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Decreased Resistance of TNF Receptor p55- and p75-Deficient Mice to Chronic Toxoplasmosis Despite Normal Activation of Inducible Nitric Oxide Synthase In Vivo

George S. Yap,[1] Tanya Scharton-Kersten, Hugues Charest, and Alan Sher

The importance of TNF-α in host defense to the intracellular parasite, *Toxoplasma gondii*, was investigated in mice lacking both the p55 and p75 receptors for this cytokine. Upon i.p. infection with the avirulent ME49 strain, knockout mice were capable of limiting acute i.p. infection, but succumbed within 3 to 4 wk to a fulminant necrotizing encephalitis. Receptor-deficient mice harbored higher cyst burdens and exhibited uncontrolled tachyzoite replication in the brain. The lack of TNF receptors did not adversely affect the development of a type 1 IFN-γ response. In vitro studies with peritoneal macrophages stimulated with IFN-γ and tachyzoites indicated that under limiting concentrations of IFN-γ, nitric oxide-mediated toxoplasmastatic activity is TNF-α dependent. However, this requirement is overcome by increasing the dose of IFN-γ. Furthermore, both ex vivo and in vivo studies demonstrated that inducible nitric oxide synthase induction in the peritoneal cavity and brain is unimpaired in receptor-deficient mice. Thus, TNF-dependent immune control of *T. gondii* expansion in the brain involves an effector function distinct from inducible nitric oxide synthase activation.   *The Journal of Immunology,* 1998, 160: 1340–1345.

In patients immunologically compromised as a consequence of AIDS or treatment with immunosuppressive drugs, infection with the intracellular protozoan parasite, *Toxoplasma gondii*, can result in a severe, life-threatening disease (1). However, in immunocompetent individuals, infection is usually asymptomatic due to the early induction of a potent cell-mediated immunity. This response drives the actively proliferating tachyzoites into quiescent bradyzoites that persist in the form of cysts in the CNS[2] and skeletal muscle of the host. Active immunity is similarly required for the maintenance of dormancy and prevention of reactivation and tachyzoite-induced lesion development. The major effector mechanism(s) that controls the acute stage of tachyzoite replication and actively suppresses reactivation of persistent tissue cysts requires IFN-γ secreted initially by NK cells and subsequently by CD4 and CD8 T cells (2–4).

In addition to IFN-γ, TNF-α has been implicated to play a major role in both innate and acquired immunity to *T. gondii*. Thus, TNF-α synergizes with IL-12 in the induction of IFN-γ from NK cells, thereby promoting the T cell-independent pathway of host resistance to the parasite (5). Moreover, the production by IFN-γ-activated macrophages of the antimicrobial metabolite NO is dependent on autocrine TNF-α (6). Finally, the in vivo importance of the cytokine in the host response to *T. gondii* is supported by studies demonstrating that treatment of mice with neutralizing anti-TNF-α Abs results in both the abrogation of acute resistance and reactivation of latent infection in the CNS (7, 8). On the other hand, TNF-α is also known to be a major mediator of tissue damage and, in *T. gondii* infection, is associated with both acute and chronic immunopathology (9–11).

To directly address the role played by TNF-α in the host response to *T. gondii*, we have studied the infection in mice produced by gene targeting, which are deficient in the two known receptors (TNF-R p55 and p75) for this cytokine (12, 13). Our results indicate that TNF-receptor signaling is not required for early control of parasite growth, but is critical for the prevention of toxoplasmic encephalitis later in infection. Surprisingly, macrophage activation, as judged by iNOS production and in vitro microbicidal function, appears to be largely unimpaired in the *T. gondii*-exposed receptor-deficient animals. The latter findings suggest that cells other than macrophages are the primary effectors of TNF receptor-dependent resistance during chronic *Toxoplasma* infection in the CNS.

Materials and Methods

Experimental animals

Mice lacking both the p55 and p75 chains of the TNF receptor were generated by interbreeding singly deficient homozygous parental mice. One parental strain lacked exons 2, 3, and part of 4 of the TNF-R p55 chain (12), while the second parental strain contained a neomycin-resistance cassette inserted into the second exon of the TNF-R p75 gene (13). Breeding pairs of these mice were generously provided by Mark Moore of Genentech (South San Francisco, CA). The TNF receptor-deficient mice used throughout these studies were maintained on a random C57BL/6 $\times$ 129 hybrid background. As wild-type (WT) controls, C57BL/6 $\times$ 129 F_2 hybrids were utilized. Animals were sex and age matched for each experiment.

