INFECTION AND IMMUNITY, Oct. 1989, p. 3254–3256
0019-9567/89/103254-03$02.00/0

Vol. 57, No. 10

Beta Interferon Inhibits *Toxoplasma gondii* Growth in Human Monocyte-Derived Macrophages

JOHN L. SCHMITZ,[1] JOSEPH M. CARLIN,[1] ERNEST C. BORDEN,[2] AND GERALD I. BYRNE[1]*

Department of Medical Microbiology and Immunology[1] and Department of Human Oncology and Medicine,[2] University of Wisconsin Medical School, Madison, Wisconsin 53706

Received 5 May 1989/Accepted 17 July 1989

Gamma interferon (IFN-γ) previously has been shown to block the replication of *Toxoplasma gondii* in fibroblasts by the induction of indoleamine 2,3-dioxygenase (IDO) activity. IFN-β also is known to induce IDO activity in monocyte-derived macrophages, but its ability to block the growth of *T. gondii* has not been demonstrated. We found not only that the combination of IFN-β and lipopolysaccharide induced greater IDO activity in monocyte-derived macrophages than did IFN-β alone but that this combination also was effective in inhibiting the growth of *T. gondii*. In addition, the inhibition was reversed by the addition of exogenous tryptophan, thus demonstrating that a mechanism by which IFN-β inhibited *T. gondii* replication was by the induction of IDO.

Gamma interferon (IFN-γ) has been shown to inhibit the multiplication of several intracellular pathogens, including *Chlamydia psittaci* (1, 11) and *Toxoplasma gondii* (10). This inhibitory effect has been attributed to the induction of indoleamine 2,3-dioxygenase (IDO) activity within cells treated with IFN-γ (2, 3, 8, 9). IDO has been shown to mediate the inhibition by catalyzing the decyclization of tryptophan to *N*-formylkynurenine (12), thus depriving the infecting organism of the essential amino acid tryptophan (9). Degradation of trytophan as the cause of intracellular pathogen inhibition has been confirmed by experiments showing that the addition of exogenous tryptophan restored the intracellular growth of the infecting organism (2, 3, 8, 9). Although IFN-α and IFN-β also have been shown to induce IDO activity (3–5), their ability to inhibit the intracellular growth of certain organisms has not been well characterized (6, 8).

IDO-mediated inhibition of the growth of intracellular pathogens has been shown to be dependent on both the host cell type and the class of IFN used. The experiments of Pfefferkorn et al. (9) provided strong evidence that the induction of tryptophan decyclization, and not direct toxic effects of IFN-γ or the tryptophan metabolites *N*-formylkynurenine and kynurenine, resulted in diminished intracellular replication of *T. gondii* in fibroblasts. In a previous report (8), type I IFNs failed both to induce IDO activity and to inhibit the growth of *T. gondii*. However, in contrast to epithelial cells and fibroblasts, peripheral blood monocytes and monocyte-derived macrophages responded to all classes of IFN with IDO induction (4, 5, 7). Although Murray et al. (6) were unable to demonstrate IFN-β-mediated inhibition of *T. gondii* in human macrophage cultures, subsequent work with *Chlamydia psittaci* (3) showed that the combination of IFN-β and lipopolysaccharide (LPS) was effective in inhibiting the intracellular growth of this organism in monocyte-derived macrophages. To determine if the combined effects of IFN-β and LPS are specific for *C. psittaci* or are more general, we examined the effect of IFN-β plus LPS on the growth of *T. gondii*. We found that the combination effectively inhibited the growth of this intracellular parasite in human monocyte-derived macrophages. Furthermore, this effect appeared at least in part mediated by the induction of IDO activity.

Human peripheral blood mononuclear cells were isolated from Ficoll-sodium diatrizoate (Histopaque 1077; Sigma Chemical Co., St. Louis, Mo.) gradients, washed, and suspended to a density of 10^7 cells per ml in RPMI 1640 medium (Hazelton Research Products, Inc., Lenexa, Kans.) supplemented with 10% fetal bovine serum (Sterile Systems, Logan, Utah), 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer, 100 μg of streptomycin sulfate per ml, and 10 μg of gentamicin sulfate per ml (Sigma). Aliquots (100 μl) were added to 12-mm glass cover slips and incubated for 2 h at 37°C. Nonadherent cells were removed by washing with Hanks balanced salt solution (HBSS), and cover slips with adherent monocytes were incubated in 24-well tissue culture plates at 37°C in 5% CO_2–95% air for 10 to 14 days to allow maturation of macrophages.

