Association of CD4$^+$ T Cell–dependent, Interferon-γ-mediated Necrosis of the Small Intestine with Genetic Susceptibility of Mice to Peroral Infection with *Toxoplasma gondii*

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Summary

Since there is a remarkable difference in susceptibility to peroral infection with *Toxoplasma gondii* among inbred strains of mice, we performed studies to examine the mechanism(s) of this difference in susceptibility. After peroral infection with the ME49 strain of *T. gondii*, C57BL/6 (B6) mice all died whereas BALB/c mice all survived. At day 7 of infection (when B6 mice began dying), massive necrosis of the villi and mucosal cells in the ilea were observed in B6 but not in BALB/c mice. To analyze the role of T cells in resistance against death and development of necrosis in the ilea after infection, studies were performed using athymic nude and euthymic control B6 and BALB/c mice. Athymic B6 mice all died after infection, but surprisingly, they survived significantly longer than control B6 mice, indicating that T cells predispose to early death in these mice. Necrosis in the ilea was observed in control B6 but not in athymic B6 mice; however, significantly less numbers of tachyzoites were observed in the ilea of the former than the latter mice. These results indicate that necrosis in the ilea of the B6 mice was not due to destruction of tissue by tachyzoites but was mediated by T cells. This deleterious effect of T cells appears to contribute to early death in these mice. In contrast, T cells conferred resistance against death in BALB/c mice but did not cause necrosis in their ilea. To analyze the T cell subset(s) that induces necrosis of the ilea in B6 mice, we examined histological changes of the small intestines after infection of mutant mice deficient in different T cell subsets (with the same H-2^b haplotype as B6 mice). Mice deficient in α/β or CD4$^+$ T cells did not develop necrosis in the ilea, whereas wild-type control mice and mice deficient in γ/δ or CD8$^+$ T cells did, suggesting that the cells that induce necrosis in the ilea after infection are CD4$^+$ α/β T cells. Since interferon (IFN)-γ has been shown to be critical for survival of BALB/c mice after infection with *T. gondii*, we examined the role of this cytokine in resistance/susceptibility of infected B6 mice. Treatment of B6 mice with anti-IFN-γ monoclonal antibody shortly before they developed illness prolonged time to death and prevented necrosis in the ilea in these mice. These results indicate that IFN-γ mediates necrosis in the ilea of B6 mice after infection. This CD4$^+$ T cell–dependent, IFN-γ–mediated necrosis of the small intestines appears to be a mechanism that underlies the genetic susceptibility of B6 mice to peroral infection with *T. gondii*, whereas the same cytokine plays a critical role in the resistance of genetically resistant BALB/c mice.

Remarkable differences in mortality after acute infection with the obligate intracellular protozoan parasite, *Toxoplasma gondii*, have been observed among inbred strains of mice (1–8). Multiple genes, including that linked to the H-2 complex, were found to be involved in regulation of resistance against death after infection (2, 6, 8). However, the mechanism(s) that underlies the differences in mortality among inbred strains of mice after the infection is unknown; the majority of studies that have addressed the question of the mechanisms of protective immunity against acute infection were performed using strains of mice that are genetically resistant to *T. gondii* rather than comparing resistant and susceptible strains of mice, as done by McLeod et al. (5).

Most studies that have analyzed protective immunity against *T. gondii* were performed using the intraperitoneal route of infection (9–13) rather than the peroral route, which is the natural route of infection with this parasite. Johnson (3) and Blackwell et al. (8) reported that mortality after acute infection in inbred strains of mice differs depending on the route of infection. Thus, their observations suggest that the mechanism(s) of resistance against acute toxoplasmosis in these mice may differ depending on the route of infection. McLeod et al. (4–6) reported remarkable differences in mortality among inbred strains of mice that had been infected perorally.

In the present study, we analyzed the mechanism(s) that underlies the remarkable difference in susceptibility to peroral infection with *T. gondii* in genetically resistant and susceptible strains of mice. $CD4^+$ T cell–dependent, IFN-γ–mediated necrosis of the villi of the small intestines was noted to occur in association with mortality in susceptible C57BL/6 (B6)[1] mice after infection, suggesting that this IFN-γ–mediated pathology in the small intestine predisposes to death in these mice. In contrast, IFN-γ was required for survival in infected, genetically resistant BALB/c mice.

Materials and Methods

Mice. Female B6, BALB/c mice (Bantin and Kingman Inc., Fremont, CA), B6-background athymic nude mice (Taconic Farms Inc., Germantown, NY), BALB/c-background athymic nude mice (The Jackson Laboratory, Bar Harbor, ME), α/β (14) and γ/δ T cell–deficient mice (15; The Jackson Laboratory), MHC class II– (which lack $CD4^+$ T cells; 16) and β2-microglobulin–deficient mice (which lack $CD8^+$ T cells; 17) (Taconic Farms, Inc.) were 7–9-wk-old when used. The mutant mice deficient in T cell subsets described above have the $H\text{-}2^b$ haplotype as do B6 mice (14–17). There were at least five mice in each experimental group to study mortality; three or four mice were used for histological studies.

Infection with T. gondii. Cysts of the ME49 strain were obtained from brains of B6 mice that had been infected intraperitoneally with 10 cysts for 2–3 mo, as previously described (18). For peroral infection, mice were infected with 100 cysts by gavage.

Histopathology. Groups of three or four mice were euthanized by asphyxiation with CO_2 at 3, 5, and 7 d after peroral infection with the ME49 strain. Their brains, lungs, hearts, livers, spleens, and small and large intestines were removed and immediately fixed in a solution containing 10% formalin, 70% ethanol, and 5% acetic acid. Two to four 5-μm-thick sections (50- or 100-μm distance between sections) of each organ from each mouse were stained with hematoxylin and eosin (H&E) and by the immunoperoxidase method with rabbit anti-*T. gondii* IgG antibody (19). Sections stained with H&E were evaluated for inflammatory changes and sections stained by the immunoperoxidase method were evaluated for the number of parasitophorous vacuoles containing *T. gondii* tachyzoites. Histological changes were consistent between individual mice in the same group and between sections from the same organ of each mouse. Each slide was evaluated by three investigators. The results of each investigator were essentially the same.

