The Journal of Immunology
RESEARCH ARTICLE | JANUARY 15 1991

Tumor necrosis factor-independent IL-6 production during murine listeriosis. FREE

E A Havell; ... et. al

J Immunol (1991) 146 (2): 756–761.
https://doi.org/10.4049/jimmunol.146.2.756

0022-1767/91/1462-0756$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 146, 756–761, No. 2, January 15, 1991
Printed in U.S.A.

TUMOR NECROSIS FACTOR-INDEPENDENT IL-6 PRODUCTION DURING MURINE LISTERIOSIS[1]

EDWARD A. HAVELL[2]* AND PRAVINKUMAR B. SEHGAL†

*From the *Trudeau Institute, Inc., Saranac Lake, NY 12983, and †The Rockefeller University, New York, NY 10021*

We report that TNF, IL-6, and IFN-α/β are produced by mice during either sublethal or lethal *Listeria monocytogenes* infections. The quantities of these cytokines in infected spleens increase and decrease in concordance with bacterial numbers in these organs. While all of these cytokines were present in *Listeria*-infected spleens, only IL-6 and IFN-α/β were found in the peripheral circulation. Inasmuch as TNF has been reported to be responsible for the production of IL-6 in vivo following the inoculation of a lethal dose of the Gram-negative bacterium, *Escherichia coli* (Fong *et al.*, 1989. *J. Exp. Med. 170: 1627*), experiments were undertaken to determine whether IL-6 production elicited by the Gram-positive bacterium, *L. monocytogenes*, was also TNF-dependent. It was found that the passive immunization of mice with neutralizing antibodies specific for TNF shortly before i.v. injection of a lethal or sublethal *Listeria* inoculum resulted in the complete neutralization of endogenously produced TNF, and in the progressive multiplication of bacteria in infected organs. It was also found that the anti-TNF IgG treatment resulted in a progressive increase in the amounts of *Listeria*-induced IL-6 present in spleen and blood, until the death of the host. These findings indicate that *Listeria*-induced IL-6 production in mice occurs primarily through a TNF-independent pathway, and correlates directly with the severity of the infection.

The production of TNF during bacterial infections can be either beneficial or detrimental to the host. On the one hand, it has been reported that the presence of TNF in the peripheral circulation during overwhelming Gram-negative bacterial infections or experimental endotoxemia causes septic shock (1–3). On the other hand, a growing body of evidence indicates that relatively small amounts of TNF produced and localized within infected tissues play an important protective role in host defense against bacteria (4–8). This has been shown by studies in which the administration of neutralizing antibodies specific for TNF exacerbated *Listeria monocytogenes* (4–6), *Mycobacterium bovis* (7), and *Chlamydia trachomatis* (8) infections in mice. The results of experiments presented in these studies also revealed that TNF plays an essential role in antibacterial resistance at an early stage of infection (4–7).

TNF induces the production of cytokines that could participate in the response of the host to bacterial infection. For example, the i.v. injection of TNF elicits the appearance of IL-6 in the blood (9). Moreover, IL-6 has been found in human serum during experimental endotoxemia (10), as well as in the serum of patients exhibiting clinical signs of bacterial infection (11). The cytokines TNF, IL-1, and IL-6 can, directly or indirectly, induce the symptoms associated with bacterial infections, such as fever and the acute-phase plasma protein responses (12) (reviewed in Refs. 13 and 14). Recently, Fong et al. (15) reported that TNF, followed by IL-1β and IL-6, appeared in the sera of baboons shortly after the intra-aortic infusion of a lethal inoculum of *Escherichia coli*. In addition, it was also reported that the quantities of *E. coli*-induced IL-1β and IL-6 appearing in the circulation of anti-TNF IgG-treated baboons were greatly reduced. Based on this finding, Fong et al. (15) concluded that TNF is primarily responsible for the appearance of IL-1β and IL-6 during a lethal Gram-negative bacterial infection. In view of these findings, experiments were carried out in our laboratories to determine whether IL-6 production also occurs through a TNF-dependent pathway during a Gram-positive bacterial infection. The results of these experiments establish that IL-6 production in mice undergoing either a sublethal or lethal infection with the Gram-positive pathogen, *L. monocytogenes*, occurs primarily through a TNF-independent mechanism(s).

MATERIALS AND METHODS

Mice. Male AB6F1 (A/Tru × C57BL/6Tru) mice 9 to 12 wk of age were used in these studies. The AB6F1 mice were supplied by the Trudeau Institute Animal Breeding Facility (Saranac Lake, NY) and were subjected to routine screening for common viral pathogens by Charles River Professional Services (Wilmington, MA).

Listeria monocytogenes. *L. monocytogenes* (Strain EGD, serotype 3b) having a 50% lethal dose (LD_{50}) in AB6F1 mice of 4×10^4 CFU was grown in Trypticase-soy broth (BBL Microbiology Systems, Cockeysville, MD) after passage in vivo. It was dispensed as stock cultures at 2×10^8 CFU/ml in Trypticase-soy broth and stored at −70°C. Enumeration of *Listeria* in spleens was performed as previously described (16). The procedure for *Listeria* killing by means of UV irradiation has been described in detail (17).

