Role of Tumor Necrosis Factor- α and Interferon- γ in Newborn Host Defense against *Listeria Monocytogenes* Infection

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ABSTRACT. The fetus and newborn are particularly susceptible to *Listeria monocytogenes* infection. We used a newborn rat animal model to investigate neonatal host defense against *Listeria*. In this animal model, newborn (3-d-old) rats are more susceptible to *L. monocytogenes* than older animals. Juvenile (23-d-old) *L. monocytogenes*-infected rats pretreated with lipopolysaccharide (LPS) had a lower bacterial load in blood than control animals, whereas LPS pretreated newborn rats had a higher bacterial load. Because LPS is a potent inducer of tumor necrosis factor (TNF) and TNF enhances host defense against this organism in adult animals, we assessed TNF content in splenic homogenates for animals of different ages. The age at which TNF was detectable in *L. monocytogenes*-infected rats corresponded to the age at which LPS became active in preventing severe bacteremia. TNF was less than 1 unit/mL in splenic homogenates taken from rats less than 8 d of age, whereas 16-d-old rats infected with *L. monocytogenes* 1 d earlier had greater than 80 units/mL ($p < 0.0001$ for 3-d-old versus 16-d-old rats). We also assessed the responsiveness of rats to exogenous TNF- α . Juvenile rats pretreated with TNF- α before *L. monocytogenes* infection had decreased bacterial load in spleen ($p < 0.02$ versus controls) and better survival at 7 d ($p < 0.05$ versus controls), whereas newborn rats did not improve with TNF- α pretreatment ($p > 0.05$ treated versus controls for splenic bacterial load and 7-d survival). However, when newborn rats were pretreated with both interferon- γ (IFN- γ) and TNF- α , splenic bacterial content was decreased compared to animals pretreated with PBS, IFN- γ , or TNF- α alone ($p < 0.005$ combination versus TNF- α , IFN- γ , or PBS). Similarly, survival also was improved for newborn rats pretreated with both IFN- γ and TNF- α compared to controls pretreated with either of these agents alone ($p < 0.02$ combination versus control, $p > 0.05$ TNF- α or IFN- γ versus controls). We conclude that TNF production is decreased among newborn rats challenged with *L. monocytogenes* compared to TNF production in older animals. TNF- α does not enhance resistance of newborn rats to *L. monocytogenes* unless they are pretreated with IFN- γ as well. (*Pediatr Res* 32: 460–464, 1992)

Abbreviations

TNF, tumor necrosis factor
IFN, interferon
rR-IFN- γ , recombinant rat interferon- γ
LPS, lipopolysaccharide

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CFU, colony forming unit
i.p., intraperitoneal
LD₂₀, 20% lethal dose

Listeria monocytogenes infections in the fetus and newborn continue to be a major cause of morbidity and mortality (1). For the newborn infant, factors that predispose to infection with this organism are poorly understood. For adults, however, the processes leading to elimination of intracellular pathogens have been studied extensively and are clear: IFN and cytokines appear to play a key role. IFN- γ enhances adult host defense against many intracellular bacterial pathogens including *L. monocytogenes* (2–4). Recent investigations have also established that TNF- α plays a prominent role in defense of adult animals against *L. monocytogenes* infection (5–8). It now appears that IFN- γ and TNF- α act by independent mechanisms to enhance host resistance against primary and secondary *L. monocytogenes* infection (9). Other cytokines such as colony stimulating factors also contribute to defense against *Listeria* (10). Although IFN- γ can protect neonatal mice and rats from subsequent challenge with a lethal dose of *L. monocytogenes* (11, 12), the role that TNF- α may play in newborn host defense mechanisms is not known.

Products secreted by host cells are not alone in their ability to increase resistance to infection. Bacterial products such as LPS also increase resistance to *L. monocytogenes* in adult mice if injected 24 h before a lethal challenge (13). LPS has the same effect in adult rats (11). However, pretreatment of 3-d-old rats with LPS does not increase resistance (11). Because LPS is a potent stimulant for the production of TNF- α , this latter observation suggests that newborn rats may lack the contribution of its critical role.

