INFECTION AND IMMUNITY, May 1992, p. 1979–1983
0019-9567/92/051979-05$02.00/0

Vol. 60, No. 5

A Protective Role for Endogenous Tumor Necrosis Factor in *Toxoplasma gondii* Infection

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Received 8 November 1991/Accepted 2 March 1992

The involvement of tumor necrosis factor (TNF) in resistance to *Toxoplasma gondii* infection was examined by means of experiments in which mice were treated with anti-TNF antibodies prior to infection with ME49, a low-virulence *Toxoplasma* strain. In (BALB/cBy × C57BL/6J)F_1 ($CB6F_1$) mice, which are highly resistant to intraperitoneal (i.p.) infection with *T. gondii* ME49, 10^4 neutralizing units of anti-TNF caused a significant increase in trophozoite numbers in the peritoneal cavities of infected mice and transient signs of illness but no deaths. i.p. infection of anti-TNF-treated C57BL/6J (B6) mice, which are more susceptible to *T. gondii* and develop a chronic progressive toxoplasmosis, resulted in death for some of the mice. If the mice were infected perorally, however, and treated with anti-TNF, mortality was extensive in B6 mice but not in $CB6F_1$ mice. Although it was not detected in their sera, TNF was found in the peritoneal fluids of i.p.-infected $CB6F_1$ and B6 mice. Endogenously produced TNF thus appears to be an important mediator of resistance to *T. gondii*.

Immunocompetent individuals who ingest cysts of the protozoan parasite *Toxoplasma gondii* ordinarily experience asymptomatic infections. Organisms released from ingested cysts invade host cells, proliferate, disseminate, and eventually form new quiescent cysts in the brain and other tissues (12). However, immunocompromised individuals may develop a severe, potentially fatal disease as a result of *T. gondii* infection. Fetuses and AIDS patients are especially at risk, the latter often experiencing toxoplasmic encephalitis believed to result from the reactivation of previously quiescent cysts as the patient's immune system deteriorates. There is considerable interest, therefore, in understanding mechanisms that may protect individuals against *T. gondii*.

Work in other laboratories has demonstrated that gamma interferon (IFN-γ) plays a crucial role in protecting mice against acute infection with ME49, a low-virulence strain of *T. gondii* (20, 21). Thus, mice given an intraperitoneal (i.p.) injection of a monoclonal antibody that neutralizes IFN-γ (17) before being infected i.p. with ME49 cysts die within as few as 8 days. Antibody-treated mice may have more than 10^7 trophozoites in their peritoneal cavities shortly before death (20).

Although it has been suggested that IFN-γ is the major mediator of resistance (21), the cytokine tumor necrosis factor (TNF) has also been studied with regard to a possible role in resistance to acute *T. gondii* infection. Mice treated with recombinant TNF were not protected against a lethal challenge with *T. gondii* (3), nor was there evidence that fibroblasts or macrophages treated with recombinant TNF in vitro could control *T. gondii* better than control-treated cells (1, 5). Another study (4) revealed that mice treated for 8 days with recombinant murine TNF during infection with trophozoites of a virulent strain of *T. gondii* were protected to some extent (40% mortality within 60 days versus 100% mortality for controls). In light of this demonstration that exogenously administered TNF may have a protective effect in vivo, the experiments described in this report were undertaken to determine whether TNF produced endogenously in response to infection with *T. gondii* is protective. It was reasoned that a protective role for endogenously produced TNF would be revealed most clearly by exacerbation of infection in its absence. Therefore, in the present experiments, in contrast to those that investigate the effects of exogenously administered TNF during infection with a virulent strain of *T. gondii*, resistance to an ordinarily avirulent strain was studied by using a TNF-neutralizing antibody.