Parasites, Ags, and experimental infections

The avirulent ME49 strain of *T. gondii* was used to infect animals. Brain suspensions were prepared from infected C57BL/6 mice. Each experimentally infected mouse received 20 cysts in a volume of 0.5 ml by the i.p. route. For in vitro infection and stimulation of cells, tachyzoites of the RH strain, maintained by passage in human foreskin fibroblasts, were utilized. Soluble tachyzoite Ag (STAg) was prepared as previously described (3).

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[2] Abbreviations used in this paper: CNS, central nervous system; iNOS, inducible nitric oxide synthase; KO, knockout; NO, nitric oxide; RT-PCR, reverse-transcriptase polymerase chain reaction; STAg, soluble tachyzoite antigen; WT, wild-type.

Histopathology

The extent of active replication of tachyzoites was assessed by harvesting peritoneal cells of mice 5 days postinfection. Methanol-fixed cytocentrifuge preparations were stained using a modified Wright Giemsa procedure, per manufacturer's instruction (Diff-Quik, Baxter, McGraw, Park, IL).

At indicated times after infection, brain as well as lung, liver, spleen, and heart tissue samples were fixed in 4% buffered Formalin and processed for paraffin embedding and sectioning. Five-micron sections were stained using the periodic acid schiff (PAS) procedure to aid in the visualization of tissue cysts. The average number of cysts per mouse was determined from two noncontiguous sections, in a blinded fashion.

Measurement of cytokine production by spleen cells

Spleens were homogenized by passing through a sterile fine wire mesh, and the resulting single cell suspensions were depleted of red cells by hypotonic lysis. Three million spleen cells/ml were plated in 24-well plates and stimulated with STAg (10 μg/ml). Supernatants were harvested 24 h later for IL-12 determination and 48 h later for measurement of IFN-γ.

Determination of IFN-γ and IL-12 p40 levels in tissue culture supernatants was conducted using previously described sandwich ELISA protocols (4).

Macrophage toxoplasmastatic assay

Peritoneal exudates were harvested from WT and KO mice 4 days after elicitation, and plated at 2×10^6 cells/ml. Cells were preincubated with 100 U/ml of murine rIFN for 2 h. RH tachyzoites were then added at a multiplicity of infection of 0.2 parasites per cell. After an overnight incubation, 50 μl of supernatant was harvested from each well for determination of NO production. Cultures were then pulsed with 0.5 uCi of [^{3}H]uracil and harvested the next day using an automatic cell harvester. Radioactivity incorporated into the cells was measured by liquid scintillation counting. The percent inhibition of tachyzoite growth was calculated as follows: {1 − [(IFN + RH) − (IFN − RH)/(medium + RH) − (medium − RH)]} $\times$ 100%.

In some experiments, a neutralizing polyclonal rabbit anti-mouse TNF-α antiserum (Genzyme Corp., Boston, MA) or preimmune rabbit serum was added together with IFN-γ at a 1/100 dilution. In other experiments, 1, 10, and 100 U/ml of IFN-γ was used to activate and compare the in vitro response of WT and KO peritoneal cells.

Measurement of NO

Nitrite levels were measured in the culture supernatants of peritoneal exudate cells using the Griess reagent (14). Fifty-microliter aliquots of culture medium were mixed in 96-well plates with an equal volume of 0.5% sulfanilamide dihydrochloride and 0.05% naphthylethylenediamide dihydrochloride in 2.5% phosphoric acid. A standard curve (4–250 μM) of NaNO$_2$ in complete medium was prepared and read together with the samples at a wavelength of 550 nm.

RT-PCR analysis

RNA was extracted from brain tissue suspended in RNAzol, as previously described (8). RT-PCR analysis was performed to detect changes in the mRNA levels of IFN-γ, iNOS, and hypoxanthine phosphoribosyl transferase using primers and probes described previously (8). Thirty cycles of amplification were used for IFN-γ mRNA detection, and 33 cycles for the other genes.