Because TNF- α appears to play an essential role in host defense against *L. monocytogenes* in adult animals and its role in newborn infection is unknown, we undertook a series of experiments to examine the endogenous production of TNF- α and the host's response to exogenous TNF- α by newborn animals.

MATERIALS AND METHODS

Bacteria. *L. monocytogenes* serotype 4b (strain 15U) was isolated originally from the blood of a 1-d-old newborn infant with neonatal listeriosis (14, 15). Vials of bacteria were maintained in suspension at -20°C in 20% glycerol. The day before use, bacteria were inoculated into brain heart infusion broth (Difco Laboratories, Detroit, MI) and incubated overnight at 37°C . The bacteria were washed, then suspended in PBS. Bacterial concentration was estimated spectrophotometrically and the number of viable CFU was verified using pour plate technique.

Reagents. Recombinant murine TNF- α was supplied by Genentech Inc. (San Francisco, CA). It was more than 99% pure

(as determined by SDS-PAGE analysis) and contained <1.0 ng endotoxin/mg protein as tested by the limulus amoebocyte lysate assay. Stock TNF- α was diluted to the desired concentration with PBS containing 0.1% BSA.

rR-IFN- γ , kindly provided by H. Schellekens (TNO, Netherlands), is the product of transfection with a plasmid carrying the chromosomal rat IFN- γ gene as described elsewhere (16). The rR-IFN- γ was purified to >98% purity by immunoaffinity chromatography using MAb bound to sepharose. LPS (*Escherichia coli* 055:B5) was obtained from Difco Laboratories and diluted to the desired concentration with PBS.

Animals. Timed pregnant Sprague-Dawley rats (Canadian Hybrid Farms, Hall's Harbour, Canada) were obtained at approximately d 15 of gestation. Pregnant animals were housed under standardized conditions and allowed food and water *ad libitum*. The time of parturition was recorded and the litter was left undisturbed until the time of study. To minimize the risk of cross-contamination, litters were studied at one time only. At a preselected age, animals were injected with the test products or PBS-BSA as placebo. The desired concentration of TNF- α or rR-IFN- γ was prepared in 0.1 mL of PBS-BSA for i.p. injection, either 1 d before or on the same day as the i.p. bacterial inoculation. Within each litter, animals were randomized to receive either PBS-BSA plus bacteria or cytokine(s) plus bacteria. To minimize interlitter variability, no more than three animals in each litter received the same treatment regimen.

For experiments that required estimation of splenic CFU, rats were injected i.p. with the LD₅₀ of *L. monocytogenes* (17). The LD₅₀ was determined separately for each experimental protocol. For 3-d-old rats and 16-d-old rats, the LD₅₀ was approximately 10^{4.5} and 10^{6.5} CFU, respectively (the LD₅₀ varied depending on the protocol). Animals were killed 3 d after bacterial injection by CO₂ inhalation. Spleens were dissected aseptically, weighed, and homogenized in PBS. Pour plates were prepared using serial dilutions from the splenic homogenates. To minimize the effect of variation in animal and splenic size for animals at different ages, bacterial content was expressed as CFU/100 mg of spleen. Pour plates of tissue were made using brain heart infusion agar containing 1.5 μ g/mL of cefuroxime to suppress any contaminating bacteria. This concentration of antibiotic did not affect *L. monocytogenes* viability (minimum growth inhibitory concentration was >25 μ g/mL).

For animal survival and TNF production experiments, the inoculum was increased to approximately the 90% lethal dose appropriate for the protocol and the animal age studied. To assess TNF production, animals were killed 1 d after i.p. injection with *L. monocytogenes*. The spleen was then dissected aseptically, weighed, and homogenized with 2.5 mL of PBS. The homogenate was sedimented at 800 $\times$ g for 10 min, and the supernatant collected, filtered through a 0.22- μ m filter (Millex-GS, Millipore, Bedford, MA) to remove bacteria, and then stored at -70°C until testing.