Reagents. An anti-murine TNF-neutralizing IgG was raised in a rabbit against pure murine rTNF (18, 19), purified from the serum, and characterized as to its capacity to specifically neutralize TNF cytotoxic activity, as previously reported (20). The anti-TNF IgG neutralizing titer (neutralizing U/ml) is defined as the reciprocal of the highest serial twofold dilution of IgG that, when reacted with an equal volume of test sample having 20 TNF cytotoxic U/ml, neutralizes 50% or more of the cytotoxic activity on actinomycin D-treated murine L929B cells (see *TNF assay* below).

Cytokine bioassays. Immediately after the plating procedure to enumerate bacteria in the spleens, the spleen homogenates were

Received for publication August 20, 1990.
Accepted for publication October 19, 1990.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] This work was supported by National Institutes of Health research Grants AI23544, RR50705, and CA44365.
[2] Address correspondence and reprint requests to Edward A. Havell, Trudeau Institute, P.O. Box 59, Saranac Lake, NY 12983.

centrifuged to remove tissue debris, and the supernatants were rendered free of bacteria by filtration through a 0.45-μm filter. The spleen homogenate supernatants and sera were stored at −70°C until they were assayed to determine TNF, IFN-α/β, and IL-6 biological activities (U/ml).

TNF assay. The assay used for the quantitation of TNF cytotoxic activity on actinomycin D-treated murine L929B fibroblasts has been previously described in detail (20). The TNF cytotoxicity titer, expressed as U/ml, is defined as the reciprocal of the highest dilution of test sample that causes 50% or more destruction of actinomycin D-treated L929B cells. Included in each TNF assay was an internal laboratory murine TNF standard that had been calibrated against the World Health Organization human rTNF standard (86/659), which was prepared by the National Institute for Biological Standards and Control (Hertfordshire, England).

IFN assay. The microtiter IFN assay procedure of Havell (17), which employs L929B cells and vesicular stomatitis virus, was used to determine IFN concentrations in the test samples. The IFN antiviral activity titer (U/ml) is the reciprocal of the highest dilution of test sample that protects 50% or more of L929B cells from the cytopathic effect of vesicular stomatis virus. All IFN titers are expressed in terms of the National Institutes of Health (NIH) G-002-904-511 international murine IFN-α/β standard. The antiviral activities of the test samples were shown to be exclusively due to IFN-α/β, through the use of anti-murine IFN-α/β neutralizing antibodies, according to previously published procedures (17).

IL-6 assay. The IL-6 content of test samples was assayed by monitoring the ability of the samples to cause proliferation of the IL-6-dependent murine hybridoma cell line B9 (21). Briefly, duplicate sets of serial threefold dilutions of test samples were added to B9 cells in 96-well plates (final volume 200 μl, containing 5000 cells). The cultures were incubated 92 to 96 h, and growth of B9 cells was then quantitated by the uptake of 3-[4,5 dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide (Sigma Chemical Co., St. Louis, MO) (22) and measured at 570/690 wavelengths using an EL312 Biotek microplate reader. Neutralizing rat anti-murine rIL-6 6B4 mAb (23) confirmed that the proliferative response of B9 cells to the test samples was due to IL-6.

IL-6 titers, expressed as laboratory reference units (U/ml), are the reciprocals of the highest dilutions of test samples that produced a half-maximal proliferative response in the B9 assay. Each assay is calibrated in terms of the biological activity of an IL-6 laboratory reference standard (purified IL-1-induced human fibroblast IL-6) (24). One laboratory U/ml corresponds to 0.3 U/ml of the 88/514 interim reference standard for IL-6 (obtained from the Biological Response Modifiers Program, National Cancer Institute, Frederick, MD).

Cell cultures. Pulmonary AM were obtained by lung lavage performed on AB6F1 mice immediately after cervical dislocation. Briefly, the trachea was exposed and intubated, and lungs were lavaged with 6 sequential 1-ml volumes of Ca^{2+}- and Mg^{2+}-free HBSS purchased from Flow Laboratories (McLean, VA) containing 3 mM EDTA (HBSS-EDTA). The cells in the pooled lavage fluids were pelleted by low speed centrifugation, HBSS-EDTA decanted, and the cell pellet suspended in complete medium, which consisted of RPMI 1640 medium (GIBCO, Grand Island, NY) containing 5% (vol:vol) FCS (GIBCO) and 5 μg/ml of gentamicin sulfate (GIBCO). Typically, at least 95% of the cells recovered from the lungs were determined to be macrophages according to staining and morphology on cell smears that were produced by cytocentrifuge (Shandon, Pittsburg, PA) and stained with Diff-Quick (American Scientific Products, McGraw Park, IL). In addition, it was found that, on average, 96% of the recovered alveolar cells internalized antibody-opsonized SRBC. One ml of cell suspension (4×10^5 cells/ml) was placed in 35-mm plastic tissue culture dishes. The cultures were then incubated at 37°C in a 5% CO_2 humidified incubator. After 2 h, the nonadherent cells were removed by aspirating the culture medium, and washing the cultures three times with complete RPMI 1640 medium while the culture dishes were gently agitated by shaking. Following the washing procedure, the adherent cells were immediately used in the experiments.

Primary cultures of MEF[3] were established from 15-to-17-day-old AB6F1 embryos, and propagated as previously described (25). At the second to third passage (1:3 splits), 10^5 MEF in 2 ml of complete RPMI 1640 medium were placed into 35-mm culture dishes. Confluent MEF monolayers (6×10^5 cells) resulted 3 to 4 days later, and were used in experiments at this time.