MATERIALS AND METHODS

Mice. Inbred female C57BL/6J (abbreviated B6) and (BALB/cBy × C57BL/6J)F_1 ($CB6F_1$) mice between 8 and 10 weeks old were used. The mice were bred in a barrier-sustained facility at the Trudeau Institute from founder stocks obtained from the Jackson Laboratory, Bar Harbor, Maine. Periodic serological tests (performed by the Research Animal Diagnostic and Investigative Laboratory, University of Missouri, Columbia, Mo.) revealed no evidence of exposure of mice bred in this facility to common pathogens of mice, including murine hepatitis virus.

Preliminary experiments performed in this laboratory have shown that $CB6F_1$ females are very resistant to infection with strain ME49 cysts (see below). No signs of sickness or deaths were evident for months after i.p. infection with 20 ME49 cysts, nor were many cysts detectable in brain suspensions. In contrast, B6 mice are chronically susceptible to an i.p. infection with 10 or more ME49 cysts. Sickness (piloerection, hunched posture, and loss of motor control) was often evident within 6 weeks of infection, and deaths occurred from 8 weeks on. At death, the brains of B6 mice contained 10^3 or more cysts. This difference between $CB6F_1$ and B6 mice in innate susceptibility is probably attributable, at least in part, to resistance mechanisms controlled by major histocompatibility complex genes (14, 23).

Toxoplasma. The ME49 strain of *T. gondii* was maintained by i.p. inoculation of B6 female mice with a brain suspension containing 20 cysts in a volume of 0.2 ml of Hanks balanced salt solution (GIBCO, Grand Island, N.Y.) buffered to pH 7.4 with HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid). Cysts for use in experiments were obtained from mice, killed by CO_2 asphyxiation 6 or more weeks after infection, by gentle manual disruption of brains in a plastic tube (Falcon 2057; Becton Dickinson, Lincoln Park, N.J.) with a loose-fitting Teflon pestle. The brains were suspended in 2.0 ml of Hanks balanced salt solution, and a 20-μl sample

TABLE 1. Effect of anti-TNF antibody, given to $CB6F_1$ mice prior to i.p. infection with *T. gondii*, on trophozoite number, serum IFN-γ, and survival

Treatment[a]	Log_{10} trophozoites	Serum antiviral activity (U/ml) following treatment with[b]		Survival (days postinfection) (n = 5)
		Medium alone	Anti-IFN-γ[c]	
Control IgG (5.8 mg)	4.9 ± 0.16	128 (78–209)	<18	>28, >28, >28, >28, >28
Anti-IFN-γ (5.8 mg)	6.7 ± 0.13	56 (41–76)	<16	8, 8, 9, 9, 10
Anti-TNF (0.65 mg)	6.7 ± 0.63	169 (44–653)	<16	>28, >28, >28, >28, >28

[a] $CB6F_1$ females were given i.p. injections of immune rabbit IgG of the indicated specificities or control IgG 6 h prior to challenge with 20 strain ME49 cysts. Some mice were killed 7 days later and bled for serum, and their peritoneal cavities were flushed to obtain trophozoites for enumeration. The survival of additional mice from each group was monitored.

[b] Means of antiviral activities were calculated from log_2-transformed data. Numbers in parentheses are activities corresponding to ±1 standard deviation of the log-transformed means. In groups in which one or more activities were <16, the detection limit of the assay, a value of 16 was used to calculate the mean. Standard deviations could not be meaningfully calculated for the groups with numbers preceded by <. Serum IFN-γ activities in uninfected $CB6F_1$ controls were less than 16 U/ml.

[c] Neutralization capacities greatly in excess of amounts of IFN-γ detected.

was placed under a coverslip for counting microscopically with a 10× phase-contrast objective. Thus, 1 cyst in a 20-μl sample corresponds to 100 cysts in the entire 2-ml suspension, making 100 cysts per brain the limit of detection. The infections were initiated, as indicated, either by injection of cysts i.p. or by gavage via a 19-gauge intubation needle (Popper and Son, New Hyde Park, N.Y.).

