INFECTION AND IMMUNITY, Dec. 1992, p. 5107–5112
0019-9567/92/125107-06$02.00/0

Vol. 60, No. 12

Endogenous Tumor Necrosis Factor Alpha Is Required for Enhanced Antimicrobial Activity against *Toxoplasma gondii* and *Listeria monocytogenes* in Recombinant Gamma Interferon-Treated Mice

JAN A. M. LANGERMANS, MARIËLLE E. B. VAN DER HULST, PETER H. NIBBERING, AND RALPH VAN FURTH*

Department of Infectious Diseases, University Hospital, Building 1 C5P, P.O. Box 9600, 2300 RC Leiden, The Netherlands

Received 15 May 1992/Accepted 5 October 1992

In vitro studies have shown that macrophages stimulated with recombinant gamma interferon (rIFN-γ) produce tumor necrosis factor alpha (TNF-α), which in an autocrine fashion activates these cells. The aim of the present study was to determine whether endogenously formed TNF-α also is required for rIFN-γ-induced macrophage activation and enhanced antimicrobial activity in vivo. After an intraperitoneal injection of rIFN-γ into CBA/J mice, their peritoneal macrophages released enhanced amounts of NO_2^- and inhibited the intracellular proliferation of *Toxoplasma gondii*. Injection of neutralizing antibodies against TNF-α simultaneously with the rIFN-γ completely inhibited both the release of NO_2^- by macrophages and their toxoplasmastatic activity. Similar results were observed after intraperitoneal injection of a competitive inhibitor of L-arginine, N^G-monomethyl-L-arginine, together with rIFN-γ, demonstrating that in vivo L-arginine-derived reactive nitrogen intermediates are essential for the induction of toxoplasmastatic activity. Intravenous injection of rIFN-γ inhibited the growth of *Listeria monocytogenes* in the livers and spleens of mice; this effect was abrogated by antibodies against TNF-α. Intravenous injection of a large dose of rTNF-α resulted in a decrease in the number of bacteria in the liver and spleen, but an injection of rIFN-γ and rTNF-α did not result in enhanced inhibition of the proliferation of *L. monocytogenes*. Together, the results of the present study are the first to demonstrate that endogenous TNF-α is required in vivo for the expression of macrophage activation with respect to the release of reactive nitrogen intermediates and toxoplasmastatic activity and for enhanced listericidal activity in the livers and spleens of mice stimulated with rIFN-γ.

Of the cytokines which mediate the host response against infections with intracellular pathogens, gamma interferon (IFN-γ) is one of the most important (29). Administration of recombinant IFN-γ (rIFN-γ) increases resistance against various intracellular pathogens (16, 23, 31), and neutralization of endogenous IFN-γ with antibodies decreases the resistance of mice against infections with intracellular pathogens such as *Leishmania major*, *Toxoplasma gondii*, and *Listeria monocytogenes* (3, 4, 35). One of the major functions of IFN-γ is activation of macrophages (22). Activation causes changes in a number of the biochemical and functional activities of murine macrophages, such as enhanced release of reactive oxygen intermediates and reactive nitrogen intermediates (RNI), enhanced microbicidal activities, and enhanced release of various cytokines, e.g., tumor necrosis factor alpha (TNF-α) (26, 28, 29, 34).

It has been demonstrated that administration of TNF-α can protect mice against infection with *L. major* (20), *T. gondii* (6), and *L. monocytogenes* (14), and injection of antibodies against TNF-α causes increased growth of *L. monocytogenes* and bacillus Calmette-Guérin (*Mycobacterium bovis* BCG) in the livers of mice (17, 24). In vitro, rTNF-α synergizes with rIFN-γ in the induction of both antiprotozoal activity and the production of RNI by murine peritoneal macrophages (8, 9, 19).

Although the microbicidal mechanisms by which macrophages control the proliferation of intracellular pathogens are not yet fully understood, it is apparent that in mice RNI derived from L-arginine play an important role in resistance to various pathogens, such as helminths (15), protozoa (2, 12, 19), bacteria (1, 5), and fungi (10). Recently, it was shown that in vitro TNF-α is an essential mediator of the rIFN-γ-induced, L-arginine-mediated microbicidal activity of murine macrophages against *L. major* and *T. gondii* (11, 19). However, it is not known whether the central role of TNF-α in resistance to protozoa in vitro also applies for the rIFN-γ-induced effects on protozoa and bacteria in vivo. The aim of the present study was to determine whether TNF-α is also required for in vivo activation of murine peritoneal macrophages, their toxoplasmastatic activity, and inhibition of the proliferation of *L. monocytogenes* in the livers and spleens of mice treated with rIFN-γ.