TNF assay. Bioactivity of TNF was determined using an L929 cell cytotoxicity assay (18). Briefly, TNF was tested by the ability of dilutions of the supernatants from splenic homogenates to kill actinomycin D-treated L929 murine fibroblasts. L929 cells were seeded at 5 $\times$ 10⁴/well into 96-well flat bottom microtiter plates in 100 μ L RPMI/640 containing penicillin (100 U/mL) and streptomycin (100 μ g/mL). After 12 h incubation, duplicates of serially diluted supernatants from splenic homogenates were added to the wells together with actinomycin D at a final concentration of 1.0 μ g/mL. Recombinant TNF- α standards were used in each plate to quantitate TNF activity. The cells were incubated for an additional 18 to 24 h. The plates were extensively washed with warm PBS to remove dead cells, and viable adherent cells were subsequently fixed with 30% ethanol. The plates were allowed to dry and were finally stained with crystal violet (0.5% in 20% ethanol) for 10 min. After washing off the dye, stained cells were solubilized with 1% SDS. The degree of cytotoxicity was determined spectrophotometrically (540 nm)

using a computerized microplate reader (Biotech Corporation, Winooski, VT).

Results of TNF content in spleen were expressed as units of TNF per mL of splenic homogenate supernatant.

Statistical methods. Analysis of groups was done using a two sided paired *t* test for comparison of bacteria in spleen for test and placebo litter mates. A Fisher exact test was used to compare survival of various groups. Significance was accepted as *p* < 0.05. A software package was used for statistical calculations (Statistix Analytical Software, St. Paul, MN).

RESULTS

We have demonstrated previously that newborn 3-d-old rats respond differently than older rats to LPS pretreatment in *L. monocytogenes* infection; 30-d-old rats are protected, whereas 3-d-old rats are not (11). In other experiments, the age at which LPS altered susceptibility to *L. monocytogenes* appeared to develop was between 7 and 14 d (Bortolussi R, unpublished data). Therefore, we wished to see if the newborn's susceptibility to *L. monocytogenes* may be related to TNF production during infection. For 16-d-old rats, TNF content in splenic homogenate supernatants was directly related to the number of viable organisms injected and to splenic bacterial load at the time the animals were killed (Table 1). For this age group, the subset of rats with high bacterial content in spleen (over 10⁴ CFU/100 mg tissue) had elevated levels of TNF in supernatant fluid (128 $\pm$ 118 U/mL, *n* = 20) compared to animals with the lower bacterial content (29 $\pm$ 45 U/mL, *n* = 7; *p* < 0.05). In contrast, 3-d-old rats had less than 1 U/mL TNF detectable in splenic homogenates at similar bacterial inocula and at high and low splenic bacterial loads. The difference in TNF activity between these two age groups was highly significant (*p* < 0.0001). TNF content in splenic homogenates was significantly greater for 16-d-old rats compared to 11- or 3-d-old rats over several inoculum concentrations (Fig. 1). To be sure the TNF deficiency at 3 d of age was not merely due to a delay in its production, we also assessed animals 2 or 3 d after bacterial challenge. In these experiments, TNF concentration in splenic homogenates was less than 1.0 (data not shown).

To determine more accurately the age at which TNF content in spleen increases, rats of various ages were inoculated i.p. with the same *L. monocytogenes* concentration. TNF content in splenic homogenate supernatants increased at approximately 10 d of age (Fig. 2).

Although TNF production appears to be "turned on" after 8 d of age, the responsiveness of animals to exogenous TNF- α at different ages is unknown. Accordingly, a series of experiments was carried out using rats pretreated with TNF- α . Animals were injected i.p. with 1 to 100 μ g/kg TNF- α either 24 h or 1 h before injection with *Listeria*. For 3-d-old rats, there was no significant change in splenic bacterial load at any of the TNF- α concentrations tested. Higher concentrations of TNF- α were toxic to the animals. For 16-d-old rats, splenic bacterial load was modestly decreased among animals injected with 100 μ g/kg TNF- α 1 h before bacterial challenge (geometric mean log 3.88 CFU/100 mg for TNF-treated animals versus log 4.33 CFU/100 mg for