For quantitation of IDO activity in macrophages, the medium from triplicate wells was aspirated and cells were incubated with culture medium alone, medium containing recombinant human IFN-γ (20 ng/ml; Biogen, Cambridge, Mass.), medium containing recombinant human IFN-β_{Ser} (10 ng/ml; Triton Biosciences, Alameda, Calif.), medium containing LPS (0.1 ng/ml; *Escherichia coli* O26:B6; Difco Laboratories, Detroit, Mich.), or medium containing LPS and IFN-β at the same concentrations as those added to wells separately. Treated cells were incubated for 48 h. The culture medium was aspirated and replaced with 0.4 ml of medium containing 25 μM tryptophan in HBSS with 1 μCi of [^{3}H]tryptophan (specific activity, 20 Ci/mmol; Research Products International Corp., Mount Prospect, Ill.) per ml. Three additional wells without cells received radiolabeled medium to determine the amount of nonspecific tryptophan degradation. A reversed-phase high-performance liquid chromatographic technique (13) was used to detect tryptophan degradation. Aliquots (50 μl) of culture supernatant fluids were injected into a μBondapak C_{18} column (inner diameter, 30 cm by 3.9 mm; Waters Associates, Inc., Milford, Mass.) and eluted with 10% methanol in 1 mM KH_2PO_4 buffer (pH 4.0) at a flow rate of 1.6 ml/min. Tryptophan metabolites were detected by measuring the A_{265} and by flowthrough scintillation spectrometry. Specific tryptophan

* Corresponding author.

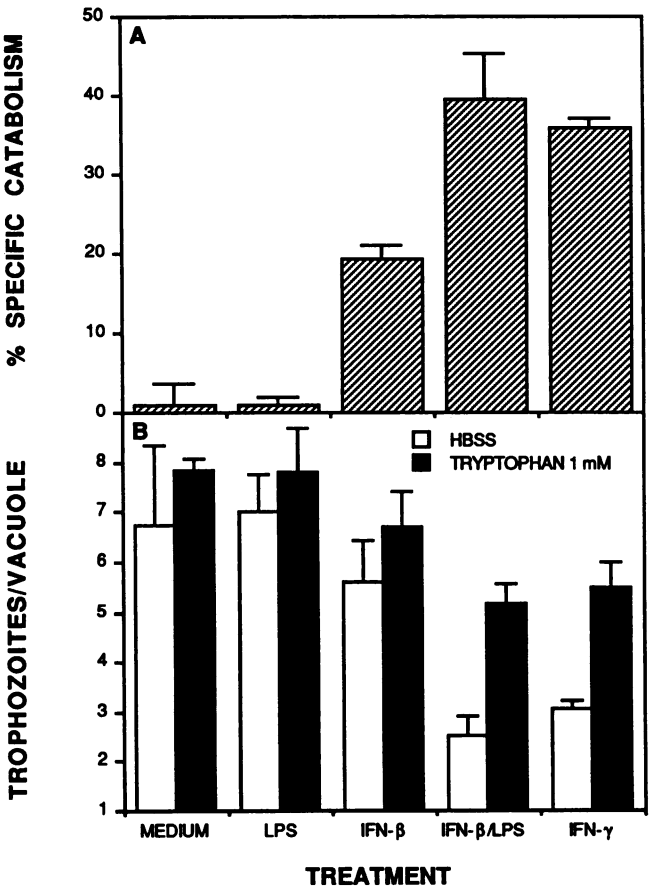

FIG. 1. (A) Effect of IFN treatment on the induction of IDO activity in monocyte-derived macrophages. IDO activity is expressed as percent specific tryptophan catabolism. IDO activity was measured in cells treated with medium alone, LPS at 0.1 ng/ml, IFN-β at 10 ng/ml, IFN-γ at 20 ng/ml, or LPS and IFN-β at the same concentrations as in controls. (B) Effect of IFN treatment on *T. gondii* growth in monocyte-derived macrophages. Cells were plated, treated as in panel A, infected with *T. gondii* for 30 h, and fixed, and the number of *T. gondii* organisms per 100 vacuoles was counted. Each bar represents the mean ± standard deviation of triplicate determinations. Solid bars, Cells that were treated with 1 mM tryptophan during infection; open bars, cells that received an equal volume of HBSS during infection. Statistical comparisons were made with two-tailed *t* tests for independent samples.

