INFECTION AND IMMUNITY, Jan. 1988, p. 283–286
0019-9567/88/010283-04$02.00/0

Vol. 56, No. 1

Recombinant Murine Gamma Interferon Inhibits *Chlamydia trachomatis* Serovar L1 In Vivo†

GUANGMING ZHONG,[1] ELLENA M. PETERSON,[1] CHRISTINE W. CZARNIECKI,[2] AND LUIS M. DE LA MAZA[1]*

Department of Pathology, University of California Irvine, Irvine, California 92717,[1] and Department of Pharmacological Sciences, Genentech, Inc., South San Francisco, California 94080[2]

Received 31 July 1987/Accepted 23 September 1987

***Chlamydia trachomatis* serovar L1 injected intravenously in mice resulted in systemic nonlethal infections of the animals. Treatment of mice with recombinant murine gamma interferon resulted in a decrease in the number of infectious *C. trachomatis* organisms recovered from the lungs, spleens, and livers as well as in a decrease of the inflammatory reaction in those organs when assessed 3 and 5 days after challenge.**

The genus *Chlamydia* consists of two species, *Chlamydia psittaci* and *Chlamydia trachomatis*. *C. trachomatis* is the most prevalent sexually transmitted pathogen in the Western world and can cause both local and systemic infections (15). In 1963, when chlamydiae were still classified as viruses, Sueltenfuss and Pollard demonstrated that the intracellular replication of *C. psittaci* could be inhibited in cells treated with interferons (IFNs; 17). Since then, evidence from several laboratories, including our own, indicates that the growth and infectious yield of both *C. psittaci* and *C. trachomatis* can be inhibited in cell cultures treated with either crude (3, 10, 14) or recombinant (5, 6, 13) IFN preparations. Specifically, we have shown that the replication of *C. trachomatis* can be significantly reduced in murine (McCoy strain) cells that have been treated with recombinant murine gamma IFN (rMuIFN-γ) either before or after infection (6). Like IFN-induced antiviral activities, the in vitro antimicrobial activities of IFN-γ appear to be mediated through effects on the host cell rather than on the pathogen. This study was designed to assay the in vivo antichlamydial activity of rMuIFN-γ with a nonlethal murine model of systemic infection with *C. trachomatis* (1).

rMuIFN-γ was cloned and expressed in *Escherichia coli* (2, 8) at Genentech, Inc., South San Francisco, Calif. Endotoxin levels, measured by a *Limulus* amoebocyte lysate assay, were <2 endotoxin units per mg. For injection into mice, the rMuIFN-γ preparations were diluted with phosphate-buffered saline (PBS; 0.01 M, pH 7.4) immediately before administration. *C. trachomatis* serovar L1 (strain 440) was grown in HeLa 229 cells and was stored as previously described (6, 7). BALB/c female mice 6 to 7 weeks old and weighing 15 to 18 g were obtained from Simmonsen Laboratories, Gilroy, Calif. Mice were injected intraperitoneally with 10 μg of rMuIFN-γ or PBS 24 h before and 24 h after infection. At the time of infection, rMuIFN-γ (10 μg per mouse) or PBS was mixed with an inoculum of 10^6 chlamydial inclusion-forming units (IFUs) and injected into the tail vein. Since it has been clearly demonstrated that the inhibitory effects of IFNs on chlamydial replication are exerted through effects on the host cell and not through effects on the organism (5, 9, 10), the number of infectious organisms delivered to control and rMuIFN-γ-treated mice was assumed to be equivalent. Groups of eight or nine animals were sacrificed by ether administration on day 3 or 5 postinfection, and the liver, spleen, and lungs of each animal were aseptically removed. These days were chosen to allow examination of the effects of treatment on the primary burst of infection and on reinfection (judged by in vitro data, the duration of the chlamydial growth cycle is approximately 48 h). Organs were weighed and suspended in 5 ml of sucrose phosphate buffer (1). Tissues were homogenized with a Stomacher Lab Blender 80 (Dynatech Laboratories, Inc., Alexandria, Va.) and were sonicated for 15 s with a Braun-Sonic 2000 ultrasonic system. Gross debris was removed by centrifugation at 300 × *g* for 10 min at room temperature. Infectious chlamydiae recovered from these organs were quantified in the supernatant by titration in McCoy cells (Viromed, Minneapolis, Minn.) and HeLa 229 cells (American Type Culture Collection, Rockville, Md.) grown in Eagle minimal essential medium with 5% fetal bovine serum and 50 μg of gentamicin per ml. Samples run in parallel in McCoy cells and in HeLa 229 cells, a human cell line in which murine IFN shows no biological activity (16), gave similar results, indicating that no significant amounts of circulating murine IFN were present in the samples. Because of these results, we performed the rest of the assays in McCoy cells. The McCoy cell monolayers were grown in 24-well plastic plates and infected by the addition of inoculum (100 μl per well) and centrifugation at 1,000 × *g* for 1 h at room temperature. Following centrifugation, 1 ml of Eagle minimal essential medium with 5% fetal bovine serum and 50 μg of gentamicin and 2 μg of cycloheximide per ml was added to each well. At 48 h postinfection, the cells were fixed with methanol, stained, and counted as previously described (5, 6). To analyze the histopathological changes, portions of the liver, spleen, and lungs were fixed in 10% Formalin, embedded in paraffin, sectioned, and stained with hematoxylin-eosin. To evaluate serological responses, blood was collected from the animals by retro-orbital puncture when they were sacrificed. Serum samples were titrated for specific antichlamydial antibodies by an indirect immunofluorescence assay, with McCoy cells infected with *C. trachomatis* serovar L1 as an antigen and fluorescein isothiocyanate-conjugated rabbit anti-mouse immunoglobulin. The two-tailed Student *t* test was used for comparison of IFN-treated and control animals. Both IFUs and antibody titers were transformed to $\log_{10}$ for statistical analysis.