Table 1. TNF- α content in spleen 1 d after *L. monocytogenes* infection

Age (d)	Log CFU inoculated (no. of rats)	Log CFU $\pm$ SD per 100 mg spleen	Splenic TNF $\pm$ SD (units/mL)
3	6.41 (5)	3.82 $\pm$ 0.11	0.71 $\pm$ 0.14*
	6.81 (5)	4.43 $\pm$ 0.08	0.74 $\pm$ 0.15
16	4.86 (6)	3.78 $\pm$ 0.155	10.1 $\pm$ 3.36
	5.86 (6)	4.20 $\pm$ 0.242	40.3 $\pm$ 41.01
	6.86 (6)	4.43 $\pm$ 0.58	81.7 $\pm$ 35.1
	7.86 (9)	4.71 $\pm$ 0.94	218.7 $\pm$ 108.1

**p* < 0.00001 3-d-old vs 16-d-old.

Fig. 1. TNF content in spleen for rats inoculated at different ages with *L. monocytogenes*. Content of TNF (units/mL) in supernatant of homogenized spleen was measured 24 h after i.p. inoculation with various numbers (log CFU) *L. monocytogenes* at animal ages as indicated.

Fig. 2. TNF content in spleen for rats inoculated with *L. monocytogenes*. TNF content (TNF units/mL) was measured in supernatants of splenic homogenates taken from rats 24 h after i.p. challenge with *L. monocytogenes* (approximately 10^6 CFU/10 g animal weight). Age of the animals is indicated at the time of bacterial challenge. Mean and SD of TNF content in spleen is indicated for each animal age group. Number of animals inoculated is shown in parentheses above SD bar.

PBS controls, $p < 0.05$, Mann-Whitney test); other concentrations of TNF- α were not protective.

Animal survival was assessed using rats pretreated with TNF- α that were then given a lethal inoculum of *L. monocytogenes* (Fig. 3). In these experiments, animal survival to 7 d was improved among 16-d-old rats pretreated 1 d earlier with 100 μ g/kg TNF- α ($p < 0.05$). Survival at 7 d was not significantly improved for 3-d-old rats at any concentration of TNF- α ; however, survival was better at d 6 ($p < 0.05$) for animals injected with 100 μ g/kg TNF- α . Animals at both ages pretreated with TNF on the same day as bacterial challenge were not protected from the lethal effects of this organism (data not shown).

TNF and rIFN- γ in combination. In previous experiments, we have shown that rIFN- γ pretreatment of 3-d-old rats dramatically improved survival and splenic content of bacteria among *L. monocytogenes*-inoculated animals (11). Because rIFN- γ treatment may alter TNF- α production (9, 19, 20) and cellular response to TNF- α (20–24), we carried out a series of experiments using rIFN- γ at a concentration that we predicted would be, by itself, suboptimal for animal protection. Splenic content of TNF in bacteria-challenged 3-d-old rats was not

increased for rIFN- γ -pretreated animals (Table 2). In addition, TNF content was not increased among animals given three injections of rIFN- γ before bacterial challenge.

When 3-d-old rats were pretreated with what we predicted would be a suboptimal dose of rIFN- γ (10^3 U/kg) 1 d before or TNF- α (0.3 μ g/kg) 1 h before bacterial challenge, there was minimal difference in splenic bacterial content compared to controls (Fig. 4). However, newborn rats pretreated with both rIFN- γ and TNF- α had decreased splenic bacterial content ($p < 0.005$) compared to controls injected with rIFN- γ , TNF- α alone, or PBS. For 16-d-old rats, this combination was not superior to rIFN- γ alone. Survival of both 3-d-old and 16-d-old rats pretreated with both rIFN- γ and TNF- α was greater than animals treated with TNF- α alone or PBS ($p < 0.02$ combination versus TNF- α or PBS for 3-d-old rats, $p < 0.05$ combination versus TNF- α or PBS for 16-d-old rats) (Fig. 5).