ME49 cysts were obtained originally from Jack Remington, Research Institute, Palo Alto Medical Foundation, Palo Alto, Calif. Trophozoites were obtained by flushing the peritoneal cavities of mice infected with cysts 7 days earlier with 5 ml of supplemented minimum essential medium (MEM) (see below). Extracellular trophozoites were counted with a hemacytometer after the peritoneal washes were forced three times through a blunted 27-gauge needle.

Supplemented MEM is MEM (GIBCO [catalog no. 330-143AJ]) containing 1% (vol/vol) L-glutamine stock solution (GIBCO [catlog no. 320-5030AG]), 2% (vol/vol) stock 5.5% $NaHCO_3$, 5% (vol/vol) fetal calf serum (GIBCO), 1% (vol/vol) antibiotic-antimycotic stock solution (GIBCO [catalog no. 600-5240AG]), 1% (vol/vol) stock buffer (15.87 g of HEPES plus 5.97 g of Tricine per 100 ml [pH 7.6]) and 0.05% (vol/vol) gentamycin stock (10 mg/ml) (GIBCO [catalog no. 600-5710AD]).

Antibodies. Monospecific polyclonal rabbit anti-murine IFN-γ immunoglobulin G (IgG) and rabbit anti-recombinant murine TNF alpha (TNF-α) IgG were kindly supplied by Edward A. Havell of the Trudeau Institute. Details of the production and purification of the antibodies are given elsewhere (6, 8). As a control, IgG from an unimmunized rabbit was similarly prepared. Antibody preparations for use in vivo were diluted in phosphate-buffered saline such that 0.2 ml contained 10^4 neutralizing units. The amounts of protein contained in each preparation are stated in the various tables. The amount of control IgG given was equal to the greater of the amounts in the anti-IFN-γ and anti-TNF preparations in each experiment. Antibodies were administered i.p. 6 h prior to challenge with *T. gondii*.

Cytokine assays. TNF cytotoxicity activity was assayed as described by Havell (8). Briefly, 1.5×10^4 L929B cells in 100 μl of supplemented MEM were incubated in individual wells of 96-well flat-bottomed microtiter dishes at 37°C in a 5% CO_2 atmosphere. Materials to be assayed (washes of peritoneal cavities or sera) were diluted sequentially twofold in supplemented MEM containing 2 μg of actinomycin D per ml (Calbiochem, La Jolla, Calif.), and 100 μl was added to wells containing L929B cells. After 24 h, the death of the L929B cells was scored microscopically. The TNF titer (in units per milliliter) is defined as the highest sample dilution for which 50% or more of the L929B cells were killed. The cytotoxic activity was shown to be due entirely to TNF by neutralization with an excess of polyclonal rabbit anti-murine TNF. Sera to be assayed were diluted 1/16 in the first well, because less-dilute sera frequently cause nonspecific death of L929B cells. Thus, the minimum detectable level of TNF in serum samples was 16 U/ml.

IFN-γ was assayed as previously described in detail (7). This IFN assay allows antiviral activity attributable to IFN-γ to be revealed by using a specific neutralizing antibody, R4-6A2 (6). Appropriate IFN or TNF standards (described in reference 10) were included in each assay. Differences in cytokine levels of two- to fourfold are not considered significant in these assays because of the subjective nature of scoring.

RESULTS

Effect of anti-TNF IgG on $CB6F_1$ mice infected i.p. with *T. gondii*. To determine whether endogenous TNF is involved in resistance to *T. gondii*, groups of 10 $CB6F_1$ females were treated either with IgG antibodies against TNF or IFN-γ or with control IgG. Anti-IFN-γ is known to convert a nonlethal infection with strain ME49 into a lethal one (21). Thus, mice treated with anti-IFN-γ served as positive controls. Seven days after initiation of infection, five mice in each group were killed for study; the remainder were monitored for survival. The results shown in Table 1 reveal that treatment with either anti-TNF or anti-IFN-γ resulted in almost 100-fold more trophozoites i.p. than were found in rabbit IgG-treated controls. Anti-IFN-γ-treated mice all died within 8 to 10 days of infection. In contrast, no anti-TNF-treated mice died, although all exhibited piloerection and hunched posture between days 10 and 12 of infection, unlike IgG-treated controls, which appeared nearly normal. Serum antiviral activity, which was entirely neutralized by anti-IFN-γ, was found to be at least as great in anti-TNF-treated mice as in controls.

