INFECTION AND IMMUNITY, Dec. 1989, p. 3936–3939
0019-9567/89/123936-04$02.00/0

Vol. 57, No. 12

NOTES

Human Recombinant Tumor Necrosis Factor Alpha Protects Susceptible A/J Mice against Lethal *Plasmodium chabaudi* AS Infection

MARY M. STEVENSON* AND ESFANDIAR GHADIRIAN

Centre for the Study of Host Resistance, McGill University, Montreal, Quebec H3A 2T5, and The Montreal General Hospital Research Institute, 1650 Cedar Avenue, Montreal, Quebec H3G 1A4, Canada

Received 19 June 1989/Accepted 30 August 1989

The effect of intravenous treatment with human recombinant tumor necrosis factor alpha (rTNF-α) on infection of susceptible A/J and resistant C57BL/6 mice with *Plasmodium chabaudi* AS was examined. Treatment of A/J mice with 10^3 or 10^5 U of rTNF-α on days 0, 3, 5, 7, and 9 after intraperitoneal infection with 10^6 parasitized erythrocytes resulted in 80% survival and a significant decrease in the peak parasitemia level. Treatment of susceptible A/J hosts with 10^5 but not 10^3 U of rTNF-α resulted in increased survival but did not alter the peak parasitemia level following infection with 10^7 parasitized erythrocytes. Moreover, all surviving A/J mice completely eliminated the parasite by approximately 4 weeks and were fully protected against a secondary infection. Except at a dose of 5×10^5 U of rTNF-α, which resulted in 100% mortality of infected animals, rTNF-α did not alter the course or outcome of infection with *P. chabaudi* AS in resistant C57BL/6 mice.

Soluble mediators, produced as a result of immune responses, have recently been shown to play an important role in host defense against infection. Of particular interest is the monokine tumor necrosis factor alpha (TNF-α), or cachectin, which is produced and released primarily by activated macrophages following exposure to lipopolysaccharide derived from gram-negative bacteria (17). In addition to its cytotoxic effect on tumor cells, TNF-α has a broad spectrum of immunomodulatory effects on a variety of cells, including macrophages, neutrophils, lymphocytes, and endothelial cells, as well as hematopoietic progenitor cells (20, 28; for a review, see reference 1).

Since the original description of this molecule as tumor-necrotizing activity in the sera of mice infected with *Mycobacterium bovis* BCG, and injected with lipopolysaccharide (4) and as cachectin in the sera of rabbits infected with *Trypanosoma brucei brucei* (21), both the endogenous and lipopolysaccharide-induced molecules have been detected in the sera of mice infected with *Listeria monocytogenes* (14, 19) and with various species of *Plasmodium* (5, 9, 11) as well as in the sera of patients with malaria (22). With the availability of the recombinant molecule, the ability of TNF-α to mediate enhanced resistance in vivo to listeriosis and malaria has been documented (8, 10, 26).

In the present investigation, we have examined the effect of treatment with human recombinant TNF-α (rTNF-α) on host resistance to infection with *Plasmodium chabaudi* AS in susceptible A/J and resistant C57BL/6 hosts. Our laboratory has previously demonstrated that the level of resistance to infection with this rodent malaria species among inbred strains of mice is genetically determined by a major dominant, autosomal, non-*H-2*-linked gene which we have designated *Pchr* (23, 24). This observation was initially made in the segregating backcross and hybrid populations derived from resistant C57BL-derived B10.A and susceptible A/J parental mice. We have subsequently confirmed unigenic control of resistance to *P. chabaudi* AS by typing AXB/BXA recombinant inbred mouse strains derived from progenitor C57BL/6 and A/J mice (25). Susceptible mouse strains, such as A/J, develop a fulminant parasitemia; 100% of the animals succumb to infection, with a mean survival time (MST) of less than 10 days. Resistant hosts, such as C57BL/6 mice, develop a moderate level of peak parasitemia, eliminate the acute infection by 4 weeks, and are immune to reinfection.