Statistical analysis of data. Data from different experimental groups were compared using Student's *t*-test.

[3] Abbreviations used in this paper: MEF, murine embryo fibroblasts; AM, alveolar macrophages.

RESULTS

The effect of anti-TNF IgG on the course of a sublethal or lethal Listeria infection. It was shown previously that after i.v. injection of a sublethal immunizing *Listeria* inoculum into mice, TNF appears in infected organs on days 1 to 3 of infection, the time when the greatest numbers of bacteria are present in these organs (6). Convincing evidence that TNF has a protective function in anti-*Listeria* resistance was obtained by showing that a sublethal *Listeria* infection can be converted to a lethal one by giving mice specific anti-TNF IgG at the end of the first day of infection (4, 6). A similar experimental approach was used to determine whether IL-6 production during a sublethal or lethal *Listeria* infection in mice is TNF-dependent. Mice were treated i.p. with rabbit anti-TNF IgG or an equivalent concentration (mg) of control rabbit IgG. Two hours later, groups of these mice were injected i.v. with either a sublethal (4×10^3 CFU) or lethal (4×10^5 CFU) inoculum, and *Listeria* was enumerated in the spleens at progressive times thereafter. It can be seen in Figure 1 that *Listeria* numbers in the spleens of the sublethally infected (0.1 LD_{50}) mice that were treated with control rabbit IgG increased until the third day of infection and declined thereafter. In contrast, *Listeria* numbers in the spleens of anti-TNF IgG-treated mice that received the same *Listeria* inoculum increased exponentially, until the death of the host. Again, anti-TNF treatment also enhanced the growth of bacteria in mice that received a lethal (10 LD_{50}) *Listeria* inoculum.

TNF, IFN-α/β, and IL-6 production during lethal listeriosis. After the plating procedure to determine *Listeria* numbers in the spleens of the different experimental groups of mice at progressive times of infection (Fig. 1), the spleen homogenates and sera from these mice were assayed for TNF, IFN-α/β, and IL-6 activities. The quantities of these cytokines in the spleens and sera of the two experimental groups of mice that received 10 LD_{50} of bacteria (Fig. 1) are presented in Table I. Here, it can be seen that whereas TNF, IFN-α/β, and IL-6 were not detected in the spleens or sera of the different groups of noninfected control mice, these cytokines were present in the spleens of control IgG-treated mice throughout the lethal infection. IFN-α/β and IL-6 were also present in the sera of these mice. Moreover, the concentration of IL-6 in the spleens and sera increased with the progression of morbidity. It was shown in Figure 1 that the passive administration of anti-TNF IgG resulted in increased *Listeria* growth in the spleens of mice given a lethal *Listeria* inoculum. The results presented in Table I also reveal that *Listeria*-induced TNF was not present in the spleens of mice treated with anti-TNF IgG. However, immediately before death, the amounts of IFN-α/β and IL-6 in the spleens and sera of these mice were greater than those of the lethally infected mice given control IgG. These findings establish that *Listeria*-induced production of IFN-α/β and IL-6 during the course of a lethal *Listeria* infection is not dependent on TNF.

IL-6 and IFN-α/β production in anti-TNF IgG-treated mice challenged with a sublethal Listeria inoculum. The concentrations of TNF, IL-6, and IFN-α/β in the spleens and sera of mice during a sublethal *Listeria* infection (Fig. 1) are presented in Table II. The quantities of these cytokines increased and decreased concordantly

