Immunology 1995 **86** 256–262

Tumour necrosis factor, but not interferon-γ, is essential for acquired resistance to *Listeria monocytogenes* during a secondary infection in mice

J. N. SAMSOM,* J. A. M. LANGERMANS,* H. F. J. SAVELKOUL† & R. VAN FURTH* *Department of Infectious Diseases, University Hospital Leiden and †Department of Immunology, Erasmus University, Rotterdam, the Netherlands

SUMMARY

Mice with a secondary *Listeria monocytogenes* infection eliminate the bacteria much faster and more efficiently from their organs than mice with a primary infection. During the course of a secondary infection, serum concentrations of interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF) are higher than during a primary infection. The aim of the present study was to determine whether these cytokines are involved in the acquired resistance to *L. monocytogenes* during a secondary infection in mice. In order to neutralize cytokines, alginate-encapsulated cells, which form anti-cytokine monoclonal antibodies, were injected into the nuchal region of mice during a *Listeria* infection. Mice recovered from a sublethal primary *Listeria* infection, which acquired cell-mediated immunity, received a subcutaneous injection of anti-IFN-γ-forming cells, or anti-TNF-forming cells, and 4 days later received an intravenous injection with 10 50% lethal dose (LD_{50}) *L. monocytogenes*. The number of bacteria recovered from the liver and spleen of immune mice treated with anti-IFN-γ-forming cells was slightly larger (~1 $\log_{10}$) than that found for immune mice treated with anti-β-galactosidase-forming cells, called immune control mice. The organs of immune mice treated with anti-TNF-forming cells yielded significantly more (~4 $\log_{10}$) bacteria than those of immune control mice, more than those of immune mice treated with anti-IFN-γ-forming cells, and comparable numbers to those of non-immune mice. Taken together, these results demonstrate that TNF is essential in acquired resistance to *L. monocytogenes* during a secondary infection in mice, while IFN-γ plays a minor role.

INTRODUCTION

Listeria monocytogenes is a facultative intracellular pathogen that is often used as a model to study cellular immunity in mice. During a primary infection with *L. monocytogenes* in mice, the bacteria proliferate in the liver and spleen during the first 3 days and are eliminated from these organs during the next 7 days,[1,2] mainly due to macrophage activation occurring during the development of cell-mediated immunity.[3] Macrophages are activated by T-lymphocyte derived cytokines, interferon-γ (IFN-γ) being one of the most important.[4,5] The effect of IFN-γ is primarily mediated by endogenous tumour necrosis factor (TNF) production by macrophages.[6] Both of these cytokines are needed to overcome a primary infection with *L. monocytogenes*.[4,7]

Mice that survive a primary infection, i.e. immune mice, possess acquired resistance to *L. monocytogenes*.[1,3] This implies that during a *Listeria* infection in immune mice bacterial proliferation in the organs occurs only during the first 24 hr, and elimination of *Listeria* takes place during the next 4 days.[1] During the course of such a secondary infection, the concentrations of IFN-γ and TNF in serum are higher than during a primary infection,[5] but the role of these cytokines in the enhanced elimination of bacteria in immune mice has not yet been elucidated.

The role of cytokines during an infection in mice can be investigated by neutralization with cytokine-specific antibodies. Recently, a method was developed using encapsulated monoclonal antibody (mAb)-forming cells, which were injected subcutaneously (s.c.) into the nuchal region of the mouse.[8] This approach has the advantage that antibody titres remain high over a prolonged period, as the encapsulated cells continue to secrete antibodies, remain viable and are not rejected.

The aim of the present study was to assess the role of IFN-γ and TNF in the acquired resistance to *L. monocytogenes* during a secondary infection in mice.

MATERIALS AND METHODS

Mice

Female, specific pathogen-free CBA/J mice, aged 6 to 8 weeks, were purchased from IFFA Credo (Saint Germaine-sur-l'Abersle, France) and given dry food (Hope Farms, Woerden, the Netherlands) and water *ad libitum*.

Received 19 January 1995; revised 27 April 1995; accepted 8 June 1995.

Correspondence: J. A. M. Langermans, University Hospital, Department of Infectious Diseases, Building 1, C5-P, PO Box 9600, 2300 RC Leiden, the Netherlands.

Bacteria

Listeria monocytogenes strain EGD was kept virulent by repeated passage through CBA/J mice and stored on blood agar plates at 4°. The bacteria were cultured in tryptose phosphate broth for 18 hr at 37°, collected by centrifugation (10 min, 900 *g*), washed in phosphate-buffered saline, pH 7·4 (PBS), and resuspended in pyrogen-free saline.

