Neonatal *Listeria monocytogenes* Infection is Refractory to Interferon

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ABSTRACT. Despite aggressive treatment, early onset neonatal *Listeria monocytogenes* infection continues to have high morbidity and mortality. We recently showed that pretreatment of newborn *L. monocytogenes*-infected rats with interferon (IFN)- α/β or recombinant rat IFN- γ dramatically improves survival. However, in the present experiment, when newborn rats were treated with IFN- α/β or recombinant rat IFN- γ after intraperitoneal injection with *Listeria* there was no benefit. Because most deaths occurred at or before 3 d in this animal model, we reasoned that the effect of interferon may be evident if animals survived longer. To accomplish this and test this hypothesis, ampicillin (20 mg/kg/d) was given 48 h after bacterial challenge. When ampicillin-treated *Listeria*-infected rats were randomized to receive PBS, IFN- α/β , or recombinant rat IFN- γ , mortality rates were 79, 76, and 69%, respectively ($p > 0.05$ versus PBS). Animals treated in a similar fashion after a lower bacterial inoculum (25% lethal dose) were killed 5 d after bacterial challenge. Bacterial concentrations in the spleen were higher for IFN-treated animals than controls. We conclude that no direct benefit of IFN is found if it is given after bacterial infection has been established. (*Pediatr Res* 29: 400-402, 1991)

Abbreviations

IFN, interferon
rRIFN- γ , recombinant rat interferon- γ
CFU, colony forming units
i.p., intraperitoneal(ly)
LAM, lipoarabinomannan
LD₂₅ or LD₉₀, 25% and 90% lethal dose, respectively

Listeria monocytogenes is the second or third most common cause of neonatal sepsis and meningitis (1). This short gram-positive motile rod is thought to be pathogenic by virtue of its ability to survive intracellularly (2). Resistance to *Listeria* infection appears to be mediated by IFN- α/β and IFN- γ , which induce increased macrophage, lymphocyte, and natural killer cell activity *in vivo* and improve survival among pretreated *Listeria*-infected animals (3-12). Some investigators, however, have questioned the direct effect of IFN, suggesting instead that its role may be to enhance the activities of other lymphokines (13, 14). Despite this controversy, the effect of IFN pretreatment acting either directly or indirectly on *Listeria*-infected animals seems well established.

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In mature animals, *L. monocytogenes* infection induces production of IFN- α/β and IFN- γ . IFN- γ is produced by immunologically active cells and can be induced by a variety of substances including *L. monocytogenes* (3, 5). The production of IFN- γ is modulated or augmented by IL-1 (15) and lipopolysaccharide (5). IFN- α/β is produced by fibroblasts or leukocytes and is induced by *L. monocytogenes* infection as well as by other IFN inducers (7-9).

The human newborn is unusually susceptible to infection with *L. monocytogenes* (1). The reason for this is not entirely clear; however, immunologic elements important for host defense against *Listeria* are poorly developed in neonates (16-18). Human neonatal cells produce little IFN when stimulated with mitogens and respond poorly to IL-2 stimulation (19). Despite this decreased activity and production of IFN, we recently showed that newborn rats have increased resistance to *L. monocytogenes* infection when pretreated with IFN or some IFN inducers (12). Because neonatal listeriosis has high mortality despite aggressive antibiotic treatment, we investigated the possibility that IFN might have an adjunctive therapeutic role. Here, we report the results of these studies using a newborn animal model. In contrast to our earlier studies in which IFN had a beneficial effect when given before infection, we found no improvement in animal survival or in bacterial load if animals were treated with IFN after infection was established.

MATERIALS AND METHODS

Reagents. Rat IFN- α/β was purchased from Lee Biophysics, San Diego, CA. rRIFN- γ is the product of transfection with a plasmid carrying the chromosomal rat IFN- γ gene as described elsewhere (20). IFN- α/β and rRIFN- γ were diluted in pyrogen free sterile PBS containing 0.1% albumin to a concentration of 10⁵ U/mL of stock solution. Lipopolysaccharide was not detectable in rRIFN- γ (<1.0 ng/mL) and was present in low concentration in IFN- α/β (~10 ng/mL). A strain of *L. monocytogenes* type 4b (designated 15U previously) isolated originally from the blood of an infant with early onset neonatal sepsis was used in these studies (21, 22). Aliquots of a stock culture were stored in 20% glycerol and frozen at -20°C until required for animal studies. A portion of the frozen aliquot was inoculated into brain heart infusion broth (BHI; Difco Laboratories, Detroit, MI) and incubated at 37°C overnight. The organism was sedimented (3000 $\times$ g for 10 min) and washed once in PBS. The number of bacteria were estimated spectrophotometrically and confirmed for each experiment using the pour plate technique. Minimal inhibitory concentration of ampicillin against the organism as determined by serial dilution was 0.46 μ g/mL. The minimal bactericidal concentration was 3.20 μ g/mL (22).