Sections of livers were evaluated for the numbers of inflammatory foci at a magnification of 100×, and the numbers are indicated by the number per 4 mm^2 field of each section. Numbers of parasitophorous vacuoles were counted at a magnification of 400× and are indicated by the number per 1 cm^2 field of each section. Small intestine was cut into two pieces and rolled on itself in order to make a "Swiss roll."The entire length of the small intestine was examined histologically. The length of ileum with necrosis of villi was measured by a scale after microscopic evaluation. Numbers of parasitophorous vacuoles that contained tachyzoites in the ileum were counted at a magnification of 400× in two areas of 1-cm lengths (one in the proximal and another in the distal part) chosen at random for each section.

Flow cytometry. mAbs used for flow cytometry were obtained from PharMingen (San Diego, CA). Single cell suspensions of spleen cells were prepared as previously described (20). The same method was used for preparing single cell suspensions from Peyer's patches excised from the small intestines. 10^6 spleen or Peyer's patch cells were pretreated on ice for 10 min with 10 μl of a predetermined optimal concentration of anti-FcγII/III receptors (2.4G2) to block nonantigen-specific binding of antibodies to the FCγII/III receptors. Thereafter, the cells were incubated on ice for 30 min with 10 μl of optimal concentrations of PE-conjugated anti-CD4 mAb (RM4-5) and FITC-conjugated anti-CD8 mAb (53-6.7). Analysis of stained cells was performed with a FACScan® (Becton Dickinson & Co., Mountain View, CA). Dead cells were gated out on the basis of propidium iodide staining.

Treatment with mAb against IFN-γ. B6 and BALB/c mice were injected intraperitoneally with 2 mg of rat anti–mouse IFN-γ mAb (XMG1.2) every 5 d beginning 1 d before infection. Activity of the mAb has been described previously (21). The dose of mAb was determined based on results of our previous studies (18). Control mice were injected with 2 mg of control IgG (GL113; rat anti–*Escherichia coli* β-galactosidase mAb; these hybridomas were kindly provided by Dr. John Abrams, DNAX Research Institute, Palo Alto, CA). In a separate study, B6 mice were injected intraperitoneally with 2 mg of either anti-IFN-γ mAb or control IgG at 5, 7, and 9 d after infection.

Depletion of $CD4^+$ T Cells In Vivo. B6 mice were injected intraperitoneally with 1 mg of rat anti–mouse CD4 mAb (GK1.5) (22) daily for 3 d beginning 3 d before infection and thereafter every other day. Control mice were injected with 1 mg of control IgG (GL113) in the same manner.

Statistical Analysis. Levels of significance for differences in mortality were determined using Fisher's Exact test. Levels of significance for time to death of mice, length of ileum with necrosis, numbers of inflammatory foci and parasitophorous vacuoles containing tachyzoites in the histological sections, and numbers of T cells in the Peyer's patches were determined using Student's *t*, Wilcoxon Two Sample Rank Sum, or Mann-Whitney test.

Results

Mortality of B6 and BALB/c Mice after Infection

Whereas B6 mice all died from 7 to 13 d after peroral infection with 100 cysts of the ME49 strain, all BALB/c mice survived ($P < 0.001$) (Fig. 1). Of interest is that B6 mice appeared healthy until 5 d after infection. Thereafter, they quickly developed piloerection, huddled, and lost mobility.

[1] *Abbreviations used in this paper:* B6, C57BL/6.

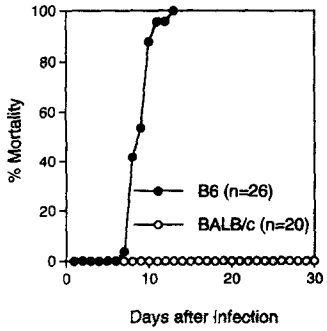

Figure 1. Mortality in B6 and BALB/c mice after peroral infection with *T. gondii*. Mice were infected with 100 cysts of the ME49 strain. Numbers in parentheses indicate numbers of mice used.

Comparison of Histological Changes in Organs of Infected B6 and BALB/c Mice

Because of the remarkable difference in mortality between B6 and BALB/c mice, we examined their brains, hearts, lungs, spleens, livers, small intestines, and large intestines at 3, 5, and 7 d after infection to determine whether histological changes differ between infected B6 and BALB/c mice. The duration of the study was 7 d since B6 mice began to die at that time.

Brains, Lungs, Hearts, Spleens, and Large Intestines. Except for similar mild infiltration of inflammatory mononuclear cells observed in the lungs of both strains of mice at 7 d after infection, there were no inflammatory changes in these organs of either strain of mouse.

Livers. Inflammatory foci were observed in livers of both strains of mice as early as 3 d after infection, and numbers of inflammatory foci increased in both strains of mice for 7 d after infection. The inflammatory foci were observed in both the portal areas and parenchyma in both strains of mice and the numbers of inflammatory foci were significantly greater in livers of B6 mice than in BALB/c mice at each time point. They were at days 3, 5, and 7, respectively: 3.9 ± 1.5 vs. 0.9 ± 1.2; 8.9 ± 2.5 vs. 1.8 ± 1.2; and 43.6 ± 15.6 vs. 17.7 ± 6.2; $P < 0.0001$ for each time point. Immunoperoxidase staining revealed small numbers of tachyzoites in association with the inflammatory foci in B6 mice only at 7 d after infection. This was not observed in BALB/c mice.

Small Intestines. At 3 d after infection, there were no demonstrable inflammatory changes or tachyzoites in either strain of mouse.

At 5 d after infection, elevation of the intact epithelial layer from the lamina propria was observed in villi of the ilea of B6 mice, whereas no histological changes were observed in the ilea of BALB/c mice. Inflammatory cells were not observed in either strain of mouse. Tachyzoites were detectable by immunoperoxidase staining in the ilea of B6 mice but not of BALB/c mice ($P < 0.004$; Table 1). Most of the tachyzoites were observed in the lamina propria; less numbers were observed in the epithelium and submucosa.

