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Murine Gamma Interferon Fails To Inhibit *Toxoplasma gondii* Growth in Murine Fibroblasts

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Although treatment of human macrophages or fibroblasts with human gamma interferon results in the inhibition of intracellular *Toxoplasma gondii*, murine gamma interferon stimulated only murine macrophages, not murine fibroblasts, to inhibit *T. gondii*. This species difference may be important in understanding the control of acute and chronic toxoplasmosis.

Gamma interferon (IFN-γ) is a multifunctional molecule with species-specific immunomodulation and antiviral and antiprotozoal activities which likely function by different mechanisms (6). The mechanism of IFN-γ antiviral activity has been at least partially defined (6). It has been suggested recently that IFN-γ is the key factor in the in vivo control of *Toxoplasma gondii* in the mouse (16). Human IFN-γ has been shown to inhibit *T. gondii* in both human macrophages (5) and human fibroblasts (10). The mechanism of inhibition of *T. gondii* growth in human fibroblasts is the induction of an indoleamine 2,3-dioxygenase which degrades tryptophan and starves the obligate intracellular parasite (7, 10). The antitoxoplasma state induced by IFN-γ in human macrophages may be similar, or other mechanisms may be involved. We have examined the effect of murine recombinant IFN-γ on the growth of intracellular *T. gondii* in mouse fibroblasts and found that this IFN-γ does not suppress intracellular growth and does not induce indoleamine 2,3-dioxygenase in these cells.

Murine IFN-γ produced in *Escherichia coli* was donated by Schering Corp. as part of the American Cancer Society Program on Interferon Research. IFN-γ batch 18563-051 was dissolved in 5 mM Tris hydrochloride–0.25 M NaCl–0.05% 2-mercaptoethanol–50% glycerol, pH 7.4, and stored at −20°C at a final concentration of 0.6 mg/ml. The N terminus of the IFN-γ was Cys-Tyr-Cys. The IFN-γ was assayed for antiviral activity by its ability to prevent lysis of L-929 cells or primary ICR mouse fibroblasts by vesicular stomatitis virus and was found to be equivalent to 1.0 U/ng when compared with National Institute of Allergy and Infectious Disease international reference murine IFN-γ (National Institutes of Health catalog no. Gg02-901-533).

T. gondii isolates of the recloned RH strain were grown in human foreskin fibroblasts as described previously (11). For assay of interferon effect, parasites just released from host cells were counted in a hemacytometer; approximately 5×10^4 PFU were transferred to 4-cm^2 monolayers of L-929 or normal ICR mouse fibroblasts that had been treated for 24 h with various concentrations of IFN-γ diluted in RPMI 1640 (GIBCO Laboratories) supplemented with 2% fetal bovine serum (HyClone Laboratories, Inc.). The parasites were allowed to infect the cells, and the intracellular growth of *T. gondii* was measured by the incorporation of [5,6-^{3}H]uracil (2 μCi/ml, 48.6 Ci/mmol; Dupont, NEN Research Products). The incorporation of this compound is specific for the parasite and correlates with intracellular growth (12). After 4 to 18 h, the monolayers were lysed in 1% sodium dodecyl sulfate–100 mM uracil in Hanks balanced salt solution and processed for the recovery of acid-precipitable radioactivity as described previously (14).

The degradation of tryptophan by intact cells treated with IFN-γ was measured in confluent monolayers grown in 0.3-cm^2 wells. IFN-γ and 0.6 μCi of L-[methylene-^{14}C] tryptophan (53.5 mCi/mmol; Amersham Corp.) per ml were added to the medium at the same time. Four days later, the medium was analyzed by ascending paper chromatography in 0.1 N HCl and the distribution of radioactivity between tryptophan and kynurenine was determined (9). Indoleamine 2,3-dioxygenase was assayed in sonic extracts of L-929 cells or ICR mouse fibroblasts by a sensitive radiometric procedure that depended on the enzymatic conversion of L-[methylene-^{14}C]tryptophan to *N*-[^{14}C]formylkynurenine and subsequent acid degradation of this product to [^{14}C]kynurenine (13).

Figure 1 shows the results of two representative independent experiments using Schering IFN-γ to assay *T. gondii* inhibition in L-929 cells. Over a broad range of IFN-γ concentrations, there was no statistically significant inhibition of *T. gondii* growth compared with untreated controls. In other experiments (data not shown), IFN-γ concentrations of up to 2,000 U/ml had no inhibitory effect. IFN-γ from the same lot assayed on the same cell line in the same medium showed the expected antiviral effect against vesicular stomatitis virus (3).