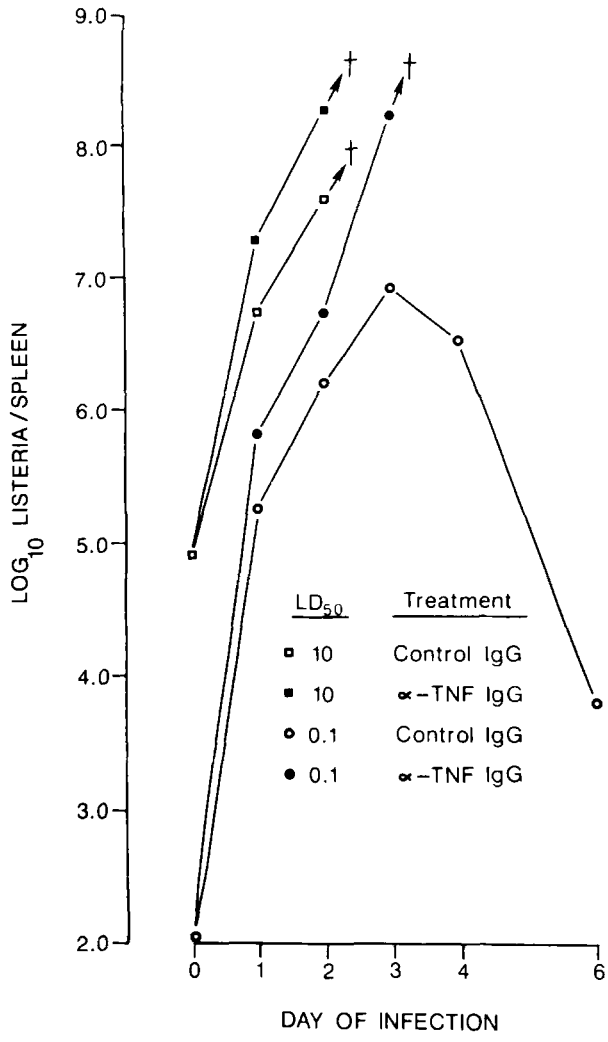

Figure 1. Infection-enhancing effect of rabbit anti-TNF IgG on a sublethal or lethal *Listeria* infection in mice. Mice were injected i.p. with anti-TNF IgG (2×10^4 TNF-neutralizing units; 4.7 mg protein), or an equivalent concentration (mg) of control rabbit IgG. After 2 h, groups of these mice were injected i.v. with *Listeria* at doses of 4×10^3 CFU (0.1 LD_{50}), or 4×10^5 CFU (10 LD_{50}), and thereafter, *Listeria* numbers (CFU) in the spleens were determined at progressive times. Initial implantation of *Listeria* in the spleens was determined 30 min after initiation of infection. *Listeria* CFU/spleen were calculated from results obtained by plating serial 10-fold dilutions of spleen homogenates (3 spleens/group homogenized in 5 ml of PBS containing 10% FCS). The symbol † indicates death.

TABLE I

Effect of anti-TNF IgG treatment on the concentrations of TNF, IFN-α/β, and IL-6 in the sera and spleens of mice during a lethal Listeria infection

Treatment		Day	Cytokine activity[a]					
			Spleen (Total U)			Serum (U/ml)		
−2 h (i.p.)	0 h (i.v.)		TNF	IFN-α/β	IL-6	TNF	IFN-α/β	IL-6
PBS	PBS	1	<53	<53	<13	<32	<32	<8
Control IgG	PBS	1	<53	<53	<13	<32	<32	<8
α-TNF IgG	PBS	1	<53	<53	<13	<32	<32	<8
Control IgG	*Listeria*[b]	1	214	855	3,761	<32	256	1,111
		2	214	214	5,857	<32	128	1,364
α-TNF IgG	*Listeria*[b]	1	<53	1,710	2,692	<32	512	590
		2	<53	428	10,538	<32	128	34,218

[a] Immediately after the plating procedure to enumerate *Listeria* in the spleens (Fig. 1), the spleen homogenates and sera from the respective experimental groups of mice were processed as described in *Materials and Methods*, before the performance of the cytokine assays.
[b] *Listeria*, 4×10^5 CFU.

TABLE II

Effect of anti-TNF IgG treatment on the concentrations of TNF, IFN-α/β, and IL-6 in the sera and spleens of mice infected with a sublethal Listeria inoculum

Treatment		Day	Cytokine activity[a]					
			Spleen (Total U)			Serum (U/ml)		
−2 h (i.p.)	0 h (i.v.)		TNF	IFN-α/β	IL-6	TNF	IFN-α/β	IL-6
PBS	PBS	1	<53	<53	<13	<32	<32	<8
Control IgG	PBS	1	<53	<53	<13	<32	<32	<8
α-TNF IgG	PBS	1	<53	<53	<13	<32	<32	<8
Control IgG	*Listeria*[b]	1	<53	<53	43	<32	<32	15
		2	107	214	1,466	<32	64	206
		3	<53	107	863	<32	<32	118
		4	<53	<53	466	<32	<32	127
		6	<53	<53	55	<32	<32	61
α-TNF IgG	*Listeria*[b]	1	<53	<53	94	<32	<32	14
		2	<53	641	3,564	<32	256	409
		3	<53	428	10,058	<32	256	4,540

[a] As in Table I.
[b] *Listeria*, 4×10^3 CFU.

with *Listeria* numbers in the spleens of control IgG-treated mice (Fig. 1), with the greatest quantities being present in the spleens on day 2 of infection. Interestingly, IL-6 was present in the spleens and sera of these mice by day 1 of infection, and persisted for several days after the disappearance of TNF from the *Listeria*-infected spleens. Moreover, *Listeria*-induced TNF was not present at any time in the serum of control IgG-treated mice, although serum IFN-α/β was detected on day 2 of infection.

As shown in Figure 1, anti-TNF IgG treatment resulted in the conversion of a sublethal *Listeria* infection into a lethal one. The quantities of TNF, IL-6, and IFN-α/β present in the spleens and sera of the anti-TNF IgG-treated mice that were injected with a sublethal *Listeria* inoculum are also presented in Table II. As anticipated, the anti-TNF IgG treatment resulted in no detectable *Listeria*-induced TNF in the spleens of these mice. However, IL-6 and IFN-α/β were present both in the spleens and sera of the anti-TNF IgG-treated mice after the first day of infection. Thereafter, the quantities of IL-6 in spleen and serum increased until death of the host. Moreover, immediately before death, the amounts of IL-6 and IFN-α/β in the spleens of the anti-TNF IgG-treated mice that received a sublethal *Listeria* inoculum (Table II) were similar to those present in the spleens of mice that received a lethal *Listeria* inoculum (Table I).