At 7 d after infection, whereas inflammatory changes were not observed in the small intestines of BALB/c mice (Fig. 2, *A* and *B*), severe necrosis of the ilea, predominantly within the villi, was observed in B6 mice (Fig. 2, *E* and *F*).

Table 1. *Necrosis and Numbers of Parasitophorous Vacuoles Containing Tachyzoites in the Ilea of B6 and BALB/c Mice after Peroral Infection with T. gondii*

Days after infection*	Strain of mouse	Length of ileum with necrosis (cm) (H&E stain)‡	Number of parasitophorous vacuoles/cm of ileum (immunoperoxidase stain)§
3	BALB/c	0	0
	B6	0	0
5	BALB/c	0	0
	B6	0	12.1 ± 18.9
7	BALB/c	0	14.9 ± 22.5
	B6	10.5 ± 0.5‖	265 ± 16.1¶

*Mice were perorally infected with 100 cysts of the ME49 strain of *T. gondii*. Three mice from each strain were examined for each time point.
‡Two sections of the entire length of small intestine from each mouse were examined. A total of six sections were examined for each group.
§Numbers of parasitophorous vacuoles containing tachyzoites in the ileum were counted by microscopy at × 400 in 1-cm length of ileum chosen at random. Three sections from each mouse were examined. A total of nine sections were examined for each experimental group.
‖ $P < 0.004$ vs. BALB/c mice at the same time point.
¶ $P < 0.0001$ vs. BALB/c mice at the same time point.

Villi were destroyed in most parts of the ilea of B6 mice (Fig. 2 *F*, Table 1), and necrosis was observed not only in areas where there were many tachyzoites but also in areas in which tachyzoites were not demonstrable. In contrast to the ileum, necrosis of villi was not observed in the duodenum and jejunum of B6 mice although small numbers of tachyzoites were detectable. Distribution of tachyzoites in the ilea was in association with inflammatory foci (Fig. 2 *G*). Most tachyzoites in these foci were in the lamina propria (Fig. 2 *G*); less were in the epithelium and submucosa. In contrast to B6 mice, only small numbers of tachyzoites were demonstrable in the small intestines of BALB/c mice, and then only in the ilea (Fig. 2 *C*, Table 1). The numbers of parasitophorous vacuoles that contained tachyzoites were markedly and significantly greater in the ilea of B6 than in BALB/c mice ($P < 0.0001$; Table 1).

Peyer's Patches. The general structure of the Peyer's patches of infected BALB/c mice was similar to that of uninfected control mice at each time point. Typical germinal centers were observed (Fig. 2 *D*). In contrast, germinal centers were not discernible in Peyer's patches of B6 mice at 7 d after infection (Fig. 2 *H*). At that time, markedly and significantly greater numbers of parasitophorous vacuoles containing tachyzoites were observed in Peyer's patches of B6 mice than of BALB/c mice (19.2 ± 10 vs. 2.8 ± 7 per section of the Peyer's patch; $P < 0.0001$).

Changes in Numbers of $CD4^+$ and $CD8^+$ T Cells in the Peyer's Patches after Infection

Because of the marked differences in morphology of Peyer's patches between B6 and BALB/c mice after infec-

