NOTES

Effects of Gamma Interferon and Indomethacin in Preventing *Brucella abortus* Infections in Mice

MARK G. STEVENS,* GEORGE W. PUGH, JR., AND LOUISA B. TABATABAI

*Brucellosis Research Unit, National Animal Disease Center, Agricultural Research Service,
United States Department of Agriculture, 2300 Dayton Avenue, Ames, Iowa 50010*

Increased resistance to infection with *Brucella abortus* 2308 resulted when recombinant murine gamma interferon (rMuIFN-γ) was given to mice both before and during infection but not when given only before infection. Mice given rMuIFN-γ had enhanced peritoneal and splenic macrophage bactericidal activity against *B. abortus*. Treatment of mice with rMuIFN-γ plus indomethacin did not further enhance resistance to infection or macrophage bactericidal activity compared with that after treatment of mice with rMuIFN-γ alone.

Recombinant murine gamma interferon (rMuIFN-γ) therapy enhances resistance of mice to infection with intracellular bacterial pathogens such as *Listeria monocytogenes* (11), *Mycobacterium tuberculosis* (10), and *Mycobacterium intracellulare* (6). Murine macrophages infected in vivo and in vitro with *Mycobacterium avium*, *M. intracellulare*, and *Mycobacterium leprae* also produce suppressive factors and become unresponsive to stimulation by rMuIFN-γ (5, 6, 17–19). However, this suppressive effect in *Mycobacterium*-infected mice and macrophages can be partially or completely removed by treatment with the cyclooxygenase inhibitor indomethacin (INDO) (5, 22). Macrophages from *L. monocytogenes*-infected mice also produce an immunosuppressive factor that can be blocked by incubating the cells with INDO (15). In addition, studies have shown that mice treated with INDO have increased resistance to infection with *L. monocytogenes* (3) and *M. intracellulare* (6). Collectively, these studies suggest that *L. monocytogenes*, *M. intracellulare*, and *M. avium* establish persistent infections by inducing macrophage production of prostaglandin E_2 or other immunosuppressive cyclooxygenase metabolites (5, 6, 15, 18, 22).

Brucella abortus is also an important bacterial pathogen that survives within murine, human, and bovine macrophages (14). Bovine infections with *B. abortus* are an economically important problem in the United States (4) and a worldwide public health concern, especially in countries where brucellosis in cattle remains a widespread zoonotic problem (13, 23). It is unknown whether rMuIFN-γ therapy can enhance resistance of mice to infection with *B. abortus* as has been shown to occur with infections with *L. monocytogenes* (11) and *M. intracellulare* and *M. tuberculosis* (6, 10). The modulatory role of suppressive cyclooxygenase-derived prostaglandins in murine immunity to *Brucella* infection is also unknown. In the current study, the role of IFN-γ and prostaglandins in murine immunity to *Brucella* infection was investigated by examining the capacity of rMuIFN-γ and INDO therapy to enhance resistance of mice to infection with *B. abortus*.

Drug preparation and treatment of mice. The nonglycosylated rMuIFN-γ (lot no. 2271-54-F2) was a gift from Genentech Inc. (South San Francisco, Calif.), had a specific activity of 8.0×10^6 U/ml, and contained less than 1 ng of endotoxin per ml. INDO (Sigma Chemical Co., St. Louis, Mo.) was solubilized in 95% ethanol at 10 mg/ml, filter sterilized, and stored in the dark at 4°C for no longer than 2 days before use. The rMuIFN-γ was diluted with NaCl saline (0.15 M, pH 7.2), and INDO was diluted with phosphate-buffered saline (PBS; 0.01 M, pH 7.2) immediately before the mice were injected. Male 8-week-old BALB/c mice were obtained from Charles River Breeding Laboratories Inc. (Wilmington, Mass.) and used in the experiments when they were 10 to 12 weeks old. Mice received rMuIFN-γ by an intramuscular (i.m.) injection in the femoral muscles (0.1 ml; 5,000, 50,000, or 500,000 U per mouse) and INDO by a subcutaneous injection in the dorsal lumbosacral region (0.1 ml; 2 mg/kg of body weight). Control mice were given an i.m. injection with 0.1 ml of NaCl saline and a subcutaneous injection with 0.1 ml of 0.5% ethanol in PBS.

