MyD88-Dependent Pathways Mediate Resistance to Cryptosporidium parvum Infection in Mice

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Cryptosporidium spp. cause diarrheal disease worldwide. Innate immune responses mediating resistance to this parasite are not completely understood. To determine whether MyD88-dependent pathways play a role in resistance to Cryptosporidium parvum, we compared the course of infection in MyD88−/− mice to that in their wild-type (WT) littermate controls. Three- to 4-week-old mice were infected with C. parvum, and infection was monitored by quantifying fecal oocyst shedding. Twelve days postinfection, the histology of the intestines was examined to quantify intestinal parasite burden and to determine if there were any pathological changes. Fecal oocyst shedding and intestinal parasite burden were significantly greater in MyD88−/− mice than in littermate controls. Nonetheless, both WT and MyD88−/− mice cleared the infection within 3 weeks. These results indicate that MyD88-dependent pathways are involved in mediating initial resistance to C. parvum. Since gamma interferon (IFN-γ) is known to mediate resistance to C. parvum, we also studied infection in MyD88−/− mice and WT controls in which this cytokine was temporarily neutralized. Fecal oocyst shedding, as well as intestinal parasite burden, intestinal inflammation, and mortality, was significantly greater in MyD88−/− mice in which IFN-γ was neutralized than in IFN-γ-neutralized WT mice or in MyD88−/− mice in which this cytokine was active. These results suggest that MyD88 and IFN-γ had an additive effect in conferring protection from C. parvum infection. While this study confirms the importance of IFN-γ in conferring resistance to infection with C. parvum, it suggests that MyD88-mediated pathways also play a role in innate immunity to this parasite.

Cryptosporidium spp. are apicomplexan parasites that cause cryptosporidiosis, a diarrheal illness, in animals and humans worldwide (15, 24). In immunocompromised individuals, infection is usually asymptomatic or mild and self-limiting. However, in immunocompromised individuals, such as AIDS patients, infection can be severe, chronic, and life threatening. In developing countries, cryptosporidiosis is a significant cause of persistent diarrhea in children, particularly those who are malnourished (15). In these countries, cryptosporidial infection in early childhood has been reported to be associated with subsequent impairment in growth and physical fitness (5, 17).

Immune responses to C. parvum infection are not completely understood. However, it is clear that cell-mediated immunity is necessary for control of infection (34, 38). The cytokine gamma interferon (IFN-γ) is an critically important in adaptive as well as innate immune responses to C. parvum infection in mice. Immunocompetent adult mice, which are normally resistant to C. parvum infection (13), develop an acute infection when IFN-γ is temporarily neutralized (39). C. parvum infection in IFN-γ−/− mice results in acute infection which is rapidly lethal in IFN-γ−/− C57BL/6 mice (37, 39) but resolves in IFN-γ−/− BALB/c mice (37). The cytokines interleukin-4 (IL-4) and tumor necrosis factor alpha have been implicated in resolution of infection in the latter strain of mice (37). Furthermore, SCID mice develop a delayed-onset chronic infection, whereas IFN-γ−/− mice carrying the scid mutation succumb to acute infection, indicating the importance of this cytokine in the activation of innate immune responses to cryptosporidiosis in mice (18). We have recently shown that IFN-γ is produced by intestinal CD8+ intraepithelial lymphocytes as early as 24 h after C. parvum infection in mice (25).

In humans, the role of IFN-γ is less clear. Immunocompetent human volunteers with prior exposure to Cryptosporidium, but not those without previous infection, expressed IFN-γ in the intestine following experimental challenge with the parasite. Expression of IFN-γ mediate resistance to infection in sensitized individuals (41). Subsequent studies with human volunteers reported intestinal IL-15 expression, which was associated with relative resistance to infection in individuals not expressing IFN-γ, implicating IL-15 in IFN-γ-independent immune responses (35).