MATERIALS AND METHODS

Animals and macrophages. Specific-pathogen-free female CBA/J mice weighing 20 to 30 g each were obtained from IFFA-Credo, Saint-Germain-sur-l'Abresle, France. The animals were maintained under strict specific-pathogen-free conditions during the experiments and received sterilized food and water ad libitum. Peritoneal macrophages were collected from the mice by lavage with 2 ml of ice-cold phosphate-buffered saline (PBS) (pH 7.4) containing 50 U of heparin per ml (37).

Cytokines and antibodies. Rat rIFN-γ, produced in Chinese hamster ovary cells (36), was generously provided by P. H. van der Meide (Institute of Applied Radiobiology and

* Corresponding author.

Immunology, TNO, Rijswijk, The Netherlands). Lyophilized rIFN-γ was diluted in pyrogen-free saline containing 1% fetal calf serum (FCS) and stored at −70°C; aliquots were thawed once immediately before use. Mouse rTNF-α, produced in *Escherichia coli*, was kindly provided by P. De Waele (Innogenetics NV, Ghent, Belgium). rTNF-γ was stored at −70°C in pyrogen-free saline containing 100 μg of bovine serum albumin per ml; aliquots were thawed once immediately before use. Rabbit anti-mouse TNF-α antiserum containing approximately 2×10^6 neutralizing units (NU) per ml was a generous gift from P. De Waele. Normal rabbit serum (NRS), used as a control rabbit immunoglobulin, was obtained from specific-pathogen-free New Zealand White rabbits. The cytokines and antibodies contained less than 0.01 ng of lipopolysaccharide per ml, as determined by the *Limulus* lysate assay.

Microorganisms. The virulent RH strain of *T. gondii* was maintained by biweekly intraperitoneal (i.p.) passage through CBA/J mice. The protozoa were collected by peritoneal lavage 2 to 3 days after i.p. injection into the mice, counted, and suspended at a concentration of 10^6 tachyzoites per ml in RPMI 1640 medium (Flow Laboratories, Rockville, Md.) supplemented with 10% heat-inactivated FCS (Flow Laboratories, Irvine, Scotland), 100 U of penicillin per ml, and 50 μg of streptomycin per ml (hereafter called medium).

Virulent *L. monocytogenes* EGD (50% lethal dose, 5×10^3 CFU for CBA/J mice) was kept virulent by repeated passage through CBA/J mice and stored on blood agar plates at 4°C. A colony was inoculated in tryptose phosphate broth (Oxoid Ltd.) and cultured for 18 h at 37°C. Bacteria were collected by centrifugation (10 min, 1,500 × *g*), washed twice with PBS, and suspended at the appropriate bacterial concentrations in pyrogen-free saline.

Measurement of NO_2^- production. The amount of NO_2^- released by peritoneal macrophages in culture supernatant was determined with Griess reagent (1% sulfanilamide, 0.1% naphthylethylene-diamide-dihydrochloride, 2.5% H_3PO_4) as described elsewhere (30). In short, 50-μl samples of the culture supernatant were mixed with 50 μl of Griess reagent and incubated for 10 min at room temperature. The A_{550} of the reaction product, which reflects the concentration of NO_2^-, was read on a Titertek Multiscan Plus ELISA reader (EFLAB, Helsinki, Finland).

To investigate the role of L-arginine in the production of NO_2^- by peritoneal macrophages and induction of toxoplasmastatic activity in vivo, mice received an i.p. injection of 1 mM N^G-monomethyl-L-arginine (NMA) (Calbiochem, San Diego, Calif.), a competitive inhibitor of L-arginine, 15 min before i.p. injection of 10^4 U of rIFN-γ. The macrophages were harvested 18 h later and incubated for 24 h in medium at 37°C in 7.5% CO_2; the amount of NO_2^- released by the macrophages was assessed as described above.

Intracellular proliferation of *T. gondii*. Peritoneal macrophages were infected with *T. gondii* as described previously (19). In short, macrophages were suspended in medium at a concentration of 10^6 cells per ml, 1 ml was plated onto 35-mm-diameter plastic culture dishes (Falcon, Lincoln Park, N.J.) containing three 12-mm-diameter round glass coverslips, and after adherence of the cells for 2 h at 37°C in 7.5% CO_2, nonadherent cells were removed by washing. Next, 1 ml of medium containing 10^6 *T. gondii* tachyzoites per ml was added to the cells, incubated for 30 min at 37°C in 7.5% CO_2, and then washed three times with warm PBS to remove noningested *T. gondii*. The macrophages on one coverslip were fixed in methanol and then stained with Giemsa stain to determine the percentage of macrophages that were infected at the start of the assay. Fresh medium was added to the other coverslips, which were incubated for another 18 h at 37°C in 7.5% CO_2; the cells were then fixed and stained. The number of *T. gondii* tachyzoites per 100 macrophages at the start of the assay and after 18 h of incubation was assessed microscopically. Whenever the percentage of macrophages that were infected after 18 h of incubation increased by more than 7% relative to that at the start of the assay, the results were discarded. The results were expressed as the fold increase in the number of *T. gondii* tachyzoites, i.e., the ratio of the number of *T. gondii* tachyzoites per 100 macrophages after 18 h of incubation to the number of *T. gondii* tachyzoites per 100 macrophages at the start of the assay.