Elimination of malarial parasites has been hypothesized to be due to the development of acquired immunity. In the case of infection with parasites of the species *P. chabaudi*, acquired immunity has been demonstrated to occur by an antibody-independent, cell-mediated mechanism requiring the presence of T cells and an intact spleen. The importance of T cells and an intact spleen in acquired resistance to *P. chabaudi adami* was observed by Grun and his colleagues (12, 13). Recently, these investigators have produced T-cell clones, identified as $L3T4^+$, which can adoptively transfer immunity to naive hosts and produce gamma interferon and interleukin-2 (2, 3). Our laboratory has confirmed the importance of T cells and an intact spleen in acquired immunity to *P. chabaudi* AS (M. M. Stevenson and D. Rae, submitted for publication). In addition, we have demonstrated the importance of macrophages as effector cells in this mechanism (M. M. Stevenson, E. Ghadirian, N. C. Phillips, D. Rae, and J. Podoba, Parasite Immunol., in press).

Age- and sex-matched mice 6 to 8 weeks old were used in all experiments. A/J mice were purchased from Jackson Laboratories, Bar Harbor, Maine; C57BL/6 mice were purchased from either Jackson Laboratories or Charles River, Inc., St. Constant, Quebec, Canada. Animals were infected

* Corresponding author.

intraperitoneally (i.p.) with 10^6 *P. chabaudi* AS parasitized erythrocytes (PRBC) maintained and prepared as previously described (23). In some experiments, A/J mice were infected with 10^7 PRBC. rTNF-α (2.51 × 10^7 U/ml) was obtained from Michael Shepard, Genentech, San Francisco, Calif. On days 0, 3, 5, 7, and 9 after infection with *P. chabaudi* AS, experimental mice were injected intravenously (i.v.) with 0.2 ml of various doses of rTNF-α diluted in sterile phosphate-buffered saline (GIBCO, Burlington, Ontario, Canada). Control animals were untreated. The course and outcome of infection were monitored in control and experimental mice. To determine the course of infection, blood samples were collected by bleeding via the retro-orbital plexus at the indicated times. Parasitemias of individual mice were determined by counting 100 erythrocytes on Wright-stained thin blood smears prepared in duplicate. The parasitemia is expressed as the mean percentage of PRBC ± the standard error of the mean for each group of mice. The outcome of infection was determined by daily observation and calculation of the MST ± the standard error of the mean.

Following i.p. infection with 10^6 *P. chabaudi* AS PRBC, susceptible A/J mice exhibited a course of infection characterized by a prepatent period of approximately 3 days (Fig. 1A). This was followed by a patent period during which the parasite multiplied and there were increasing numbers of parasites in the peripheral blood, resulting in a fulminant level of parasitemia of approximately 50% PRBC and the death of 100% of the animals, with a MST of 10 days (Fig. 1B).

The ability of rTNF-α to enhance the resistance of susceptible hosts to *P. chabaudi* AS infection was investigated by treating A/J mice with 10^5 U administered i.v. on days 0, 3, 5, 7, and 9 after i.p. infection with 10^6 PRBC. Treatment with 10^5 U of rTNF-α resulted in a significant difference in the level of peak parasitemia, which occurred on day 7, between treated and untreated (control) animals (Fig. 1A). Control mice had a peak parasitemia of 49.6 ± 1.6% PRBC, while mice treated with 10^5 U of rTNF-α had a peak parasitemia of 38.6 ± 2.0% PRBC ($P < 0.001$). In addition, 80% of the rTNF-α-treated A/J mice survived the normally lethal infection (Fig. 1B).

The effect of a dose of 10^3 U of rTNF-α on the resistance of susceptible A/J mice to infection with 10^6 *P. chabaudi* AS PRBC was also examined in the same experiment (Table 1). As with mice treated with 10^5 U of rTNF-α, animals treated with this lower dose exhibited both 80% survival and a significant decrease in the level of peak parasitemia, which was evident on day 7 postinfection ($P < 0.001$).

In order to determine whether treatment with rTNF-α is protective against a larger inoculum of the malarial parasite, A/J mice were infected i.p. with 10^7 PRBC and treated i.v. with either 10^3 or 10^5 U of rTNF-α on days 0, 3, 5, 7, and 9 postinfection (Table 1). Treatment with 10^3 U of rTNF-α was ineffective in protecting A/J mice against infection with 10^7 *P. chabaudi* AS PRBC. There was no significant difference between treated and control mice in the MST or the peak parasitemia level. All of the mice in the treated group died, with a MST equal to 11.2 ± 3.9 days and a range of survival times between 7 and 23 days. One mouse appeared to have eliminated the parasite such that <1% PRBC was apparent in the peripheral blood on day 14 postinfection. However, on day 21, the parasitemia was 37.0 ± 4%, and the mouse succumbed to infection on day 23. Mice treated with 10^5 U of rTNF-α exhibited 50% survival but no significant difference in peak parasitemia level, which occurred on day 5 with this dose of PRBC. The parasitemia in the surviving animals of this group of mice maintained a plateau through day 7. Thereafter, the parasites were rapidly eliminated such that the parasitemia was <2% on day 11 postinfection.