While innate immune responses are clearly involved in the
resistance of mice to *C. parvum* infection, the mechanisms by which the innate immune system is able to recognize and respond to *C. parvum* are unknown. Myeloid differentiation protein 88 (MyD88) is an adaptor protein recruited upon stimulation of several pathways of the innate immune system, including the IL-1 receptor, IL-18 receptor, and Toll-like receptor (TLR) pathways (2, 3). These pathways, which involve receptors containing the Toll/IL-1 receptor domain, are important in mediating resistance to a number of pathogens, including protozoan parasites (reviewed in reference 14). The TLRs are a family of pattern recognition receptors critical for the detection of a wide array of pathogens, including parasites (3, 27). Most TLRS signal by recruiting MyD88. Recognition of pathogen-associated molecular patterns by TLRs and subsequent recruitment of MyD88 activate a cascade of kinases, resulting in the nuclear translocation of NF-κB, the activation of c-Jun, and the expression of inflammatory cytokine genes. In addition to mediating resistance to infection, the inflammatory response may contribute to the pathogenesis of disease.

MyD88−/− mice infected with other parasites, such as *Leishmania major* (10, 29), *Toxoplasma gondii* (6, 36), or *Trypanosoma cruzi* (4), exhibit enhanced susceptibility to infection, increased parasite burden, altered cytokine production, and impaired immune responses. The aim of this study was to determine whether MyD88-dependent pathways play a role in resistance to *C. parvum* infection. To test this hypothesis, we used mice with a targeted disruption in the MyD88 gene. Since IFN-γ is a mediator of resistance to *C. parvum*, we also assessed the effect of neutralization of this cytokine on the susceptibility of MyD88-deficient mice to infection with this parasite.

**MATERIALS AND METHODS**

**Parasites.** *C. parvum* oocysts of the Moredun isolate (30) were obtained from Saul Tzipori, Tufts University School of Veterinary Medicine, North Grafton, MA. Oocysts were treated with 10% sodium hypochlorite for 10 min on ice and washed three times with sterile phosphate-buffered saline (PBS) containing 10 mM sodium phosphate (pH 7.2), 150 mM sodium chloride prior to infection.

**Mice.** MyD88−/− mice on a mixed C57BL/6 and 129X1/SvJ (B6;129X1) background were generated at Osaka University, Japan (19), and backcrossed onto the C57BL/6 background for three generations at the University of Massachusetts Medical Center, Worcester, MA, and for two additional generations at Tufts-New England Medical Center, Boston, MA. MyD88+/− mice and MyD88+/+ littermate controls were obtained by intercross of MyD88 heterozygotes. MyD88 inheritance was determined by PCR. Genomic DNA was extracted from ear punches using a DNeasy kit (QIAGEN Inc., Valencia, CA). PCRs used the forward primer MyD88F (5′ TGGCATGCTCCTGATGTTAACC 3′) and the reverse primer MyD88R (5′ GTGAAAGACCCCAACCATTGCT 3′), and a primer targeted to the neomycin cassette NeoR (5′ ATGCCCTTCTCATGC CTTCCTGACG 3′) (23). PCR conditions were as follows: 95°C for 1 min, 65°C for 1 min, and 72°C for 30 s for 35 cycles. All mice were housed in specific-pathogen-free/viral antibody-free facilities at Tufts University School of Medicine. The protocol was approved by the Tufts-New England Medical Center Institutional Animal Care and Use Committee according to National Institutes of Health guidelines.

**Infection of mice.** Three- to 4-week-old male and female mice were infected with 107 *C. parvum* oocysts in 100 μl of PBS by oral gavage. Two independent experiments were performed. In the first experiment, six or seven mice were infected per group, and infection was monitored for 24 days. In the second experiment, 10 to 14 mice were infected per group, and infection was monitored for 33 days. Each experiment consisted of four groups. The first and second groups consisted of MyD88−/− and wild-type (WT) littermate control mice, respectively. The third and fourth groups consisted of MyD88−/− and WT littermates that were administered, 2 hours prior to infection, a single intraperitoneal injection of 1 mg of XMG 1.2, an IFN-γ neutralizing rat anti-mouse immunoglobulin monoclonal antibody (8). This antibody was obtained from Robert Coffman (DNAx Research Institute, Palo Alto, CA). Fecal pellets were collected from each mouse for 30 min three times a week. Pellets were weighed and suspended in PBS (4× wt/vol). Ten-μl portions of slurry were smeared onto microscope slides in duplicate. The slides were heat fixed and stained with Kinyoun carbol fuchsin stain (Becton Dickinson, Sparks, MD) and oocysts enumerated microscopically in 30 oil-immersion 100× objective fields as previously described (39). For each mouse, two smears were examined (i.e., a total of 60 fields) per time point, and the total number of oocysts was determined.