Effect of anti-TNF IgG on B6 mice infected i.p. Because anti-TNF produced an effect on trophozoite numbers and caused transient illness in $CB6F_1$ mice, it was predicted that more susceptible mice might succumb to *T. gondii* infection if given anti-TNF. The results presented in Table 2 show that this is so, in that two of five B6 mice treated with anti-TNF IgG died 10 days after infection. Although average numbers

TABLE 2. Effect of anti-TNF antibody given to B6 mice prior to i.p. infection with *T. gondii*

Treatment[a]	Log_{10} trophozoites	Serum antiviral activity (U/ml) following treatment with		Survival (days postinfection)
		Medium alone	Anti-IFN-γ	
Control IgG	<4.8 ± 1.13	56 (40–78)	<16	>28, >28, >28[b]
Anti-IFN-γ	7.2 ± 0.18	<16	<16	8, 9, 9, 9
Anti-TNF	5.7 ± 0.91	223 (64–771)	<16	10, 10, >28, >28, >28

[a] Conditions of treatment and challenge with ME49 were identical to those described in the footnotes to Table 1.
[b] An additional mouse survived 14 days in apparently good health but was killed accidentally.

of trophozoites recovered from TNF-treated mice were above control numbers, there was considerable variability between individual mice, which may be reflected in the mortality data. As in the previous experiment, serum IFN-γ levels were higher in the anti-TNF-treated group than in controls.

Effect of anti-TNF antibody on mice infected perorally. Natural infections with *T. gondii* are usually acquired per os. Furthermore, peroral infection with *T. gondii* results in a higher rate of mortality and more cysts per brain than does infection of mice with the same number of cysts i.p. (10a, 13). Therefore, to determine whether TNF plays a protective role in infection via the natural route, which may be a more sensitive setting in which to detect effects of anti-TNF treatment, mice were challenged perorally with 20 ME49 cysts. Survival data are shown in Table 3. Only anti-IFN-γ treatment caused deaths in both $CB6F_1$ and B6 mice. However, all B6 mice treated with anti-TNF died. Uninfected control B6 mice given anti-TNF survived in good health.

In additional experiments, groups of five B6 mice were treated with rat IgG1 monoclonal antibodies specific for horseradish peroxidase (control), IFN-γ (R4-6A2; 6), or TNF (XT3.11; DNAX Corp., Palo Alto, Calif.). Results virtually identical to those shown in Table 3 were obtained (data not shown). Thus, the effect of anti-TNF on survival of *T. gondii*-infected mice is not peculiar to polyclonal rabbit antibodies.

TNF is produced in response to i.p. infection with *T. gondii* ME49. In the experiments shown in Table 1, serum samples were collected from mice in the various groups and assayed for TNF. In all groups, the levels were below 16 U/ml, the lower limit of detection of the TNF assay (data not shown). This result differs from that reported by Chang et al. (4), who found detectable levels of TNF in the sera of mice infected with a more virulent strain of *T. gondii* and much higher levels in the sera of infected mice treated with lipopolysaccharide (LPS) just prior to serum collection. Thus, it was deemed important to verify that TNF is produced during infection with the ME49 strain of *T. gondii* to corroborate results obtained in experiments using anti-TNF antibodies. Peritoneal fluids from $CB6F_1$ mice were assayed for TNF at various times after i.p. infection with 20 ME49 cysts. The data shown in Fig. 1 indicate that TNF was indeed present at the site of infection. Control mice given an i.p. injection of an equivalent mass of brain suspension from an uninfected mouse did not produce detectable amounts of TNF (data not shown). TNF was also found in the peritoneal fluids of infected B6 mice treated with control IgG described in Table 2 (data not shown).