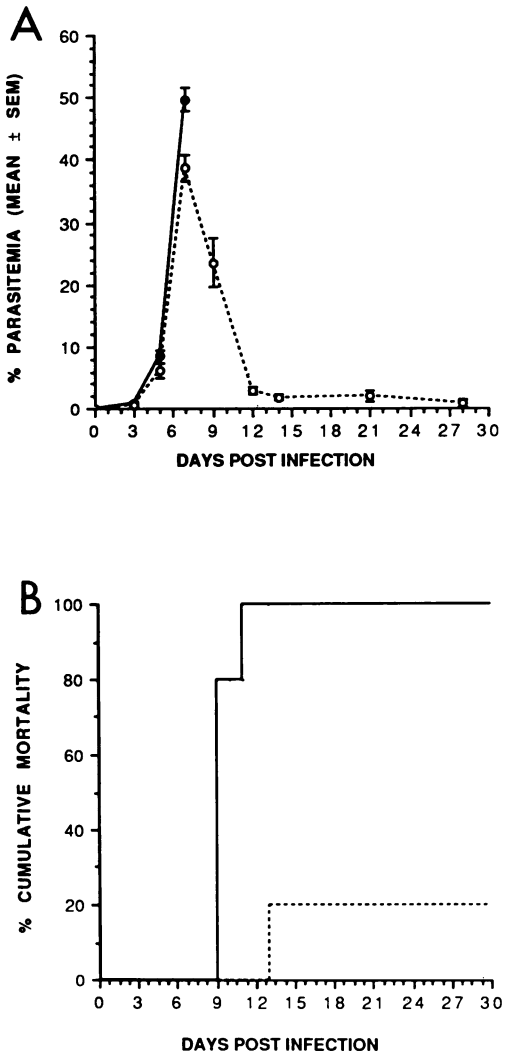

FIG. 1. Effect of treatment with rTNF-α on the course and outcome of infection with *P. chabaudi* AS in susceptible A/J mice. Groups of five mice were injected i.v. with either 0.2 ml of sterile phosphate-buffered saline (●) or 10^5 U of rTNF-α (○) on days 0, 3, 5, 7, and 9 following i.p. infection with 10^6 PRBC. The course of infection (A) was determined as described in the text by quantitating the percent PRBC in the peripheral blood on the days indicated. The outcome of infection (B) was determined by daily observation and calculation of the MST.

All surviving A/J mice infected with either 10^6 or 10^7 PRBC and treated with rTNF-α had 0 to 2% PRBC at 21 days postinfection. Four weeks later, the surviving animals were reinfected i.p. with the same number of PRBC used to initiate the primary infection. The mice were fully protected against the secondary malaria infection such that the parasitemia levels of these animals were 0 to 1% at days 7 and 14 after reinfection (Table 1).

Resistant C57BL/6 mice were also treated with rTNF-α, and the course of infection with 10^6 *P. chabaudi* AS PRBC was examined. In contrast to susceptible A/J mice, who succumbed to fulminant parasitemia and died after this dose

TABLE 1. Effect of treatment with various doses of human rTNF-α on resistance to *P. chabaudi* AS infection in susceptible A/J mice

Treatment[a] (U of rTNF-α)	% Survival	MST[b] (days)	Day of death[c]	% Peak parasitemia[d] (mean ± SEM)	Resistance to reinfection[e]
10^6 PRBC infection (n = 5)					
0 (Control)	0	9.4 ± 0.4 (9–11)		49.6 ± 1.6	ND
10^3	80		11	34.4 ± 2.8[f]	+
10^5	80		13	38.6 ± 2.0[f]	+
10^7 PRBC infection (n = 4)					
0 (Control)	0	7.7 ± 0.5 (7–9)		41.0 ± 1.6	ND
10^3	0	11.2 ± 3.9 (7–23)		41.5 ± 3.9	ND
10^5	50		7	34.4 ± 5.9	+