Antibody-forming cells

Hybridoma cell lines forming either rat anti-mouse IFN-γ mAb (XMG1.2; IgG1)[9] or rat anti-mouse TNF mAb (XT22; IgG1)[10] were a gift from DNAX Research Institute (Palo Alto, CA). A rat anti-β-galactosidase (anti-β-gal) mAb-producing hybridoma cell line (GL113; DNAX)[11] was used as an IgG1 isotype control. The cells were cultured in HEPES-buffered RPMI-1640 (Flow Laboratories, Irvine, UK) supplemented with 10% heat-inactivated fetal calf serum (Gibco Laboratories, Grand Island, NY), 2 mM L-glutamine (Flow Laboratories), 50 μg/ml streptomycin (Biochemie GmbH Vienna, Austria), 1000 U/ml natrium penicillin-G (Gist Brocades, Leiderdorp, the Netherlands) and 5×10^{-5} M β-mercaptoethanol (Sigma, St Louis, MO). The cells were collected by centrifugation at 300 *g* for 8 min at 4°.

Encapsulation of cells and injection into mice

Cells were encapsulated as described elsewhere.[8] In short, about 1×10^7 antibody-forming cells were washed with cold pyrogen-free saline and a suspension containing 3×10^6 cells/ml was mixed with alginate entrapment medium (FMC Bioproducts-Europe, Vallensbaek Strand, Denmark) at a volume ratio of 1:2. The solution of cells and alginate was transferred to a polypropylene syringe with a 21 g needle, which was squirted into a dish containing 80 mM $CaCl_2$. The resulting capsules, which entrap the antibody-forming cells, were washed with pyrogen-free saline. Endotoxin levels of the solution of alginate-encapsulated mAb-forming cells (antibody-FC) were below 10 pg/ml, as determined with a Limulus assay. It has been shown that this procedure enables *in vivo* neutralization of cytokine activity analogous to injection of purified mAb.[8]

Mice were injected s.c. with 1 ml of a suspension of antibody-FC in the nuchal region 4 days before the *L. monocytogenes* infection. Each mouse received 1×10^6 or 2×10^6 antibody-FC, depending on the kind of hybridoma cell line and the type of experiment.

Induction of primary or secondary L. monocytogenes *infection in mice*

A primary *L. monocytogenes* infection was induced with an intravenous (i.v.) injection of 5×10^2 *L. monocytogenes* [(0·1 50% lethal dose LD_{50})].[12] A secondary infection was induced in mice that had survived the primary infection with *L. monocytogenes* by injecting 10 LD_{50} *L. monocytogenes* i.v. 3 weeks later. On various days of the primary or secondary infection, the spleen and liver were removed and homogenized with a tissue homogenizer (type X-1020; Ystral GmbH, Döttingen, Germany). Serial 10-fold dilutions of the organ suspensions were plated onto blood agar plates and incubated for 24 hr at 37°; the number of colonies was used to calculate the number of viable *L. monocytogenes* per organ.

Enzyme-linked immunosorbent assay (ELISA) for determination of rat IgG in mouse serum

From each mouse a blood sample was taken by heart puncture, collected in a heparinized tube and stored as plasma at −20° until use. The concentration of rat anti-mouse IgG present in plasma was assessed with a sandwich ELISA. The wells of microtitre plates (Nunc A/S, Roskilde, Denmark) were coated with 100 μl of a solution containing 1 μg/ml mouse anti-rat IgG mAb (Pharmingen, San Diego, CA), and kept overnight at 4°. The remaining aspecific binding sites were then blocked by incubation for 60 min at room temperature in phosphate-buffered saline (PBS) containing 0·05% Tween-20 and 1% heat-inactivated newborn calf serum (Gibco), hereafter referred to as blocking buffer. Next, 100 μl of either a plasma sample diluted with blocking buffer or a serial dilution of purified rat IgG1 κ immunoglobulin (Pharmingen) with blocking buffer was added to the wells and incubated for 3 hr at room temperature. After multiple washes with PBS containing 0·05% Tween-20 (PBS–Tween), 100 μl of biotin-conjugated mouse anti-rat κ mAb (Pharmingen) at a concentration of 0·5 μg/ml blocking buffer was added to the wells and incubated for 60 min at room temperature. Next, the wells were washed with PBS–Tween and 100 μl of peroxidase-conjugated streptavidine (Jackson Immunoresearch Laboratories Inc., Westgrove, PA), at a concentration of 0·25 μg/ml blocking buffer, was added to the wells and incubated for 60 min at room temperature. As substrate, 3,3′,5,5′ tetramethylbenzidine (Sigma) was added; after incubation for 15 min at room temperature the optical density (OD) at 450 nm was measured with a Titertek Multiskan (Eflab Oy, Helsinki, Finland).

Inhibition of Toxoplasma gondii *proliferation*

On various days of a primary or secondary *L. monocytogenes* infection in mice, peritoneal macrophages were collected by peritoneal lavage with 2 ml ice-cold saline containing 50 U/ml heparin,[13] and cultured at a concentration of 1×10^6 macrophages/ml. The state of activation of the peritoneal macrophages was evaluated with a *T. gondii* proliferation assay, as described earlier.[14] The increase of the number of *T. gondii* was calculated as the ratio of the number of *T. gondii* per 100 infected macrophages after 18 hr of incubation to the number of *T. gondii* per 100 infected macrophages at the start of the assay. The results are expressed as the percentage fold increase of *T. gondii* in peritoneal macrophages from infected mice relative to the fold increase in macrophages from naive control mice.