Western blot detection of brain-associated iNOS protein

Brain tissue from uninfected or day 20 infected mice was homogenized in 1× Laemmli solubilizing buffer containing 2 μM leupeptin, 1 μM pepstatin, and 1 mM PMSF using a polytron tissue disrupter. Five microliters of solubilizing buffer were used per mg tissue. Extracts were diluted 1 to 5 in sample buffer and boiled for 5 min. Samples were separated on 4 to 12% Tris-glycine gels and blotted onto nitrocellulose paper. Membranes were blocked and probed with a rabbit anti-iNOS polyclonal Ab (0.1 μg/ml) (Upstate Biotech, Lake Placid, NY) and developed for chemiluminescence detection using horseradish peroxidase anti-rabbit IgG. Extracts from iNOS-deficient mice were included as negative controls.

Statistical analysis

An unpaired student *t* test was used for comparison of cyst counts and cytokine secretion in WT and TNF receptor-deficient mice.
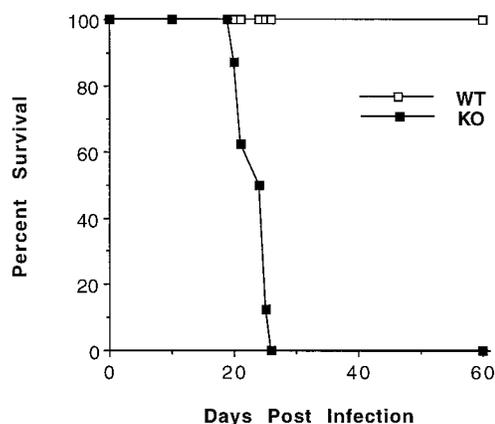
FIGURE 1. Survival of TNF R1/R2-deficient and WT (C57BL/6 $\times$ 129/J F$_2$) mice infected i.p. with 20 cysts of *T. gondii* (ME49 strain). The data shown involved nine mice per group and are representative of three experiments performed.

Results

Decreased resistance of TNF R1/R2-deficient mice to T. gondii *infection*

To assess their susceptibility to *T. gondii*, TNF R1/R2-deficient and WT C57BL/6 $\times$ 129/J F$_1$ mice were inoculated i.p. with 20 cysts of the avirulent ME49 strain, and the survival of the animals was monitored. Whereas the WT mice survived for at least 60 days postinfection, the receptor-deficient animals all succumbed within 20 to 26 days (Fig. 1). This survival pattern, which was observed repeatedly in three independent experiments, is clearly distinct from that of IFN-γ-deficient mice, which succumb to acute infection within 10 to 12 days after inoculation with the same parasite strain (4, 15). The resistance of the TNF R1/R2-deficient animals to acute infection was confirmed by microscopic examination of cells harvested from the peritoneal inoculation site at day 5. In both these and WT animals, only 0.4% of the cells was infected with tachyzoites vs greater than 25% in populations recovered at the same time point from IFN-γ-deficient mice.

Examination of periodic acid Schiff-stained sections from brains of infected WT and mutant mice on days 15 and 21 revealed a 10-fold increase in the number of parasite cysts in the TNF R1/R2 KO animals (Fig. 2*A*). Whereas the tissues from the WT animals at these time points showed only intact cysts and inflammatory nodules (Fig. 2*B*), active tachyzoite replication (Fig. 2*C*), combined with extensive parenchymal cell necrosis (Fig. 2*D*), was clearly evident within the brain sections from infected TNF R1/R2-deficient mice. Moreover, numerous extracellular tachyzoites were evident within the fully developed necrotizing lesions (Fig. 2*E*). Examination of other organs, including the lungs, spleen, and liver of moribund mutant mice, failed to reveal systemic dissemination of the infection (data not shown). Thus, the dominant pathologic lesion in the TNF R1/R2-deficient animals appears to be the development of a fulminant toxoplasmic encephalitis.