Listeria-induced TNF, IFN-α/β, and IL-6 production by MEF. *Listeria* enters and proliferates in nonprofessional phagocytic cells both in vivo (6) and in vitro (25–27). The results of earlier studies showed that IFN-α/β was produced in vitro by *Listeria*-infected MEF (25). Therefore, experiments were carried out to determine whether *Listeria*-infected MEF also produce TNF and IL-6. The TNF, IL-6, and IFN-α/β activities (U/ml) present in the culture media from confluent MEF cultures infected with viable *Listeria* are presented in Table III. It can be seen that TNF, IL-6, and IFN-α/β were produced by MEF infected with viable *Listeria* and incubated with a concentration (5 μg/ml) of gentamicin sulfate that is listericidal for extracellular, but not intracellular bacteria (27). In contrast, killed *Listeria* induced MEF to produce only TNF and IL-6. It was also found that untreated MEF produced low amounts of IL-6 during the 24-h incubation period, and that IL-6 production by these cultures was

TABLE III

Listeria-induced TNF-independent and TNF-dependent IL-6 production in MEF cultures

24-h MEF[a] treatment	+24 h culture medium activity[b] (U/ml)		
	TNF	IFN-α/β	IL-6
None	<4	<4	221
rTNF (200 U/ml)	≤4	<4	620
rTNF (200 U/ml) + α-TNF IgG[c]	<4	<4	51
Killed *Listeria*[d]	256	<4	12,749
Killed *Listeria* + α-TNF IgG[c]	<4	<4	5,178[e]
Viable *Listeria*[f]	21	512	66,983
Viable *Listeria* + α-TNF IgG[c]	<4	512	34,275[g]

[a] Confluent MEF cultures (35 mm) incubated with 1 ml of RPMI 1640 medium containing 5% FCS (vol:vol) and gentamicin sulfate (5 μg/ml).

[b] Mean of 3 replicate cultures.

[c] Anti-TNF IgG was added to a final concentration of 4×10^2 TNF neutralizing U/ml of culture medium.

[d] 10^6 UV-irradiated *L. monocytogenes* in 1 ml medium/culture dish.

[e] IL-6 activity significantly less ($p < 0.01$) than in corresponding cultures not treated with α-TNF IgG.

[f] Viable *Listeria monocytogenes* (10^7 CFU) were added to the cultures in 1 ml of antibiotic-free RPMI 1640 medium. After 2 h, the medium was removed, cultures washed three times, and then the cultures were incubated for 24 h with 1 ml of medium containing 5 μg/ml of gentamicin sulfate.

[g] IL-6 activity significantly less ($p < 0.001$) than in corresponding cultures not treated with α-TNF IgG.

TABLE IV

Listeria-induced TNF-independent and TNF-dependent IL-6 production in murine pulmonary AM cultures

24-h AM[a] treatment	+24 h culture medium activity (U/ml)[b]		
	TNF	IFN-α/β	IL-6
None	<4	<4	2
rTNF (200 U/ml)	<4	<4	3
rTNF (200 U/ml) + α-TNF IgG[b]	<4	<4	2
Killed *Listeria*[b]	9	<4	74
Killed *Listeria* + α-TNF IgG[b]	<4	<4	50[c]
Viable *Listeria*[d]	171	<4	278
Viable *Listeria* + α-TNF IgG[b]	<4	<4	80[e]

[a] 35 mm culture dishes containing 4×10^5 AM incubated in 1 ml of RPMI 1640 medium containing 5% FCS (vol:vol) and gentamicin sulfate (5 μg/ml).

[b] As in Table III.

[c] IL-6 activity significantly less ($p < 0.02$) than from corresponding cultures not treated with α-TNF IgG.

[d] As in Table III.

[e] IL-6 activity significantly less ($p < 0.05$) than from corresponding cultures not treated with α-TNF IgG.

enhanced threefold by the addition of rTNF (200 U/ml) to the culture medium. It should be noted that TNF activity was absent from the culture medium of MEF cultures incubated for 24 h with 200 U/ml rTNF. It has been observed that under the culture conditions employed in this experiment, MEF as well as AM cause the disappearance of 256 U of TNF from the culture media (E. A. Havell, unpublished observations). Therefore, the concentrations of *Listeria*-induced TNF found in the culture media at the end of the 24-h incubation period probably represent less than the total amount produced during the 24-h incubation period. The addition of anti-TNF IgG to the culture media of MEF that were infected with viable *Listeria*, or MEF that were incubated with killed *Listeria*, resulted in a twofold decrease in IL-6 production. These findings indicate that *Listeria*-induced IL-6 production in cultures of nonprofessional phagocytic cells occurs through both TNF-independent and TNF-dependent pathways.

Listeria-induced TNF-independent and TNF-dependent IL-6 production by murine pulmonary AM. Macrophages are responsible for the eradication of *Listeria* from host tissues (28), and exposure of these professional phagocytes to bacteria or endotoxin induces them to produce TNF (29–31), IFN-α/β (32–34), and IL-6 (11) (reviewed in Ref. 13). Therefore, to determine whether *Listeria* induces macrophage IL-6 production through TNF-independent and/or TNF-dependent mechanisms, freshly isolated AM were treated under the experimental conditions employed in the above MEF experiments. The results of this experiment are presented in Table IV. They show that viable or killed *Listeria* induced AM to produce TNF and IL-6, but failed to induce IFN-α/β production. Moreover, the amounts of IL-6 produced in response to bacteria were much lower than that produced by identically treated MEF (compare Tables III and IV). The finding that anti-TNF IgG partially reduced the production of *Listeria*-induced IL-6 established that *Listeria*-induced IL-6 production in AM cultures also occurs through TNF-independent and TNF-dependent pathways.