[a] Groups of four or five mice were injected i.v. with 10^3 or 10^5 U of rTNF-α on days 0, 3, 5, 7, or 9 after i.p. infection with 10^6 or 10^7 PRBC.
[b] Ranges of survival times are shown in parentheses.
[c] For groups in which survival was <100%.
[d] Occurred on day 7 for mice infected with 10^6 PRBC and on day 5 for those infected with 10^7 PRBC.
[e] Seven weeks postinfection, surviving animals were reinfected i.p. with the same number of PRBC used to initiate the primary infection. +, Resistant; ND, Not done.
[f] $P < 0.001$.

of PRBC, C57BL/6 hosts were resistant and survived. C57BL/6 mice exhibited a course of infection characterized by a prepatent period, parasite multiplication between day 3 and the peak parasitemia level (which was less than 50%), and then elimination of the parasite, during which time there was control of the infection and decreasing parasitemia (Fig. 2). C57BL/6 mice treated with 10^5 U of rTNF-α had a course of infection similar to that of control animals.

The effect of treatment with various doses of rTNF-α on the course of *P. chabaudi* AS infection was also examined in resistant C57BL/6 mice. Lower doses (10^3 or 10^4 U of rTNF-α) were without effect on the course of infection in these animals (data not shown). Treatment of C57BL/6 mice with 5×10^5 U of rTNF-α on day 4 postinfection with 10^6 PRBC, however, resulted in the death of 100% (five of five) of the animals within a few hours after treatment (data not shown). At the time of treatment, the parasitemia of these animals was approximately 4%. Clark and his colleagues (5, 8) have likewise observed that high doses of rTNF-α had an adverse effect on mice infected with either *P. chabaudi adami* or *P. vinckei*, with parasitemias as low as 1%.

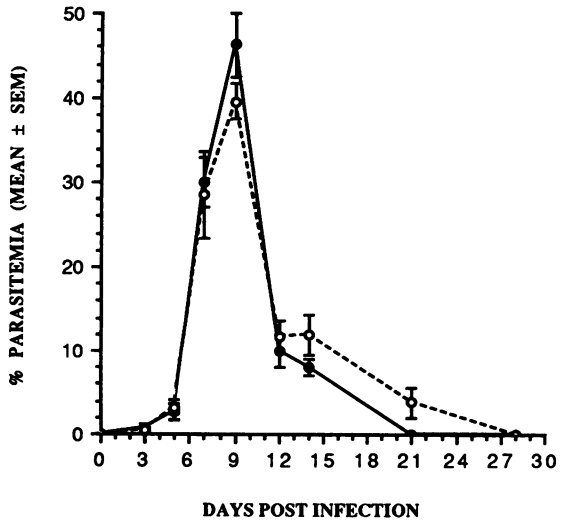

FIG. 2. Effect of treatment with rTNF-α on the course of infection with *P. chabaudi* AS in resistant C57BL/6 mice. Groups of five mice were injected i.v. with either 0.2 ml of sterile phosphate-buffered saline (●) or 10^5 U of rTNF-α (○) on days 0, 3, 5, 7, and 9 following i.p. infection with 10^6 PRBC. The course of infection was determined as described in the text by quantitating the percent PRBC in the peripheral blood on the days indicated.

Intravenous treatment with rTNF-α thus enhanced the resistance of susceptible A/J mice to infection with *P. chabaudi* AS but did not alter the level of resistance of resistant C57BL/6 hosts. Treatment of A/J mice with 10^5 as well as 10^3 U resulted in 80% survival and a significant decrease in the peak parasitemia level following infection with 10^6 PRBC. We observed that 10^5 but not 10^3 U resulted in increased survival but did not alter the peak parasitemia level following infection with 10^7 PRBC. All surviving A/J mice, however, completely eliminated the parasite by approximately 4 weeks after infection and were fully protected against a secondary infection, indicating the development of both primary and secondary immunity to this normally lethal infection.

Treatment of resistant C57BL/6 mice with a high dose of rTNF-α resulted in the death of 100% of the animals even though they had low levels of parasitemia. This observation suggests that C57BL/6 hosts can produce a level of endogenous TNF-α adequate for elimination of the infection. The toxicity of the endogenous plus the exogenous TNF-α suggests that in excess this molecule is harmful to the host. Clark and his colleagues (5–7) and several other investigators have concluded that TNF-α mediates much of the pathology associated with malaria, including cerebral malaria (11) and anemia (5, 18). Thus, TNF-α is effective in mediating resistance to infection but is deleterious to the host in excess.