Normal development of type 1 cytokine responses in TNF R1/R2 KO mice

As IL-12-dependent IFN-γ production is the major response that controls *T. gondii* infection, we asked whether both cytokines are produced normally in the absence of TNF receptor signaling. As shown in Figure 3, spleen cells from WT and receptor-deficient mice spontaneously produced comparable levels of IFN-γ and IL-12p40 on day 5 postinfection. Moreover, when restimulated in
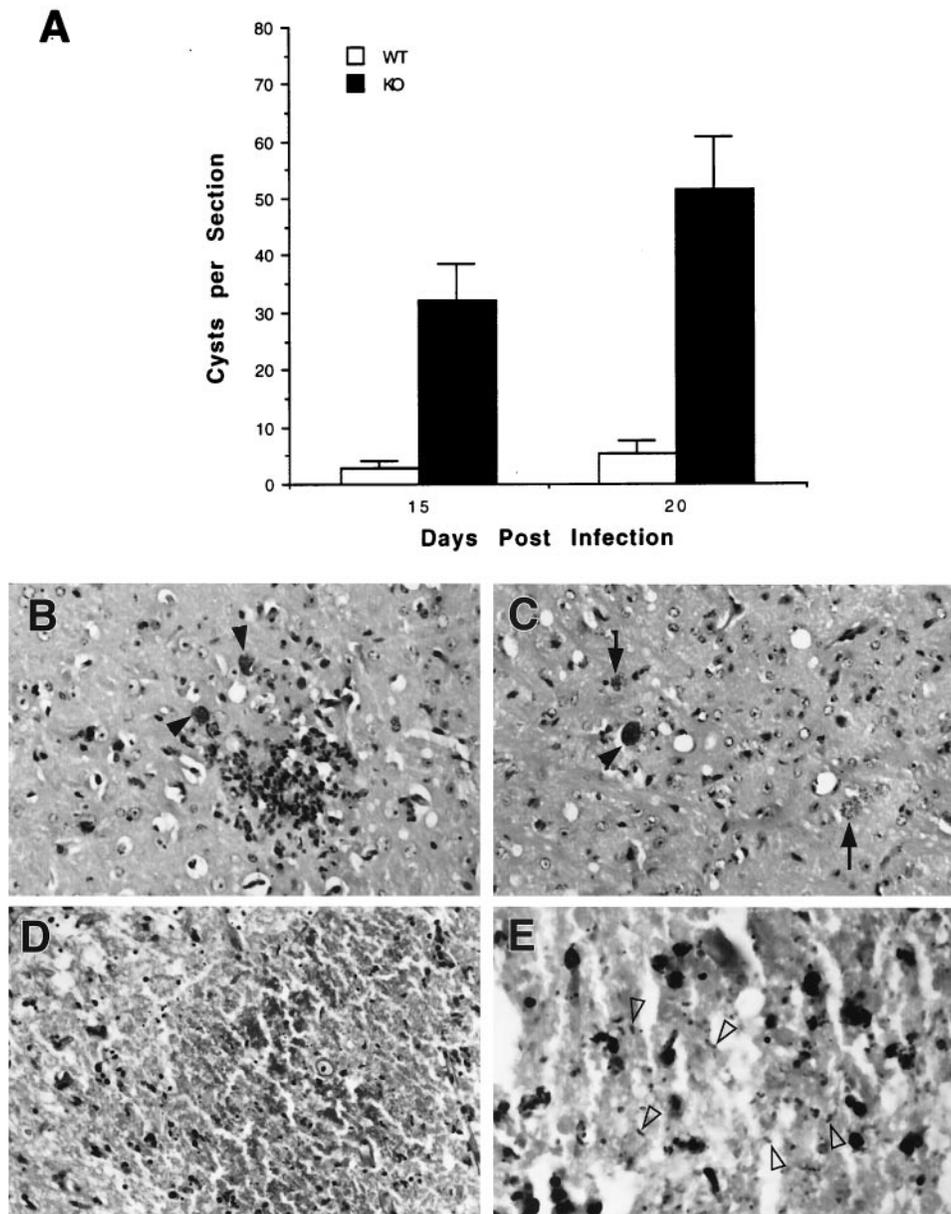
FIGURE 2. Increased cyst counts and severe necrotizing encephalitis in *T. gondii*-infected TNF R1/R2-deficient mice. WT and TNF R1/R2-deficient mice were infected i.p. with 20 cysts and brains harvested 15 and 21 days later for histopathology and quantitation of cysts. *A*, Mean cyst counts and SE are shown for each group of 7 to 11 animals. Cyst counts were significantly different between WT and TNF R1/R2-deficient mice at both time points ($p <$ 0.01). *B–E*, Photomicrographs of periodic acid Schiff's-stained brain sections from WT (*B*) and receptor-deficient mice at 21 days postinfection. *C–E*, An inflammatory nodule with two dormant cysts (solid arrowheads) is shown in *B*. Active tachyzoite replication (arrows) and a cyst (solid arrowhead) are evident in the brain of an infected TNF R1/R2-deficient mouse (*C*). In addition, an extensive area of necrosis (*D*) with tachyzoites (unfilled arrowheads) in the cellular debris is shown at a higher magnification in *E*.

vitro with STAg, the control and mutant splenocytes synthesized indistinguishable quantities of IFN-γ both before and on days 5, 15, and 20 after parasite inoculation. Similarly, no difference was apparent in the STAg-induced IL-12 p40 responses of the WT and TNF R1/R2-deficient mice on day 5 postinfection.