In vivo studies. Timed pregnant outbred Sprague Dawley rats (Canadian Hybrid Farms, Halls Harbour, Nova Scotia, Canada) were obtained at the 13th d of gestation, fed rat food *ad libitum*, and housed in a controlled environment (12 h light/d) until parturition (usually on the 21st or 22nd d of gestation).

For studies in which animals were pretreated with IFN, rat pups were injected i.p. with PBS or 10^5 U/kg of IFN- α/β or rRIFN- γ at 48 and 24 h before i.p. challenge with *L. monocytogenes* [$1.0 \pm 0.5 \times 10^5$ CFU (mean $\pm$ SEM) per animal] which was given on d 3 of life. This inoculation was equivalent to approximately LD₂₅ in this schedule of injections. In these experiments, animals were killed by CO₂ inhalation 3 d after bacterial challenge. The spleen was dissected free, weighed, and homogenized in PBS. Duplicate pour plates were prepared using serial dilutions in sterile water from the splenic homogenates and bacterial content expressed as CFU/100 mg of spleen. In all experiments, animals were returned to their mother when not being treated.

In studies in which animals were treated with IFN after bacterial challenge, 3-d-old rats were injected i.p. with $1.0 \pm 0.5 \times 10^6$ CFU for survival experiments or $1.8 \pm 0.1 \times 10^5$ CFU for dissection experiments (LD₉₀ and LD₂₅, respectively, in this experimental protocol). Forty-eight and 72 h later, they were injected s.c. with ampicillin (20 mg/kg/d). In preliminary experiments, we established that this schedule and dose prevented death in approximately 40% of animals for an inoculum of 1.0×10^6 CFU. Median survival was approximately 4 d for ampicillin-treated *versus* 3 d for untreated animals. Antibiotic-treated animals were randomly allocated to receive rRIFN- γ or IFN- α/β (10^5 U/kg) i.p. at 48 and 72 h after bacterial challenge. Animals were killed 5 d after bacterial challenge and the spleens processed as described above.

Statistical analysis. Analysis of groups was done using a two-sided paired *t* test for comparison of concentration of bacteria in tissue. A χ^2 test with Yates correction was used to compare survival of various groups (23).

RESULTS

In preliminary experiments, we found no improvement in survival or in bacterial content of spleen when newborn animals were injected with *Listeria* followed later by treatment with IFN- α/β . This was despite the fact that IFN- α/β was 100% protective when administered before infection at this dose (12). Inasmuch as in this animal model most deaths occurred at or before the 3rd d of infection, we reasoned that no effect was evident because insufficient time had elapsed for IFN to act. We therefore modified the model by treating with antibiotics to prolong survival. A subcurative dose of ampicillin (20 mg/kg/d) was deliberately chosen because we wished to assess the potential additive effects of IFN.

As shown in Figure 1, survival of *Listeria*-infected animals was better ($p < 0.05$ on d 4 after challenge) if they were treated with ampicillin 48 and 72 h after bacterial challenge. However, addition of IFN- α/β or rRIFN- γ to therapy did not improve survival any further for these animals. For all ampicillin-treated animals, including IFN- α/β , rRIFN- γ , and placebo groups, median survival was approximately 4 d.

Fig. 1. Percent survival of 3-d-old rats after *L. monocytogenes* infection. Animals were injected with 1×10^6 CFU *Listeria* i.p. on d 0 followed by treatment on d 2 and 3 with: PBS ($\blacktriangle$, 38 animals), PBS and ampicillin ($\circ$, 85 animals), IFN- γ and ampicillin ($\times$, 78 animals), or IFN- α/β and ampicillin ($\bullet$, 74 animals).

Table 1. Effect of IFN given after bacterial challenge on *Listeria*-infected ampicillin-treated newborn rats

Treatment*	No. of animals	Splenic bacterial concentration†
PBS:PBS	8	2.66 $\pm$ 0.8
IFN- α/β :IFN- α/β	8	3.53 $\pm$ 1.1‡
rRIFN- γ :rRIFN- γ	8	3.46 $\pm$ 1.0‡
IFN- α/β :rRIFN- γ	9	3.21 $\pm$ 0.6‡
PBS:rRIFN- γ	8	3.08 $\pm$ 1.1

* IFN- α/β , rRIFN- γ , or PBS were injected i.p. at 48 and 72 h after i.p. injection of *Listeria*.

† Log CFU per 100 mg splenic tissue ($\pm$ SD) for animals killed.

‡ $p < 0.05$ vs PBS controls.

Table 2. Effect of IFN given before bacterial challenge on *Listeria*-infected newborn rats

Treatment*	No. of animals	Splenic bacterial concentration†
PBS:PBS	8	3.9 $\pm$ 0.45
IFN- α/β :IFN- α/β	7	3.5 $\pm$ 0.69
rRIFN- γ :rRIFN- γ	7	2.7 $\pm$ 0.39‡
IFN- α/β :rRIFN- γ	7	2.6 $\pm$ 0.56‡
PBS:rRIFN- γ	5	3.5 $\pm$ 0.79

* IFN- α/β , rRIFN- γ , or PBS were injected i.p. 48 and 24 h before i.p. injection of *Listeria*.