The induction of indoleamine 2,3-dioxygenase was measured in L-929 cells and ICR fibroblasts. Cultures were treated with up to 2,000 U of IFN-γ per ml for 2 days before sonic extracts were prepared. No significant enzyme activity was detected. Both control and IFN-γ-treated cells had activities of <0.05 nmol of *N*-formylkynurenine/min per mg of protein. In contrast, treatment of human fibroblasts for 2 days with 32 U of human IFN-γ per ml raises the enzyme level from undetectable to 1.5 nmol/min per mg of protein (9). Consistent with the inability of IFN-γ to induce murine indoleamine 2,3-dioxygenase, we observed no interferon-induced degradation of tryptophan in the medium of L-929 cells treated for 4 days with 200 U of murine IFN-γ per ml. Human fibroblasts treated with 4 U of human IFN-γ per ml for 2 days show complete degradation of tryptophan in the medium (7). The same lot of IFN-γ had the expected inhibitory effect on intracellular replication of *T. gondii* in resident peritoneal macrophages of ICR mice that were

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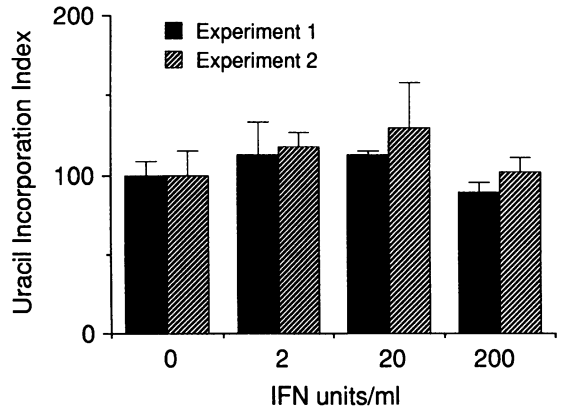

FIG. 1. Two independent experiments with various concentrations of IFN-γ on monolayers of L-929 cells infected with equal inocula of *T. gondii*. The uracil incorporation data are normalized to 100 against the control lacking IFN-γ. Error bars represent standard deviations of three replicates.

treated with 20 U of IFN-γ per ml; 2.96 ± 1.8 (standard deviation) parasites per cell were counted in controls, compared with 0.11 ± 0.17 parasites per cell in treated cultures after 24 h.

Previous work has established the activity and mode of action of human IFN-γ against *T. gondii* in human fibroblasts (5, 7, 9, 10, 13); IFN-γ activates human macrophages to inhibit *T. gondii* (5), but the mechanism of inhibition may not be the same as has been demonstrated in nonmacrophage cell lines because supplementation of tryptophan reverses only a small portion of IFN-γ-mediated inhibition, and mouse macrophages stimulated by IFN-γ inhibit *T. gondii* but do not degrade tryptophan (4). Our results indicate that IFN-γ does not directly empower mouse fibroblasts to inhibit intracellular *T. gondii*. Our findings are in conflict with previous reports (4, 15) that murine type II interferon inhibits *T. gondii* in infected L-929 cells. The interferon used in these earlier experiments was produced from mouse spleen cells stimulated by *T. gondii*, concanavalin A, or mycobacterial antigens. Our results suggest that pure IFN-γ may not be responsible for the reported effect. Data similar to those presented here, but using mouse 3T3 cells and recombinant murine IFN-γ prepared by Genetech Inc., were noted in a review of the antitoxoplasma activity of human IFN-γ (8).

It has been shown recently that antibody to IFN-γ can modulate in vivo survival of acute *T. gondii* infection in BALB/c mice (16). Our studies suggest that mice can control acute (but not necessarily chronic) *T. gondii* infection entirely by macrophages, that infection of cells other than macrophages is not important to the survival of the host, or that infection of nonmacrophage cells is controlled by a mechanism other than the direct production of an antiparasitic state by IFN-γ. IFN-γ-stimulated macrophages may produce a monokine that inhibits *T. gondii* intracellular growth in fibroblasts and other cell types. Such an inhibitor, possibly specific for *T. gondii*, has been demonstrated in the mouse model (2). Lymphokines and monokines may therefore inhibit *T. gondii* replication by more than one mechanism. Susceptibility of inbred mouse strains to *T. gondii* infection varies (1). Species and intrastrain differences may give useful clues to the pathobiology of this infection.

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