To assess IFN-γ response development in vivo, the cytokine was measured by RT-PCR in the brains of mice on days 5, 15, and 21 postinfection. As shown in Figure 5*A*, on day 5, little or no cytokine message was detected. By days 15 and 21, high levels of IFN-γ mRNA were present in brain tissue from both receptor-deficient and WT mice, and no consistent quantitative differences were evident. Thus, *T. gondii*-infected TNF R1/R2-deficient mice display unimpaired IFN-γ and IL-12 synthesis, and therefore, their

inability to control tachyzoite replication in the CNS cannot be explained by a defect in type 1 cytokine expression.
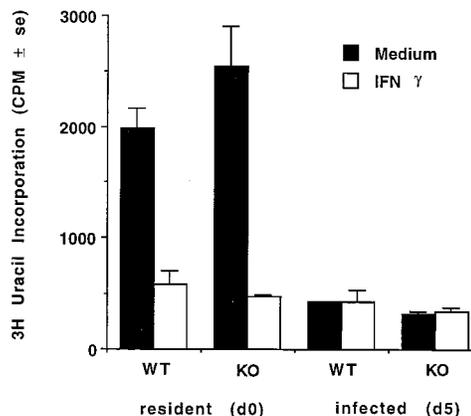
TNF R1/R2-deficient macrophages exhibit a conditional defect in NO production and toxoplasmastatic activity in vitro

The in vitro toxoplasmastatic activity of IFN-γ-activated macrophages is strictly dependent upon the antimicrobial metabolite, NO, a product of the iNOS enzyme (16, 17). Previous studies have reported that iNOS activation requires autocrine stimulation by TNF-α (6). We, therefore, assessed whether NO production and in vitro control of tachyzoite replication can be triggered in macrophages in the absence of TNF receptors. In the absence of
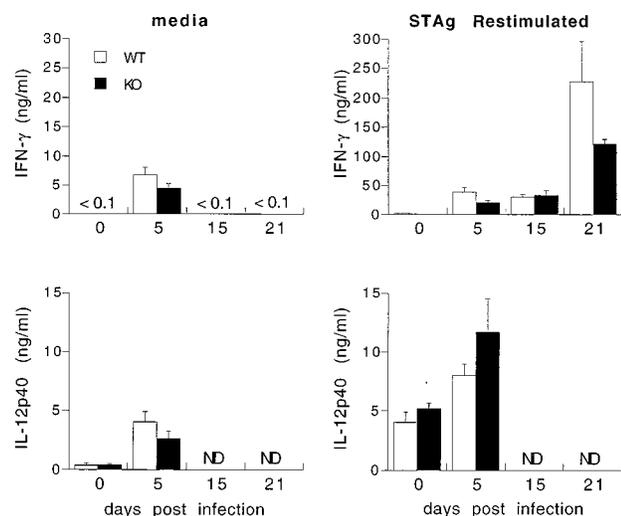
FIGURE 3. IFN-γ and IL-12 p40 synthesis by splenocytes from WT and TNF R1/R2-deficient mice in vitro. Spleen cells were cultured and cytokine levels in supernatant were tested, as detailed in *Materials and Methods*. Data shown are mean and SE of four to five mice per group. Similar results were obtained in two additional experiments. No statistically significant difference in production of either cytokine between WT and KO cells was observed.

FIGURE 4. In vitro replication of *T. gondii* tachyzoites in peritoneal cells harvested from uninfected and 5 day infected TNF R1/R2-deficient or WT mice. Animals were infected with 20 ME49 cysts, and 5 days later peritoneal cells were harvested. The cells were then stimulated with 100 U/ml of IFN-γ or left untreated, infected, and cultured, as described in *Materials and Methods*. Data shown are representative of three experiments performed.

signaling by autocrine TNF-α emerges as a requirement for iNOS induction in macrophages.