The failure of anti-TNF to cause death in infected CB6 mice may have been due to administration of insufficient amounts of antibody to neutralize all of the protective TNF the mice produce. To investigate this possibility, groups of three $CB6F_1$ females were given 10^4 neutralizing units of rabbit polyclonal anti-TNF i.p. and infected with 20 ME49 cysts i.p. 6 h later. Two groups were left uninfected as controls. Sera were collected 1 or 4 days later, the time at which peak TNF levels are seen at the site of infection (Fig. 1), and assayed for anti-TNF activity in vitro. Mean serum antibody levels decayed from 2,580 neutralizing units per ml on day 1 to 1,625 neutralizing units per ml on day 4 in uninfected mice and from 2,896 neutralizing units per ml on day 1 to 645 neutralizing units per ml on day 4 in infected

TABLE 3. Effect on host survival of anti-TNF antibody given to $CB6F_1$ and B6 mice prior to peroral infection with *T. gondii*

Treatment[a]	Survival (days postinfection) of	
	$CB6F_1$	B6
Rabbit IgG (5.6 mg)	>28, >28, >28, >28, >28	11, >28, >28, >28, >28
Anti-IFN-γ (5.6 mg)	8, 9, 10, 11	10, 10, 10, 10, 11
Anti-TNF (1.2 mg)	>28, >28, >28, >28, >28	10, 10, 10, 11, 11

[a] Female mice were challenged perorally with 20 strain ME49 cysts 6 h after i.p. injections of the indicated IgG preparations.

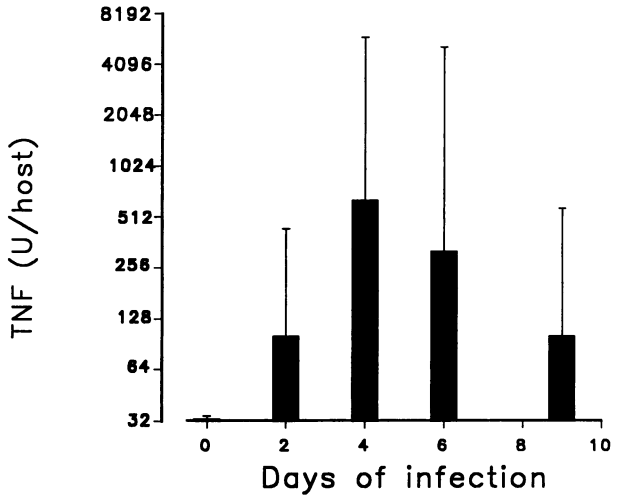

FIG. 1. TNF in peritoneal fluids of $CB6F_1$ mice at various times of i.p. infection with *T. gondii*. Groups of three $CB6F_1$ female mice were given 20 strain ME49 cysts i.p. At the indicated times, their peritoneal cavities were flushed with 2 ml of supplemented MEM and the fluids were assayed for TNF as described in Materials and Methods. Values are mean (log_2) amounts of TNF per host ± 1 standard deviation.

mice. Uninfected, untreated controls had no anti-TNF activity whatsoever in their sera, as expected. Thus, although serum antibody levels in infected mice were slightly reduced on day 4 relative to those in uninfected controls, presumably as a result of binding to TNF produced in response to *T. gondii*, there was still a measurable level of TNF-neutralizing activity in the serum well after the beginning of infection. Thus, it is unlikely that the failure of anti-TNF antibody to cause death in $CB6F_1$ mice was due to administration of insufficient amounts of antibody to completely neutralize the TNF.

DISCUSSION

The reported results provide evidence that TNF produced endogenously during *T. gondii* infection plays a role in restraining trophozoite numbers and, in B6 mice infected perorally, may prevent death. Thus, *T. gondii* joins *Listeria monocytogenes* (8–10), *Chlamydia trachomatis* (24), *Histoplasma capsulatum* (19), *Mycobacterium bovis* BCG (11), and *Leishmania major* (22) as organisms for which infection is exacerbated by host treatment with anti-TNF.