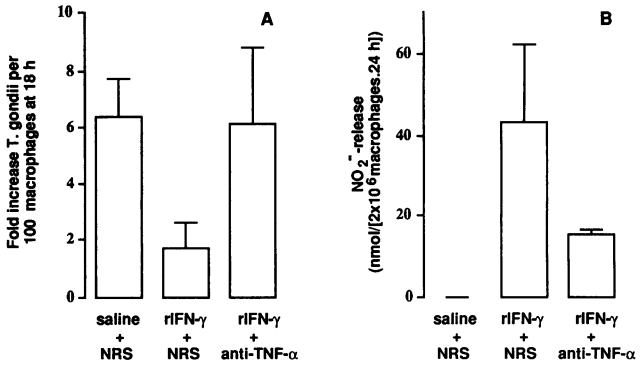

FIG. 1. Intracellular proliferation of *T. gondii* (A) and generation of RNI (B) by peritoneal macrophages after treatment of mice with anti-TNF-α. Mice received an i.p. injection of 6×10^4 NU of anti-TNF-α or an equal volume of NRS 30 min before i.p. injection of 1×10^4 U of rIFN-γ. The peritoneal macrophages were harvested 18 h after injection of rIFN-γ and infected with *T. gondii*; after an 18-h infection period, the fold increase in toxoplasmas and the amount of NO_2^- in the medium, which serves as a measure for RNI generation, were determined. Values are the means of three experiments; error bars indicate SDs.

Proliferation of *L. monocytogenes* in the liver and spleen. Mice received an intravenous (i.v.) injection of 10^4 CFU of *L. monocytogenes* (2 times the 50% lethal dose). At various times during the infection, several animals were killed, and their livers and spleens were isolated and homogenized in 2 ml of saline in a tissue homogenizer (model X-1020; Ystral GmbH, Dottingen, Germany). Serial 10-fold dilutions of the organ suspensions were plated onto blood agar plates; after incubation for 18 to 24 h at 37°C, the number of colonies was counted to obtain the number of CFU of *L. monocytogenes* per organ.

Statistical analysis. Results are expressed as the mean ± standard deviation (SD). Differences between various groups were assessed by a one-way analysis of variance with a Dunnett *t* test. Values below 0.05 were considered significant.

RESULTS

Effect of anti-TNFα on rIFN-γ-induced macrophage activation. Peritoneal macrophages from mice that had received an i.p. injection of NRS and 10^4 U of rIFN-γ 18 h before harvesting of the cells inhibited the intracellular proliferation of *T. gondii* (Fig. 1A) and generated greater amounts of NO_2^- (Fig. 1B) than peritoneal macrophages from control mice that had received an i.p. injection of NRS and saline.

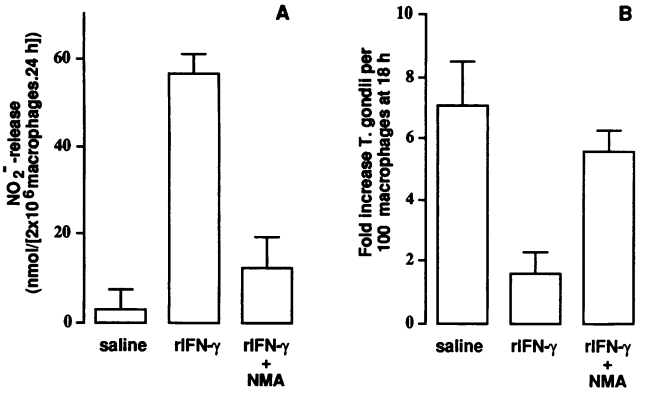

FIG. 2. Generation of RNI by (A) and intracellular proliferation of *T. gondii* in (B) peritoneal macrophages after treatment of mice with NMA. Mice received an i.p. injection of 1 mM NMA 15 min before i.p. injection of 10^4 U of rIFN-γ. The peritoneal macrophages were harvested 18 h after injection of rIFN-γ and infected with *T. gondii*; after an 18-h infection period, the fold increase in toxoplasmas and the amount of NO_2^- in the medium, which serves as a measure for RNI generation, were determined. Values are the means of three experiments; error bars indicate SDs.