DISCUSSION

IFN- γ is induced during *L. monocytogenes* infection among adult rats and is thought to make the animals resistant to the organism by activating their monocytes and macrophages (2, 3, 25). Previous studies in our laboratory have demonstrated that susceptibility of newborn rats to *L. monocytogenes* is dramatically decreased when 3-d-old rats are pretreated with rIFN- γ (11). Because newborn animals are particularly susceptible to systemic *Listeria* infection, this observation suggests that the production of IFN- γ may not be increased among newborns. It does, however, indicate that intact receptors for IFN- γ are present.

In the present study, we have examined the possibility that production of TNF- α or response to TNF- α by newborn animals may be defective and contribute to the animal's susceptibility to *L. monocytogenes*. When 3-d-old rats are injected with LPS 24 h before their challenge with *L. monocytogenes*, there is no improvement in animal survival (11) even though adult rats do have increased survival. We therefore examined in more detail the ontogeny of LPS-induced protection. Because LPS is a potent inducer of TNF- α production and *L. monocytogenes* produces LPS-like toxins (26, 27), we next assessed the production of TNF- α by animals at different ages during *L. monocytogenes* infection. Reports on the ability of newborn animals to produce TNF- α provide conflicting information. Some have found TNF- α is decreased (28, 29), whereas others have found it to be normal (30–32). The dynamics of its production have not been studied in newborn animals with infection. Among animals over 10 d of age in our study, TNF- α content in spleen was directly related to the concentration of bacteria in this organ as well as the bacterial inoculum (Fig. 1). However, among animals less than 10 d of age, TNF- α was not detected in splenic homogenates over a range of bacterial inocula and splenic content (Table 1).

The recent observation that TNF- α , IFN- γ , and IL-1 may act synergistically prompted us to assess the effects of combining TNF- α and IFN- γ (9, 19, 20, 22, 33–35). In addition, a defect in neonatal polymorphonuclear leukocyte activation and movement is corrected with IFN- α *in vitro* (36). In our experiments, however, we could demonstrate no increase in TNF- α content in spleens among 3-d-old rats pretreated with IFN- γ (Table 2). Because the dose and schedule of IFN- γ used in these animals was previously shown to be protective against *L. monocytogenes*, we conclude that IFN- γ acts by a mechanism independent of TNF- α production in its protective activity. Similarly, Nakane *et al.* (9), using adult mice, concluded that endogenous IFN- γ and TNF- α acted by independent mechanisms to increase host resistance against primary and secondary *L. monocytogenes* infection.

Although TNF- α production may be depressed in newborn rats, such animals may still respond to exogenous TNF- α . We therefore assessed the bacterial content in the spleen of animals injected with TNF- α either 24 h or 1 h before i.p. injection with

Fig. 3. Effect of TNF- α pretreatment on survival for newborn (3-d-old) or juvenile (16-d-old) rats after i.p. challenge with *L. monocytogenes*. Animals were pretreated with an i.p. injection of TNF- α at a concentration of 100 μ g/kg (—□—), 10 μ g/kg (---○---), or 1 μ g/kg (·····△·····) or PBS (—●—) 1 d before an i.p. injection with *L. monocytogenes* at a concentration equivalent to the 90% lethal dose. *, $p < 0.05$ test vs control animals.

Table 2. Effect of IFN- γ pretreatment on TNF content in spleen among *L. monocytogenes*-infected 3-d-old rats

Pretreatment	Splenic CFU (log CFU/ 100 mg $\pm$ SD)	Splenic TNF- α (units/mL) (mean $\pm$ SD)
PBS + LM*	4.45 $\pm$ 0.24	2.01 $\pm$ 1.89
IFN $\times$ 1 + LM†	4.07 $\pm$ 0.12	1.14 $\pm$ 0.59
IFN $\times$ 3 + LM‡	4.28 $\pm$ 0.1	1.07 $\pm$ 0.38
IFN $\times$ 3 + PBS	Not detectable	0.95 $\pm$ 0.22

* LM, *L. monocytogenes*.

† Animals pretreated with an i.p. injection of rR-IFN- γ 1 d before *L. monocytogenes* challenge.

‡ Animals pretreated with rR-IFN- γ on three occasions 2 d before, 1 d before, and the same day as *L. monocytogenes* challenge.