From the results shown in Tables 1 and 2, it can be seen that anti-TNF-treated mice had IFN-γ in their sera. Nonetheless, these mice had greater trophozoite burdens and in some cases died. This suggests that serum IFN-γ level, by itself, is not necessarily predictive of the ability to control trophozoite numbers, at least under the conditions of these experiments.

Although the results demonstrate a role for TNF in resistance to acute *T. gondii* infection, it is not clear what that role is. One possibility is that TNF is directly toxic to extracellular or intracellular *T. gondii*; however, in vitro studies indicate that this is unlikely (1, 3, 5). A more likely explanation is that TNF potentiates a cellular anti-*T. gondii* mechanism. One such mechanism involves macrophages activated with IFN-γ. Although it has been reported that anti-*T. gondii* activity of macrophages can be elicited by IFN-γ alone (2, 15, 16), a recent study (18) provides evidence that IFN-γ by itself is an insufficient stimulus. However, LPS or TNF was able to activate IFN-γ-primed macrophages to exhibit anti-*T. gondii* activity in vitro. To explain this discrepancy between studies regarding the sufficiency of IFN-γ as an activator of anti-*T. gondii* activity, it was suggested (18) that low-level LPS contamination of culture media may have masked a requirement for cytokines other than IFN-γ in the earlier studies.

In keeping with a role for TNF in activation of IFN-γ-primed macrophages to control *T. gondii*, it might be expected that IFN-γ-treated, *T. gondii*-infected macrophages themselves would produce the needed TNF. However, an in vitro study suggests that this is not the case; TNF had to be provided exogenously (18). If activated macrophages are indeed responsible for resistance to acute *T. gondii* infection in vivo, it will be interesting to determine the cellular source of needed TNF.

ACKNOWLEDGMENTS

I thank Edward A. Havell for providing the polyclonal antibodies used in this study and for guidance with regard to the assays for IFN-γ and TNF. Thanks are also given to Jack Remington and Fausto Araujo, who kindly provided the ME49 strain and information regarding various experimental procedures involving *T. gondii*. The capable technical assistance of Paula Lanthier is also gratefully acknowledged.

This work was supported by funds provided by the Trudeau Institute.

REFERENCES

1. **Black, C. M., L. E. M. Bermudez, L. S. Young, and J. S. Remington.** 1990. Co-infection of macrophages modulates interferon-γ and tumor necrosis factor-induced activation against intracellular pathogens. J. Exp. Med. **172:**977–980.
2. **Black, C. M., J. R. Catterall, and J. S. Remington.** 1987. In vivo and in vitro activation of alveolar macrophages by recombinant interferon-γ. J. Immunol. **138:**491–495.
3. **Black, C. M., D. M. Israelski, Y. Suzuki, and J. S. Remington.** 1989. Effect of tumor necrosis factor on acute infections in mice with *Toxoplasma gondii* or *Trypanosoma cruzi*. Immunology **68:**570–574.
4. **Chang, H. R., G. E. Grau, and J. C. Pechère.** 1990. Role of TNF and IL-1 in infection with *Toxoplasma gondii*. Immunology **69:**33–37.
5. **DeTitto, E. H., J. R. Catterall, and J. S. Remington.** 1986. Activity of recombinant tumor necrosis factor on *Toxoplasma gondii* and *Trypanosoma cruzi*. J. Immunol. **137:**1342–1345.
6. **Havell, E. A.** 1986. Purification and further characterization of an anti-murine interferon-γ monoclonal neutralizing antibody. J. Interferon Res. **6:**489–497.
7. **Havell, E. A.** 1986. Augmented production of interferons during *Listeria monocytogenes* infection. J. Infect. Dis. **153:**960–969.
8. **Havell, E. A.** 1987. Production of tumor necrosis factor during murine listeriosis. J. Immunol. **139:**4225–4231.
9. **Havell, E. A.** 1989. Evidence that tumor necrosis factor has an important role in antibacterial resistance. J. Immunol. **143:**2894–2899.
10. **Havell, E. A., and P. B. Sehgal.** 1991. Tumor necrosis factor-independent IL-6 production during murine listeriosis. J. Immunol. **146:**756–761.