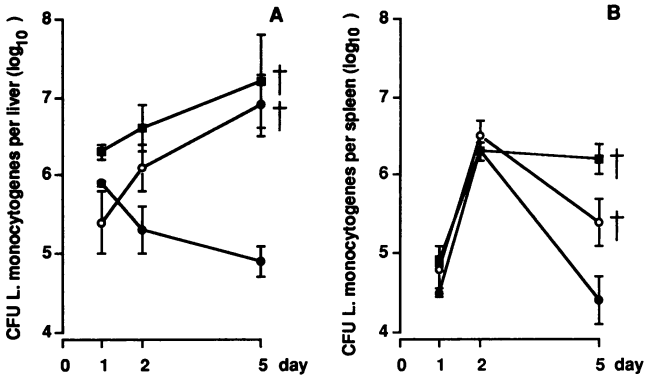

FIG. 3. Proliferation of *L. monocytogenes* in the livers (A) and spleens (B) of mice treated with rIFN-γ with or without anti-TNF-α. Mice received an i.v. injection of 6×10^4 NU of anti-TNF-α (■) or an equal volume of NRS (○ and ●) 30 min before i.v. injection of 1×10^5 U of rIFN-γ (● and ■) or saline (○); 18 h later, the mice received an i.v. injection of 1×10^4 CFU of *L. monocytogenes*. The number of bacteria in the liver and spleen of each mouse was determined on days 1, 2, and 5 of infection. Each value is the mean ± SD for six mice. †, mice died after day 5 of infection.

Macrophages from mice that had received an i.p. injection of 6×10^4 NU of anti-TNF-α 30 min before the i.p. injection of 1×10^4 U of rIFN-γ did not inhibit the proliferation of *T. gondii* (Fig. 1A) and generated less NO_2^- (Fig. 1B) than macrophages from mice that had received rIFN-γ and NRS. In these experiments, anti-TNF-α caused a reduction in NO_2^- of 66.5% ± 18.7%. Injection of anti-TNF-α 3 h after injection of rIFN-γ resulted in a 48.6% ± 28.6% decrease in NO_2^-; when anti-TNF-α was injected 6 h after rIFN-γ, generation of NO_2^- by the macrophages was inhibited by only 14.1% ± 14.2% relative to that by control macrophages. i.p. injection of NRS did not affect the rIFN-γ-induced generation of NO_2^- by the macrophages. Infection of macrophages from rIFN-γ-treated mice with *T. gondii* did not result in increased generation of NO_2^- by the cells relative to that by noninfected macrophages from these mice (data not shown), a result which is comparable to the results from our in vitro study (19).

Involvement of RNI in rIFN-γ-induced toxoplasmastatic activity. To investigate the involvement of L-arginine-derived RNI in the toxoplasmastatic activity induced by rIFN-γ, mice were treated with NMA, a competitive inhibitor of L-arginine. Macrophages from mice that had received an i.p. injection of 1 mM NMA 15 min before the i.p. injection of 10^4 U of rIFN-γ generated less NO_2^- than macrophages from mice injected with rIFN-γ alone (Fig. 2A). The intracellular proliferation of *T. gondii* in macrophages from mice that received an i.p. injection of rIFN-γ and NMA was significantly higher than that in macrophages obtained from mice injected with only rIFN-γ and similar to that in control macrophages (Fig. 2B).

Effect of anti-TNF-α on rIFN-γ-induced inhibition of proliferation of *L. monocytogenes*. After i.v. injection of 10^4 CFU of *L. monocytogenes*, the proliferation of the bacteria in the livers of mice that had received an i.v. injection of 10^5 U of rIFN-γ and NRS 18 h earlier was less than that in control mice. After day 6 of infection, all mice that had received rIFN-γ were still alive, but all of the control mice had died (Fig. 3). When mice received an i.v. injection of 6×10^4 NU of anti-TNF-α simultaneously with rIFN-γ, the proliferation of *L. monocytogenes* in the liver was not inhibited and was even slightly enhanced relative to that in control mice; moreover, all mice died (Fig. 3A). During the first 2 days of infection, the rate of proliferation of *L. monocytogenes* in the spleens of mice that had received anti-TNF-α and rIFN-γ was similar to that found for mice that had received NRS and rIFN-γ. On day 5 of infection, significantly ($P < 0.05$) fewer bacteria were recovered from the spleens of mice that had received rIFN-γ and NRS than from the spleens of control mice. The number of bacteria in the spleens of mice injected with anti-TNF-α and rIFN-γ was significantly higher than that in the spleens of rIFN-γ-treated as well as control mice (Fig. 3B).