L. monocytogenes. In this experiment, the bacterial load in the spleen was only moderately affected by prior TNF- α treatment among 16-d-old animals. In a separate experiment using animals injected with a lethal inoculum of *L. monocytogenes*, survival was significantly improved among 16-d-old rats pretreated with 100 μ g/kg of TNF- α 1 d before bacterial challenge (Fig. 3). Survival was not significantly improved among 16-d-old rats pretreated with other concentrations of TNF- α nor among 3-d-old rats pretreated with a range of concentrations of TNF- α .

We also conducted experiments to assess the protective value for animals treated with a combination of IFN- γ and TNF- α . The concentrations of TNF- α and IFN- γ used in these experiments were both predicted to have minimal effect on bacterial content in spleen and on survival for the animals. In this experiment, bacterial content in the spleen was lowest for 3-d-old rats pretreated with a combination of IFN- γ followed by TNF- α before bacterial challenge (Fig. 4). For 16-d-old rats, no additive effect was found when TNF- α was combined with IFN- γ . Finally, survival of animals challenged with a lethal inoculum of *L. monocytogenes* was significantly improved only for newborn and 16-d-old animals that were pretreated with a combination of IFN- γ and TNF- α (Fig. 5).

On the basis of these observations, we conclude that TNF is decreased among newborn rats challenged with *L. monocytogenes*. TNF was detectable only among animals over 8 d of age. The age at which TNF was measurable corresponds to the approximate age at which LPS becomes active in preventing *L. monocytogenes* infection (11). The studies also suggest that 3-d-old rats do not resist *L. monocytogenes* after treatment with exogenous TNF- α . However, pretreatment of 16-d-old rats with exogenous TNF- α before challenge with *L. monocytogenes* results in improved survival and a lower bacterial load in the spleen. In spite of this, 3-d-old rats did develop toxicity to higher

Fig. 4. Effect of IFN- γ and TNF- α pretreatment on splenic bacterial content. The mean number of CFU of *L. monocytogenes* (log CFU/100 mg spleen $\pm$ SD) was estimated for newborn (3-d-old) or juvenile (16-d-old) rats. Animals were pretreated with either PBS alone, TNF- α (0.3 μ g/kg), IFN- γ (10^3 U/kg), or a combination of rR-IFN- γ and TNF- α (I+T) before an LD₂₀ infection with *L. monocytogenes*. rR-IFN- γ and TNF- α were injected at 24 h and 1 h, respectively, before bacterial challenge. The rats were killed 3 d after bacterial inoculum was injected. *, $p < 0.05$ test vs PBS control; **, $p < 0.005$ rR-IFN- γ plus TNF- α vs rR-IFN- γ or TNF- α or PBS; #, $p < 0.01$ rR-IFN- γ plus TNF- α vs TNF- α -injected animals.

concentrations of TNF- α , suggesting that its toxic effects may not be age limited. Finally, the observations suggest that IFN- γ may permit newborn rats to respond to exogenous TNF- α or to TNF- α in concert with other cytokines such as IFN- α , IL-1, and colony stimulating factors, which are reportedly produced endogenously during *L. monocytogenes* infection. The experiments have not identified the regulatory mechanisms for TNF- α production in very young animals. Further studies along this line may provide insight into the regulation of TNF- α *in vivo*.

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Fig. 5. Effect of rR-IFN- γ and TNF- α pretreatment on survival. Rats were infected with rR-IFN- γ and TNF- α as described in Figure 4, challenged with a 90% lethal dose of *L. monocytogenes*, and then followed for 7 d to monitor survival. Lines represent rR-IFN- γ + TNF- α (---□---), IFN- γ alone (---○---), TNF- α alone (---△---), and PBS (—). ▼, $p < 0.02$ rR-IFN- γ plus TNF- α vs TNF- α ; ▽, $p < 0.02$ rR-IFN- γ plus TNF- α vs PBS controls; and *, $p < 0.05$ rR-IFN- γ plus TNF- α vs PBS control or TNF- α .