Statistical analysis

Results are expressed as the mean ± SEM for at least eight mice per time-point, unless otherwise indicated. Analysis of experiments with two groups was performed with two-sample Student's *t*-tests. Differences between various groups at one time-point were assessed using a one-way ANOVA with a Fisher's least-square differences (LSD) comparison report. The course of infection of *L. monocytogenes* in the different groups was compared by two-way ANOVA. For all analyses the level of significance was set at 0·05. When a mouse died during infection, the number of *L. monocytogenes* could not be determined; therefore the number of bacteria was set at 9·72 $\log_{10}$ for the liver and 8·25 $\log_{10}$ for the spleen, on the basis of the mean of the maximum numbers of bacteria determined in the respective organs of live animals.

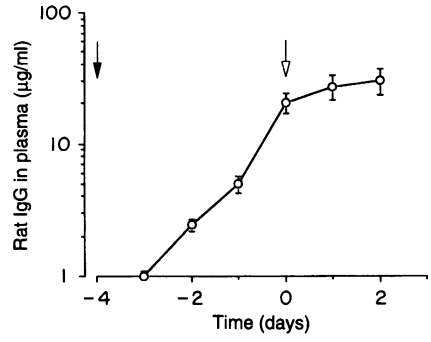

Figure 1. Course of rat IgG concentration in plasma of immune mice treated with 1×10^6 anti-TNF-FC during a secondary *L. monocytogenes* infection. Black arrow indicates injection of anti-TNF-FC, white arrow indicates injection with 5×10^4 *L. monocytogenes*. Results of a representative experiment are expressed as mean serum concentrations ± SD ($n = 5$).

RESULTS

Concentrations of rat anti-cytokine IgG in plasma of mice

After s.c. injection of antibody-FC, the concentration of rat IgG in the plasma of mice increased gradually during the first 4 days, and then remained at an almost constant high level (Fig. 1). The level of rat IgG varied between experiments but was always higher than the threshold, allowing effective neutralization of the cytokine.[15]

Effect of anti-IFN-γ-FC and anti-TNF-FC on a primary *L. monocytogenes* infection in mice

Treatment of mice with either 1×10^6 anti-IFN-γ-FC (Fig. 2a) or 1×10^6 anti-TNF-FC (Fig. 2b) during a primary infection with 5×10^2 *L. monocytogenes* resulted in a significant increase in the number of bacteria in the liver and spleen on day 4 of infection relative to that in the organs of mice treated with 2×10^6 anti-β-gal-FC. Combined treatment with 1×10^6 anti-IFN-γ-FC and 1×10^6 anti-TNF-FC resulted in an increase in the number of *L. monocytogenes* in the organs of the mice that was comparable to that in the organs of mice treated with anti-TNF-FC alone (Fig. 2b). Two out of four anti-TNF-FC treated mice and one out of four mice receiving the combination of anti-IFN-γ-FC and anti-TNF-FC died before day 4 of infection, while no deaths occurred in the anti-IFN-γ-FC-treated group. The bacterial counts for dead animals were set at the maximum value, as described in the Materials and Methods. Mean rat IgG concentrations in the plasma, on day 4 of infection, of anti-IFN-γ-FC, anti-TNF-FC, anti-IFN-γ plus anti-TNF-FC, or anti-β-gal-FC treated mice were 40 μg/ml, 82 μg/ml, 53 μg/ml and 55 μg/ml, respectively. Neither alginate-entrapment medium nor anti-β-gal-FC had an effect on the proliferation of *L. monocytogenes* in the liver and spleen of mice in comparison with non-treated infected mice (data not shown).

Effect of anti-IFN-γ-FC and anti-TNF-FC on the course of a *L. monocytogenes* infection in immune mice

Immune mice that had recovered from a primary *L. monocytogenes* infection first received a s.c. injection of 2×10^6 anti-IFN-γ-FC, 1×10^6 anti-TNF-FC or a combination of 2×10^6 anti-IFN-γ-FC and 1×10^6 anti-TNF-FC, and then 10 LD_{50} *L. monocytogenes* 4 days later. Immune mice treated with 2×10^6 anti-β-gal-FC and infected with 10 LD_{50} *L. monocytogenes* served as immune control mice, while naive mice with a primary infection following an injection of 10 LD_{50} *L. monocytogenes* served as non-immune control mice. Mean rat IgG concentrations in the plasma of immune mice treated with antibody-FC, killed at 24, 48 or 72 hr of infection, varied between 64 and 134 μg/ml (Fig. 3).