* Corresponding author.
† Genentech contribution number 854.

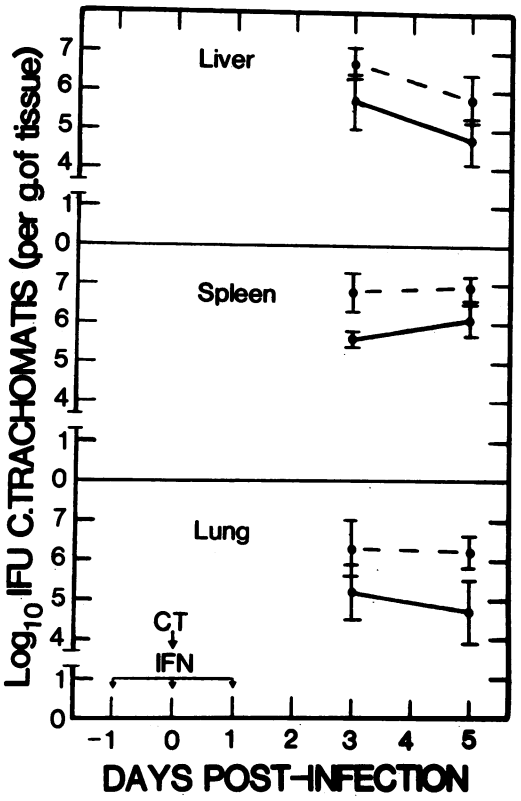

FIG. 1. Effect of rMuIFN-γ on the growth of *C. trachomatis* in mice tissues. Values are titers of *C. trachomatis* in control mice (- - - -) and rMuIFN-γ-treated mice (——). Values are means from eight or nine mice per time point, and vertical lines indicate 1 standard deviation from the mean. The experiments were performed twice with groups of four or five animals each. CT, *C. trachomatis* infection.