To determine when TNF-α is involved in the rIFN-γ-induced effects on the proliferation of *L. monocytogenes*, anti-TNF-α was injected at various times during the infection, and the number of bacteria in the liver and spleen was determined on day 4 of infection. In mice that received an i.v. injection with anti-TNF-α together with the injection of bacteria at 0 h, i.e., 18 h after injection of rIFN-γ, the rIFN-γ-induced effects on bacterial proliferation in the liver and spleen were abrogated, as was also the case when rIFN-γ and anti-TNF-α were both injected 18 h before injection of the bacteria (Fig. 4). Injection of anti-TNF-α at 48 h, i.e. 66 h after injection of rIFN-γ, did not affect the rIFN-γ-induced decrease in the number of *L. monocytogenes* cells in the spleen, but the number of bacteria in the liver exceeded that in mice treated with rIFN-γ alone (Fig. 4).

Effect of rTNF-α alone and together with rIFN-γ on the number of *L. monocytogenes* cells in the liver and spleen. Two days after i.v. injection of 10^4 CFU of *L. monocytogenes*, the number of bacteria recovered from the livers and spleens of mice that had received an i.v. injection of 10^5 U of rIFN-γ 18 h before injection of the bacteria was significantly less than that in control mice (Table 1). i.v. injection of 10^4 U of rTNF-α 18 h before injection of the bacteria also inhibited the growth of *L. monocytogenes*, and the number of bacteria recovered from the liver and spleen was similar to that after an i.v. injection of 10^5 U of rIFN-γ. The number of *L. monocytogenes* cells in the organs of mice that had received

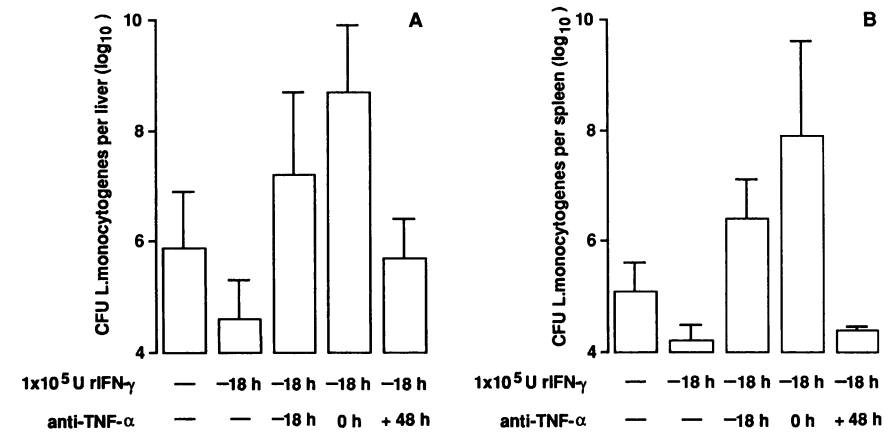

FIG. 4. Proliferation of *L. monocytogenes* in the livers (A) and spleens (B) of mice treated with rIFN-γ with or without anti-TNF-α. Mice received an i.v. injection of 6×10^4 NU of anti-TNF-α at various times. Eighteen hours before i.v. injection of 10^4 CFU of *L. monocytogenes*, i.e., at 0 h, the mice received an i.v. injection of 10^5 U of rIFN-γ (−18 h). The number of bacteria in the liver and spleen of each mouse was determined on day 4 of infection. Values are the means for four to six mice; error bars indicate SDs.

an i.v. injection of 10^5 U of rIFN-γ together with 10^3 U of rTNF-α was similar to that in mice that had received rIFN-γ alone (Table 1); similar results were found after an i.v. injection of 10^5 U of rIFN-γ together with 10^4 U of rTNF-α (data not shown).

DISCUSSION

The main conclusion from the present study is that activation of peritoneal macrophages and enhanced listericidal activity in the livers and spleens of mice that were treated with rIFN-γ requires TNF-α. Endogenous TNF-α probably activates peritoneal macrophages and induces enhanced listericidal activity in the liver and spleen in an autocrine fashion. This conclusion is based on the finding that in vivo administration of anti-TNF-α together with rIFN-γ both inhibits the rIFN-γ-induced activation of macrophages, as expressed by the reduced generation of RNI and the inhibition of toxoplasmastatic activity, and permits unrestricted growth of *L. monocytogenes* in the livers and spleens of mice.