Liver

At all times the number of *L. monocytogenes* recovered from the liver of anti-IFN-γ-FC treated immune mice was larger

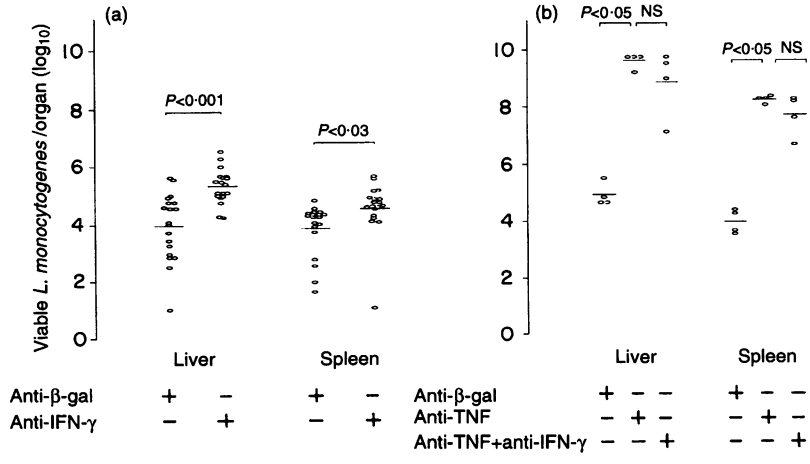

Figure 2. Proliferation of *L. monocytogenes* in liver and spleen of mice treated with anti-cytokine-forming cells during a primary infection. Mice received a s.c. injection of 1×10^6 anti-IFN-γ-FC (a), 1×10^6 anti-TNF-FC (b), or 2×10^6 anti-β-gal-FC as a control. Four days later these mice were infected with 5×10^2 *L. monocytogenes*. The number of bacteria in the liver and spleen was determined on day 4 of infection. Dots represent values of individual mice from five (a) and one (b) experiments; horizontal bars represent mean values. NS, a non-significant difference.

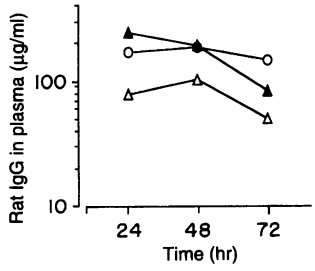

Figure 3. Course of rat IgG concentrations in plasma of immune mice treated with either 2×10^6 anti-IFN-γ-FC (△), 1×10^6 anti-TNF-FC (▲) or 2×10^6 anti-β-gal-FC (○) during secondary *L. monocytogenes* infection. Results are expressed as mean serum concentrations ($n = 8$).

($P < 0{\cdot}05$) than that recovered from immune control mice and smaller ($P < 0{\cdot}05$) than that recovered from non-immune control mice (Fig. 4a). However, the course of infection in the liver of anti-IFN-γ-FC treated immune mice was not different ($P = 0{\cdot}26$) from that in the liver of immune control mice.

The number of *L. monocytogenes* recovered from the liver of anti-TNF-FC treated immune mice was larger ($P < 0{\cdot}05$) than that recovered from both immune control mice and non-immune control mice. In the liver of anti-TNF-FC treated immune mice, the course of infection was significantly different ($P < 0{\cdot}001$) from that in immune control mice, and the same ($P = 0{\cdot}31$) as that in non-immune control mice (Fig. 4b).

The number of bacteria in the liver of immune mice treated with a combination of anti-IFN-γ-FC and anti-TNF-FC did not differ significantly from that in immune mice treated with anti-TNF-FC alone and differed to the same extent from immune and non-immune control mice as anti-TNF-FC treated mice (Fig. 4c).

Spleen

The number of *L. monocytogenes* recovered from the spleen of anti-IFN-γ-FC treated immune mice during the first 48 hr of infection was not significantly larger ($P > 0{\cdot}05$) than that recovered from immune control mice. Only at 72 hr was the number of *L. monocytogenes* recovered from the spleen of anti-IFN-γ-FC treated immune mice significantly larger ($P < 0{\cdot}05$) than that recovered from the spleen of immune control mice (Fig. 4d). The course of infection in the spleen of anti-IFN-γ-FC treated immune mice was significantly different from that in the spleen of immune control mice ($P = 0{\cdot}035$), as well as non-immune control mice ($P < 0{\cdot}001$) (Fig. 4d).

The number of bacteria recovered from the spleen of anti-TNF-FC treated immune mice during the first 48 hr of infection was larger than that recovered from the spleen of immune control mice ($P < 0{\cdot}05$) and comparable ($P > 0{\cdot}05$) to that found for non-immune control mice (Fig. 4e). At 72 hr the number of *L. monocytogenes* recovered from the spleen of anti-TNF-FC treated mice was larger than that recovered from the spleen of immune control mice but tended to be smaller ($P > 0{\cdot}05$) than that found for non-immune control mice (Fig. 4e). The course of infection in the spleen of anti-TNF-FC treated immune mice was different ($P < 0{\cdot}001$) from that seen in immune control mice but similar ($P = 0{\cdot}34$) to that in non-immune control mice (Fig. 4e).