catabolism was calculated by using the following equation: percent specific catabolism = $[(cpm_{test} - cpm_{spontaneous})/(cpm_{total} - cpm_{spontaneous})] \times 100$, where cpm_{test} represented the cpm present in *N*-formylkynurenine- and kynurenine-containing fractions, $cpm_{spontaneous}$ represented the cpm of nonspecific tryptophan degradation products, and cpm_{total} was the sum of cpm from all fractions. A representative experiment (Fig. 1A) demonstrated that cells cultured with LPS or medium alone showed no decyclization of tryptophan. In contrast, cells treated with 10 ng of INF-β per ml showed a significant amount of tryptophan catabolism ($P < 0.01$). When LPS was added to the IFN-β-containing wells, a significant augmentation of the percentage of specific catabolism of tryptophan over that measured in wells treated with IFN-β alone was observed ($P < 0.01$). The amount of tryptophan catabolism in the wells containing both IFN-β and LPS was similar to that seen in the wells treated with IFN-γ. These results confirmed earlier work (5) showing that IFN-β was capable of inducing IDO activity in monocyte-derived macrophages.

To determine if IFN-γ and IFN-β inhibited the intracellular growth of *T. gondii*, we set up two sets of triplicate wells in parallel to those used to determine tryptophan catabolism. Subsequent to the 48-h treatment period, 50 μl of *T. gondii* RH (10^6 triphozoites per ml), which had been maintained by passage in mice, was added to the culture medium. One parallel set of wells received exogenous tryptophan at a final concentration of 1 mM, while the other set received an equal volume of HBSS. After the addition of *T. gondii*, the plates were incubated for 30 h, the medium was aspirated from the wells, and the cells were fixed for 15 min with 2.5% glutaraldehyde. Cover slips were removed from the plates, inverted in 1 drop of fixative on a microscope slide, sealed, and examined by phase-contrast microscopy. For each cover slip the number of *T. gondii* organisms per 100 vacuoles was counted in cells containing only one vacuole. Cells treated with medium alone or with 0.1 ng of LPS per ml showed similar, high numbers of trophozoites per vacuole (Fig. 1B). When IFN-β alone was included in the culture medium, a slight decrease ($P > 0.05$) in the number of trophozoites per vacuole was seen. However, when macrophages were stimulated with LPS plus IFN-β, a greatly reduced number of trophozoites per vacuole was observed ($P < 0.001$). The reduction in *T. gondii* numbers with the LPS–IFN-β treatment was similar to that measured in cells treated with IFN-γ. Thus, IFN-β plus LPS inhibited intracellular growth in a manner analogous to that observed when cells were treated with IFN-γ.

The addition of tryptophan to the third set of wells provided strong evidence that the effects of IFN-γ and IFN-β plus LPS were due to IFN-mediated induction of tryptophan catabolism. The addition of 1 mM tryptophan resulted in an increase in the number of *T. gondii* organisms per vacuole in both the IFN-γ- and IFN-β–LPS-treated wells when compared with corresponding wells without tryptophan. Although the counts were not restored to control values, there was a dramatic and significant ($P < 0.01$) increase in trophozoite numbers.

The antiviral effects of the IFN system have been known for some time, and interest in the role of IFNs in controlling nonviral infections has been increasing during the past few years. IFN-γ has been shown to inhibit the growth of a variety of intracellular bacterial and parasitic pathogens, but a similar role for IFN-β has not been consistently observed. Previous work in this laboratory (2, 3) demonstrated the bacteriostatic effects of IFN-γ on chlamydial replication. More recently, the combination of IFN-β and LPS was shown to inhibit the intracellular growth of *C. psittaci* (3). The ability of IFN-β to inhibit *C. psittaci* growth was dependent on both the additional effect of LPS and the use of macrophages as host cells. Thus, the capacity of macrophage cultures to respond to IFN-β–LPS treatment with high levels of IDO activity and the inability of fibroblasts to respond in this manner may account for the lack of effect of IFN-β treatment on *T. gondii* growth in fibroblasts (9). The present study demonstrates the effectiveness of IFN-β plus LPS in inhibiting the intracellular multiplication of *T. gondii* in monocyte-derived macrophages, in contrast to the results of Murray et al. (6). The most obvious difference and reasonable explanation for the contradicting results is the addition of LPS to the culture medium, which potentiated IFN-β-mediated induction of IDO and may have provided an unknown additional stimulus to help block *T. gondii* growth. The concentration of IFN-β used and the length of time