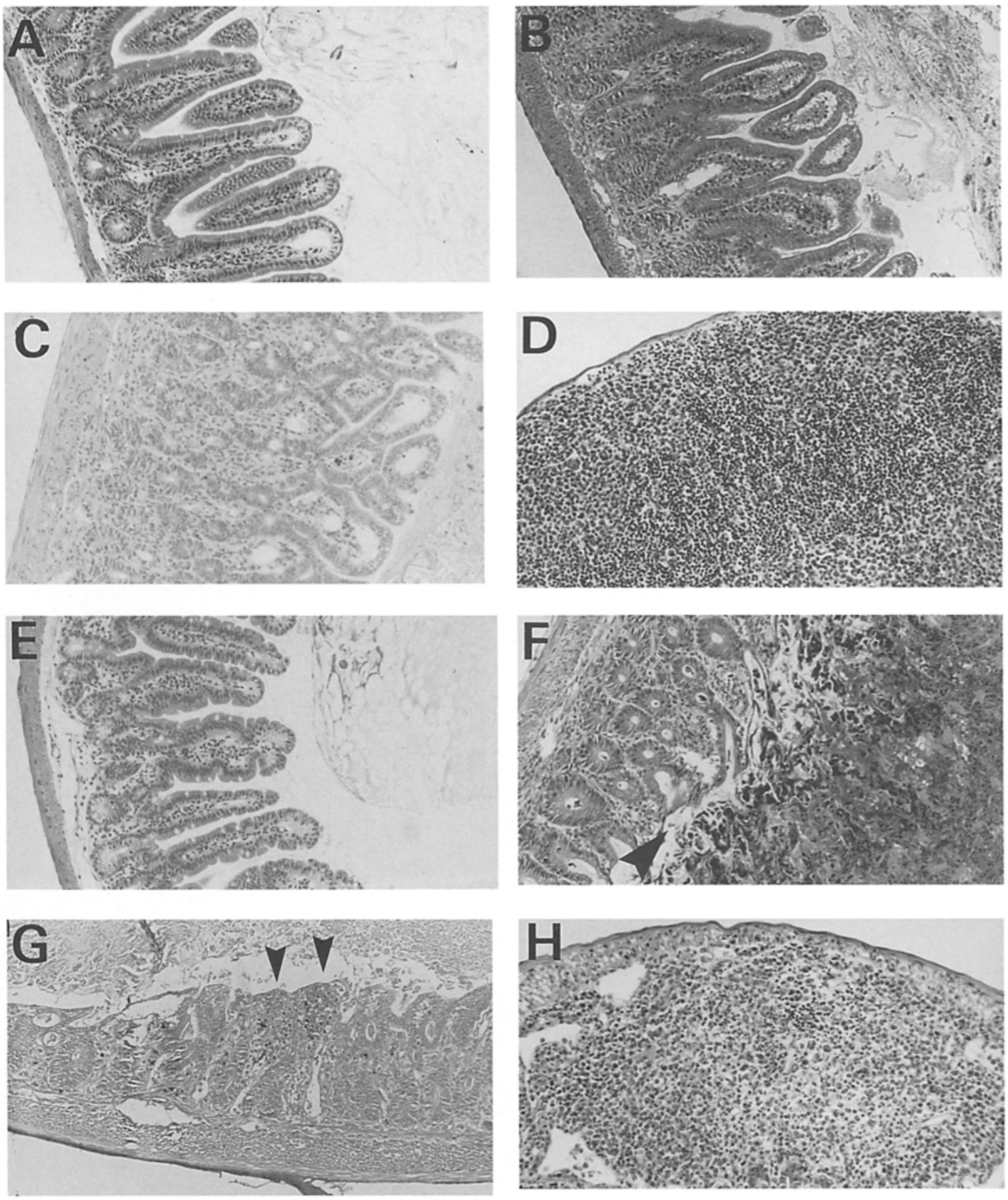

Figure 2. Histological changes in the ilea and Peyer's patches of BALB/c and B6 mice after peroral infection with *T. gondii*. Mice were infected with 100 cysts of the ME49 strain, and 7 d later, histological studies were performed on their small intestines. (*A*) ileum of normal BALB/c mouse, (*B* and *C*) ileum of infected BALB/c mouse, (*D*) Peyer's patch of infected BALB/c mouse, (*E*) ileum of normal B6 mouse, (*F* and *G*) ileum of infected B6 mouse. An arrow in *F* indicates the junction of the relatively unaffected lamina propria and the necrotic villi (the villi clearly visible in *E* are almost indiscernible in *F* due to the necrosis). (*H*) Peyer's patch of infected B6 mouse. (*A*, *B*, *D*, *E*, *F*, and *H*) hematoxylin and eosin stain. (*C* and *G*) immunoperoxidase stain (dark stained spots indicate tachyzoites and antigens; *Arrows* in *G* indicate foci containing numerous tachyzoites).

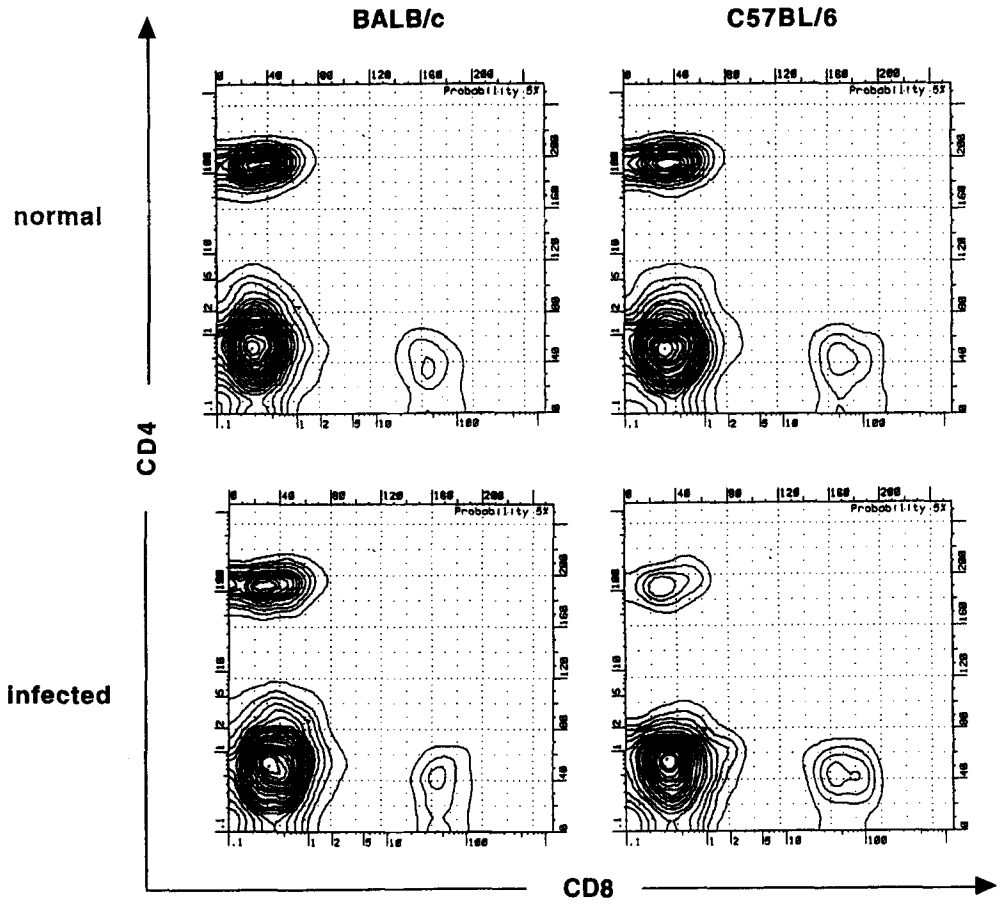

Figure 3. Changes in the relative percentages of $CD4^+$ and $CD8^+$ T cells in Peyer's patches of B6 and BALB/c mice after peroral infection with *T. gondii*. Mice were infected with 100 cysts of the ME49 strain and the numbers of $CD4^+$ and $CD8^+$ T cells in their Peyer's patches were examined individually at 7 d after infection as described in Materials and Methods. Essentially identical results were obtained in each mouse of the same experimental group.

tion, we examined the numbers of $CD4^+$ and $CD8^+$ T cells in the Peyer's patches of both strains of mice by flow cytometry after infection. At 3 and 5 d after infection, significant changes were not observed in numbers of $CD4^+$ or $CD8^+$ T cells in Peyer's patches in both strains of mice (data not shown). However, at 7 d after infection, the numbers of $CD4^+$ T cells in Peyer's patches were significantly lower in infected ($0.4 \pm 0.2 \times 10^7$) than uninfected B6 mice ($1.3 \pm 0.6 \times 10^7$, $P = 0.0101$; Fig. 3), whereas their numbers did not differ significantly between infected and uninfected BALB/c mice (2.2 ± 0.6 vs. 2.9 ± 1.3, $P = 0.1403$; Fig. 3). There were no significant differences observed in the numbers of $CD8^+$ T cells between infected and uninfected mice of both strains (Fig. 3).