In vitro studies have demonstrated that macrophages activated with rIFN-γ with or without a second stimulation produce TNF-α, which in an autocrine fashion activates the macrophages (11, 19). The present study is the first to demonstrate that there is a similar pathway in vivo; i.e., endogenous TNF-α, probably produced upon injection of rIFN-γ, is essential for macrophage activation. TNF-α is required in an early phase of macrophage activation, since antibodies against TNF-α inhibited the generation of RNI by and toxoplasmastatic activity of peritoneal macrophages only when administered to the mice within 6 h of the rIFN-γ injection; such an interval was also found in a previous in vitro study (19).

In vivo, endogenous TNF-α is essential to limit the proliferation of *L. monocytogenes* in rIFN-γ-treated mice as well, since administration of anti-TNF-α antibodies over a period of 2 to 3 days after administration of rIFN-γ results in a significant increase in the number of bacteria in the liver and spleen. This indicates that in these mice, TNF-α is produced for several days, probably because of the infection with *L. monocytogenes* since such an infection in naive mice induces the production of this cytokine (24, 25).

Involvement of TNF-α in resistance to *L. monocytogenes* was also shown by the decrease in bacterial proliferation in rTNF-α-treated mice (references 17 and 24 and this study), but injection of high doses of both rTNF-α and rIFN-γ has no additional effect on the proliferation of *L. monocytogenes* in the liver and spleen (reference 32 and this study). During an infection of mice with *L. monocytogenes*, high levels of TNF-α are detectable on day 2 or 3 of infection (25), which might limit the effects of exogenously added rTNF-α. In our model, we injected mice with rIFN-γ and rTNF-α before infection with *L. monocytogenes*, and thus it is unlikely that the lack of a synergistic effect of these two cytokines is due to TNF-α that is produced during the infection.

TNF-α is required for the generation of RNI by murine macrophages both in vitro (11, 19) and in vivo (this study). In vitro, RNI have been shown to be involved in inhibition of intracellular proliferation and killing of many microorgan-

TABLE 1. Number of CFU of *L. monocytogenes* in the livers and spleens of mice treated with rIFN-γ or rTNF-α

Organ	$\log_{10}$ CFU of *L. monocytogenes* in mice treated with[a]:				
	Saline	10^5 U of rIFN-γ	10^3 U of rTNF-α	10^4 U of rTNF-α	10^5 U of rIFN-γ and 10^3 U of rTNF-α
Liver	5.4 ± 0.3	4.4 ± 0.1[b]	5.2 ± 0.3	4.6 ± 0.4[b]	4.4 ± 0.2[b]
Spleen	5.9 ± 0.3	4.8 ± 0.3[b]	5.9 ± 0.4	5.5 ± 0.3[b]	4.6 ± 0.3[b]

[a] Mice received an i.v. injection of rIFN-γ or rTNF-α 18 h before an i.v. injection of 10^4 CFU of *L. monocytogenes*. The number of bacteria in the liver and spleen of each mouse was assessed on day 2 of infection. Each value is the mean ± SD for four to six mice.

[b] Value is significantly ($P < 0.05$) less than that for saline-treated mice.

isms (27). The present finding that i.p. injection of NMA, an inhibitor of L-arginine-dependent microbicidal mechanisms, inhibits both the production of RNI and the toxoplasmastatic activity of murine macrophages demonstrates that RNI play also an important role in vivo in resistance to *T. gondii*.

Activation of macrophages is considered to be essential for protection against *L. monocytogenes*, and TNF-α is involved in macrophage activation in vitro and in vivo (references 11 and 19 and this study). One of the possible mechanisms for the reduced proliferation of *L. monocytogenes* due to TNF-α is the generation of RNI. It has been demonstrated that RNI are involved in the killing of various species of mycobacteria (1, 5). We have recently shown that generation of RNI correlates with the rate of intracellular killing of *L. monocytogenes* by BCG-activated macrophages (33).

As yet, the mechanism by which TNF-α protects mice against infections with *L. monocytogenes* is not clear. Activated Kupffer cells release proinflammatory products, e.g., TNF-α (7). These cytokines activate hepatocytes to generate RNI, and such activated cells might be involved in inhibition of proliferation of *L. monocytogenes* (13). TNF-α is also involved in the formation of granulomas in the livers of listeria-immune mice stimulated with heat-killed bacteria (21) and in the livers of BCG-infected mice (17). Recently, we found that anti-TNF-α inhibits rIFN-γ-induced development of granulomatous lesions and activation of cells in the liver during an infection of mice with *L. monocytogenes* (18). Treatment of mice with anti-TNF-α has been reported to result in extensive proliferation of *L. monocytogenes* in hepatocytes with little influx of mononuclear cells (14). These results indicate that TNF-α is required for recruitment of phagocytes to the site of infection.

ACKNOWLEDGMENTS

We thank Bernard van de Berg and Ireen van Graas for their excellent technical assistance.