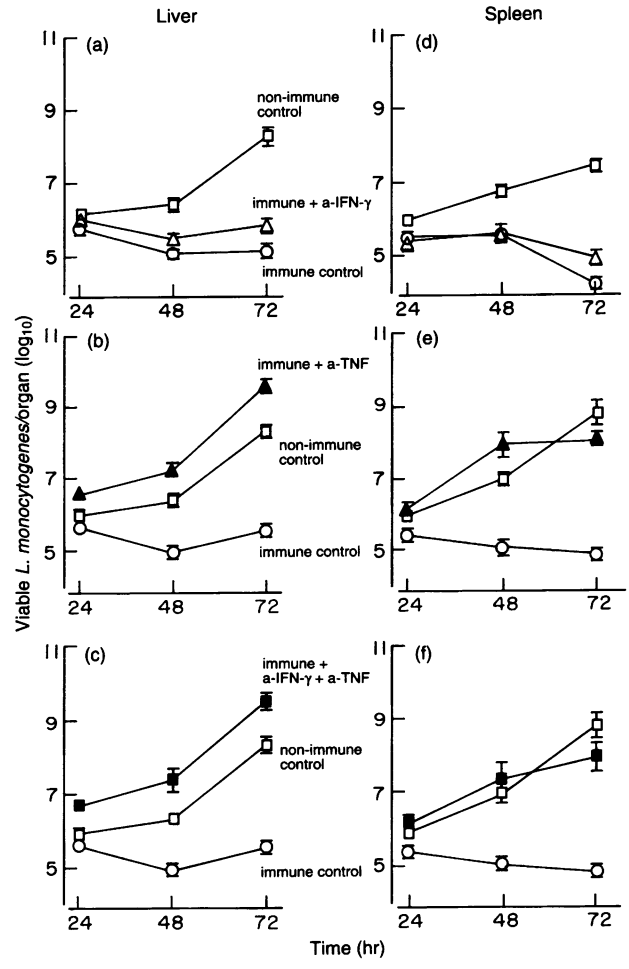

Figure 4. Course of *L. monocytogenes* infection in liver (left panels) and spleen (right panels) of immune mice treated with anti-cytokine-forming cells during a secondary infection. Mice were immunized with 5×10^2 *L. monocytogenes*; 3 weeks later they received a challenge inoculum of 5×10^4 *L. monocytogenes*. Non-immune control mice were naive mice that received an inoculum of 5×10^4 *L. monocytogenes* (□). Prior to the second infection immunized groups received 2×10^6 anti-IFN-γ-FC (△), 1×10^6 anti-TNF-FC (▲) or 1×10^6 anti-TNF-FC + 2×10^6 anti-IFN-γ-FC (■). The immunized group that received 2×10^6 anti-β-gal-FC served as immune control mice (○). The number of bacteria in the liver and spleen was determined at 24, 48 and 72 hr of infection. Values are the mean ± SEM for eight mice. At 72 hr of infection, two out of eight anti-TNF-FC treated mice had died.

The number of bacteria in the spleen of immune mice treated with a combination of anti-IFN-γ-FC and anti-TNF-FC did not differ significantly from that in immune mice treated with anti-TNF-FC alone, and differed to the same extent from immune and non-immune control mice as anti-TNF-FC treated immune mice (Fig. 4f).

Inhibition of *T. gondii* proliferation by peritoneal macrophages derived from mice with a primary or secondary *L. monocytogenes* infection

As earlier studies had shown that during a primary *Listeria* infection peritoneal macrophages are activated[1,16] their ability to inhibit *T. gondii* proliferation was assessed to check the state

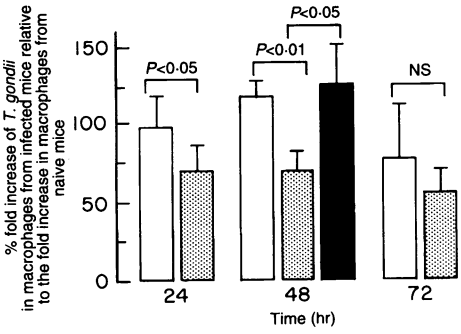

Figure 5. Inhibition of *T. gondii* proliferation by peritoneal macrophages from mice with a primary or secondary *L. monocytogenes* infection. Mice were given a primary infection with 5×10^4 *L. monocytogenes* (□), or were immunized with 5×10^2 *L. monocytogenes* and received a challenge inoculum of 5×10^4 *L. monocytogenes* 3 weeks later (▨). Some immune mice received 1×10^6 anti-TNF-FC 4 days prior to the second infection (■). On various days of primary or secondary infection, peritoneal macrophages were collected and their toxoplasmastatic activity assessed. Values are means ± SD ($n = 5$).

of activation of the mice under study. To standardize the results, proliferation of *T. gondii* in macrophages from infected mice was expressed relative to the proliferation in macrophages from naive mice. Peritoneal macrophages from mice with a primary *Listeria* infection did not inhibit *T. gondii* proliferation at 24 and 48 hr, but at 72 hr of infection *T. gondii* proliferation was decreased by 23% (Fig. 5). Peritoneal macrophages from mice with a secondary *Listeria* infection inhibited *T. gondii* proliferation by 30% at 24 and 48 hr, and by 44% at 72 hr of *Listeria* infection (Fig. 5). Treatment of mice with anti-TNF-FC during a secondary infection abrogated macrophage activation with respect to the inhibition of *T. gondii* proliferation at 48 hr (Fig. 5).