**Histology.** After 12 days postinfection, three to seven mice per group were euthanized by CO2 inhalation, and intestinal specimens were collected for histological examination. In addition, in the second experiment, samples for histology were collected from surviving mice (three to five per group) at day 33 postinfection. In all cases, the small intestine, cecum, and colon were removed, expressed to remove luminal contents, and fixed in 10% neutral buffered formalin. Tissues were routinely processed and 4-μm sections stained with hematoxylin and eosin. Slides were examined by a comparative pathologist blinded to sample identity. Lesions were assigned semiquantitative scores for mucosal inflammation, hyperplasia, and epithelial defects according to the following criteria. For mucosal inflammation, scores were as follows: 1, small multifocal leukocyte aggregates in the lamina propria; 2, coalescing mild to moderate leukocyte infiltrates limited to mucosa; 3, focally heavy mucosal infiltration with submucosal extension; or 4, heavy diffuse mucosal infiltration with perivascular extension into the muscularis and beyond. For hyperplasia, scores were as follows: 1, crypt of 1.5 times normal length and rare mitoses; 2, crypt of 2 times normal length and occasional mitoses; 3, crypt of 3 times normal length and common mitoses; or 4, crypt of 4 or more times normal length and abundant mitoses. For epithelial defects, scores were as follows: A score of 1 was given for hyperplasia with mild loss and mild surface defects. A score of 2 was given for focal erosions, villous blunting, atrophy, and fusion. A score of 3 was given for extensive erosions, foci ulcers, crypt abscesses, and moderate loss of crypts and villi with mild fibroplasia. A score of 4 was given for widespread and/or deep ulceration, laceral edema, and extensive loss of crypts/villi with abundant fibrous replacement. All scores were combined for each mouse and expressed as an enterocolitis index (EI).

The parasite burden in intestines was determined by counting intracellular parasitic stages (all developmental forms) in 30 oil-immersion 100× objective fields over the entire intestine using an ocular lens with a grid. The distribution of *C. parvum* infection throughout the intestine was determined at five intestinal sites: three equally spaced sites in the small intestine (terminal jejunum, proximal ileum, and distal ileum), the cecum, and the proximal colon. A semiquantitative parasite burden score was calculated for each site as described previously (16). Each site was scored as follows: 0, no infection; 1, very difficult to find parasite stages; 2, sparse but easily found parasite stages; 3, abundantly present but focally distributed parasite stages; 4, extensive presence of parasite stages covering most mucosal surfaces; or 5, extensive presence of parasite stages covering the entire mucosal surface.

**Statistical analysis.** For quantification of oocyst shedding, tissue parasite burden, parasite distribution, and histopathology, slides were examined in a blinded manner. Data were analyzed with Graph Pad Prism statistics and graphing program (Graph Pad Inc., San Diego, CA). To normalize the oocyst shedding and the parasite burden data, the numbers of oocysts detected were log transformed. To avoid taking the log of zero when no oocysts were detected, 1.0 was added to all scores before transformation (16). For the oocyst shedding and parasite burden data, the Student t test was used to compare data between groups. Mann-Whitney U tests were used to compare differences between groups for the parasite distribution and enterocolitis indices when semiquantitative analyses were used. Differences in survival between groups were compared using Kaplan-Meier survival analysis and chi-square tests. In all cases, differences were considered significant if P was less than or equal to 0.05.

**RESULTS**

*C. parvum*-infected MyD88−/− mice display greater levels of oocyst shedding, intestinal parasite burden, and enterocolitis than WT littermate controls. To determine if MyD88-dependent pathways are involved in the resistance to *C. parvum* infection, MyD88−/− mice and their WT littermate controls were infected with *C. parvum* oocysts and the courses of the infection monitored by quantification of fecal oocysts (Fig. 1a). In the WT mice, oocyst shedding was minimal. In contrast,
MyD88−/− mice shed more oocysts than WT mice. We determined the area under the concentration-time curve to integrate oocyst shedding as a function of time. This analysis revealed that MyD88−/− mice had an overall oocyst shedding level (P = 0.004) greater than that of WT littermate controls (Table 1). However, both WT and MyD88−/− mice stopped shedding oocysts by 3 weeks postinfection.