Livers from mice receiving PBS yielded 3.9×10^6 IFU/g of tissue at 3 days postinfection and 5.7×10^5 IFU/g of tissue at 5 days postinfection (Fig. 1). In contrast, mice receiving rMuIFN-γ had a significant decrease in the yield of chlamydial infectious particles. The yield at 3 days postinfection was 4.9×10^5 IFU/g of tissue ($P < 0.01$), and at 5 days the yield was 4.9×10^4 IFU/g of tissue ($P < 0.002$). Similar results were obtained with spleens and lungs. We recovered 5.9×10^6 chlamydial IFU/g of spleen from the control mice at 3 days, whereas the yield from animals treated with rMuIFN-γ was 3.8×10^5 IFU/g of tissue ($P < 0.001$). At 5 days, there was a 0.69 $\log_{10}$ reduction of chlamydial IFUs between the rMuIFN-γ-treated and control groups of mice ($P < 0.001$). From the lungs of the PBS-treated mice, the yields were 2.2×10^6 and 1.5×10^6 IFU/g of tissue at 3 and 5 days, respectively, whereas from the mice treated with rMuIFN-γ, we recovered only 1.6×10^5 ($P < 0.01$) and 5.1×10^4 ($P < 0.001$) IFU/g of tissue, respectively. Serum samples collected on days 3 and 5 postinfection were assayed for the presence of specific chlamydial antibodies. At 3 days postinfection, lower antibody titers were obtained from mice treated with rMuIFN-γ (geometric mean titer, 10.7 ± 3.2) than from the PBS-treated control group (36.2 ± 2; $P < 0.05$). There was no statistically significant difference between the geometric mean titers of the chlamydial antibodies of the two groups at 5 days (control group, 67.3 ± 2.05, versus rMuIFN-γ group, 56.5 ± 1.45; $P < 0.60$). Liver sections from the control mice treated with PBS showed numerous foci of polymorphonuclear leukocytes and mononuclear cells in the liver parenchyma, particularly in the animals sacrificed on day 3 postinfection (Fig. 2A and B). The animals treated with rMuIFN-γ showed a minimal inflammatory reaction (Fig. 2C). Similarly, sections from the lungs of the control animals showed marked to moderate focal infiltrates of neutrophilic leukocytes and mononuclear cells (Fig. 2D and E), whereas the animals treated with rMuIFN-γ had only mild focal inflammatory reactions (Fig. 2F). Sections from the spleen also had more marked infiltrates, with neutrophils and hyperplasia of the white pulp, in the control animals than in those treated with rMuIFN-γ.

Although the inhibitory effects of IFNs on the replication of chlamydiae have been convincingly demonstrated in vitro (3–7, 10, 13, 14, 17), little is known about the in vivo effects of IFNs on chlamydial replication. In the early 1970s, Oh et al. (12) and Kazar et al. (10) observed the effects of poly(dI-dC) and Newcastle disease virus, both IFN inducers, on in vivo chlamydial infections. Oh et al. (12) injected the B trachoma strain into the anterior chamber of the rabbit eye and produced ocular lesions similar to those of human trachoma. When rabbits were injected intravenously with poly(dI-dC), there was a suppression of the ocular lesions, but there was no change in the amount of infectious *C. trachomatis* recovered from tissues. These authors also found that a single injection of poly(dI) or poly(dC) was also protective, although there was no increase in circulating IFN. From these findings, they concluded that factors other than IFNs may play a role in the suppression of the trachoma agent by poly(dI-dC). Similarly, Kazar et al. (10) showed that intranasal treatment of mice with poly(dI-dC) or Newcastle disease virus delayed by 2 or 3 days the death of mice given aerosolized *C. trachomatis* MRC1/G. However, the yields of infectious chlamydiae from the lungs of mice treated with poly(dI-dC) or Newcastle disease virus were no different from those of the controls. Furthermore, when Newcastle disease virus was injected intravenously, death from chlamydial infection was accelerated despite increasing levels of IFN in the lungs. Because of this apparent contradiction, these authors also postulated that the protection resulting from IFN inducers was mediated by mechanisms other than IFN.

Our results strongly indicate that IFN-γ plays a significant role in limiting in vivo infection caused by *C. trachomatis*. Furthermore, the fact that we were able to obtain a significant reduction in the number of *C. trachomatis* infective units found in the three organs assayed suggests that the rMuIFN-γ-induced activity can be expressed at multiple sites. The apparent contradiction between our results and those of Oh et al. (12) and Kazar et al. (10) may be due to the types or concentrations of IFNs achieved in the tissues. It is also possible that the *C. trachomatis* serovars assayed vary in their response in the three systems. The *C. trachomatis* L1 serovar used in this study infects mononuclear cells, and IFN-γ has been shown to be a profound activator of the antimicrobial activity of this type of cell (11). The trachoma serovars grow mainly in epithelial cells that may not be highly sensitive to the antichlamydial activity of the IFNs. In support of a role for IFN-γ in the control of chlamydial infections, Brunham et al. (1) found that resistance to infection by *C. trachomatis* could be passively transferred with spleen cells but not with serum and postulated that IFN-γ released by T lymphocytes could be the active mediator. Similarly, with athymic nude mice, Williams et al. (18) have shown that T cells play an important role in