The observation that A/J mice, which we have previously shown to be susceptible to infection with *P. chabaudi* AS because of the inheritance of *Pchr*^{s}, the susceptibility allele of the putative *Pchr* gene (23, 24), can be protected against an otherwise lethal infection by treatment with rTNF-α suggests that production of this molecule in response to *P. chabaudi* AS infection is the basis of the genetic difference between A/J and C57BL/6 mice. However, such a conclusion cannot be made without formal proof of genetic linkage between the traits of resistance to *P. chabaudi* AS and the ability to produce TNF-α. As a preliminary step to such studies, we are currently examining the ability of A/J mice to produce TNF-α in response to infection.

The mechanism of action of endogenous TNF-α on host resistance to infection is not yet defined. Treatment with

rabbit anti-murine TNF-α immunoglobulin was found to abrogate the resistance of mice to a sublethal inoculum of *Listeria* spp. (14, 19). Nakane and colleagues (19) further demonstrated that anti-TNF-α-treated mice produced normal levels of alpha and beta interferons in the bloodstream but did not develop activated, bactericidal macrophages in the peritoneal cavity during infection. This observation suggests that endogenous TNF-α directly activates macrophages. In concurrence with this observation is the finding that in vitro treatment of infected macrophage monolayers with rTNF-α inhibits the intracellular multiplication of *Trypanosoma cruzi* but has no effect on extracellular trypomastigotes (27).

It is likely that TNF-α has a similar mechanism of action during malaria, that is, activation of another cell type rather than a direct cytotoxic effect on the parasite. It has been observed that the recombinant molecule enhances host resistance in vivo but is not cytotoxic to the intraerythrocytic parasites in vitro (15, 16, 26). The mechanism by which TNF-α enhances resistance to malaria may occur via activation of macrophages and thus may be nonspecific in nature. Conversely, since TNF-α-treated A/J mice which survived infection with *P. chabaudi* AS were immune to reinfection, TNF-α may activate T lymphocytes, thereby imparting specificity to the enhanced resistance. We are currently investigating these alternatives by using the model of *P. chabaudi* AS-resistant C57BL/6 and -susceptible A/J mice.

We thank Darlene Rae and Mi Fong Tam for expert technical assistance.

This work was supported by the Medical Research Council of Canada (grant MA-7785).