To determine whether the increase in oocyst shedding by MyD88−/− mice resulted from an increase in parasite burden in the intestine, the number of intracellular parasites in the intestines was quantified at day 12 postinfection. The intestinal parasite burden in MyD88−/− mice was 3.7 times that of WT controls (P = 0.004; Table 1). Consistent with the low level of oocyst shedding in WT mice, intestinal parasites were rarely detected in these mice (Fig. 1b). In contrast, parasites were readily observed in intestinal sections from MyD88−/− mice (Fig. 1b). Intracellular parasites were evident in epithelial cells of both villi (small intestine) and crypts. In the second experiment, we examined intestinal histology in a group of mice at day 33 postinfection. In parallel with the cessation of fecal oocyst shedding, intestinal parasites were absent from WT mice and rarely detected in the intestine of MyD88−/− mice (not shown).

To determine the distribution of C. parvum infection in the intestine, we compared the degrees of infection in the terminal jejunum, proximal ileum, distal ileum, cecum, and proximal colon using a semiquantitative scoring system. In both WT and MyD88-deficient mice, parasites were present throughout these sites in the small and large intestines. However, parasite burdens in the small intestine of MyD88 mice were higher than those in WT controls at levels that approached or exceeded statistical significance. For the terminal jejenum, the medians (25th, 75th percentiles) were 1.5 (0.5, 2.5) for the MyD88−/− mice and 0 (0, 1) for the WT mice, with a P value of 0.07; for the proximal ileum, the medians (25th, 75th percentiles) were 1 (0.5, 2.5) for MyD88−/− mice and 0 (0, 1) for WT mice, with a P value of 0.06; and for the distal ileum, the medians (25th, 75th percentiles) were 2 (1, 3) for MyD88−/− mice and 0 (0, 1) for WT mice, with a P value of 0.008.

To determine if there were histopathological changes in the intestines of C. parvum-infected MyD88−/− mice, we compared the histologies of the small and large intestines from

![FIG. 1. C. parvum fecal oocyst shedding and parasite burden in the intestines of WT and MyD88−/− mice during infection. (a) Time course of shedding from day 1 to day 21 postinfection by WT and MyD88−/− mice. Data from the second experiment are shown. (b) Intracellular stages of C. parvum in representative WT (a) and MyD88−/− (b) mice. Mice were euthanized 12 days postinfection and the intestines processed for histological analysis. Arrows point to examples of intracellular forms of the parasite. Scale bars = 10 μm.](http://iai.asm.org/)

**TABLE 1. Comparison of C. parvum infection in untreated MyD88−/− and WT mice and in those pretreated with a neutralizing antibody to IFN-γ**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Oocyst sheddingb</th>
<th>Intestinal parasite burdenc</th>
<th>Enterocolitisd</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT</td>
<td>MyD88−/−</td>
<td>P</td>
</tr>
<tr>
<td>None</td>
<td>1.2 ± 0.35</td>
<td>3.3 ± 0.43</td>
<td>0.004</td>
</tr>
<tr>
<td>Anti-IFN-γ</td>
<td>5.7 ± 0.84</td>
<td>9.5 ± 0.90</td>
<td>0.004</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
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a Oocyst shedding, intestinal parasite burden, and enterocolitis data from both experiments were combined.
b Mean ± standard error of the mean area under the curve of log number of oocysts per day.
c Mean ± standard error log number of parasites per field.
d Enterocolitis indices for ileum and cecum combined and expressed as median (25th, 75th percentiles).
* N.S., not significant.
whether the neutralization of this cytokine would alter the innate immune responses to *C. parvum* MyD88 (not shown).

Between the enterocolitis indices for the two groups of mice postinfection, histological changes were minimal or absent in MyD88-/- mice and WT mice. In agreement with the marginal levels of fecal differences between the enterocolitis indices for MyD88-/- and WT mice (Table 1). Nonetheless, oocyst shedding was much greater in MyD88-/- mice pretreated with the anti-IFN-γ mAb than in mAb-treated WT mice over the course of the infection (Fig. 3a) and by area-under-the-curve analysis (P = 0.004; Table 1). Shedding was also significantly greater in these mice than in untreated MyD88-/- mice (P < 0.0001; Table 1). Nonetheless, both WT and MyD88-/- mice pretreated with the anti-IFN-γ mAb cleared the infection within 3 weeks (Fig. 3a).

