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Different Strains of *Toxoplasma gondii* Induce Different Cytokine Responses in CBA/Ca Mice

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To investigate the role that cytokines may have in the development of toxoplasmic encephalitis (TE), the levels of gamma interferon (IFN-γ), tumor necrosis factor alpha (TNF-α), interleukin-12 (IL-12]p40[), IL-10, IL-6, IL-4, and IL-2 in serum were examined in CBA/Ca mice infected with a type II strain (ME49 or FORT) of *Toxoplasma gondii*. These strains caused severe (ME49) or mild (FORT) TE in CBA/Ca mice. From weeks 1 to 8 of infection, the levels of IL-6, IL-10, IL-12, IFN-γ, and TNF-α in serum were significantly higher in the ME49-infected mice than in the FORT-infected mice, suggesting a role for these cytokines in the severity of TE in CBA/Ca mice. Since the ME49 and FORT strains are of the same type, our results suggest a role for the parasite in the development of severe TE through the increased production of proinflammatory cytokines and indicate that not all type II strains cause TE.

Toxoplasmic encephalitis (TE) caused by *Toxoplasma gondii* remains an important human disease, particularly in immunosuppressed individuals (12). Murine models employing the ME49 strain of *T. gondii* have been developed and used to study the pathogenesis and therapy of TE (2, 3, 18). The ME49 strain of *T. gondii* (1) is of type II, which has been reported as the type most frequently associated with human disease (7, 8, 15). The availability in our laboratory of strains of different types, including type II isolated from humans with TE, prompted us to attempt to develop a murine model of TE by using strains of *T. gondii* other than the ME49 strain and including strains of human origin. For this study, groups of CBA/Ca mice were infected orally with equal numbers of cysts of 1 strain from among either 15 strains isolated from humans or 3 strains isolated from animals (Table 1). Infected mice were not treated and were monitored for 6 weeks. At that time, representative numbers of mice were euthanized, and sections of lung, intestine, and brain were examined for histopathology to determine the degrees of infection and of TE. Human strains CAST, VEL, MOO, and SOU and animal strain C56 caused 80 to 100% mortality in the first 2 weeks of infection (Table 1). Death was due to severe lung and intestinal inflammation. In contrast, infection with each of the other strains resulted in low or zero mortality (Table 1). Lung and intestinal inflammations were present in these mice but were usually mild. Reinfection of the survivors with tachyzoites of the highly virulent RH strain resulted in 70 to 100% survival, indicating that an effective protective immune response had been induced by the primary infection. The histopathology of the brain tissue of the mice following the primary infection with each of the strains of *T. gondii* (4) revealed that only the mice infected with the ME49 strain had severe TE (Table 1 and Fig. 1). This observation revealed that severe TE developed in ME49-infected CBA/Ca mice but not in mice of the same genotype infected with other type II strains of *T. gondii*, including strains isolated from human cases of TE (Table 1).

To investigate the reasons for this observation, the levels of the cytokines gamma interferon (IFN-γ), tumor necrosis factor alpha (TNF-α), interleukin-12 (IL-12 [p40]), IL-10, IL-6, IL-4, and IL-2 in serum in CBA/Ca mice experiencing severe or mild TE were examined. These cytokines were chosen because of their reported roles in resistance to toxoplasmosis and in the pathogenesis of TE (9–11, 14, 17–19). For this study, strains ME49 and FORT were used. Although previous work (16) reported severe and mild TE in CBA/Ca mice infected with strainsME49 and DAG, respectively, the FORT strain, instead of the DAG strain, was chosen for this study. A preliminary

TABLE 1. Strains of *T. gondii* according to genotype, origin, and capacity to cause TE and mortality in CBA/Ca mice

Strain	Genotype	Origin	Infectivity for CBA/Ca mice	
			TE[a]	Mortality (%)[b]
CAST	I	Human with TE	+	100
VEL	I	Human with TE	+	100
WIL	II	Human with TE	±	<10
SAV	II	Human with TE	−	<10
MOY	II	Human with TE	+	<10
HART	II	Human with TE	+	<10
DAG	II	Human with TE	±	0
FORT	II	Human with TE	+	10
DEY	II	Human with TE	±	<10
VEG	III	Human with TE	+	30
MOO	III	Human with TE	+	100
SOU	III	Human with TE	±	80
OPE	III	Human with TE	±	<10
SS	ND[c]	Human with TE	−	<10
MEG	ND	Human with TE	−	<10
C56	I	Chicken	±	100
M7741	II	Sheep	±	<10
ME49	II	Sheep	++++	10

[a] TE infectivity ranged from absent (−) to severe (++++).
[b] After 6 weeks of infection.
[c] ND, genotype not defined at the time of the study.