allowed for macrophage maturation also may have been important factors in determining the effectiveness of IFN-β inhibition of *T. gondii* growth. Nevertheless, treatment of macrophages with quantities of IFN-β and LPS that were optimal for the inhibition of chlamydial replication also were effective against *T. gondii*. Much of the effect of the IFN-β–LPS combination was the result of the induction of IDO, since the addition of exogenous tryptophan largely restored the capacity of *T. gondii* to multiply in the treated cells. The increased tryptophan degradation in macrophages treated with IFN-β plus LPS is consistent with this mechanism. Other inhibitory mechanisms also may be involved in this system, since the number of intracellular *T. gondii* organisms did not return to full control values after the addition of tryptophan. However, the effect of IFN-β plus LPS was clearly an IDO-related activity and expands the known role for IFN-β against intracellular pathogens. It will be of interest to determine whether the combination of IFN-β and LPS can exert inhibitory effects against other intracellular pathogens.

This work was supported by grants from Triton Biosciences and CIBA-GEIGY Corp.

LITERATURE CITED

1. **Byrne, G. I., and D. A. Krueger.** 1983. Lymphokine-mediated inhibition of *Chlamydia* replication in mouse fibroblasts is neutralized by anti-gamma interferon immunoglobulin. Infect. Immun. **42**:1152–1158.
2. **Byrne, G. I., L. K. Lehmann, and G. J. Landry.** 1986. Induction of tryptophan catabolism is the mechanism for γ interferon-mediated inhibition of *Chlamydia psittaci* replication in T24 cells. Infect. Immun. **53**:347–351.
3. **Carlin, J. M., E. C. Borden, and G. I. Byrne.** 1989. Interferon-induced indoleamine 2,3-dioxygenase activity inhibits *Chlamydia psittaci* replication in human macrophages. J. Interferon Res. **9**:329–337.
4. **Carlin, J. M., E. C. Borden, P. M. Sondel, and G. I. Byrne.** 1987. Biologic response modifier-induced indoleamine 2,3-dioxygenase activity in human peripheral blood mononuclear cell cultures. J. Immunol. **139**:2414–2418.
5. **Carlin, J. M., E. C. Borden, P. M. Sondel, and G. I. Byrne.** 1988. Interferon-induced indoleamine 2,3-dioxygenase activity in human mononuclear phagocytes. J. Leukocyte Biol. **45**:29–34.
6. **Murray, H. W., A. Szuro-Sudol, D. Wellner, M. J. Oca, A. M. Granger, D. M. Libby, C. D. Rothermel, and B. Y. Rubin.** 1989. Role of tryptophan degradation in respiratory first-independent antimicrobial activity of gamma interferon-stimulated human macrophages. Infect. Immun. **57**:845–849.
7. **Ozaki, Y., M. P. Edelstein, and D. S. Duch.** 1987. The actions of interferon and antiinflammatory agents on induction of indoleamine 2,3-dioxygenase in human peripheral blood monocytes. Biochem. Biophys. Res. Commun. **144**:1147–1153.
8. **Pfefferkorn, E. R.** 1984. Interferon γ blocks the growth of *Toxoplasma gondii* in human fibroblasts by inducing the host cells to degrade tryptophan. Proc. Natl. Acad. Sci. USA **81**: 908–912.
9. **Pffefferkorn, E. R., M. Eckel, and S. Rebhun.** 1986. Interferon-γ suppresses the growth of *Toxoplasma gondii* in human fibroblasts through starvation for tryptophan. Mol. Biochem. Parasitol. **20**:215–222.
10. **Pfefferkorn, E. R., and P. M. Guyre.** 1984. Inhibition of growth of *Toxoplasma gondii* in cultured fibroblasts by human recombinant gamma interferon. Infect. Immun. **44**:211–216.
11. **Rothermel, C. D., B. Y. Rubin, and H. W. Murray.** 1983. γ Interferon is the factor in lymphokine that activates human macrophages to inhibit *Chlamydia psittaci* replication. J. Immunol. **131**:2542–2544.
12. **Shimizu, T., S. Nomiyama, R. Hirata, and O. Hayaishi.** 1978. Indoleamine 2,3-dioxygenase purification and some properties. J. Biol. Chem. **253**:4700–4706.
13. **Yong, S., and S. Lau.** 1979. Rapid separation of tryptophan, kynurenines, and indoles using reversed-phase high-performance liquid chromatography. J. Chromatogr. **175**:343–348.