Effect of Absence of T Cells on Mortality and Time to Death

Since we observed a significant decrease in $CD4^+$ T cells in Peyer's patches only in infected B6 mice, we examined the role of T cells in resistance against death due to infection in both strains of mice. B6- and BALB/c-background athymic nude mice that lack thymus-dependent T cells and euthymic control mice were infected perorally with the ME49 strain and followed for mortality and time to death. BALB/c-background athymic nude mice all died whereas euthymic control BALB/c mice all survived ($P < 0.0001$; Fig. 4). In contrast, B6-background athymic nude mice survived significantly longer than euthymic control B6 mice ($P = 0.0007$), although both died of acute infection (Fig. 4). Of interest, there were no differences in mortality or time to death between B6- and BALB/c-background athymic nude mice ($P = 0.1364$; Fig. 4). These results indicate that the presence of T cells predisposes to early death in genetically susceptible B6 mice after infection, whereas these cells confer protection against death in resistant BALB/c mice.

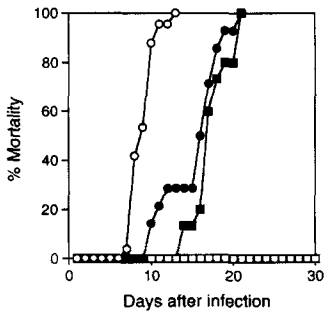

Figure 4. Mortality in B6- and BALB/c-background athymic nude and their euthymic control mice after peroral infection with *T. gondii*. Mice were infected with 100 cysts of the ME49 strain. Numbers in parentheses indicate numbers of mice used. —○—, B6 control (n = 26); —●—, B6, athymic nude (n = 14); —□—, BALB/C, control (n = 20); —■—, BALB/C, athymic nude (n= 15).

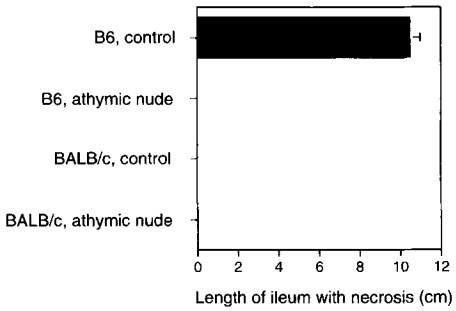

Figure 5. Length of the ileum with necrosis in B6- and BALB/c-background athymic nude mice and their euthymic controls after peroral infection with *T. gondii*. Mice were infected with 100 cysts of the ME49 strain and histological studies were performed on their small intestines at 7 d after infection. Two sections of the entire length of small intestine from each mouse were examined. Three mice were used for each group. Essentially identical results were obtained in two independent experiments. Results are expressed as mean length of ileum with necrosis in centimeters.

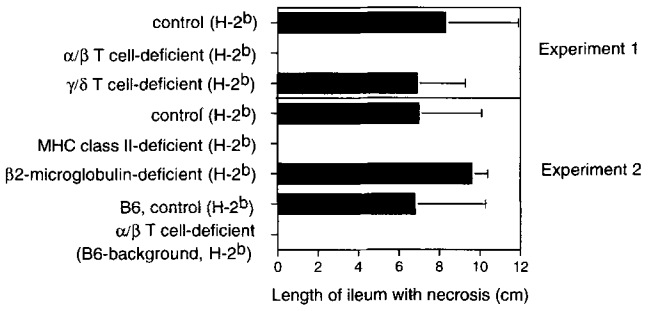

Figure 6. Length of the ileum with necrosis in mutant mice deficient in different T cell subsets after peroral infection with *T. gondii*. Mice were infected with 100 cysts of the ME49 strain and histological studies were performed on their small intestines at 7 d after infection. Two sections of the entire length of small intestine from each mouse were examined. At least three mice were used for each group. Results are expressed as mean length of ileum with necrosis in centimeters.

Effect of Absence of T Cells on Development of Necrosis in the Small Intestines in Infected B6 Mice

Since the presence of T cells was found to predispose to early death in infected B6 mice, we examined whether T cells also played a role in development of necrosis of the villi and mucosal cells in the small intestines of these mice. Athymic and control mice were infected and histological studies performed on their small intestines at 7 d after infection (when the infected control B6 mice began dying). Necrosis in the ilea was observed in the control but not the athymic B6 mice (Fig. 5). However, less numbers of tachyzoites were observed in the ilea of the former than the latter mice (numbers of parasitophorous vacuoles per centimeter of ileum = 162.6 ± 103.3 vs. 785.9 ± 155.1; $P < 0.0001$). These results indicate that necrosis in the ilea of B6 mice was not due to destruction of tissue by tachyzoites but rather, was mediated by T cells. In contrast, neither control nor athymic BALB/c mice developed necrosis of the ilea (Fig. 5). Less numbers of tachyzoites were observed in the ilea of the former than the latter mice (3.2 ± 4.1 vs. 183.9 ± 108.0; $P < 0.0001$).

Effect of Absence of Different T Cell Subsets in Development of Necrosis in the Small Intestines

Based on the results in athymic nude mice which revealed a requirement for T cells in the development of necrosis in the small intestines of infected B6 mice, further studies were performed to analyze the T cell subset(s) required for these histological changes. To determine whether α/β or γ/δ T cells are critical for development of necrosis, α/β T cell–deficient, γ/δ T cell-deficient, and control mice, which have the H-2^b haplotype as do B6 mice, were