DISCUSSION

Although during a primary infection both IFN-γ and TNF are essential for the elimination of *L. monocytogenes* from the liver and spleen, this study is the first to demonstrate that during a secondary infection TNF is the major cytokine involved in enhanced resistance to *Listeria* in mice. This conclusion is based on the observation that neutralization of TNF during a secondary *L. monocytogenes* infection completely abrogated acquired immunity, and agrees with an earlier finding that during secondary infection acquired immunity correlated with an elevated TNF serum concentration in mice.[5] The mechanism by which TNF induces enhanced resistance during a secondary infection is unknown. Possibly, TNF induces recruitment of granulocytes and monocytes to the liver and spleen during a secondary infection, as has been shown for migration of these cells to the peritoneal cavity in a *L. monocytogenes* peritonitis model.[17] This cellular influx may lead to granuloma formation, which is also dependent on TNF in secondary *L. monocytogenes* infections[18] and infections with *Mycobacterium bovis*.[19] Once granulomas have been formed, efficient limitation of proliferation and eventual elimination of the pathogen by activated phagocytic cells can occur.[20] Another mechanism by which TNF possibly induces enhanced resistance is activation of resident macrophages in an early phase of secondary infection, before granuloma formation. In the present study inhibition of *T. gondii* proliferation by peritoneal macrophages was used as evidence for macrophage activation during infection. The results indicated that during a primary *Listeria* infection macrophage activation occurred at 72 hr of infection, whereas during a secondary *L. monocytogenes* infection macrophage activation began within 24 hr of infection and continued for at least 3 days. Neutralization of TNF during secondary infection abrogated macrophage activation entirely.

Another indication that TNF-mediated resistance begins at an early phase of a secondary infection is that neutralization of TNF during a secondary infection in mice had already resulted in a significant increase in the number of *L. monocytogenes* in the liver and spleen at 24 hr of infection. This is in agreement with reports demonstrating that TNF appears in the circulation within 1 hr of a secondary infection with *L. monocytogenes* and reaches a maximum at 2 hr.[5]

During a primary *L. monocytogenes* infection in mice, IFN-γ activates macrophages.[4] Although there is controversy about the enhanced listericidal capacity of IFN-γ-activated macrophages *in vitro*,[21,22] treatment with IFN-γ has been shown to decrease proliferation of *L. monocytogenes* in mice during a primary infection[14,23] depending on the number of infecting bacteria.[14] Treatment with anti-IFN-γ increases proliferation of *Listeria* during primary infection in mice, as demonstrated previously[5,4] and confirmed by the present study. It has been demonstrated that during a secondary *L. monocytogenes* infection acquired resistance is accompanied by an enhanced IFN-γ concentration in the serum, which reaches a maximum 6 hr after bacterial challenge.[5] This suggests that IFN-γ is involved in the elimination of bacteria during a secondary *Listeria* infection. However, the present study demonstrates that IFN-γ plays only a minor role in acquired resistance to *L. monocytogenes* during secondary infection, as neutralization of IFN-γ enhanced bacterial proliferation only slightly. This finding agrees with results indicating that $CD8^+$ T lymphocytes can protect mice against *L. monocytogenes* in an IFN-γ-independent fashion,[24] in contrast to $CD4^+$ T lymphocytes which need IFN-γ to mediate acquired resistance.[23,25]

In the present study the course of *L. monocytogenes* infection in the liver of immune mice was different from that in the spleen. This may be explained by a difference in the cell type involved in acquired immunity in these organs. In the liver of immune mice both $CD4^+$ and $CD8^+$ T lymphocytes can mediate acquired immunity,[26] and hepatocytes from immune mice are able to eliminate *Listeria* rapidly during a secondary infection.[27] In the spleen of immune mice mainly $CD8^+$ T lymphocytes are involved in acquired immunity.[19,28]