Resistance of mice to infection with *B. abortus*. *B. abortus* 2308 was grown on potato infusion agar and prepared for infecting mice as previously described (16). Mice received between 1×10^4 and 2×10^4 viable *B. abortus* CFU in 0.2 ml of NaCl saline by an intraperitoneal injection. The number of *B. abortus* CFU per spleen was determined at 7 days after mice were infected as previously described (16). Mice given a single i.m. injection of 5,000, 50,000, or 500,000 U of rMuIFN-γ per day at 1 day before or both 1 and 2 days before infection with *B. abortus* did not have different numbers of *B. abortus* CFU per spleen compared with those in *B. abortus*-infected control mice (data not shown). Numbers of *B. abortus* CFU per spleen in mice given rMuIFN-γ (5,000, 50,000, or 500,000 U/day) plus INDO (2 mg/kg/day) at 1 day before or both 1 and 2 days before infection with *B. abortus* were not different from those in control mice or mice given rMuIFN-γ or INDO alone (data not shown). Treatment of mice with 5,000 or 50,000 U of rMuIFN-γ per day at 1 day before and again at 2 and 4 days after infection with *B. abortus* resulted in significantly ($P \leq 0.05$) decreased numbers of *B. abortus* CFU per spleen compared with those in *B. abortus*-infected but untreated control mice (Table 1). The

---

* Corresponding author.

TABLE 1. Effect of rMuIFN-γ and INDO when given to mice both before and during infection with *B. abortus*

Treatment[a]	*B. abortus* (CFU/spleen [10^7])[b]	Spleen wt (mg)[b]
Control	8.66 ± 1.80	147 ± 7
INDO	3.65 ± 0.48*	170 ± 10
IFN-γ (5,000 U)	4.36 ± 0.76*	146 ± 3
IFN-γ (5,000 U) + INDO	3.25 ± 0.51*	163 ± 11
IFN-γ (50,000 U)	2.51 ± 0.44*	140 ± 5
IFN-γ (50,000 U) + INDO	1.56 ± 0.28*	158 ± 9

[a] Mice received a daily i.m. injection of rMuIFN-γ at 1 day before and again at 2 and 4 days after infection by an intraperitoneal injection with *B. abortus* 2308. INDO (2 mg/kg) was given daily to mice by subcutaneous injection for 7 consecutive days beginning 1 day before animals were infected. Numbers of *B. abortus* CFU per spleen and spleen weights were measured at 7 days after infection.

[b] Results are expressed as mean ± standard error of the mean (n = 10). *, $P \leq 0.05$ versus control by analysis of variance and Tukey's honesty sum difference test for multiple group comparisons (20).

TABLE 2. Spleen weights and splenic and peritoneal macrophage bactericidal activities against *B. abortus* following treatment of mice with rMuIFN-γ

U of IFN-γ[a]	Time (h) after IFN-γ injection	Spleen wt (mg)[b]	% Bacteria killed[b] by:	
			Spleen macrophages	Peritoneal macrophages
0 (control)	24	82 ± 3	14.86 ± 2.08	20.37 ± 1.82
5,000		93 ± 5	21.30 ± 4.92	25.57 ± 1.77
50,000		104 ± 4*	19.14 ± 1.83	31.70 ± 2.45*
500,000		114 ± 2*	24.01 ± 2.61	46.63 ± 2.69*
0 (control)	48	89 ± 4	6.91 ± 3.46	16.78 ± 8.72
5,000		115 ± 14	14.67 ± 2.57	24.87 ± 3.62
50,000		122 ± 2	26.19 ± 1.75*	42.31 ± 5.94*
500,000		145 ± 11*	30.39 ± 7.83*	55.83 ± 8.38*

[a] Mice received an i.m. injection of rMuIFN-γ. Spleen weights and splenic and peritoneal macrophage bactericidal activities against *B. abortus* 2308 were measured 24 and 48 h later.