In keeping with the markedly increased oocyst shedding, the intestinal parasite burden was greater in the anti-IFN-γ mAb-treated MyD88-/- mice than in the mAb-treated WT mice (P = 0.03; Table 1) or in untreated MyD88-/- mice (P < 0.0001; Table 1). *C. parvum* was readily detected in intestinal sections from MyD88-/- and WT mice treated with the anti-IFN-γ mAb (Fig. 3b). Parasites were distributed throughout the small and large intestines in MyD88-/- and WT mice pretreated with the anti-IFN-γ mAb. However, there were significantly more parasites present in MyD88-/- mice pretreated with the anti-IFN-γ mAb than in identically treated WT mice in the terminal jejunum (P = 0.02), with a median (25th, 75th percentiles) of 4 (4, 5) for MyD88-/- mice and of 2 (3, 4) for WT mice infected with *C. parvum*.

Neutralization of IFN-γ increases the susceptibility of MyD88-/- mice to *C. parvum* infection. Since IFN-γ mediates innate immune responses to *C. parvum* (18), we determined whether the neutralization of this cytokine would alter the susceptibility of MyD88-deficient mice by infection with this parasite. To do so, we compared resistance to *C. parvum* infection in MyD88-/- and WT mice in which IFN-γ was inactivated by treatment with a neutralizing monoclonal antibody (mAb) 2 hours prior to infection. Similar to the findings of Theodos et al. (39), WT mice pretreated with the anti-IFN-γ mAb displayed high levels of oocyst shedding (P < 0.0001) compared to untreated WT mice (Table 1). However, oocyst shedding was much greater in MyD88-/- mice pretreated with the anti-IFN-γ mAb than in mAb-treated WT mice over the course of the infection (Fig. 3a) and by area-under-the-curve analysis (P = 0.004; Table 1). Shedding was also significantly greater in these mice than in MyD88-/- mice (P < 0.0001; Table 1). Nonetheless, both WT and MyD88-/- mice pretreated with the anti-IFN-γ mAb cleared the infection within 3 weeks (Fig. 3a).

In keeping with the markedly increased oocyst shedding, the intestinal parasite burden was greater in the anti-IFN-γ mAb-treated MyD88-/- mice than in the mAb-treated WT mice (P = 0.03; Table 1) or in untreated MyD88-/- mice (P < 0.0001; Table 1). *C. parvum* was readily detected in intestinal sections from MyD88-/- and WT mice treated with the anti-IFN-γ mAb (Fig. 3b). Parasites were distributed throughout the small and large intestines in MyD88-/- and WT mice pretreated with the anti-IFN-γ mAb. However, there were significantly more parasites present in MyD88-/- mice pretreated with the anti-IFN-γ mAb than in identically treated WT mice in the terminal jejunum (P = 0.02), with a median (25th, 75th percentiles) of 4 (4, 5) for MyD88-/- mice and of 2 (3, 4) for WT mice infected with *C. parvum*. MyD88-deficient and WT mice. Uninfected WT (not shown) as well as uninfected MyD88-/- (Fig. 2a [ileum] and d [cecum]) mice produced no notable histological findings. Histopathological changes were most evident in the terminal ileum and cecum of infected mice. WT mice infected with *C. parvum* displayed minimal ileitis (Fig. 2b) characterized by multifocal to coalescing aggregates of mononuclear cells and fewer polymorphonuclear cells in the lamina propria and the absence of significant typhlitis (cecal inflammation; Fig. 2e). In contrast, most of the MyD88-/- mice developed mild to moderate lymphoplasmacytic and granulocytic ileitis (Fig. 2c) and typhlitis (Fig. 2f) characterized by multifocal to coalescing aggregates of mononuclear cells in both compartments. We used a semiquantitative enterocolitis index to compare the histopathological changes in the ileum and cecum of infected mice, as well as the combined total score for the intestinal samples (Table 1). There were no significant differences between the enterocolitis indices for MyD88-/- and WT mice. In agreement with the marginal levels of fecal oocyst shedding and intestinal parasite burden at day 33 postinfection, histological changes were minimal or absent in the MyD88-/- or WT mice, respectively, with no difference between the enterocolitis indices for the two groups of mice (not shown).