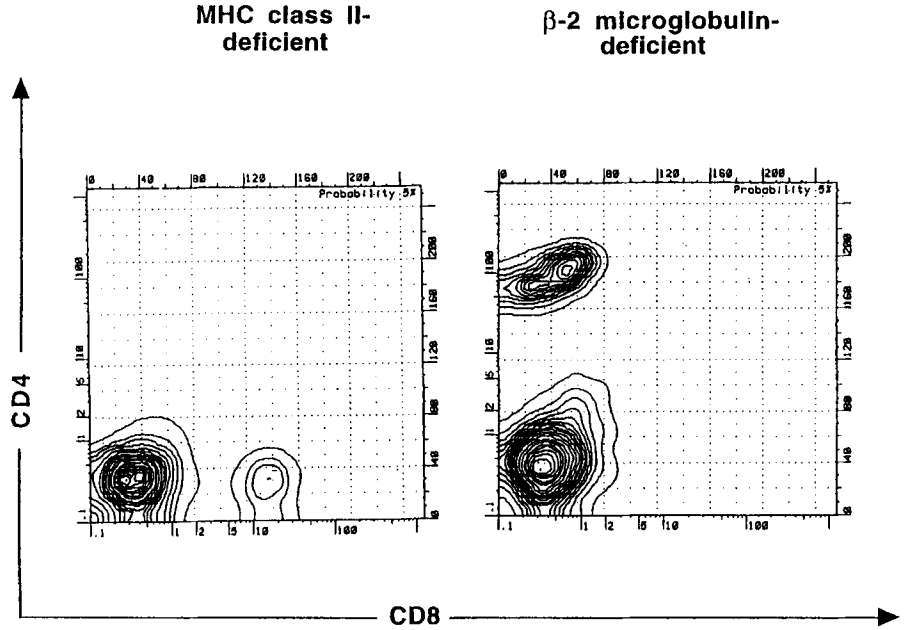

Figure 7. Flow cytometric analysis of spleens of MHC class II– or β2-microglobulin–deficient mice after infection with *T. gondii*. Mice were infected with 100 cysts of the ME49 strain and their spleen cells were examined for numbers of CD4$^+$ and CD8$^+$ T cells individually at 7 d after infection as described in Materials and Methods. Essentially identical results were obtained in each mouse of the same experimental group.

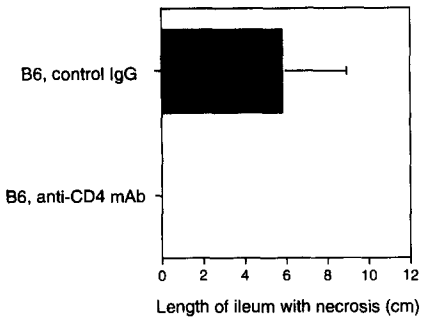

Figure 8. Effect of depletion of $CD4^+$ T cells on development of necrosis in the ilea of B6 mice after peroral infection with *T. gondii*. Mice were treated with anti-CD4 mAb and infected with 100 cysts of the ME49 strain. Control mice were treated with control IgG and infected with the ME49 strain. Histological studies were performed on their small intestines at 7 d after infection. Two sections of the entire length of the small intestines were examined. Four mice were used for each group. Results are expressed as mean length of ileum with necrosis in centimeters.

perorally infected with the ME49 strain. 7 d later, their small intestines were examined histologically. Necrosis of the villi and mucosal cells was observed in the ilea of the γ/δ T cell–deficient and control mice but not in the α/β T cell–deficient mice (Fig. 6), indicating that α/β but not γ/δ T cells are required for development of necrosis in the ilea after infection.

To determine whether $CD4^+$ or $CD8^+$ T cells are required for development of necrosis in the ilea, we used MHC class II–deficient mice and β2-microglobulin–deficient mice that were deficient in $CD4^+$ and $CD8^+$ T cells, respectively (16, 17). 7 d after infection with *T. gondii*, severe necrosis was observed in wide areas of the ilea of β2-microglobulin–deficient mice (which lack $CD8^+$ T cells [Fig. 7]) and control mice (Fig. 6). In contrast, necrosis was not observed in the small intestines of MHC class II–deficient mice (which lack $CD4^+$ T cells [Fig.7]) (Fig. 6). These results indicate that $CD4^+$ T cells induce necrosis of the ilea in genetically susceptible mice after infection.

Although the mutant mice deficient in different T cell subsets that we used have the same $H\text{-}2^b$ haplotype as do B6 mice, their genetic backgrounds are not completely the same as B6 mice. Therefore, to confirm the critical role of $CD4^+$ T cells in induction of necrosis in the ilea in B6 mice, these mice were treated with anti-CD4 mAb to deplete $CD4^+$ T cells, and infected with *T. gondii*. Depletion of $CD4^+$ T cells in the spleens and Peyer's patches of mice treated with anti-CD4 mAb was confirmed by flow cytometry (data not shown). Histological studies were performed on their small intestines at 7 d after infection. Severe necrosis of the villi and mucosal cells was observed in the ilea of mice treated with control IgG but not in the ilea of mice treated with anti-CD4 mAb ($P < 0.001$; Fig. 8).

Effect of Treatment with Anti-IFN-γ mAb on Mortality and Time to Death.

Since IFN-γ is known to be critical for resistance against death of BALB/c mice after peroral infection with *T. gondii*

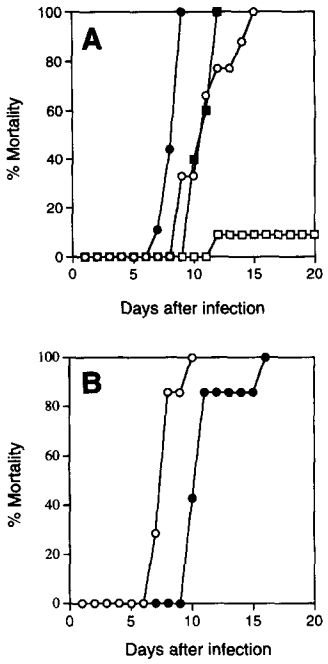

Figure 9. Mortality in BALB/c and B6 mice treated with anti-IFN-γ mAb before or after peroral infection with *T. gondii*. (*A*) Mice were injected intraperitoneally with 2 mg of anti-IFN-γ mAb beginning 1 d before infection with 100 cysts of the ME49 strain. –○–, B6, control IgG; –●–, B6, anti-IFN-γ mAb; –□–, BALB/C, control IgG; –■–, BALB/C, anti-IFN-γ mAb. (*B*) Mice were injected with anti-IFN-γ mAb at 5, 7, and 9 d after infection. For each experiment, control mice were injected with control IgG in the same manner as anti-IFN-γ mAb. 5–11 mice were used per group. –○–, B6, control IgG; –●–, B6, anti-IFN-γ mAb.