[b] Results are expressed as mean ± standard error of the mean (n = 6). *, $P \leq 0.05$ versus control at 24 or 48 h by analysis of variance and Tukey's honesty sum difference test for multiple group comparisons (20).

numbers of CFU per spleen were also significantly ($P \leq$ 0.05) decreased by daily treatment of mice with 2 mg of INDO per kg for 7 consecutive days beginning 1 day before infection of mice with *B. abortus*. Treatment of mice with 5,000 or 50,000 U of rMuIFN-γ plus 2 mg of INDO per kg did not further decrease the numbers of CFU per spleen compared with numbers in mice treated with rMuIFN-γ or INDO alone. Spleen weights in *B. abortus*-infected mice that received rMuIFN-γ, INDO, or rMuIFN-γ and INDO were not different from those in infected control mice (Table 1).

Macrophage bactericidal activity against *B. abortus*. Erythrocyte-free peritoneal and spleen cell suspensions were prepared from mice as previously described (1, 12). The cells were suspended in RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) containing 5% fetal bovine serum (HyClone Laboratories, Inc., Logan, Utah). A 100-μl aliquot containing 10^5 peritoneal cells or 5 × 10^5 spleen cells was added to three separate flat-bottom wells of a 96-well microtiter plate. Plates were incubated for 2 h at 37°C in 5% CO_2, and the nonadherent cells were removed by washing. Cultures of *B. abortus* 2308 were prepared as previously described (16). Fifty microliters of RPMI containing 5% fetal bovine serum and 2 × 10^6 *B. abortus* CFU was added to each of the three wells containing the macrophages, which resulted in approximately 20 bacteria per macrophage. Bacteria were pelleted onto the macrophage monolayers by centrifuging plates at 1,600 × g for 5 min. The plates and the *B. abortus* suspension added to macrophages were incubated for 1 h at 37°C. The macrophages were then lysed by adding 50 μl of 0.2% saponin (Sigma) in PBS. The tetrazolium compound 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT; Sigma) was added, and the number of *B. abortus* CFU that were killed by macrophages was quantitated by colorimetrically measuring conversion of MTT to formazan by live *B. abortus* as previously described (21).

Bactericidal activity in vitro against *B. abortus* by peritoneal macrophages, but not splenic macrophages, was significantly ($P \leq 0.05$) increased at 24 h after mice received 50,000 or 500,000 U of rMuIFN-γ (Table 2). At 48 h after mice received 50,000 and 500,000 U of rMuIFN-γ, both splenic and peritoneal macrophage bactericidal activities were significantly ($P \leq 0.05$) increased compared with those of macrophages from untreated control mice. Treatment of mice with 5,000, 50,000, or 500,000 U of rMuIFN-γ resulted in a dose-dependent increase in spleen weights at 24 and 48

h after treatment (Table 2). Spleen weights were significantly ($P \leq 0.05$) increased at 24 h after mice received 50,000 or 500,000 U and at 48 h after animals received 500,000 U ($P \leq$ 0.05). The enlarged spleens of mice given rMuIFN-γ were normal when examined histologically (data not shown). Spleen weights and in vitro splenic and peritoneal macrophage bactericidal activities returned to control levels at 72 h after mice received rMuIFN-γ (data not shown). Mice treated with rMuIFN-γ (5,000, 50,000, or 500,000 U) plus 2 mg of INDO per kg did not have spleen weights or in vitro splenic and peritoneal macrophage bactericidal activities at 24, 48, and 72 h after treatment that were different from those of mice given rMuIFN-γ alone (data not shown).

This study showed that rMuIFN-γ or INDO therapy enhanced resistance of mice to *B. abortus* infection as assessed by measurement of decreased numbers of *B. abortus* CFU per spleen in infected animals. Mice given rMuIFN-γ plus INDO did not have further enhanced resistance compared with mice given rMuIFN-γ or INDO alone. Effective treatment with rMuIFN-γ (5,000 or 50,000 U per mouse) occurred when it was given to mice at 1 day before and again at 2 and 4 days after infection with *B. abortus*. Mice given low (5,000-U), moderate (50,000-U), or very high (500,000-U) concentrations of rMuIFN-γ at 1 day before or both 1 and 2 days before infection with *B. abortus* did not have increased resistance to infection. These results suggest that rMuIFN-γ is an effective therapeutic agent when given both before and during infection with *B. abortus* but not when given only before infection.