Unimpaired iNOS activation in TNF R1/R2-deficient mice in vivo

If the concentrations of IFN-γ are indeed not limiting in vivo, macrophages from infected TNF R1/R2-deficient mice should exhibit the same level of toxoplasmastatic activity as cells from infected WT mice when examined ex vivo. As shown in Figure 4, resident peritoneal cells from either mutant or WT animals both supported growth of the parasite and were activated by 100 U/ml of IFN-γ to suppress tachyzoite replication. In contrast, tachyzoites grew only marginally in ex vivo derived peritoneal cell populations from 5 day infected mice, regardless of further in vitro activation with IFN-γ. The cells from infected TNF R1/R2-deficient mice were indistinguishable from WT peritoneal populations in their ability to restrict parasite growth, both spontaneously as well as after in vitro activation with IFN-γ. Thus, during the acute stage of infection, macrophages from TNF R1/R2-deficient animals appear to have unimpaired microbistatic activity against *T. gondii* in vivo.

As noted above, TNF R1/R2-deficient mice die at 20 to 26 days postinfection, and this mortality is associated with increased parasite replication and encephalitis. To assess whether this defect in

tachyzoite infection, exposure of thioglycolate-elicited macrophages from WT mice to 100 U/ml of IFN-γ for 18 h resulted in the induction of low, but significant, NO synthesis, whereas the same cell populations from TNF R1/R2-deficient mice failed to respond (Table I). Nevertheless, upon addition of RH tachyzoites to macrophage preincubated with 100 U/ml of IFN-γ, NO production was stimulated equally in cultures from WT or receptor-deficient mice, and both macrophage populations were equivalent in their ability to restrict the growth of the parasite in vitro. These results suggest that in the absence of TNF receptor function, the parasite itself may provide compensatory signals needed to activate iNOS. However, when cells were activated with only 1 U/ml of IFN-γ, NO production and toxoplasmastatic activity were found to be impaired significantly in the cells from mutant animals. In agreement with this observation, addition of neutralizing anti-TNF-α Ab diminished NO production and toxoplasmastatic activity by WT macrophages in response to IFN-γ and RH only when the lower dose (1 U/ml) of IFN-γ was used. Taken together, the above results suggest that under limiting concentrations of IFN-γ,

Table I. *NO synthesis and toxoplasmastatic activity of WT and TNFR KO macrophages[a]*

	WT			KO		
	Medium	1 U/ml IFN-γ	100 U/ml IFN-γ	Medium	1 U/ml IFN-γ	100 U/ml IFN-γ
Medium	6.3 ± 1.6[b]	5.6 ± 1.5	21.7 ± 5.2	6.3 ± 0.6	5.5 ± 1.1	8.7 ± 1.8
Medium + RH	6.3 ± 1.0	77.7 ± 13.6	95.2 ± 8.1	5.5 ± 0.4	22.2 ± 16.0	81.7 ± 5.3
% Inhibition	0[c]	93	94	0	29	90
Normal IgG	5.0 ± 0.9	4.9 ± 1.1	16.1 ± 2.3	6.1 ± 1.0	5.1 ± 0.6	6.2 ± 1.5
Normal IgG + RH	5.3 ± 1.8	79.4 ± 16.2	86.6 ± 15.5	5.8 ± 0.5	23.4 ± 19.5	82.6 ± 3.7
% Inhibition	0	84	90	0	17	88
Anti-TNF-α	5.2 ± 0.7	6.2 ± 1.9	6.5 ± 0.7	5.7 ± 1.1	5.6 ± 0.2	6.9 ± 0.6
Anti-TNF-α + RH	5.8 ± 0.9	12.8 ± 2.2	68.5 ± 2.7	6.0 ± 0.3	12.3 ± 2.4	78.1 ± 5.2
% Inhibition	0	22	78	0	16	79

[a] The values shown for % Inhibition and NO synthesis were averaged from each of three mice assayed separately.

[b] Nitric oxide production (nitrite in micromoles per liter).