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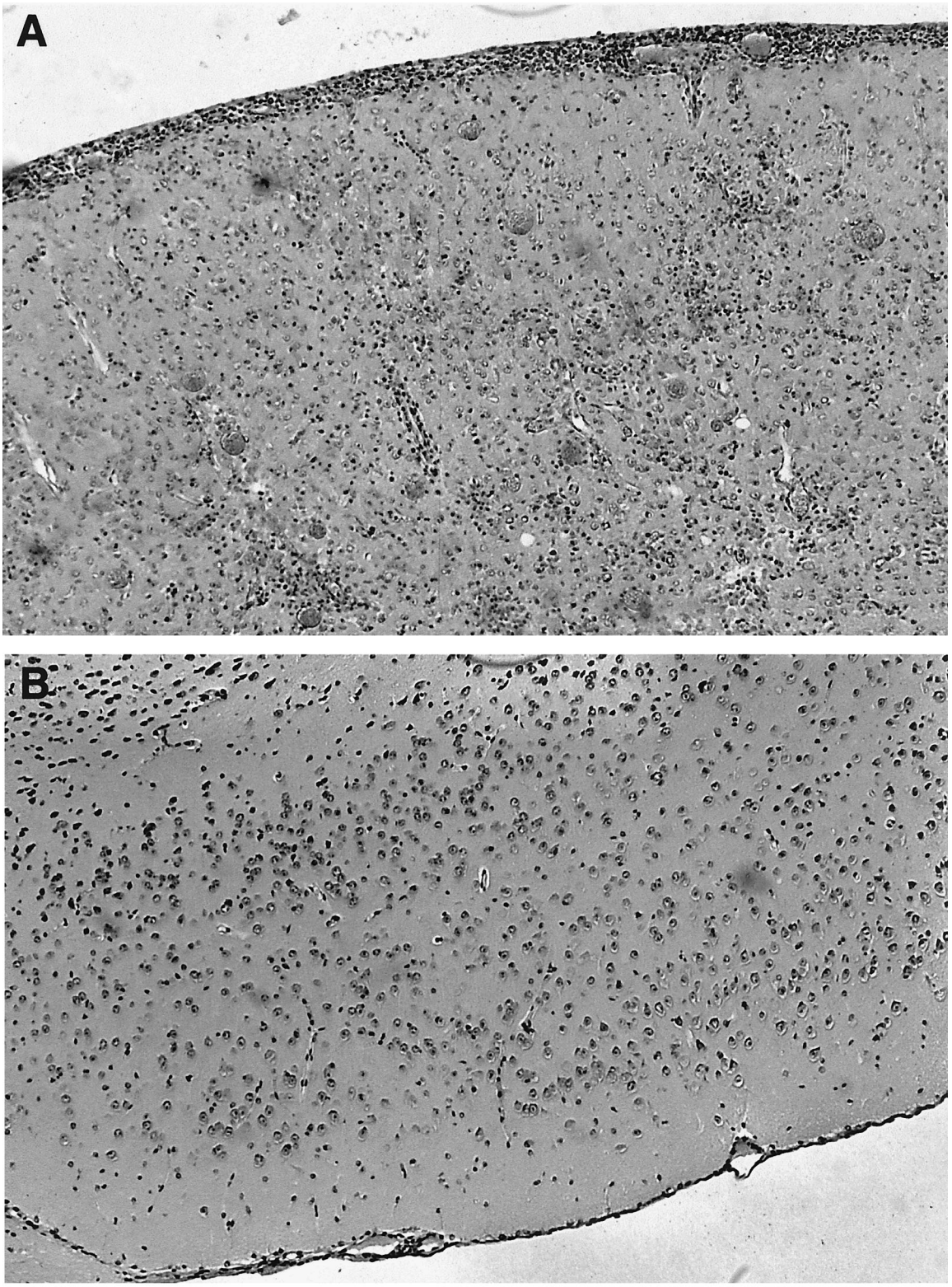

FIG. 1. Histopathology of brain tissue from CBA/Ca mice infected with the ME49 (A) or FORT (B) strain of *T. gondii*. Severe TE with extensive cellular infiltrates in the parenchyma and meninges, necrosis, and numerous cysts of *T. gondii* are present in ME49-infected mice. Mild inflammation and the absence of *T. gondii* cysts are noted in the tissue from the FORT-infected mice.