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There were also crypt abscesses containing white blood cells and apoptotic epithelial cells. Semiquantitative analysis revealed that the anti-IFN-γ mAb-treated MyD88−/− mice had enterocolitis indices greater than those of identically treated WT mice at levels that approached or exceeded statistical significance in the ileum (P = 0.006; median [25th, 75th percentiles], 4.8 [4.5, 5.5] for MyD88−/− mice and 3 [2.3, 5.3] for WT mice) and cecum (P = 0.006; 2.8 [1.3, 4.8] for MyD88−/− mice and 1 [0.5, 1] for WT mice). When the total enterocolitis indices for animals were compared, combining the scores for both the ileum and cecum, the anti-IFN-γ mAb-treated MyD88−/− mice had greater levels of enterocolitis than identically treated WT mice (P = 0.004; Table 1). Enterocolitis was also significantly more severe (P = 0.0001) in the infected IFN-γ-neutralized MyD88−/− mice than in infected MyD88−/− mice in which IFN-γ was active (not shown).

During the study, none of the IFN-γ-neutralized WT mice died. However, ~50% of IFN-γ-neutralized MyD88−/− mice died or were moribund and had to be euthanized (Fig. 5; P = 0.006). There was no significant difference in the levels of oocyst shedding in the first 12 days postinfection or in intestinal parasite burdens between mice that died spontaneously and mice that were moribund and had to be euthanized (not shown).

**DISCUSSION**

To the best of our knowledge, this is the first report on the role of MyD88-dependent pathways in murine cryptosporidion.
osis and the first report on the combined effects of MyD88 and IFN-γ disruption in any parasitic infection. We report that MyD88 mediates resistance to C. parvum infection, since MyD88−/− mice have significantly greater levels of oocyst shedding and intestinal parasite burden than their wild-type littermates. We also show that a lack of MyD88 signaling combined with neutralization of IFN-γ has additive effects on the degree of severity of C. parvum infection as well as on the associated intestinal inflammation. Indeed, MyD88−/− mice in which IFN-γ is temporarily neutralized develop enterocolitis of greater severity, have greater levels of oocyst shedding and intestinal parasite burden, and have higher mortality than identically treated WT mice or than MyD88−/− mice in which this cytokine is active. While these results confirm the importance of IFN-γ in mediating resistance to C. parvum, they suggest that MyD88-mediated pathways are also involved in innate immunity to this parasite.

MyD88-dependent pathways, particularly those involving TLRs, have been shown to play a role in host defense to infections caused by several parasitic protozoa (14). MyD88−/− mice infected with T. cruzi develop enhanced parasitemia, increased mortality, and impaired cytokine production compared to WT mice (4). T. cruzi-derived glycosylphosphatidylinositol-linked mucins and glycoinositolphospholipids interact with TLR-2 (4) and TLR-4 (31), respectively. MyD88−/− mice are more susceptible to L. major infection than WT mice (10, 11, 29). Both TLR-2 (11) and TLR-4 (21) are thought to mediate MyD88-dependent resistance to this parasite. Lipophosphoglycan from L. major has been identified as the TLR-2 ligand (11). Similarly, T. gondii infection of MyD88−/− mice results in impaired cytokine production, uncontrolled parasite replication, and increased mortality (6, 36). TLR-2 (28) and recently TLR-11, which is activated by a profilin-like protein ligand (42), have been identified as important in MyD88-dependent responses to T. gondii. MyD88−/− mice have impaired IL-12 responses, implicating MyD88-dependent pathways in innate immune responses to Plasmodium species (1). More recent studies identified glycosylphosphatidylinositol sugars from P. falciparum as ligands for TLR-2 and TLR-4 (20). Activation of TLR-9 by a P. falciparum schizont extract (32) or by hemozoin, an immunologically active poly-

![Figure 4](http://iai.asm.org/) Histopathologies of the ileum and cecum of IFN-γ neutralized WT and MyD88−/− mice infected with C. parvum. Mice were sacrificed 12 days postinfection and the intestines processed for histological analysis. Representative images at 10× magnification; scale bars = 200 μm; insets at 40× magnification. Arrows indicate crypt abscesses. (a) Uninfected IFN-γ-neutralized MyD88−/− ileum (EI = 0). (b) Infected IFN-γ-neutralized WT ileum (EI = 6.0). (c) Infected IFN-γ-neutralized MyD88−/− ileum (EI = 8.5). (d) Uninfected MyD88−/− cecum (EI = 0). (e) Infected IFN-γ-neutralized WT cecum (EI = 2.5). (f) Infected IFN-γ neutralized MyD88−/− cecum (EI = 5.0).