[c] Inhibition of [3H]uracil uptake: [1− {[(IFN + RH) − (IFN-RH)/(medium + RH)−(medium-RH)}] × 100%.
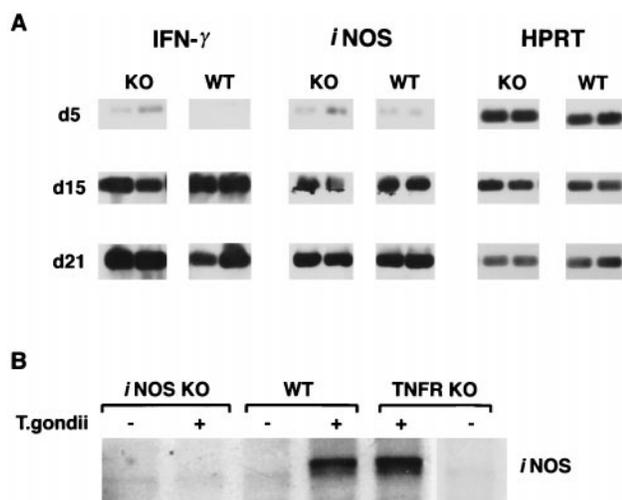
FIGURE 5. Induction of IFN-γ and iNOS gene expression in brains of TNF R1/R2-deficient and WT mice upon *T. gondii* infection. *A*, RNA and cDNA were prepared from brain tissue of WT or TNF R1/R2-deficient (KO) mice and analyzed for IFN-γ, iNOS, and hypoxanthine phosphoribosyl transferase gene expression by RT-PCR analysis. *B*, Extracts from uninfected and day 20 infected WT and TNF R1/R2-deficient mice were probed with a polyclonal rabbit anti-mouse iNOS. Samples from uninfected and day 20 infected iNOS-deficient (iNOS KO) mice were included as negative controls. Similar results were obtained from further analysis of three additional WT and KO mice.

host resistance is due to a failure in iNOS induction, mRNA levels for the enzyme were measured by RT-PCR in brain tissue on days 5, 15, and 21. As shown in Figure 5*A*, iNOS transcripts increased substantially after 15 and 21 days of infection in both WT and mutant mice. Similarly, induction of the iNOS protein in both strains of infected animals was observed by Western blot analysis of brain extracts probed with an iNOS-specific polyclonal antiserum (Fig. 5*B*). Thus, during *T. gondii* infection, the induction of iNOS activation in vivo can occur independently of signals delivered by the TNF receptor.

Discussion

The aim of the present study was to elucidate the role of TNF-α in host resistance to *T. gondii* in vivo. Toward this end, we studied avirulent infection in mice lacking both the p55 and p75 TNF receptors. Our results indicate that TNF receptor signaling is essential for survival of the infected animals. Thus, WT mice inoculated i.p. with the ME49 strain of *T. gondii* survived beyond 60 days, while TNF receptor-deficient mice succumbed within 20 to 26 days postinfection and exhibited gross as well as histopathologic manifestations characteristic of necrotizing encephalitis. This observation is consistent with previous findings indicating that administration of neutralizing anti-TNF Abs to chronically exposed mice results in rapid reactivation of latent infection and death due to encephalitis (8). Surprisingly, however, at 5 days postinoculation, we failed to observe increased parasite numbers in peritoneal cells of receptor-deficient vs WT animals. These parasitologic findings in infected TNF receptor-deficient mice are reminiscent of those recently reported for iNOS-deficient mice, which also survive acute, but not chronic, toxoplasmosis (17). The latter phenotype is quite distinct from that observed in IFN-γ-deficient mice that succumb within 10 to 12 days after parasite inoculation (4). Taken together, the above findings indicate that TNF receptor signaling and iNOS activity are not required for early resistance to *T.*

gondii, but are essential for control of chronic infection in the CNS.