In initial studies, it was established that daily treatment of mice with 2 mg of INDO per kg for 2 days decreased cyclooxygenase activity in spleen cells by 80 to 90% as assessed by measuring cyclooxygenase production of prostaglandin E_2 by a radioimmunoassay (unpublished data). Mice were given 2 mg of INDO per kg daily for no longer than 7 consecutive days because this is the maximum nontoxic treatment regimen that can be used in these animals (3, 9). Daily treatment of mice for 7 consecutive days with INDO beginning 1 day before animals were infected with *B. abortus* decreased the number of CFU per spleen. These results suggest that inhibition of cyclooxygenase activity by INDO in *B. abortus*-infected mice increases clearance of

bacteria from the spleen. The reduction of bacteria in the spleen that occurred with seven consecutive daily injections of mice with INDO in our study was very similar to that seen when we gave mice three injections of rMuIFN-γ during the same 7-day interval. Similar results have been reported in other studies that have shown that INDO when given daily for 7 days to mice decreases *M. intracellulare* infection of the spleen and lung in a similar manner as did daily treatment of mice for 7 days with rMuIFN-γ (6). It has also been shown that mice given INDO daily for 6 days have enhanced survival of infection with *L. monocytogenes* (3). These studies indicate that INDO treatment of *Listeria*- and *Mycobacterium*-infected mice increases bacterial clearance. Our results suggest that INDO also provides a similar benefit when given to *Brucella*-infected mice.

INDO is a potent inhibitor of cyclooxygenase activity (7), and it is widely believed that its immunostimulatory action results from inhibiting production of prostaglandin E_2 and other cyclooxygenase-derived suppressive prostaglandins (2, 8). In the current study, INDO or rMuIFN-γ therapy alone effectively enhanced resistance of mice to infection with *B. abortus*. However, combined therapy with INDO and rMuIFN-γ was no more effective than either of these treatments used alone. A possible explanation for these results is that INDO, by presumably blocking production of cyclooxygenase-derived suppressive prostaglandins, enabled increased endogenous cytokine production to occur in *B. abortus*-infected mice. Thus, INDO-treated mice may have been able to produce sufficient levels of endogenous cytokines to combat the *B. abortus* infection effectively; therefore, the additional treatment of these animals with exogenous rMuIFN-γ was unable to produce any further therapeutic effect. Others have proposed that INDO enhances resistance of mice to *Mycobacterium* infection by blocking cyclooxygenase production of prostaglandin E_2, which subsequently enhances endogenous production of IFN-γ and other cytokines that stimulate macrophage bactericidal activity against *Mycobacterium* infection (6).

We showed that a single injection of 50,000 or 500,000 U of rMuIFN-γ per mouse stimulated the immune system, as evident by splenomegaly and increased splenic and peritoneal macrophage bactericidal activity against *B. abortus*. However, these effects were transient in that they persisted for no longer than 48 h. This transiently enhanced macrophage bactericidal activity induced by rMuIFN-γ may explain why rMuIFN-γ enhanced resistance when given to mice both before and during the *B. abortus* infection but not when given only before infection. Perhaps continued periodic treatment of *B. abortus*-infected mice with rMuIFN-γ during the infection is required to sustain enhanced macrophage bactericidal activity that aids in eliminating the bacteria.