REFERENCES

1. **Adams, L. B., S. G. Franzblau, Z. Vavrin, J. B. Hibbs, Jr., and J. L. Krahenbuhl.** 1991. L-arginine-dependent macrophage effector functions inhibit metabolic activity of *Mycobacterium leprae*. J. Immunol. **147:**1642–1646.
2. **Adams, L. B., J. B. Hibbs, Jr., R. R. Taintor, and J. L. Krahenbuhl.** 1990. Microbistatic effect of murine activated macrophages for *Toxoplasma gondii*. Role for synthesis of inorganic nitrogen oxides from L-arginine. J. Immunol. **144:** 2725–2730.
3. **Belosovic, M., D. S. Finbloom, P. H. van der Meide, M. V. Slayter, and C. A. Nacy.** 1989. Administration of monoclonal anti-IFN-γ antibodies in vivo abrogates natural resistance of C3H/HeN mice to infection with *Leishmania major*. J. Immunol. **143:**266–274.
4. **Buchmeier, N. A., and R. D. Schreiber.** 1985. Requirement of endogenous interferon-γ production for resolution of *Listeria monocytogenes* infection. Proc. Natl. Acad. Sci USA **82:**7404–7408.
5. **Chan, J., Y. Xing, R. S. Magliozzo, and B. Bloom.** 1992. Killing of virulent *Mycobacterium tuberculosis* by reactive nitrogen intermediates produced by activated murine macrophages. J. Exp. Med. **175:**1111–1122.
6. **Chang, H. R., G. E. Grau, and J. C. Pechere.** 1990. Role of TNF and IL-1 in infections with *Toxoplasma gondii*. Immunology **69:**33–37.
7. **Curran, R. D., T. R. Billiar, D. J. Stuehr, K. Hofmann, and R. L. Simmons.** 1989. Hepatocytes produce nitrogen oxides from L-arginine in response to inflammatory products of Kupffer cells. J. Exp. Med. **170:**1769–1774.
8. **Ding, A. H., C. F. Nathan, and D. J. Stuehr.** 1988. Release of reactive nitrogen intermediates and reactive oxygen intermediates from mouse peritoneal macrophages. J. Immunol. **141:** 2407–2412.
9. **Drapier, J.-C., J. Wietzerbin, and J. B. Hibbs, Jr.** 1988. Interferon-γ and tumor necrosis factor induce the L-arginine-dependent cytotoxic effector mechanism in murine macrophages. Eur. J. Immunol. **18:**1587–1592.
10. **Granger, D. L., J. B. Hibbs, Jr., J. R. Perfect, and D. T. Durack.** 1987. Specific amino acid (L-arginine) requirement for the microbiostatic activity of murine macrophages. J. Clin. Invest. **81:**1129–1136.
11. **Green, S. J., R. M. Crawford, J. T. Hockmeyer, M. S. Meltzer, and C. A. Nacy.** 1990. *Leishmania major* amastigotes initiate the L-arginine-dependent killing mechanism in IFN-γ-stimulated macrophages by induction of tumor necrosis factor-α. J. Immunol. **145:**4290–4297.
12. **Green, S. J., M. S. Meltzer, J. B. Hibbs, Jr., and C. A. Nacy.** 1990. Activated macrophages destroy intracellular *Leishmania major* amastigotes by an L-arginine-dependent killing mechanism. J. Immunol. **144:**278–283.
13. **Gregory, S. H., L. K. Barczynski, and E. J. Wing.** 1992. Effector function of hepatocytes and Kupffer cells in the resolution of systemic bacterial infections. J. Leukocyte Biol. **51:**421–424.
14. **Havell, E. A.** 1989. Evidence that tumor necrosis factor has an important role in antibacterial resistance. J. Immunol. **143:**2894–2899.
15. **James, S. L., and J. Glaven.** 1989. Macrophage cytotoxicity against schistosomula of *Schistosoma mansoni* involves arginine-dependent production of reactive nitrogen intermediates. J. Immunol. **143:**4208–4212.
16. **Kiderlen, A. F., S. H. E. Kaufmann, and M. L. Lohmann-Matthes.** 1984. Protection of mice against the intracellular bacterium *L. monocytogenes* by recombinant immune interferon. Eur. J. Immunol. **14:**964–967.
17. **Kindler, V., A.-P. Sappinno, G. E. Grau, P.-F. Piguet, and P. Vassali.** 1989. The inducing role of tumor necrosis factor in the development of bacterial granulomas during BCG infection. Cell **56:**731–740.
18. **Langermans, J. A. M., D. M. Mayanski, P. H. Nibbering, M. E. B. van der Hulst, J. S. van de Gevel, and R. van Furth.** Immunocytochemical analysis of the protection of mice against an infection with L. monocytogenes by IFN-γ and endogenous TNF-α. Submitted for publication.
19. **Langermans, J. A. M., M. E. B. Van der Hulst, P. H. Nibbering, P. S. Hiemstra, L. Fransen, and R. Van Furth.** 1992. IFN-γ-induced L-arginine-dependent toxoplasmastatic activity in murine peritoneal macrophages is mediated by endogenous tumor necrosis factor-α. J. Immunol. **148:**568–574.
20. **Liew, F. Y., C. Parkinson, S. Millot, A. Severn, and M. Carrier.** 1990. Tumour necrosis factor (TNFα) in leishmaniasis. I. TNFα mediates host protection against cutaneous leishmaniasis. Immunology **69:**570–573.
21. **Mielke, M. E. A., H. Rosen, S. Brocke, C. Peters, and H. Hahn.** 1992. Protective immunity and granuloma formation are mediated by two distinct tumor necrosis factor alpha- and gamma interferon-dependent T cell-phagocyte interactions in murine listeriosis: dissociation on the basis of phagocyte adhesion mechanisms. Infect. Immun. **60:**1875–1882.
22. **Murray, H. W.** 1988. Interferon-γ, the activated macrophage, and host defense against microbial challenge. Ann. Intern. Med. **108:**595–608.
23. **Nacy, C. A., A. H. Fortier, M. S. Meltzer, N. A. Buchmeier, and R. D. Schreiber.** 1985. Macrophage activation to kill *Leishmania major*: activation of macrophages for intracellular destruction of amastigotes can be induced by both recombinant interferon-γ and non-interferon lymphokines. J. Immunol. **135:**3505–3511.
24. **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.
25. **Nakane, A., A. Numata, and T. Minagawa.** 1992. Endogenous tumor necrosis factor, interleukin-6, and gamma interferon