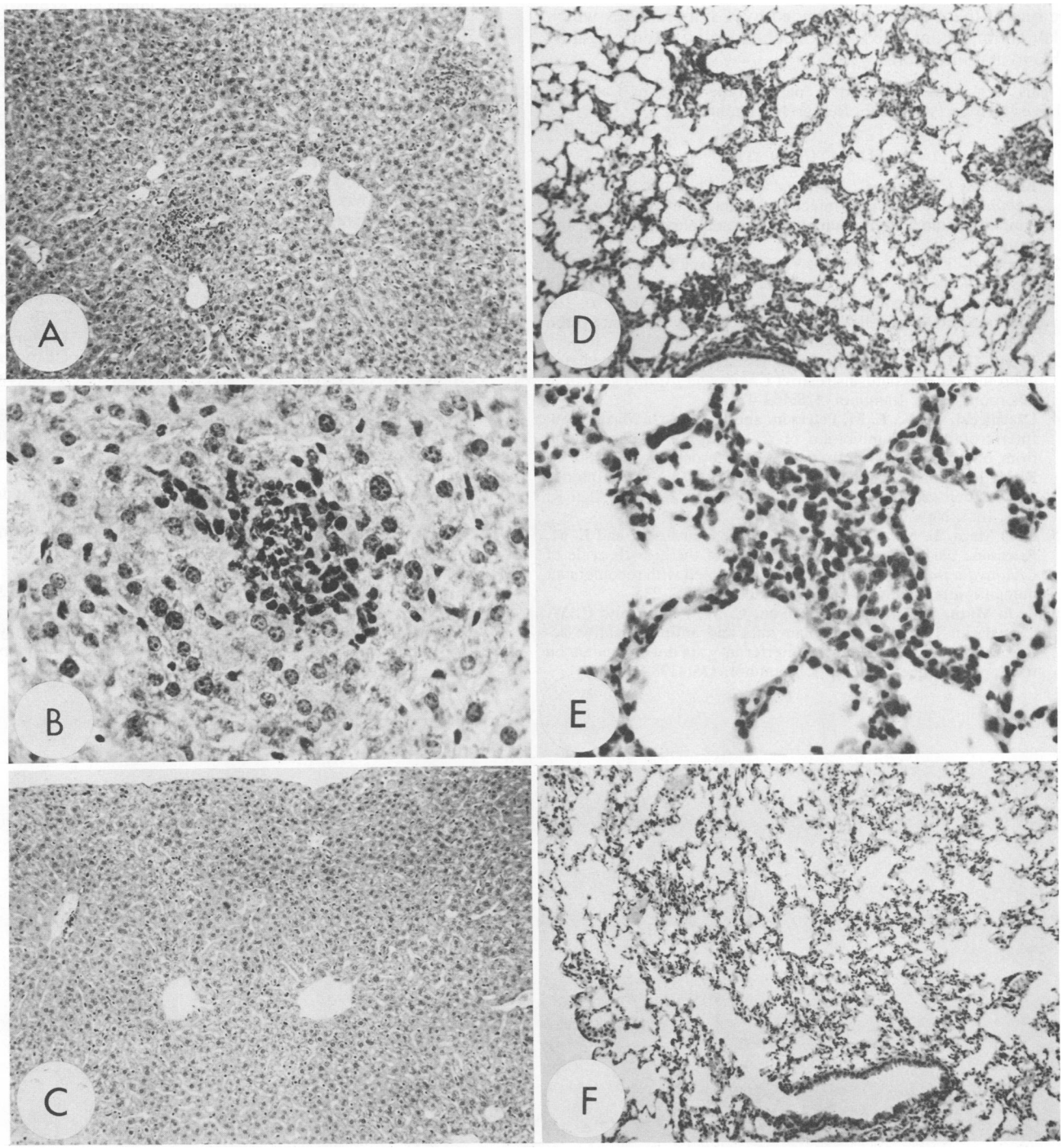

FIG. 2. Histological sections of livers from control (A [×300] and B [×800]) and rMuIFN-γ-treated (C [×300]) animals sacrificed 3 days postinfection. Focal infiltrates of polymorphonuclear leukocytes and mononuclear cells can be observed in sections from the control animals. Lung sections of control (D [×300] and E [×800]) and rMuIFN-γ-treated (F [×300]) mice sacrificed 5 days postinfection are also shown. Patchy interstitial infiltrates can be seen in the lungs of PBS-treated mice.

defense against *C. trachomatis* pneumonia. IFN-γ has been shown to effectively activate macrophages and other cells of the immune system to destroy chlamydiae in vitro (3, 13, 14). These reports and our results suggest that IFN-γ may indeed be one of the mediators produced by T lymphocytes which play an important role in the mechanism of defense against chlamydial infections.

This work was supported in part by the State of California Universitywide Pacific Rim Research Program.