† Log CFU per 100 mg splenic tissue ($\pm$ SD) for animals killed 72 h after last treatment.

‡ $p < 0.05$ vs PBS controls.

We next considered the possibility that the inoculum of bacteria (approximately LD₉₀) was too high to demonstrate any potential benefit of IFN. In a second experiment, bacterial challenge of animals was lowered to 1.8×10^5 CFU (approximately LD₂₅). As shown in Table 1, bacterial content of spleen in *Listeria*-infected ampicillin-treated animals was not improved by treatment with two doses of 10^5 U/Kg of IFN- α/β or rRIFN- γ or a combination of these two IFN. In these experiments, bacterial load was lower ($p < 0.05$ two-sided *t* test) for control animals that were not treated with IFN compared with groups of animals treated with IFN- α/β , rRIFN- γ , or IFN- α/β plus rRIFN- γ .

In contrast to this, newborn rats treated with IFN- α/β , rRIFN- γ , or IFN- α/β plus rRIFN- γ before bacterial challenge had decreased ($p < 0.05$) bacterial concentration in the spleen (Table 2). In this experiment the bacterial inoculum was lower than in the first experiment and antibiotics were not given. The inoculum was approximately equivalent to a LD₂₅ under these conditions. In addition, it is noteworthy that a single inoculation of rRIFN- γ produced lower bacterial loads only if it was preceded by IFN- α/β and not by PBS.

DISCUSSION

L. monocytogenes is an intracellular pathogen that resides chiefly within the cells of the mononuclear phagocytic system. Although the underlying defect in newborn immunity to *L. monocytogenes* is not clearly understood, the failure of the macrophage to kill or inhibit the organism is a central factor. The macrophage has been shown to alter its metabolic, phagocytic, and bactericidal activity when stimulated by macrophage activating factor or recombinant IFN- γ (24). Several investigators have studied the role of IFN- γ in *L. monocytogenes* infection. In 1984, Kiderlen *et al.* (6) demonstrated that IFN- γ pretreatment of mice led to their increased resistance to killing by *L. monocytogenes*. We and others have shown similar results using a newborn rat model of *L. monocytogenes* infection (12, 25).

These observations led us to speculate that IFN- γ or IFN- α/β may have a potential therapeutic role in established infection. In

the present experiments we examined this possibility. To our surprise, we showed no benefit of rIFN- γ or IFN- α/β when given by themselves or in combination with ampicillin to *Listeria*-infected newborn rats. This conclusion was drawn after studies on animal survival and bacterial load in spleens. In fact, use of IFN after bacterial challenge increased bacterial load in spleens. In contrast, the same dose of IFN given before bacterial challenge had an opposite effect. Bacterial load in the spleen was significantly decreased by pretreatment with two injections of rIFN- γ . In addition, we have demonstrated here that a single injection with rIFN- γ was effective in limiting growth of *Listeria* in the spleen if animals were pretreated with IFN- α/β even though IFN- α/β by itself had no effect, suggesting that IFN- α/β may prepare the host for response to IFN- γ .

The overall conclusion from the experiments is that IFN modifies host response to *L. monocytogenes* infection differently if it is given after rather than before infection. The explanation for such an apparent paradox is at present unknown. Several explanations for these observations should be considered; products manufactured by the bacteria or products made by the host after infection may modify host responsiveness to cytokine. Alternatively, the sequence of cytokine appearance may be a critical factor.

That some organisms may produce exotoxins *in vivo* that render cells unresponsive to IFN has been shown for *Mycobacterium leprae* (26). Macrophages from animals infected with *M. leprae* are defective in their responsiveness to lymphokines including IFN- γ even though these cells appear to be normal in other functions. The inhibiting effect of *M. leprae* is due to a major cell wall constituent, LAM, which specifically inhibits efferent functions of mouse macrophages. Preincubation with LAM resulting in uptake of LAM into cytoplasmic vacuoles of macrophages was necessary to show inhibition (26). No such product has been identified for *L. monocytogenes*. A second possibility is that a product secreted by the host may be induced during *L. monocytogenes* infection that renders the macrophages unresponsive. Such a macrophage deactivation protein, which blocks activation by IFN- γ and reverses preexisting activation, has been identified from tumor cell lines (27). Finally, the possibility that the sequence of IFN appearance is crucial should be considered. Indeed, both an agonist and an antagonist effect of IFN- α/β has been demonstrated on activation of human macrophages depending on the time of introduction and concentration of this IFN *in vitro* (28). Others have described a synergistic interaction between IFN on transcription of IFN-stimulating genes only when the sequence of IFN introduction was controlled (29).

Although our observations should not be generalized to other animal species or infections, we believe that the therapeutic potential of IFN should be critically evaluated in similar models before clinical trials of it in such circumstances are undertaken.

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