REFERENCES

1. **Antonelli, G., and F. Dianzani.** 1986. Antigen presentation by specifically sensitized macrophages in interferon-γ induction. J. Interferon Res. **6**:535–542.
2. **Bonta, I. L., and M. J. Parnham.** 1982. Immunomodulatory-antiinflammatory functions of E-type prostaglandins. Minireview with emphasis on macrophage-mediated effects. Int. J. Immunopharmacol. **4**:103–109.
3. **Boorman, G. A., M. I. Luster, J. H. Dean, and R. W. Luebke.** 1982. Effect of indomethacin on the bone marrow and immune system of the mouse. J. Clin. Lab. Immunol. **7**:119–126.
4. **Crawford, R. P., L. G. Adams, J. D. Williams, and R. A. Dietrich.** 1990. Economic losses attributable to brucellosis, p. 357–372. *In* L. G. Adams (ed.), Advances in brucellosis research. Texas A&M University Press, College Station.
5. **Denis, M., and E. O. Gregg.** 1991. Modulation of *Mycobacterium avium* growth in murine macrophages: reversal of unresponsiveness to interferon-gamma by indomethacin or interleukin-4. J. Leukocyte Biol. **49**:65–72.
6. **Edwards, C. K., H. B. Hedegaard, A. Zlotnik, P. R. Gangadharam, R. B. Johnston, and M. J. Pabst.** 1986. Chronic infection due to *Mycobacterium intracellulare* in mice: association with macrophage release of prostaglandin E_2 and reversal by injection of indomethacin, muramyl dipeptide or interferon-γ. J. Immunol. **136**:1820–1826.
7. **Flower, R. J.** 1974. Drugs which inhibit prostaglandin biosynthesis. Pharmacol. Rev. **26**:33–67.
8. **Goodwin, J. S., and J. Ceuppens.** 1983. Regulation of the immune response by prostaglandins. J. Clin. Immunol. **3**:295–315.
9. **Hart, D. A.** 1985. Increased sensitivity of *Corynebacterium parvum*-treated mice to toxic effects of indomethacin and lipopolysaccharide. Infect. Immun. **47**:408–414.
10. **Khor, M., D. B. Lowrie, A. R. M. Coates, and D. A. Mitchison.** 1986. Recombinant interferon-gamma and chemotherapy with isoniazid and rifampicin in experimental murine tuberculosis. Br. J. Exp. Pathol. **67**:587–596.
11. **Kiderlen, A. F., S. H. Kaufmann, and M. L. Lohmann.** 1984. Protection of mice against the intracellular bacterium *Listeria monocytogenes* by recombinant immune interferon. Eur. J. Immunol. **14**:964–967.
12. **Maier, T., and J. H. Holda.** 1987. Natural suppressor (NS) activity from murine neonatal spleen is responsive to IFN-γ. J. Immunol. **138**:4075–4084.
13. **Matyas, Z., and T. Fujikura.** 1984. Brucellosis as a world problem. Dev. Biol. Stand. **56**:3–20.
14. **Nicoletti, P., and A. J. Winter.** 1990. The immune response to *B. abortus*: the cell-mediated response to infections, p. 83–95. *In* K. Nielsen and J. R. Duncan (ed.), Animal brucellosis. CRC Press, Inc., Boca Raton, Fla.
15. **Petit, J. C., G., Richard, B. Burghoffer, and G. L. Daguet.** 1985. Suppression of cellular immunity to *Listeria monocytogenes* by activated macrophages: mediation by prostaglandins. Infect. Immun. **49**:383–388.
16. **Pugh, G. W., Jr., L. B. Tabatabai, B. J. Bricker, J. E. Mayfield, M. Phillips, T. J. McDonald, and E. S. Zehr.** 1989. Identification of a virulence factor for *Brucella abortus* infection in BALB/c mice. Am. J. Vet. Res. **50**:887–892.
17. **Sibley, L. D., L. B. Adams, and J. L. Krahenbuhl.** 1990. Inhibition of interferon-gamma-mediated activation in mouse macrophages treated with lipoarabinomannan. Clin. Exp. Immunol. **80**:141–148.
18. **Sibley, L. D., S. W. Hunter, P. J. Brennan, and J. L. Krahenbuhl.** 1988. Mycobacterial lipoarabinomannan inhibits gamma interferon-mediated activation of macrophages. Infect. Immun. **56**:1232–1236.
19. **Sibley, L. D., and J. L. Krahenbuhl.** 1987. *Mycobacterium leprae*-burdened macrophages are refractory to activation by gamma interferon. Infect. Immun. **55**:446–450.
20. **Steel, R. G. D., and J. H. Torrie.** 1960. Principles and procedures of statistics, p. 109–114. McGraw-Hill Book Co., New York.
21. **Stevens, M. G., M. E. Kehrli, Jr., and P. C. Canning.** 1991. A colorimetric assay for quantitating bovine neutrophil bactericidal activity. Vet. Immunol. Immunopathol. **28**:45–56.
22. **Tomioka, H., H. Saito, and Y. Yamada.** 1990. Characteristics of immunosuppressive macrophages induced in spleen cells by *Mycobacterium avium* complex infections in mice. J. Gen. Microbiol. **136**:965–973.
23. **Young, E. J.** 1983. Human brucellosis. Rev. Infect. Dis. **5**:821–842.