DISCUSSION

IL-6 is produced during an array of pathological conditions. This cytokine has been found in the cerebrospinal fluid of patients with viral (35, 36) or bacterial (11, 37) infections of the central nervous system, as well as in the sera of patients with bacterial sepsis (11) and experimental endotoxemia (10). In addition to inducing the production of IL-6 in vivo, bacteria or bacterial endotoxins elicit the production of TNF and IL-1 (15, 38). Moreover, IL-6 production in vivo can be induced by the i.v. injection of TNF (9) or IL-1 (38). Thus, it appears that evolutionary processes have endowed the host with multiple pathways through which the production of IL-6 can be induced during bacterial infections.

Fong et al. (15) reported that the production of *E. coli*-induced IL-6 in baboons occurred almost entirely through TNF-dependent mechanisms. These investigators found that TNF, IL-1β, and IL-6 appeared in the peripheral circulation of baboons shortly after the intra-aortic infusion of a lethal *E. coli* inoculum (15). Peak concentrations of blood TNF and IL-1β were reached, respectively, 1.5 and 3 h after the injection of bacteria. However, IL-6 was first detected in the circulation at 3 h, and progressively increased in concentration for at least 5 h. It was also found that treating baboons with anti-TNF antibodies 2 h before the injection of the lethal dose of *E. coli* resulted in dramatically reduced levels of IL-1β, and almost undetectable amounts of IL-6. This result indicated that TNF and/or TNF-induced IL-1β induced or amplified the production of IL-6 during *E. coli* infection. Moreover, since it has previously been shown (2) that TNF is involved in the septic shock syndrome manifested by the *E. coli*-infected baboons, Fong et al. (15) raised the possibility that TNF-induced IL-6 might also be involved in the deleterious pathophysiological consequences caused by large amounts of TNF in the peripheral circulation during Gram-negative bacterial sepsis. However, at the present time there is no evidence showing that large amounts of IL-6 per se in the peripheral circulation are deleterious.

In contrast to the results of Fong et al. (15), the results of experiments presented in this paper establish that IL-6 production in mice undergoing either lethal or sublethal listeriosis occurs largely through TNF-independent

mechanisms. That *Listeria*-induced IL-6 production might occur independently of TNF is suggested by our finding (shown in Table III) that the appearance of IL-6 preceded detectable levels of TNF, as well as those of IFN-α/β, in the peripheral circulation and spleens of sublethally infected mice. Conclusive evidence showing that *Listeria*-induced IL-6 production can occur independently of TNF was obtained by measuring the quantities of TNF and IL-6 present in the spleens and peripheral circulation at different times after the initiation of infection in mice treated with anti-TNF IgG. TNF was not detected at any time in these mice after the injection of a lethal or sublethal *Listeria* inoculum. Moreover, anti-TNF IgG treatment resulted in exacerbation of infection and enhanced amounts of IL-6. These two effects caused by the anti-TNF IgG treatment of *Listeria*-infected mice are, then, opposite to those caused by anti-TNF antibody treatment of *E. coli*-infected baboons (2, 15).

The finding that the production of *Listeria*-induced IL-6 can occur independently of TNF in mice treated with anti-TNF IgG did not exclude the possibility that TNF-induced IL-6 production occurs in *Listeria*-infected mice not treated with anti-TNF IgG. Indeed, such a possibility seems likely based on the results of in vitro experiments presented in this paper, which show that viable as well as killed *Listeria* elicited TNF and IL-6 production in cultures of MEF (Table III) and AM (Table IV). The addition of anti-TNF IgG to cultures of these cells abolished *Listeria*-induced TNF activity and caused a reduction in IL-6 titers. In view of these results, and the results of histological studies showing that *Listeria* gains entry into nonprofessional phagocytes (6) as well as professional phagocytes (28) in vivo, it is possible that both TNF-independent and TNF-dependent mechanisms may contribute to *Listeria*-induced IL-6 production in mice not treated with anti-TNF IgG.

During a sublethal immunizing *Listeria* infection, the quantities of IL-6 in the peripheral circulation and spleens of infected mice increase and decrease in concordance with bacterial numbers. IL-6 has been found to mediate a number of effects, which could prove important in host resistance to bacteria. This cytokine has been shown to induce the production of IgG, possibly through its ability to induce the terminal differentiation of B cells into plasma cells (39). IL-6 has also been shown to be involved in T cell activation. Evidence suggests that IL-6 acts in early events which result in the proliferation of T cells (reviewed in Refs. 13 and 40). Such in vivo effects on T cells may be essential in resolving a sublethal *Listeria* infection, because it is known that the eradication of this pathogen is dependent on the acquisition by the host of specifically sensitized T cells (41–43). It is also possible that IL-6 functions in nonspecific antibacterial resistance mechanisms by its ability to trigger the acute phase plasma protein response (reviewed in Ref. 13). The alterations in plasma protein composition during the acute phase response could serve to seal off the site of tissue injury caused by bacteria. For example, increased production of fibrinogen, an acute phase response protein produced by hepatocytes, could result in deposition of fibrin at sites of bacterial infection. The formation of fibrin clots could serve to restrict bacterial dissemination.