LITERATURE CITED

1. **Beutler, B., and A. Cerami.** 1987. Cachectin: more than a tumor necrosis factor. N. Engl. J. Med. **316**:379–385.
2. **Brake, D. A., C. A. Long, and W. P. Weidanz.** 1988. Adoptive protection against *Plasmodium chabaudi adami* malaria in athymic nude mice by a cloned T cell line. J. Immunol. **140**: 1989–1993.
3. **Brake, D. A., W. P. Weidanz, and C. A. Long.** 1986. Antigen-specific, interleukin 2-propagated T lymphocytes confer resistance to a murine malarial parasite, *Plasmodium chabaudi adami*. J. Immunol. **137**:347–352.
4. **Carswell, E. A., L. J. Old, R. L. Kassel, S. Green, N. Fiore, and B. Williamson.** 1975. An endotoxin-induced serum factor that causes necrosis of tumors. Proc. Natl. Acad. Sci. USA **72**: 3666–3670.
5. **Clark, I. A., and G. Chaudhri.** 1988. Tumor necrosis factor may contribute to the anaemia of malaria by causing dyserythropoiesis and erythrophagocytosis. Br. J. Haematol. **70**:99–103.
6. **Clark, I. A., G. Chaudri, and W. B. Cowden.** 1989. Roles of tumour necrosis factor in the illness and pathology of malaria. Trans. R. Soc. Trop. Med. Hyg. **83**:433–437.
7. **Clark, I. A., W. B. Cowden, G. A. Butcher, and N. H. Hunt.** 1987. Possible roles of tumor necrosis factor in the pathology of malaria. Am. J. Pathol. **129**:192–199.
8. **Clark, I. A., N. H. Hunt, G. A. Butcher, and W. B. Cowden.** 1987. Inhibition of murine malaria (*Plasmodium chabaudi*) in vivo by recombinant interferon-γ or tumor necrosis factor, and its enhancement by butylated hydroxyanisole. J. Immunol. **139**:3493–3496.
9. **Clark, I. A., J.-L. Virelizier, E. A. Carswell, and P. R. Wood.** 1981. Possible importance of macrophage-derived mediators in acute malaria. Infect. Immun. **32**:1058–1066.
10. **Desiderio, J. V., P. A. Kiener, P.-F. Lin, and G. A. Warr.** 1989. Protection of mice against *Listeria monocytogenes* infection by recombinant human tumor necrosis factor alpha. Infect. Immun. **57**:1615–1617.
11. **Grau, G. E., L. P. Fajardo, P.-F. Piquet, B. Allet, P.-H. Lambert, and P. Vassalli.** 1987. Tumor necrosis factor (cachectin) as an essential mediator in murine cerebral malaria. Science **237**:1210–1212.
12. **Grun, J. L., C. A. Long, and W. P. Weidanz.** 1985. Effects of splenectomy on antibody-independent immunity to *Plasmodium chabaudi adami* malaria. Infect. Immun. **48**:853–858.
13. **Grun, J. L., and W. P. Weidanz.** 1981. Immunity to *Plasmodium chabaudi adami* in the B-cell-deficient mouse. Nature (London) **290**:143–145.
14. **Havell, E. A.** 1987. Production of tumor necrosis factor during murine listeriosis. J. Immunol. **139**:2970–2976.
15. **Hviid, L., C. M. Reimert, T. G. Theander, S. Jepsen, and K. Bendtzen.** 1988. Recombinant human tumour necrosis factor is not inhibitory to *Plasmodium falciparum* in vitro. Trans. R. Soc. Trop. Med. Hyg. **82**:48–49.
16. **Jensen, J. B., J. A. Vande Waa, and A. J. Karadsheh.** 1987. Tumor necrosis factor does not induce *Plasmodium falciparum* crisis forms. Infect. Immun. **55**:1722–1724.
17. **Männel, D. N., R. N. Moore, and S. E. Mergenhagen.** 1980. Macrophages as a source of tumoricidal activity (tumor-necrotizing factor). Infect. Immun. **30**:523–530.
18. **Miller, K. L., P. H. Silverman, B. Kullgren, and L. J. Mahlmann.** 1989. Tumor necrosis factor alpha and the anemia associated with murine malaria. Infect. Immun. **57**:1542–1546.
19. **Nakane, A., T. Minagawa, and K. Kato.** 1988. Endogenous tumor necrosis factor (cachectin) is essential to host resistance against *Listeria monocytogenes* infection. Infect. Immun. **56**: 2563–2569.
20. **Plaetinck, G., W. Declerq, J. Travernier, M. Nabholz, and W. Fiers.** 1987. Recombinant tumor necrosis factor can induce interleukin 2 receptor expression and cytotoxic activity in a rat × mouse T cell hybrid. Eur. J. Immunol. **17**:1835–1838.
21. **Rouzer, C. A., and A. Cerami.** 1980. Hypertriglyceridemia associated with *Trypanosoma brucei brucei* infection in rabbits: role of defective triglyceride removal. Mol. Biochem. Parasitol. **2**:31–38.
22. **Scuderi, P., K. S. Lam, K. J. Ryan, E. Petersen, K. E. Sterling, P. R. Finley, C. G. Ray, D. J. Slymen, and S. E. Salmon.** 1986. Raised serum levels of tumor necrosis factor in parasitic infections. Lancet **ii**:1364–1365.
23. **Stevenson, M. M., J. J. Lyanga, and E. Skamene.** 1982. Murine malaria: genetic control of resistance to *Plasmodium chabaudi*. Infect. Immun. **38**:80–88.
24. **Stevenson, M. M., M. N. Nesbitt, and E. Skamene.** 1988. Chromosomal location of the gene determining resistance to *Plasmodium chabaudi* AS. Curr. Top. Microbiol. Immunol. **137**:325–328.
25. **Stevenson, M. M., and E. Skamene.** 1985. Murine malaria: resistance of AXB/BXA recombinant inbred mice to *Plasmodium chabaudi*. Infect. Immun. **47**:452–456.
26. **Taverne, J., J. Tavernier, W. Fiers, and J. H. L. Playfair.** 1987. Recombinant tumour necrosis factor inhibits malaria parasites in vivo but not in vitro. Clin. Exp. Immunol. **67**:1–4.
27. **Titto, 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.
28. **Vogel, S. N., E. N. Kaufman, M. D. Tate, and R. Neta.** 1988. Recombinant interleukin-1α and recombinant tumor necrosis factor α synergize in vivo to induce early endotoxin tolerance and associated hematopoietic changes. Infect. Immun. **56**:2650–2657.