![Figure 5](http://iai.asm.org/) Survival of C. parvum-infected WT and MyD88−/− mice with and without IFN-γ neutralization. Mice from both experiments which died spontaneously or were moribund and had to be euthanized were included in the analysis. *P* = 0.006.
mer from this parasite (9), also has been recently reported to mediate innate responses to this parasite. Thus far, there have been no studies reported on the role of MyD88-dependent pathways in activating a proinflammatory response to C. parvum.

IFN-γ is important in innate immunity to C. parvum infection in mice. However, it is not known whether early IFN-γ production by cells of the innate immune system is MyD88 dependent in C. parvum infection. In other parasitic infections, IL-12 production by dendritic cells or macrophages in response to TLR activation is believed to play a crucial role in IFN-γ production (14). It is possible that similar mechanisms may be operative in C. parvum infection, particularly since IL-12 is known to be important in immune responses to this parasite (12, 40). However, the finding that mice deficient in both IFN-γ- and MyD88-dependent pathways have more-severe infection than those deficient in MyD88 alone suggests that MyD88-independent pathways may be involved in IFN-γ-mediated innate resistance to C. parvum infection.

Both WT and MyD88−/− mice cleared the infection within 3 weeks, indicating that MyD88-mediated pathways are important in innate resistance to C. parvum but not in resolution of infection, which requires adaptive immune responses. Although the level of infection was much greater in IFN-γ-neutralized MyD88−/− mice than in MyD88−/− mice in which this cytokine was active, mice in the former group, which survived the acute infection, also cleared the parasite within 3 weeks. Resolution of infection in these mice is likely due to restoration of IFN-γ-mediated adaptive immunity, since we used a single dose of the anti-IFN-γ mAb (which has a half-life of 3 to 4 days [39]) in order to neutralize mainly the innate IFN-γ response.

C. parvum-infected WT mice had minimal intestinal inflammation, whereas infected MyD88−/− mice developed mild to moderate intestinal inflammation. IFN-γ-neutralized WT mice infected with C. parvum infection displayed moderate to severe mucosal damage and inflammation. However, C. parvum infection of IFN-γ-neutralized MyD88−/− mice resulted in severe enterocolitis, which most likely contributed to mortality in many of these mice. Our study confirms previous findings of intestinal inflammation in C. parvum-infected IFN-γ-deficient mice (22, 39). MyD88-dependent pathways have also recently been shown to play a critical role in intestinal homeostasis and resistance to epithelial injury and inflammation (33). The finding that the combined lack of MyD88-mediated and IFN-γ-dependent pathways leads to more-severe enterocolitis than that seen with either deficiency alone suggests that both pathways are involved in C. parvum-induced intestinal inflammation.

The mechanisms underlying the increased susceptibility to infection and the accompanying enterocolitis in the MyD88−/− mice and IFN-γ neutralized MyD88−/− mice are not known. It is possible that altered proinflammatory cytokine and chemokine expression may contribute to decreased resistance to infection as well as to the intestinal inflammation. It is also possible that MyD88-mediated NF-κB activation protects infected intestinal epithelial cells from parasite-induced cell death. In vitro studies have shown that C. parvum limits apoptosis by activation of NF-κB in infected cells (7, 26).

In conclusion, we demonstrate in a mouse model of crypto-

sporidiosis that pathways regulated by MyD88 mediate resistance to infection. The combined lack of MyD88 and IFN-γ results in a severe parasite burden, severe enterocolitis, and increased mortality. Thus, MyD88 and IFN-γ-dependent pathways are both required for resistance to C. parvum infection. Further studies directed at characterizing the cytokines, chemokines, and immune cell types mediating protective as well as pathological inflammatory responses in this model are warranted. Further studies are also required to identify which of the MyD88-dependent pathways are involved in mediating resistance to C. parvum infection.

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