REFERENCES

- Bortolussi R, Seelinger HPR 1990 Listeriosis. In: Lamsback W (ed) Infectious Diseases of the Fetus and Newborn Infant. WB Saunders, Philadelphia, pp 812-833
- Murray HW 1988 Interferon-gamma, the activated macrophage, and host defense against microbial challenge. *Ann Intern Med* 108:595-608
- Murray HW 1990 Gamma interferon, cytokine-induced macrophage activation, and antimicrobial host defense. *In vitro*, in animal models, and in humans. *Diagn Microbiol Infect Dis* 13:411-421
- Tsukada H, Kawamura I, Arakawa M, Nomoto K, Mitsuyama M 1991 Dissociated development of T cells mediating delayed-type hypersensitivity and protective T cells against *Listeria monocytogenes* and their functional difference in lymphokine production. *Infect Immun* 59:3589-3595
- Desiderio JV, Kiener PA, Lin P-F, Warr GA 1989 Protection of mice against *Listeria monocytogenes* infection by recombinant human tumor necrosis factor alpha. *Infect Immun* 57:1615-1617
- Havell EA 1987 Production of tumor necrosis factor during murine listeriosis. *J Immunol* 139:4225-4231
- Havell EA, Sehgal PB 1991 Tumor necrosis factor-independent IL-6 production during murine listeriosis. *J Immunol* 146:756-761
- Nakane A, Minagawa T, Kato K 1988 Endogenous tumor necrosis factor (cachectin) is essential to host resistance against *Listeria monocytogenes* infection. *Infect Immun* 56:2563-2569
- Nakane A, Minagawa T, Kohanawa M, Chen Y, Sato H, Moriyama M, Tsuruoka N 1989 Interactions between endogenous gamma interferon and tumor necrosis factor in host resistance against primary and secondary *Listeria monocytogenes* infections. *Infect Immun* 57:3331-3337
- Cheers C, Haigh AM, Kelso A, Metcalf D, Stanley ER, Young AM 1988 Production of colony-stimulating factors (CSFs) during infection: separate determinations of macrophage-, granulocyte-, granulocyte-macrophage-, and multi-CSFs. *Infect Immun* 56:247-251
- Bortolussi R, Issekutz T, Burbridge S, Schellekens H 1989 Neonatal host defense mechanisms against *Listeria monocytogenes* infection: the role of lipopolysaccharides and interferons. *Pediatr Res* 25:311-315
- Chen Y, Nakane A, Minagawa T 1989 Recombinant murine gamma interferon induces enhanced resistance to *Listeria monocytogenes* infection in neonatal mice. *Infect Immun* 57:2345-2349
- Galleli A, Le Garreo Y, Chadid L 1981 Increased resistance and depressed delayed-type hypersensitivity to *Listeria monocytogenes* induced by pretreatment with lipopolysaccharide. *Infect Immun* 31:88-94
- Evans JR, Allen AC, Stinson DA, Bortolussi R, Peddle LJ 1985 Perinatal listeriosis: report of an outbreak. *Pediatr Infect Dis* 4:237-241
- Nair MPN, Schwartz SA, Menon M 1985 Association of decreased natural and antibody-dependent cellular cytotoxicity and production of natural killer cytotoxic factor and interferon in neonates. *Cell Immunol* 94:159-171
- Dijkema R, van der Meide PH, Pouwels PH, Caspers M, Dubbeld M, Schellekens H 1985 Cloning and expression of the chromosomal immune interferon gene of the rat. *EMBO J* 4:761-767
- Reed LJ, Muench H 1938 A simple method of estimating fifty percent endpoints. *Am J Hyg* 27:493-497
- Bruce MC, Baley JE, Medvik KA, Berger M 1987 Impaired surface membrane expression of C3bi but not C3b receptors on neonatal neutrophils. *Pediatr Res* 21:306-311
- Reiner NE, Ng W, Wilson CB, McMaster R, Burchett SK 1990 Modulation of *in vitro* monocyte cytokine responses to *Leishmania donovani*. Interferon- γ prevents parasite-induced inhibition of interleukin-1 production and primes monocytes to respond to *Leishmania* by producing both tumor necrosis factor- α and interleukin-1. *J Clin Invest* 85:1914-1924