(23, 24), we examined the effect of anti-IFN-γ mAb on mortality and time to death in B6 mice. BALB/c mice were injected with anti-IFN-γ mAb as a control for the effect of the mAb. Mice were treated with 2 mg of anti-IFN-γ mAb every 5 d beginning 1 d before infection. Control mice received control IgG in the same manner. BALB/c mice treated with anti-IFN-γ mAb all died by day 12 whereas 91% (10/11) of control BALB/c mice survived ($P = 0.0014$; Fig. 9 *A*). B6 mice treated with anti-IFN-γ mAb all died significantly earlier than control mice which all died by day 15 ($P = 0.0023$; Fig. 9 *A*). These results indicate that IFN-γ plays a protective role in resistance against death in B6 mice, although the protective effect was partial and all mice died by day 15 of infection.

Because B6 mice developed signs of illness between 5 and 6 d after infection, we performed a separate experiment to examine the role of IFN-γ in resistance against death of these mice during this stage of their infection. For this purpose, B6 mice were treated with anti-IFN-γ mAb at 5, 7, and 9 d after infection. Control B6 mice received the control IgG. In contrast to the results obtained when treatment with anti-IFN-γ mAb was begun 1 d before infection, mice treated with anti-IFN-γ mAb at 5, 7, and 9 d after infection survived significantly longer than control mice ($P = 0.0012$; Fig. 9 *B*). These results indicate that IFN-γ predisposed to death in B6 mice through its action during the time when the mice developed clinical signs of the infection.

Effect of Treatment with Anti-IFN-γ mAb on Development of Necrosis of the Villi of the Small Intestine of B6 Mice

Since treatment of B6 mice with mAb against IFN-γ beginning 5 d after infection (shortly before they develop illness) significantly prolonged time to death, we examined

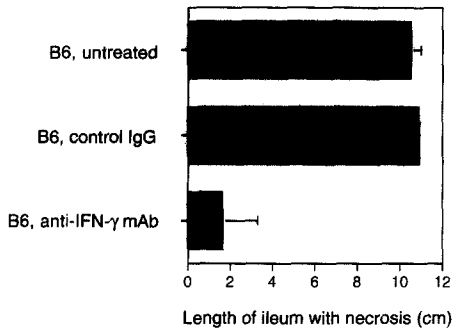

Figure 10. Effect of treatment with anti-IFN-γ mAb on development of necrosis in the ilea of B6 mice after peroral infection with *T. gondii*. Mice were injected intraperitoneally with 2 mg of anti-IFN-γ at 5 d after infection with 100 cysts of the ME49 strain, and histological studies were performed on their small intestines at 7 d after infection. Control mice were injected intraperitoneally with 2 mg of control IgG or nothing at 5 d after infection. Two sections of the entire length of small intestine from each mouse were examined. Three mice were used for each group. Results are expressed as mean length of ileum with necrosis in centimeters.

whether treatment with the mAb prevents development of necrosis in their small intestines. Mice were injected with 2 mg of anti-IFN-γ mAb at 5 d after infection and histological studies were performed at 7 d after infection (when infected B6 mice always had developed necrosis in their ilea). Whereas mice treated with control IgG had severe necrosis of the villi in most parts of the ilea, those treated with anti-IFN-γ mAb had only a few such areas of necrosis (Fig. 10). The numbers of parasitophorous vacuoles containing tachyzoites in the ilea did not differ between mice treated with control IgG and those treated with anti-IFN-γ mAb (187.6 ± 98.9 vs. 177.4 ± 69.7). These results demonstrate that development of necrosis in the ilea was mediated by IFN-γ.

Discussion

We performed studies to analyze the mechanism(s) that underlies the remarkable difference in susceptibility to peroral infection with *T. gondii* among inbred strains of mice. We found a novel deleterious effect of IFN-γ—this cytokine induced necrosis of the villi and mucosal cells in the small intestines of the infected mice. It is noteworthy that the IFN-γ–mediated necrosis of the small intestine occurred in a strain of mice that died after infection but not in a strain of mice that survived the infection.

Of all the organs studied, the small intestine had the most remarkable histological changes in B6 mice (all of which died after peroral infection). Necrosis of villi and mucosal cells occurred in most parts of the ilea. In contrast, these histological changes were not observed in the small intestines of BALB/c mice that survived the infection. In addition to necrosis of the villi and mucosal cells, marked histological changes (loss of discernible germinal centers) occurred in Peyer's patches of B6 mice but not of BALB/c mice. Flow cytometric analysis revealed a significant decrease in numbers of $CD4^+$ T cells in Peyer's patch cells in B6 but not in BALB/c mice, suggesting that the T cell response may differ between B6 and BALB/c mice after the peroral infection.

Our results in the athymic nude mice provided definitive evidence for a critical difference in the role of T cells in resistance against peroral infection with *T. gondii* between B6 and BALB/c mice. Differences in mortality and time to death after infection were observed between euthymic B6 and BALB/c mice but not between athymic B6 and BALB/c mice. It is of interest that B6 mice that have thymus-dependent T cells died earlier than B6 mice that did not have those T cells. In contrast, BALB/c mice that had thymus-dependent T cells survived infection whereas BALB/c mice that did not all died. These results reveal that after the peroral infection, thymus-dependent T cells (mostly α/β T cells) predispose to early mortality in B6 mice and confer protection in BALB/c mice.

Our results in the athymic nude mice also provide important evidence for a marked difference in the role of T cells in induction of the histological changes in the small intestines between B6 and BALB/c mice. The remarkable absence of necrosis of the villi and mucosal cells in the ilea in athymic B6 mice at a time when control B6 mice had begun dying and had necrosis in their ilea indicates that T cells induced the necrosis in the ilea of the infected B6 mice. Destruction of tissue by tachyzoites does not appear to be the mechanism by which necrosis developed since we observed less numbers of tachyzoites in ilea of control mice that developed necrosis than in ilea of athymic mice that did not develop necrosis. The fact that necrosis of the villi in ilea of infected control B6 mice was observed not only in areas where there were many tachyzoites but also in areas in which tachyzoites were not detected also supports our conclusion that necrosis is not caused by destruction of tissues by tachyzoites.