levels during *Listeria monocytogenes* infection in mice. Infect. Immun. **60:**523–528.
26. **Nathan, C. F.** 1987. Secretory products of macrophages. J. Clin. Invest. **79:**319–326.
27. **Nathan, C. F., and J. B. Hibbs, Jr.** 1991. Role of nitric oxide synthesis in macrophage antimicrobial activity. Curr. Opin. Immunol. **3:**65–70.
28. **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.
29. **Nathan, C. F., and R. Yoshida.** 1988. Cytokines: interferon-γ, p. 229–251. *In* J. I. Gallin, I. M. Goldstein, and R. Snyderman (ed.), Inflammation: basic principles and clinical correlates. Raven Press, Ltd., New York.
30. **Nibbering, P. H., J. A. M. Langermans, J. S. Van de Gevel, M. E. B. Van der Hulst, and R. Van Furth.** 1991. Nitrite production by activated murine macrophages correlates with their toxoplasmastatic activity, Ia antigen expression, and production of H_2O_2. Immunobiology **184:**93–105.
31. **Reed, S. G.** 1988. In vivo administration of recombinant IFN-γ induces macrophage activation, and prevents acute disease, immune suppression, and death in experimental *Trypanosoma cruzi* infections. J. Immunol. **140:**4342–4347.
32. **Roll, J. T., K. M. Young, R. S. Kurtz, and C. J. Czuprinsky.** 1990. Human TNF-α augments anti-bacterial resistance in mice: potentiation of its effects by recombinant human IL-1α. Immunology **69:**316–322.
33. **Stokvis, H., J. A. M. Langermans, E. de Backer-Vledder, M. E. B. van der Hulst, and R. van Furth.** 1992. Hydrocortisone-treatment of BCG-infected mice impairs the activation and enhancement of antimicrobial activity of peritoneal macrophages. Scand. J. Immunol. **36:**299–305.
34. **Stuehr, D. J., and M. A. Marletta.** 1987. Induction of nitrite/nitrate synthesis in murine macrophages by BCG infection, lymphokines, or interferon-γ. J. Immunol. **139:**518–525.
35. **Suzuki, Y., M. A. Orellana, R. D. Schreiber, and J. S. Remington.** 1988. Interferon-γ: the major mediator of resistance against *Toxoplasma gondii*. Science **240:**516–518.
36. **Van der Meide, P. H., M. Dubbeld, K. Vijverberg, T. Kos, and H. S. Schellekens.** 1986. The purification and characterization of rat gamma interferon by use of two monoclonal antibodies. J. Gen. Virol. **67:**1059–1071.
37. **Van Furth, R., and Z. A. Cohn.** 1968. The origin and kinetics of mononuclear phagocytes. J. Exp. Med. **128:**415–435.