10a. **Johnson, L. L.** Unpublished data.

11. **Kindler, V., A. P. Sappino, G. E. Grau, P.-F. Piquet, and P. Vassalli.** 1989. The inducing role of tumor necrosis factor in the development of bactericidal granulomas during BCG infection. Cell **56:**731–740.
12. **McCabe, R. E., and J. S. Remington.** 1990. *Toxoplasma gondii*, p. 2090–2103. *In* G. L. Mandell, R. G. Douglas, and J. E. Bennett (ed.), Principles and practices of infectious diseases. Churchill Livingstone, Inc., New York.
13. **McLeod, R., P. Eisenhauer, D. Mack, C. Brown, G. Filice, and G. Spitalny.** 1989. Immune responses associated with early survival after peroral infection with *Toxoplasma gondii*. J. Immunol. **142:**3247–3255.
14. **McLeod, R., E. Skamene, C. R. Brown, P. B. Eisenhauer, and D. G. Mack.** 1989. Genetic regulation of early survival and cyst number after peroral *Toxoplasma gondii* infection of AXB/BXA recombinant inbred and B10 congenic mice. J. Immunol. **143:**3031–3034.
15. **Murray, H. W., G. Spitalny, and C. F. Nathan.** 1985. Activation of mouse peritoneal macrophages in vitro and in vivo by interferon-γ. J. Immunol. **134:**1619–1622.
16. **Nathan, C. F., H. W. Murray, M. E. Wiebe, and B. Y. Rubin.** 1983. Identification of interferon-γ as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. J. Exp. Med. **158:**670–689.
17. **Schreiber, R. D., L. J. Hicks, A. Celada, N. A. Buchmeier, and P. W. Gray.** 1985. Monoclonal antibodies to murine γ-interferon which differentially modulate macrophage activation and antiviral activity. J. Immunol. **134:**1609–1618.
18. **Sibley, L. D., L. B. Adams, Y. Fukutomi, and J. L. Kranenbuhl.** 1991. Tumor necrosis factor-α triggers antitoxoplasmal activity of IFN-γ-primed macrophages.
19. **Smith, J. G., D. M. Magee, D. M. Williams, and J. R. Graybill.** 1990. Tumor necrosis-α plays a role in host defense against *Histoplasma capsulatum*. J. Infect. Dis. **162:**1349–1353.
20. **Suzuki, J., and J. S. Remington.** 1989. A method for obtaining large numbers of trophozoites of avirulent strains of *Toxoplasma gondii* using an antibody to interferon-gamma. J. Parasitol. **75:**174–176.
21. **Suzuki, Y., M. A. Orellana, R. D. Schreiber, and J. S. Reming-**

ton. 1988. Interferon-γ: the major mediator of resistance against *Toxoplasma gondii*. Science **240:**516–518.

22. **Titus, R. G., B. Sherry, and A. Cerami.** 1989. Tumor necrosis factor plays a protective role in experimental murine cutaneous leishmaniasis. J. Exp. Med. **170:**2097–2104.
23. **Williams, D. M., F. C. Grumet, and J. S. Remington.** 1978. Genetic control of murine resistance to *Toxoplasma gondii*. Infect. Immun. **19:**416–420.
24. **Williams, D. M., D. M. Magee, L. F. Bonewald, J. G. Smith, C. A. Bleicker, G. I. Byrne, and J. Schachter.** 1990. A role in vivo for tumor necrosis factor alpha in host defense against *Chlamydia trachomatis*. Infect. Immun. **58:**1572–1576.