Since necrosis of the ilea was the major pathological finding in B6 mice that died after infection, and since T cells predisposed to early death in these mice, development of necrosis, which was shown to be mediated by T cells, appears to contribute to their mortality after peroral infection with *T. gondii*. This is supported by the fact that such histological changes were not observed in BALB/c mice that survived the infection. Results of our studies using two other strains of mice provide further evidence that the necrosis contributes to the mortality in the infected mice. Necrosis of the ilea was observed in C57BL/10 mice (all of which died as early as did B6 mice after infection) but not in C3H/He mice (most of which survived after infection; data not shown). These results indicate that the term "susceptibility" to acute infection with *T. gondii* should be given a new and broader definition to include death due to inflammation (mediated by immune responses) induced by the parasite and death directly due to multiplication of the parasite.

To analyze which T cell subset(s) induced development of necrosis in the ilea of B6 mice, we performed experiments using mutant mice (with the same $H\text{-}2^b$ haplotype as B6 mice) deficient in different T cell subsets. In these stud-

ies, mice deficient in either α/β or $CD4^+$ T cells did not develop necrosis in their ilea after infection, whereas control mice and mice deficient in γ/δ or $CD8^+$ T cells did. These results indicate a requirement for α/β T cells and $CD4^+$ T cells for induction of the necrosis. Therefore, $CD4^+$ α/β T cells appear to be the primary lymphocyte subset that induces necrosis. With these primary T cells, $CD8^+$ α/β and/or $CD4^+$ γ/δ T cells may also be involved, but in a supplementary role (although the existence of $CD4^+$ γ/δ T cells has not been reported [25–28]). The requirement for $CD4^+$ T cells in development of ileal necrosis in infected B6 mice was confirmed by the fact that depletion of $CD4^+$ T cells by treatment with anti-CD4 mAb prevented the necrosis.

In contrast to our observation of the importance of $CD4^+$ T cells in determining the outcome of acute peroral infection with *T. gondii*, Brown and McLeod (29) have reported that class I MHC genes and $CD8^+$ T cells are important in determining susceptibility to cyst formation in the brains of mice during the late stage of infection. Thus, it is likely that different mechanisms are operative in determining mortality during the acute stage of infection and formation of cysts during the late stage of infection. This is supported by results of our previous studies which demonstrated that mortality during the acute stage of infection does not correlate with mortality during the chronic stage of infection in inbred strains of mice (7).

We (23) and others (24) have reported that IFN-γ is critical for survival of BALB/c mice after peroral infection with *T. gondii*. We therefore examined the role of this cytokine in resistance against death of B6 mice that died by day 15 of infection. Treatment of B6 mice with anti-IFN-γ mAb beginning 1 d before infection resulted in significantly earlier death than in controls. These results indicate that IFN-γ played a protective role against death in B6 mice after infection, although the protective effect was not sufficient to prevent death. However, when treatment with anti-IFN-γ mAb was begun 5 d after infection (shortly before mice developed clinical illness), the treated mice survived significantly longer than control mice. These results indicate that IFN-γ is detrimental during the stage of infection when the mice had developed clinical signs of illness. Thus, IFN-γ seemed a double-edged sword. It was protective in the very early stage of the infection and thereafter predisposed to death. It may be that IFN-γ is overproduced during the time when the mice develop clinical illness and thereby contributes to early death of mice.

In addition to the significant prolongation of time to death of B6 mice after treatment with anti-IFN-γ mAb that was begun 5 d after infection (shortly before mice developed clinical illness), histological studies revealed that treatment with the mAb at this stage of infection prevented necrosis of the villi and mucosal cells in the ilea. Thus, necrosis of the ilea in infected B6 mice was mediated by IFN-γ. Since necrosis of the ilea was the major pathological finding observed in infected B6 mice, and since treatment with anti-IFN-γ prevented development of the necrosis and prolonged time to death of these mice, IFN-γ–mediated necrosis of the ilea appears to be an important mechanism predisposing to early death of these mice.

IFN-γ has not previously been reported to mediate necrosis of the villi and mucosal cells in the small intestines. We could find little published information on the effect of IFN-γ on the small intestine. Przemioslo et al. (30) recently reported toxic effects of IFN-γ on human duodenal mucosal enterocytes in vitro.

Necrosis of the villi and mucosal cells in the ilea of infected B6 mice in the present study appears to have been caused by locally produced IFN-γ since the necrosis occurred only in the ilea (not in the duodena and jejuna). Mortality of infected B6 mice associated with development of necrosis in their ilea does not appear to have been due to a systemic effect of IFN-γ since their serum levels of IFN-γ were lower at 7 d after infection (when they had developed the necrosis) than that of BALB/c mice which did not develop necrosis in their ilea and survived infection (data not shown).

Since our studies revealed a requirement for $CD4^+$ T cells (most likely $CD4^+$ α/β T cells) and IFN-γ for development of necrosis in the ilea of B6 mice during the time when mice had developed clinical signs of illness, these T cells may have been a major source of IFN-γ in the small intestines of these mice. In relation to this, Powrie et al. (31) recently reported that the incidence of severe inflammatory responses in the colon of SCID mice induced by the transfer of $CD45RB^{high}$ $CD4^+$ T cells from BALB/c mice is prevented by treatment of the recipient mice with anti-IFN-γ mAb.

As reported previously (23, 24) and as shown in the present study, BALB/c mice that survived peroral infection with *T. gondii* required IFN-γ for their survival. This protective role of IFN-γ in genetically resistant BALB/c mice along with the detrimental role of this cytokine in genetically susceptible B6 mice indicate that a single cytokine, IFN-γ, plays a critical role in the mechanisms that determine early death or survival of inbred stains of mice after peroral infection with *T. gondii*.

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