It has become apparent that tissue injury results in the production of a number of cytokines that are involved in responses to tissue damage. Cytokines appear to function in an hierarchical order, with the particular order differing for different infections. In order to begin to understand the actions of a cytokine alone, or in combination with others, in the host's response to a pathogen, some basic questions must be addressed: 1) What is the anatomical location of the cytokine? 2) When during infection does it appear? 3) What is the relationship between the amount of cytokine produced and the ensuing course of infection? 4) Does the cytokine contribute to host defense, or can it be deleterious to the host? The present study continues our efforts at addressing these questions in the context of a Gram-positive bacterial infection.

Acknowledgments. The authors would like to express their appreciation to Dr. Uma Santhanam for her helpful discussion; to Lynn Stiles, Joyce Reome, and Huguette Viguet for their excellent technical assistance; and to Mary Durett for typing this manuscript.

REFERENCES

1. **Beutler, B., I. W. Milsark, and A. Cerami.** 1985. Passive immunization against cachectin/tumor necrosis factor protects mice from the lethal effect of endotoxin. *Science 229:869.*
2. **Tracey, K. J., Y. Fong, D. G. Hesse, K. R. Manogue, A. T. Lee, G. C. Kuo, S. F. Lowry, and A. Cerami.** 1987. Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. *Nature 330:662.*
3. **Mathison, J. C., E. Wolfson, and R. J. Ulevitch.** 1988. Participation of tumor necrosis factor in the mediation of Gram negative bacterial lipopolysaccharide-induced injury in rabbits. *J. Clin. Invest. 81:1925.*
4. **Havell, E. A.** 1987. Production of tumor necrosis factor during murine listeriosis. *J. Immunol. 139:4225.*
5. **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.*
6. **Havell, E. A.** 1989. Evidence that tumor necrosis factor has an important role in antibacterial resistance. *J. Immunol. 143:2894.*
7. **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.*
8. **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.*
9. **Jablons, D. M., J. J. Mule, J. K. McIntosh, P. B. Sehgal, L. T. May, C. M. Huang, S. A. Rosenberg, and M. T. Lotze.** Interleukin-6/interferon-β_2 as a circulating hormone: induction by cytokine administration in man. *J. Immunol. 142:1542.*
10. **Fong, Y., L. L. Moldawer, M. Marano, H. Weir, S. B. Tatter, R. M. Clarick, U. Santhanam, D. Sherris, L. T. May, P. B. Sehgal, and S. F. Lowry.** 1989. Endotoxemia elicits increased circulating β_2-IFN/IL-6 in man. *J. Immunol. 142:2321.*
11. **Helfgott, D. C., S. B. Tatter, U. Santhanam, R. H. Clarick, N. Bhardwaj, L. T. May, and P. B. Sehgal.** 1989. Multiple forms of IFN-β_2/IL-6 in serum and body fluids during acute bacterial infection. *J. Immunol. 142:948.*
12. **Helle, M., J. P. J. Brakenhoff, E. R. DeGroot, and L. A. Aarden.** 1988. Interleukin 6 is involved in interleukin 1-induced activities. *Eur. J. Immunol. 18:957.*
13. **Sehgal, P. B., G. Grieninger, and G. Tosato.** 1989. Regulation of the acute phase and immune responses: interleukin-6. *Ann. NY Acad. Sci. 557:1.*
14. **Sehgal, P. B.** 1990. Interleukin-6: a regulator of plasma protein gene expression in hepatic and non-hepatic tissues. *Mol. Biol. Med. 7:117.*
15. **Fong, Y., K. J. Tracey, L. L. Moldawer, D. G. Hesse, K. B. Manogue, J. S. Kenney, A. T. Lee, G. C. Kuo, A. C. Allison, S. F. Lowry, and A. Cerami.** 1989. Antibodies to cachectin/tumor necrosis factor reduce interleukin 1β and interleukin 6 appearance during lethal bacteremia. *J. Exp. Med. 170:1627.*
16. **Newborg, M. F., and R. J. North.** 1980. On the mechanism of T cell-independent anti-*Listeria* resistance in nude mice. *J. Immunol. 124:571.*
17. **Havell, E. A.** 1986. Augmented induction of interferons during *Listeria monocytogenes* infection. *J. Infect. Dis. 253:960.*
18. **Fransen, L., R. Muller, A. Marmenout, J. Tavernier, J. Van der Heyden, E. Kawashima, A. Chollet, R. Tizard, H. Van Heuverswyn,**

A. Van Uhet, M. R. Ruysscharet, and W. Fiers. 1985. Molecular cloning of mouse tumor necrosis factor cDNA and its eukaryotic expression. *Nucleic Acids Res. 13:4417.*