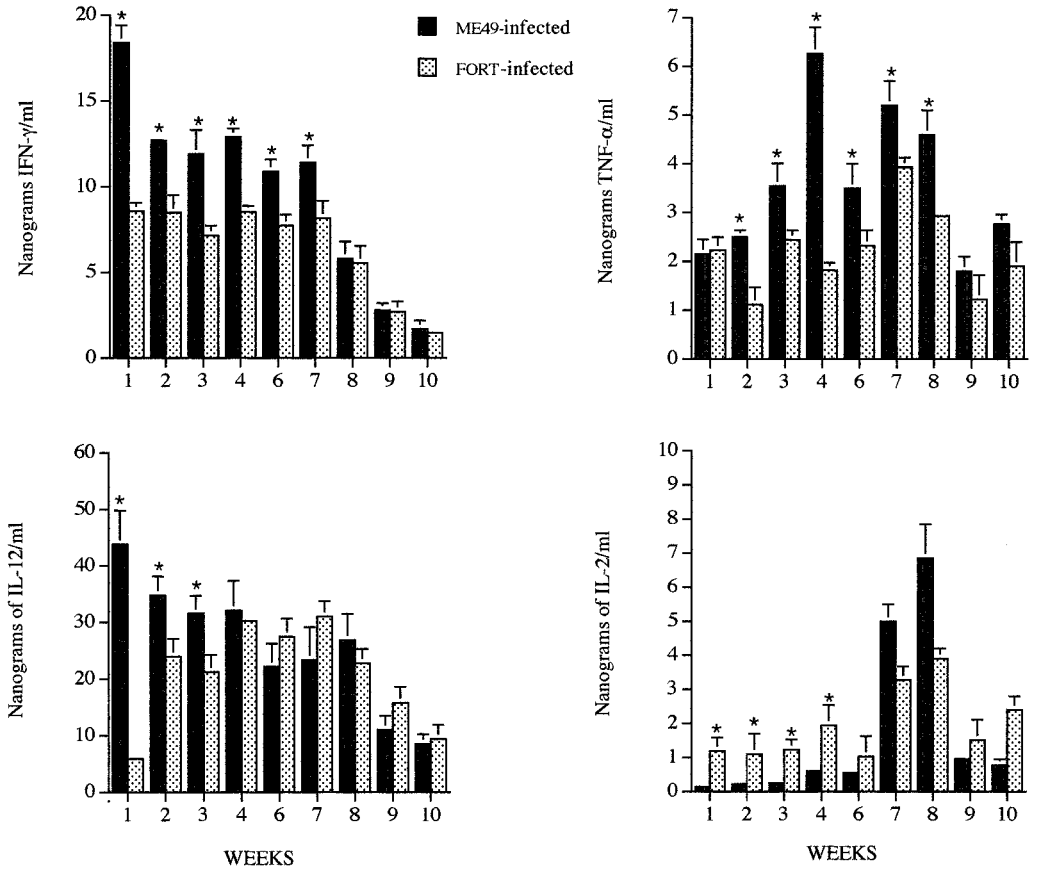

FIG. 2. Levels of IFN-γ, TNF-α, IL-12, and IL-2 in serum from CBA/Ca mice infected with the ME49 or FORT strain of *T. gondii*. Asterisks indicate *P* values of 0.01 or less.

study had revealed that the inflammation and tissue damage caused by the FORT strain in the intestines of CBA/Ca mice following oral infection with cysts were similar to those observed in mice infected with the ME49 strain. In addition, infection with the FORT strain caused mortality rates similar to the mortality rates caused by the ME49 strain (Table 1).

To examine the levels of cytokines in serum, CBA/Ca mice were infected orally with 5 cysts of either the ME49 or the FORT strain. Beginning 1 week after infection and continuing weekly for 10 weeks, three mice from each group were bled and serum samples were collected and stored at −80°C until they were tested individually in parallel by using cytokine enzyme-linked immunosorbent assay (ELISA) kits (PharMingen, San Diego, Calif.). Cytokine assays were performed in quadruplicate, and quantification was by use of a standard curve as described in the directions accompanying the kits. Welch's modified *t* test (InStat 2.0; GraphPad Software, San Diego, Calif.) was used for statistical analysis of the data. The levels of IFN-γ and TNF-α in serum, from weeks 1 to 7 and from weeks 1 to 8 of infection, respectively, were significantly ($P < 0.01$) higher in the ME49-infected mice than in the FORT-infected mice (Fig. 2). From weeks 1 to 3, the levels of IL-12 and IL-6 in serum were significantly ($P < 0.01$) higher in the ME49-infected mice (Fig. 2). At weeks 3 and 4, the levels of IL-10 in serum were also significantly ($P < 0.01$) higher in the ME49-infected mice (data not shown). The results with IL-2 were interesting since the levels of this cytokine in serum were significantly ($P < 0.01$) higher in the FORT-infected mice from weeks 1 to 4 and in week 10 (Fig. 3). No significant differences were noted in the levels of IL-4 in serum between the ME49- and FORT-infected mice (data not shown). These results indicated that ME49-infected CBA/Ca mice developed significantly higher levels of IFN-γ, TNF-α, IL-12, IL-10, and IL-6 in