- Talmadge JE, Bowersox O, Tribble H, Lee SH, Shepard M, Liggitt D 1987 Toxicity of tumor necrosis factor is synergistic with γ -interferon and can be reduced with cyclooxygenase inhibitors. *Am J Pathol* 128:410-425
- Kumaratilake LM, Ferrante A, Bates EJ, Kowanko IC 1990 Augmentation of the human monocyte/macrophage chemiluminescence response during short-term exposure to interferon-gamma and tumour necrosis factor-alpha. *Clin Exp Immunol* 80:257-262
- Perussia B, Kobayashi M, Rossi ME, Anegón I, Trinchieri G 1987 Immune interferon enhances functional properties of human granulocytes: role of Fc receptors and effect of lymphotoxin, tumor necrosis factor, and granulocyte-macrophage colony-stimulating factor. *J Immunol* 138:765-774
- Shalaby MR, Aggarwal BB, Rinderknecht E, Svedersky LP, Finkle BS, Palladino Jr MA 1985 Activation of human polymorphonuclear neutrophil functions by interferon- γ and tumor necrosis factors. *J Immunol* 135:2069-2073
- Wherry JC, Shreiber RD, Unanue ER 1991 Regulation of gamma interferon production by natural killer cells in SCID mice: roles of tumor necrosis factor and bacterial stimuli. *Infect Immun* 59:1709-1715
- Lewis DB, Wilson CB 1990 Gamma-interferon: an immunoregulatory lymphokine with immunotherapeutic potential. *Pediatr Infect Dis J* 9:642-651
- Bortolussi R, McGregor DD, Kongshavn PAL, Galsworthy S, Albritton W, Davies JW, Seeliger HPR 1984 Host defense mechanisms to perinatal and neonatal *Listeria monocytogenes* infection. *Surv Synth Pathol Res* 3:311-332
- Bortolussi R, Vandenbroucke-Grauls CMJE, Van Asbeck BS, Verhoef J 1987 Relationship of bacterial growth phase to killing of *Listeria monocytogenes* by oxidative agents generated by neutrophils and enzyme systems. *Infect Immun* 55:3197-3203
- English K, Burchett SK, English JD, Ammann AJ, Wara DW, Wilson CB 1988 Production of lymphotoxin and tumor necrosis factor by human neonatal mononuclear cells. *Pediatr Res* 24:717-722
- Bortolussi R, Campbell N, Krause V 1984 Dynamics of *Listeria monocytogenes* type 4b infection in pregnant and infant rats. *Clin Invest Med* 7:273-279
- Adams JL, Semrad SD, Czuprynski CJ 1990 Administration of bacterial lipopolysaccharide elicits circulating tumor necrosis factor-alpha in neonatal calves. *J Clin Microbiol* 28:998-1001
- Casey ML, Cox SM, Beutler B, Milewich L, MacDonald PC 1989 Cachectin/tumor necrosis factor- α formation in human decidua. *J Clin Invest* 83:430-436
- Szabo G, Miller-Graziano CL, Wu J-Y, Takayama T, Kodys K 1990 Differential tumor necrosis factor production by human monocyte subsets. *J Leukocyte Biol* 47:206-216
- Flesch IEA, Kaufmann SHE 1990 Activation of tuberculostatic macrophage functions by gamma interferon, interleukin-4, and tumor necrosis factor. *Infect Immun* 58:2675-2677
- Lewis CE, McCarthy SP, Lorenzen J, McGee JO'D 1990 Differential effects of LPS, IFN- γ and TNF- α on the secretion of lysozyme by individual human mononuclear phagocytes: relationship to cell maturity. *Immunology* 69:402-408
- Liew FY, Li Y, Millott S 1990 Tumor necrosis factor- α synergizes with IFN- γ in mediating killing of *Leishmania major* through the induction of nitric oxide. *J Immunol* 145:4306-4310
- Hill HR, Augustine NH, Jaffe HS 1991 Human recombinant interferon γ enhances neonatal polymorphonuclear leukocyte activation and movement, and increases free intracellular calcium. *J Exp Med* 173:767-770