19. **Tavernier, J., L. Fransen, A. Marmenout, J. Van der Heyden, R. Mueller, M. R. Ruysscharet, A. Van Vliet, R. Bauvdem, and W. Fiers.** 1987. Isolation and expression of the genes coding for mouse and human tumor necrosis factor (TNF) and biological properties of recombinant TNF. In *Lymphokines*, Vol. 13. D. R. Webb, and D. V. Goeddel, eds. Academic Press, New York, pp. 181–198.
20. **Havell, E. A., W. Fiers, and R. J. North.** 1988. The antitumor function of tumor necrosis factor (TNF). I. Therapeutic action of TNF against an established murine sarcoma is indirect, immunologically dependent, and limited by severe toxicity. *J. Exp. Med. 167:1067.*
21. **Brakenhoff, J. B. J., E. R. DeGroot, R. F. Evers, H. Pannekock, and L. A. Aarden.** 1987. Molecular cloning and expression of hybridoma growth factor in *E. coli*. *J. Immunol. 139:4116.*
22. **Mosmann, T.** 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods 65:55.*
23. **Vink, A., P. G. Coulie, P. Wauters, R. P. Nordan, and J. Van Snick.** 1988. B cell growth and differentiation activity of interleukin-HP1 and related murine plasmacytoma growth factors: synergy with interleukin-1. *Eur. J. Immunol. 18:607.*
24. **May, L. T., J. Ghrayeb, U. Santhanam, S. B. Tatter, A. Sthoeger, D. C. Helfgott, N. Chiorazzi, G. Grieninger, and P. B. Sehgal.** 1988. Synthesis and secretion of multiple forms of β_2-interferon/B cell differentiation factor-2-hepatocyte stimulating factor by human fibroblasts and monocytes. *J. Biol. Chem. 263:7760.*
25. **Havell, E. A.** 1986. Synthesis and secretion of interferon by murine fibroblasts in response to intracellular *Listeria monocytogenes*. *Infect. Immun. 54:787.*
26. **Gaillard, J.-L., P. Berche, J. Mounier, S. Richard, and P. Sansonetti.** 1987. In vitro model of penetration and intracellular growth of *Listeria monocytogenes* in the human enterocyte-like cell line Caco-2. *Infect. Immun. 55:2822.*
27. **Portnoy, D. A., P. S. Jacks, and D. J. Hinricks.** 1988. Role of hemolysin for the intracellular growth of *Listeria monocytogenes*. *J. Exp. Med. 167:1459.*
28. **North, R. J.** 1970. The relative importance of blood monocytes and fixed macrophages to the expression of cell-mediated immunity to infection. *J. Exp. Med. 132:521.*
29. **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.*
30. **Mannel, D. N., R. N. Moore, and S. E. Mergenhagen.** 1980. Macrophages as a source of tumoricidal activity. *Infect. Immun. 30:523.*
31. **Bancroft, G. J., K. C. Sheehan, R. D. Schreiber, and E. R. Unanue.** 1989. Tumor necrosis factor is involved in the T cell-independent pathway of macrophage activation in *scid* mice. *J. Immunol. 143:129.*
32. **Borecky, L., V. Lackovic, N. Fuchsberger, and V. Hajnicka.** 1974. Stimulation of interferon production in macrophages. In *Activation of Macrophages*. W. H. Wagner, and H. Hahn, eds. American Elsevier Publishing Co., New York, p. 111.
33. **Havell, E. A., and G. L. Spitalny.** 1980. The induction and characterization of interferon from pure cultures of murine macrophages. *Ann. NY Acad. Sci. 350:413.*
34. **Havell, E. A., and G. L. Spitalny.** 1983. Endotoxin-induced interferon synthesis in macrophage cultures. *J. Reticuloendothel. Soc. 33:191.*
35. **Houssiau, F. H., K. Bukasa, C. Sindic, J. M. Van Damme, and J. Van Snick.** 1988. Elevated levels of the 26K human hybridoma growth factor (interleukin 6) in cerebrospinal fluid of patients with acute infection of the central nervous system. *Clin. Exp. Immunol. 71:320.*
36. **Frei, K., T. P. Lirst, A. Meager, P. Gallo, D. Leppert, R. M. Zinkernagel and A. Fontana.** 1988. Production of B cell stimulatory factor and interferon γ in the central nervous system during viral meningitis and encephalitis: evaluation in a murine model infection and in patients. *J. Exp. Med. 168:449.*
37. **Waage, A., P. Brandtzaeg, A. Halstensen, P. Kierulf, and T. Espevik.** 1989. The complex pattern of cytokines in serum from patients with meningococcal septic shock. *J. Exp. Med. 169:333.*
38. **Shalaby, M. R., A. Waage, L. Aarden, and T. Espevik.** 1989. Endotoxin, tumor necrosis factor-α and interleukin 1 induce interleukin 6 production in vivo. *Clin. Immunol. Immunopathol. 53:488.*
39. **Hirano, T., K. Yasukawa, H. Harada, T. Taga, Y. Watanabe, T. Matsuda, S.-I. Kashiwamura, K. Nakajima, K. Koyamia, A. Iwamatsu, S. Taunasawa, F. Sakiyama, H. Matsui, Y. Takahara, T. Taniguchi, and T. Kishimoto.** 1986. Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. *Nature 324:73.*
40. **Van Snick, J.** 1990. Interleukin-6: an overview. *Annu. Rev. Immunol. 8:253.*
41. **Lane, F. C., and E. R. Unanue.** 1972. Requirement of thymus (T) lymphocytes for resistance to listeriosis. *J. Exp. Med. 135:1104.*
42. **Blanden, R. V., and D. J. Hinrichs.** 1972. Cell-mediated immunity to bacterial infection in the mouse: thymus-derived cells as effectors of acquired resistance to *Listeria monocytogenes*. *Scand. J. Immunol. 1:379.*
43. **North, R. J.** 1973. Importance of thymus-derived lymphocytes in cell-mediated immunity to infection. *Cell. Immunol. 7:166.*