Do the similarities in the resistance phenotype of the TNF receptor- and iNOS-deficient mice simply reflect lesions in a common or convergent effector pathway? A prevailing paradigm of macrophage activation suggests that this may indeed be the case. Thus, autocrine production of TNF-α has been shown to be required for IFN-γ to fully activate macrophages based on both iNOS induction and in vitro microbicidal activity against a variety of pathogens (18). Indeed, when tested under conditions of low dose IFN-γ priming, TNF R1/R2-deficient macrophages or macrophages from WT mice treated in vitro with neutralizing TNF-specific Ab displayed defective NO and toxoplasmastatic responses (Table I). Nevertheless, this requirement for TNF-α triggering is by no means obligatory, since it is overcome readily when the parasites are added to the cultures along with increased concentrations of IFN-γ. The latter observation is seemingly in conflict with a previous report describing a requirement for TNF-α in the toxoplasmastatic activity of macrophages activated with high doses (1000 U) of IFN-γ (6). Nonetheless, in that study, the tachyzoites were added after 24 h of preincubation with IFN-γ, whereas in our studies, infection was initiated 2 h after IFN-γ treatment. Thus, in the former series of experiments, it is possible that the initial priming signal provided by IFN-γ may have decayed by the time parasites were introduced into the cultures. This interpretation is consistent with the observations of Sibley et al. (19), that concomitant treatment with TNF-α or LPS is necessary for triggering of toxoplasmastatic activity in IFN-γ-primed macrophages. In the same experiments, neutralization of TNF-α resulted in loss of microbicidal function in cultures triggered by TNF-α, but not by LPS.

The concept that *T. gondii* can provide its own triggering signal is supported by the finding that a STAg induces NF-κB DNA-binding activity in inflammatory macrophages (20), even when TNF-α is neutralized. Thus, the requirement for endogenous TNF-α signaling in the NF-κB-dependent transcriptional activation of the iNOS promoter (21) appears to be bypassed effectively by the parasite itself, or possibly through the induction of other cytokines. That this same bypass of TNF function occurs in vivo is supported by our observation of unimpaired iNOS induction and ex vivo macrophage toxoplasmastatic activity in infected TNF R1/R2-deficient mice.

Although not necessarily involving the same mechanisms, studies on host resistance to other pathogens in TNF receptor-deficient animals also argue that the requirement for TNF-α in macrophage activation and iNOS expression can be circumvented in vivo. For example, while p55 TNF receptor-deficient mice are less resistant to *Mycobacterium tuberculosis*, they nevertheless are able to up-regulate iNOS expression in vivo (22). Similarly, the same TNF receptor-deficient animals were able to clear cutaneous infections with *Leishmania major* and exhibited unimpaired NO-dependent macrophage leishmanicidal activity in vitro (23). Interestingly, in the latter study, in common with our findings, a defect in the macrophage activation could be revealed by lowering the dose of IFN-γ used for in vitro priming. Since there are two known receptors for TNF-α, these previous studies do not preclude the possible use of the second, p75 receptor for macrophage triggering. The latter objection is not a concern in the experiments performed in this study on *T. gondii*, in which mice lacking both receptors were used, nor in a recent study, in which the same animals were infected with *Mycobacterium avium* and no impairment of host resistance was observed (24).

Although in the murine *T. gondii* infection model iNOS and TNF receptor deficiencies appear to have different effects on macrophage activation, they nevertheless result in the same host resistance phenotype in vivo. In attempting to understand the function of TNF vs iNOS in control of *T. gondii* infection, two points are worth considering. First, TNF receptors are known to be distributed ubiquitously in a wide range of nucleated cell types (25) as opposed to the more restricted expression of the iNOS gene primarily in macrophage-lineage and endothelial cells (26, 27). Secondly, the promiscuous host cell infectivity of *T. gondii* predicts that essentially all of the cell types encountered by the parasite, as well as controlling its growth, will express TNF receptors. Thus, it is likely that TNF-α-dependent resistance to *T. gondii* involves effector functions unrelated to iNOS activity. For instance, effector T cells synthesizing both IFN-γ and TNF-α (28) may trigger control of parasite growth in infected neurons, a cell type that does not express iNOS (29, 30), but that serves as a unique reservoir for bradyzoites in the brain (31). Since iNOS is also required for control of chronic infection (17), such a neuronal effector mechanism is likely to act in concert with iNOS-dependent effector functions expressed selectively by CNS-associated macrophages/microglial cells and/or endothelial cells. This model would explain why mice lacking TNF receptors remain susceptible to chronic toxoplasmosis despite apparently normal induction of iNOS in vivo. Further analysis of the cellular basis of the defect in parasite control in TNF receptor-deficient mice should provide a useful approach for identifying this tissue-specific and perhaps stage-specific mechanism of host resistance. One candidate effector function is the depletion of intracellular tryptophan pools by the enzyme, indoleamine dioxygenase, which is known to be synergistically induced by IFN-γ and TNF-α in cells of neuroglial origin (32).

Acknowledgments

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