LITERATURE CITED

1. **Brunham, C. R., C. C. Kuo, and W.-J. Chen.** 1985. Systemic *Chlamydia trachomatis* infection in mice: a comparison of lymphogranuloma venereum and trachoma biovars. Infect. Immun. **48**:78–82.
2. **Burton, L. E., P. W. Gray, D. V. Goeddel, and E. Rinderknecht.** 1984. Modifications of recombinant human and murine IFN-γ and their biochemical characteristics, p. 403–409. *In* H. Kirchner and H. Schellekens (ed.), The biology of the interferon system. Elsevier Biomedical Press, Amsterdam.
3. **Byrne, G. I., and C. L. Faubion.** 1982. Lymphokine-mediated microbistatic mechanisms restrict *Chlamydia psittaci* growth in macrophages. J. Immunol. **128**:469–474.
4. **Czarniecki, C. W., E. M. Peterson, and L. M. de la Maza.** 1986. Interferon-induced inhibition of *C. trachomatis*: dissociation from other biological activities of interferons, p. 467–480. *In* R. M. Friedman, T. Merigan, and T. Sreevalsan (ed.), Interferons as cell growth inhibitors and antitumor factors. Alan R. Liss, Inc., New York.
5. **de la Maza, L. M., J. M. Goebel, C. W. Czarniecki, and E. M. Peterson.** 1984. Ultrastructural analysis of the growth cycle of *Chlamydia trachomatis* in mouse cells treated with recombinant human α-interferons. Exp. Mol. Pathol. **41**:227–235.
6. **de la Maza, L. M., E. M. Peterson, C. W. Fennie, and C. W. Czarniecki.** 1985. The antichlamydial and antiproliferative activities of recombinant murine interferon-γ are not dependent on tryptophan concentrations. J. Immunol. **135**:4198–4200.
7. **de la Maza, L. M., E. M. Peterson, J. M. Goebel, C. W. Fennie, and C. W. Czarniecki.** 1985. Interferon-induced inhibition of *Chlamydia trachomatis*: dissociation from antiviral and antiproliferative effects. Infect. Immun. **47**:719–722.
8. **Gray, P. W., and D. V. Goeddel.** 1983. Cloning and expression of murine immune interferon cDNA. Proc. Natl. Acad. Sci. USA **80**:5842–5846.
9. **Hanna, L., T. C. Merigan, and E. Jawetz.** 1966. Inhibition of TRIC agents by virus-induced interferons. Proc. Soc. Exp. Biol. Med. **122**:417–421.
10. **Kazar, J., J. D. Gillmore, and F. B. Gordon.** 1971. Effect of interferon and interferon inducers on infections with a nonviral intracellular microorganism, *Chlamydia trachomatis*. Infect. Immun. **3**:825–832.
11. **Murray, H. W., G. L. Spitalny, and C. F. Nathan.** 1985. Activation of mouse peritoneal macrophages in vitro and in vivo by interferon-γ. J. Immunol. **134**:1619–1622.
12. **Oh, J. O., H. B. Ostler, and J. Schachter.** 1970. Protective effect of a synthetic polynucleotide complex (poly I:C) on ocular lesions produced by trachoma agent in rabbits. Infect. Immun. **1**:566–573.
13. **Rothermel, C. D., B. Y. Rubin, E. A. Jaffe, and H. W. Murray.** 1986. Oxygen independent inhibition of intracellular *Chlamydia psittaci* growth by human monocytes and interferon-γ-activated macrophages. J. Immunol. **137**:689–692.
14. **Rothermel, C. D., B. Y. Rubin, and H. W. Murray.** 1983. γ-Interferon is the factor in lymphokine that activates human macrophages to inhibit intracellular *Chlamydia psittaci* replication. J. Immunol. **131**:2542–2544.
15. **Schachter, J., and C. R. Dawson.** 1978. Human chlamydial infections. PSG Publishing Co., Littledon, Mass.
16. **Stewart, W. E.** 1979. The interferon system, p. 134–145. Springer-Verlag, New York.
17. **Sueltenfuss, E. A., and M. Pollard.** 1963. Cytochemical assay of interferon produced by duck hepatitis virus. Science **139**: 595–596.
18. **Williams, D. M., J. Schachter, D. J. Drutz, and C. V. Sumoya.** 1981. Pneumonia due to *Chlamydia trachomatis* in the immunocompromised (nude) mouse. J. Infect. Dis. **143**:238–241.