In our experiments, simultaneous neutralization of IFN-γ and TNF in immune mice did not result in enhanced *L. monocytogenes* proliferation compared to TNF neutralization alone. Others have reported that combined injection of purified polyclonal anti-TNF antibody and anti-IFN-γ mAb during a secondary *Listeria* infection resulted in a larger increase in proliferation than injection of anti-TNF alone.[5] In these studies, injection of purified anti-TNF was not lethal to any of the immune mice that received 25 LD_{50} *L. monocytogenes*, whereas in our experiments treatment of immune mice with anti-TNF-FC during infection with only 10 LD_{50} was already lethal for 25% of the mice at day 3 of infection. This

discrepancy might be explained by a difference in susceptibility of the mouse strain used or a difference in the method to neutralize cytokines. In the present study neutralization was accomplished by injection of antibody-FC into mice, which resulted in a constant very high level of mAb in the circulation. In contrast, an injection of purified mAb results in a steadily declining concentration in the serum,[8] which can reach a concentration below the level that neutralizes endogenous cytokines. The plateau level of rat IgG concentration in plasma during antibody-FC treatment differs with the type of hybridoma.[8] In our study the plateau level reached with anti-IFN-γ-FC was lower than that reached with anti-TNF-FC. We determined whether there is a relationship between increasing concentrations of anti-IFN-γ rat IgG, ranging from 1 to 180 μg/ml, and the number of *Listeria* in the liver and spleen. The concentration of rat IgG did not correlate with the proliferation in the liver ($r = -0{\cdot}3$, $P = 0{\cdot}3$) or spleen ($r = -0{\cdot}08$, $P = 0{\cdot}8$), demonstrating that all rat IgG concentrations above 1 μg/ml gave a maximal effect on *L. monocytogenes* proliferation. Therefore, it is unlikely that the difference in effect of anti-IFN--FC and anti-TNF-FC on *Listeria* proliferation is due to the difference in antibody concentration.

Treatment with anti-TNF-FC or anti-IFN-γ-FC during a primary *L. monocytogenes* infection was performed to confirm earlier findings based on injection of purified antibodies.[4,5,7,29] The numbers of bacteria in the liver and spleen of anti-TNF-FC or anti-IFN-γ-FC treated mice were significantly higher than in control mice. In addition, neutralization of IFN-γ during primary infection had a more pronounced effect on *L. monocytogenes* proliferation in the liver than in the spleen. This finding agrees with earlier reports demonstrating that IFN-γ inhibits bacterial proliferation in the liver but not in the spleen of mice with a primary *L. monocytogenes* infection.[14]

Another way to study the role of TNF during a *Listeria* infection is to use knockout mice lacking TNF receptor type-1. These TNF receptor-deficient mice are unable to limit *L. monocytogenes* proliferation in their organs during a sublethal primary infection.[30] Injection of mice with anti-TNF-FC prior to a sublethal primary infection gave similar results. Because TNF receptor-deficient mice succumb to infection with very low numbers (250 colony-forming units) of *L. monocytogenes* and thus do not acquire cell-mediated immunity, these animals cannot be used to study the role of TNF during a secondary infection.

ACKNOWLEDGMENTS

The authors wish to express their gratitude to Drs Peter Nibbering, Wouter Hazenbos, and Paul Groeneveld for critically reading the manuscript, Dr J. Hermans for his assistance with the statistical analysis, and Connie Hoogstraten for technical assistance.

