of C57BL/6 control mice could thus be due to a lack of $\gamma\delta$ T cells located within the intestinal epithelium and the resultant loss of cytotoxic activity and/or cytokine production. Another intracellular parasite, *Listeria monocytogenes*, induces gamma interferon production through intraepithelial $\gamma\delta$ T cells (28). As stated above, gamma interferon is important in limiting the severity of *C. parvum* infection in mice (27). Thus, more specifically, the delayed clearance in $\gamma\delta$ -T-cell-deficient mice could reflect the loss of gamma interferon production by $\gamma\delta$ T cells within the intestinal epithelium.

In conclusion, it is clear that both $\alpha\beta$ and $\gamma\delta$ T cells are important in defense against *C. parvum* infection in neonatal mice. $\gamma\delta$ T cells appear to be important for the initial control of *C. parvum* infection, whereas $\alpha\beta$ T cells appear to be required for complete clearance of the parasite.

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TABLE 2. Histology results of 20-week-old mice challenged with 2×10^5 *C. parvum* oocysts at 8 weeks of age

Mouse group (n)	% Positive ^a	Mean infectivity score ^b $\pm$ SEM of section type:	
		Ileum-cecum	Proximal colon
C57BL/6 (16)	0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
TCR- δ deficient (24)	0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
TCR- α deficient (31)	77 ^c	0.81 $\pm$ 0.10 ^c	0.55 $\pm$ 0.09 ^c

^a Mice were considered positive if *C. parvum* was seen in either ileum-cecum or proximal colon histologic sections.

^b Infectivity scores for histologic sections were determined as follows: 0, no *C. parvum* seen; 1, few *C. parvum* seen; 2, many *C. parvum* seen.

^c $P < 0.05$ (compared with C57BL/6 control mice and TCR- δ -deficient mice).

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