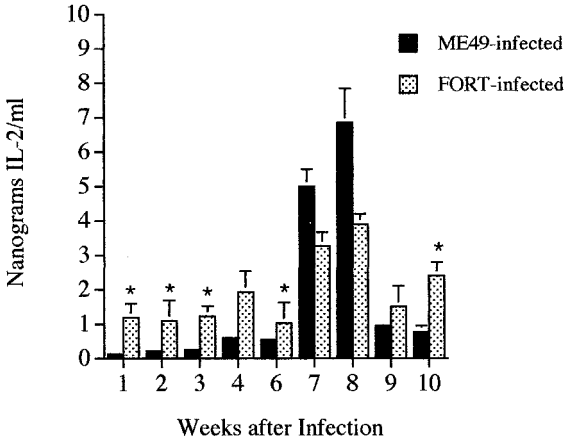

FIG. 3. Levels of IL-2 in serum from CBA/Ca mice infected with the ME49 or FORT strain of *T. gondii*. Asterisks indicate *P* values of 0.01 or less.

serum than did mice of the same genotype infected with the FORT strain. It is of interest that although it has been reported that IFN-γ is the major cytokine involved in resistance to *T. gondii* (9, 18), our results revealed that mice with severe TE had levels of IFN-γ in serum that were significantly higher than those of mice with mild TE. Moreover, it has been reported that the administration of recombinant IFN-γ to CBA/Ca mice infected with the ME49 strain of *T. gondii* resulted in an improvement in the brain inflammation in these mice (17). However, it should be noted that recent reports have shown that IFN-γ can have a detrimental effect on intestinal toxoplasmosis (10, 11).

The virulence of *T. gondii* appears to be influenced by the type of the parasite strain; type I strains cause rapid death in mice, whereas the outcome of infections with type II strains depends on the challenge dose and on the genotype of the host (13). Our results revealed that each of the three type I strains examined caused rapid and high mortality, whereas mortality in mice infected with each of the eight type II strains was very low. In addition, of the type II strains, only the ME49 strain caused severe TE, indicating that not all type II strains are capable of producing severe TE in CBA/Ca mice. A recent report demonstrated that sexual recombination in distinct and competing clonal lines of *T. gondii* resulted in a significant increase in the virulence of the F_1 progeny for mice (6). Data on the degree of sexual recombination of the strains used in the present study was not available when the work was conducted.

The two type II strains employed in this study caused low mortality, and histopathology did not reveal any differences in degree of intestinal and lung infections caused by these strains. Thus, the capacity to disseminate rapidly and cause death was apparently not the reason for the differences in the cytokine levels in serum as has been suggested previously (13). Similar to the results for IFN-γ, the levels of TNF-α in serum were higher in the ME49-infected mice than in the FORT-infected mice. TNF-α has been reported as important for preventing TE in mice. Its in vivo neutralization resulted in acute TE in C57BL/6 mice (which have the same genotype as CBA/Ca mice) infected with *T. gondii* ME49 (5). In contrast, our results revealed that severe TE was noted in those mice with the highest levels of TNF-α in serum. However, the levels of this cytokine in the brains of infected mice were not measured in the present study.

The levels of IL-2 in serum were significantly higher in mice with mild TE than in mice with severe TE. This observation is of interest because the administration of recombinant IL-2 to mice infected with a lethal inoculum of *T. gondii* promoted their survival (14).

At this point, the high levels of cytokines, especially of IFN-γ and TNF-α, that we found in the serum of mice experiencing severe TE may not be considered the main cause of the TE. However, given the roles of IFN-γ and TNF-α in enhancing the severity of toxoplasmosis in mice (13) and in the development of necrosis (10) and induction of apoptosis of intestinal T cells (11), it is possible that these cytokines participated in the development of the severe TE noted in the ME49-infected mice. The fact that mice of the same genotype that were infected with the FORT strain did not develop severe TE indicates a role for the strain of *T. gondii* in the generation of severe TE. In addition, our results indicated that not all type II strains of *T. gondii*, including those isolated from humans with TE, are capable of causing severe TE in CBA/Ca mice.

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