REFERENCES

1. Mackaness G.B. (1962) Cellular resistance to infection. *J Exp Med* **116,** 381.
2. Mitsuyama M., Takeya K., Nomoto K. & Shimotori S. (1978) Three phases of phagocyte contribution to resistance against *Listeria monocytogenes*. *J Gen Microbiol* **106,** 165.
3. Hahn H. & Kaufmann S.H.E. (1981) The role of cell-mediated immunity in bacterial infections. *Rev Infect Dis* **3,** 1221.
4. Buchmeier N.A. & Schreiber R.D. (1985) Requirement of endogenous interferon-γ production for resolution of *Listeria monocytogenes* infection. *Proc Natl Acad Sci USA* **82,** 7404.
5. Nakane A., Minagawa T., Kohanawa M. *et al.* (1989) Interactions between endogenous gamma interferon and tumor necrosis factor in host resistance against primary and secondary *Listeria monocytogenes* infections. *Infect Immun* **57,** 3331.
6. Langermans J.A.M., van der Hulst M.E.B., Nibbering P.H. & van Furth R. (1992) Endogenous TNF-α is required for enhanced antimicrobial activity against *Toxoplasma gondii* and *L. monocytogenes* in r-IFN-γ treated mice. *Infect Immun* **60,** 5107.
7. Havell E.A. (1989) Evidence that tumor necrosis factor has an important role in antibacterial resistance. *J Immunol* **143,** 2894.
8. Savelkoul H.F.J., van Ommen R., Vossen A.C.T.M., Breedland E.G., Coffman R.L. & van Oudenaren A. (1994) Modulation of systemic cytokine levels by implantation of alginate encapsulated cells. *J Immunol Meth.* **170,** 185.
9. Cherwinski H.M., Schumacher J.H., Brown K.D. & Mosmann T.M. (1987) Two types of mouse helper T cell clone. III. Further differences in lymphokine synthesis between Th1 and Th2 clones revealed by RNA hybridization, functionally monospecific bioassays, and monoclonal antibodies. *J Exp Med* **166,** 1229.
10. Abrams J.S., Roncarolo M.G., Yssel H., Andersson U., Gleich G.J. & Silver J.E. (1992) Strategies of anti-cytokine monoclonal antibody development: immunoassay of IL-10 and IL-5 in clinical samples. *Immunol Rev* **127,** 5.
11. Savelkoul H.F.J., Seymour B.W.P., Sullivan L. & Coffman R.L. (1991) IL-4 can correct defective IgE production in SJA/9 mice. *J Immunol* 146, 1801.
12. van Dissel J.T., Stikkelbroeck J.J.M., van den Barselaar M.T., Sluiter W., Leijh P.C.J. & van Furth R. (1987) Divergent changes in antimicrobial activity after immunologic activation of mouse peritoneal macrophages. *J Immunol* **139,** 1665.
13. van Furth R. & Cohn Z.A. (1968) The origin and kinetics of mononuclear phagocytes. *J Exp Med* **132,** 813.
14. Langermans J.A.M., van der Hulst M.E.B., Nibbering P.H., van der Meide P.H. & van Furth R. (1992) Intravenous injection of IFN-γ inhibits the proliferation of *Listeria monocytogenes* in the liver but not in the spleen and peritoneal cavity. *Immunology* **77,** 354.
15. van ommen R., Vredendaal A.E.C.M. & Savelkoul H.F.J. (1994) Supression of polyclonal and antigen-specific murine IgG1 but not IgE responses by neutralizing interleukin-6 *in vivo*. *Eur J Immunol* **24,** 1396.
16. Langermans J.A.M., van der Hulst M.E.B., Nibbering P.H. & van Furth R. (1990) Activation of mouse peritoneal macrophages during infection with *Salmonella typhimurium* does not result in enhanced intracellular killing. *J Immunol* **144,** 4340.
17. van Furth R., van Zwet T.R., Buisman A.M. & van Dissel (1994) Anti-tumor necrosis factor antibodies inhibit the influx of granulocytes and monocytes into an inflammatory exudate and enhance growth of *Listeria monocytogenes* in various organs. *J Infect Dis* **170,** 234.
18. Mielke M.E.A., Rosen H., Brocke S., Peters C. & Hahn H. (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.
19. Kindler V., Sappino A.-P., Grau G.E., Piguet P.-F. & Vassalli P. (1989) The inducing role of tumor necrosis factor in the development of bactericidal granulomas during BCG infection. *Cell* **56,** 731.
20. Mielke M.E.A., Ehlers S. & Hahn H. (1988) T-cell subsets in delayed type hypersensitivity protection and granuloma formation in primary and secondary *Listeria* infection in mice: superior role of Lyt2$^+$ cells in acquired immunity. *Infect Immun* **56,** 1920.
21. van Dissel J.T., Stikkelbroeck J.J.M., Michel B.C., van den

BARSELAAR M. T., LEIJH P.C. & VAN FURTH R. (1987) Inability of recombinant IFN-γ to activate the antibacterial activity of mouse peritoneal macrophages against *Listeria* and *Salmonella typhimurium*. *J Immunol* **139,** 1673.

22. CAMPBELL P.A., CANONO B.P. & COOK J.L. (1988) Mouse macrophages stimulated by recombinant gamma interferon to kill tumor cells are not bactericidal for the facultative intracellular bacterium *Listeria monocytogenes*. *Infect Immun* **56,** 1371.
23. KIDERLEN A.F., KAUFMANN S.H.E. & LOHMAN-MATTHES M.L. (1984) Protection of mice against the intracellular bacterium *Listeria monocytogenes* by recombinant immune interferon. *Eur J Immunol* **14,** 964.
24. HARTY J.T., SCHREIBER R.D. & BEVAN M.J. (1992) $CD8^+$-T-cells can protect against an intracellular bacterium in an interferon-γ independent fashion. *Proc Natl Acad Sci USA* **89,** 11612.
25. MAGEE D.M. & WING E.J. (1988) $L3T4^+$ T-lymphocytes protect mice against *Listeria monocytogenes* by secreting IFN-γ. *J Immunol* **141,** 3203.
26. RAKHMILEVICH A.L. (1994) Evidence for a significant role of $CD4^+$ T cells in adoptive immunity to *Listeria monocytogenes* in the liver. *Immunology* **82,** 249.
27. GREGORY S.H., BARCZYNSKI L.K. & WING E. (1992) Effector function of hepatocytes and Kupffer cells in the resolution of systemic bacterial infections. *J Leuk Biol* **51,** 421.
28. KAUFMANN S.H.E. (1993) Immunity to intracellular bacteria. *Annu Rev Immunol* **11,** 129.
29. NAKANE A., NUMATA A. & MINAGAWA T. (1992) The role of TNF in listeriosis. In: *Mononuclear Phagocytes, Biology of Monocytes and Macrophages* (ed. R. van Furth), p.626. Kluwer, Dordrecht.
30. ROTHE J., LESSLAUER W., LÖTSCHER H. (1993) Mice lacking the tumour necrosis factor receptor 1 are resistant to TNF mediated toxicity but highly susceptable to infection by *L. monocytogenes*. *Nature* **364,** 798.