Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 7144–7148, September 1989
Medical Sciences

Interferon γ-resistant mutants are defective in the induction of indoleamine 2,3-dioxygenase

(mutagenesis/L-tryptophan/tumor cells)

GEN-SHENG FENG AND MILTON W. TAYLOR*

Department of Biology, Indiana University, Bloomington, IN 47405

Communicated by Ruth Sager, May 22, 1989 (received for review December 20, 1988)

ABSTRACT **Several mutants of the human cell line ME-180 resistant to the cytotoxic effect of interferon γ (IFN-γ) were isolated after mutagenesis with nitrosoguanidine. Two of the mutant lines (ME-IR3b and ME-IR6g) examined had significantly lower induction levels of the L-tryptophan (L-Trp) degradative enzyme indoleamine 2,3-dioxygenase activity in response to IFN-γ. Moreover, culture medium supplemented with low concentrations of L-Trp reversed the cytotoxic effect of IFN-γ, whereas higher concentrations of L-Trp in the medium were extremely toxic to both parental and mutant cells. These mutants were still protected against herpes simplex virus infection by IFN-γ and expressed the *HLA-DRα* gene normally in the presence of this lymphokine. Thus, the mutation in these cells is specific to indoleamine 2,3-dioxygenase and not a global effect of an IFN-γ-induced gene. This genetic evidence indicates that the major pathway of IFN-γ cytotoxicity in this cell line is mediated primarily by induction of indoleamine 2,3-dioxygenase and deprivation of L-Trp.**

Interferon γ (IFN-γ) was originally identified in lymphocyte cultures after induction with the mitogen phytohemagglutinin (1). IFN-γ is distinct from IFN-α and IFN-β on the basis of inducer, antigenicity, molecular structure, cell receptor, and producing cells (for review, see ref. 2). IFN-γ inhibits the proliferation of various malignant cells and is pivotal in regulating immune functions—for example, IFN-γ is more effective than either IFN-α or IFN-β in activating macrophage and natural killer cell activity (3, 4). The major interest in IFN-γ is not in its antiviral effect but rather in its immunoregulatory and potential antitumor function.

Although the antiviral activity of the IFNs has been extensively studied, the mechanism whereby the IFNs exert their antiproliferative effect remains poorly understood. Treatment of cells with IFNs induces (2′-5′)oligoadenylate synthetase and a protein kinase (5, 6). Both of these enzymes are recognized as playing a central role in inhibiting viral replication. However, the inhibitory effect by IFN-γ on growth of the intracellular parasites *Toxoplasma gondii* and *Chlamydia psittaci* was shown to be due to starvation for L-tryptophan (L-Trp) within the host cell (7, 8), rather than to induction of the enzymes involved in antiviral function. More recently, two groups (9, 10) have reported that the antiproliferative effect of IFN-γ on some human tumor cell lines *in vitro* could be partly explained by IFN-γ induction of indoleamine 2,3-dioxygenase (IDO), the first enzyme in a major pathway for degradation of L-Trp. IFNs have been shown to induce the enzyme at the transcriptional level (11–15) and, therefore, IDO is the third identified IFN-inducible enzyme that mediates IFN biological action. However, how IFN-γ regulates the expression of the IDO gene remains unclear, and the generality and importance of this pathway in the anticellular activity of IFN-γ need evaluation. IFN-γ still manifests some cytotoxic effect on certain cell lines in which IDO is not induced (9), and supplementing the medium with L-Trp could not completely reverse IFN-γ cytotoxicity in some cases (9, 10, 16), suggesting that IDO induction is not the only mechanism for anticellular activity of IFN-γ.

We report the isolation of IFN-γ-resistant mutants from a human cervical carcinoma cell line, ME-180 and show that such mutants are defective in the inducibility of IDO activity. This is direct genetic evidence correlating the induction of IDO activity by IFN-γ with the anticellular effect of IFN-γ. We believe that these mutants will be useful in analysis of the molecular basis of IFN-γ resistance and its synergy with other lymphokines.

MATERIALS AND METHODS

Materials and Cell Line. Purified human recombinant IFN-γ, 1.6×10^7 units/mg of protein, was provided by H. M. Shepard (Genentech); *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine and L-Trp were purchased from Sigma; and the ME-180 human cervical carcinoma cell line was obtained from the American Type Culture Collection. ME-180 cells were grown in McCoy's 5A medium (GIBCO) supplemented with 10% fetal bovine serum (HyClone), penicillin and streptomycin each at 100 units/ml, and 100 mM glutamine.

Mutagenesis and Mutant Isolation. ME-180 cells were seeded in 100-mm plastic Petri dishes at a density of 10^6 cells per dish and incubated overnight to form monolayers. *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine was added at 1 μg/ml and removed after 24 hr. Cells were washed twice with phosphate-buffered saline, pH 7.2, and incubated in McCoy's 5A medium for 5 days to allow recovery from mutagenesis and expression of the mutant phenotype. IFN-γ was then added at 50 units/ml, and IFN-γ-resistant clones were selected. After 2 weeks, the resistant clones were individually isolated. The cloned cells were reselected in IFN-γ at 50 units/ml and maintained routinely in nonselective medium.

Cytotoxic Activity of IFN-γ. The cytotoxicity of IFN-γ was tested as reported (17) with slight modifications. Briefly, cells were cultured in 96-well plates (3×10^4 cells per well) with different concentrations of IFN-γ for 72 hr. Then, the cell monolayers were stained with 0.5% crystal violet in 20% (vol/vol) methanol, and the cell-bound dye was eluted with 200 μl of Sorenson's citrate buffer [0.1 M sodium citrate, pH 4.2/50% (vol/vol) ethanol]. The absorbance was measured at 590 nm with a microplate reader. Cytotoxic efficiency was expressed as the ratio of treated-versus-untreated cells.

Induction and Assay of IDO Activity. Cell monolayers grown in plastic 60-mm dishes were treated with different concentrations of IFN-γ for 48 hr. The cells were washed twice with phosphate-buffered saline, trypsinized, and pel-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: IFN, interferon; IDO, indoleamine 2,3-dioxygenase; L-Trp, L-tryptophan.

*To whom reprint requests should be addressed.

leted by centrifugation. The cell pellets were washed twice in cold phosphate-buffered saline, frozen at −70°C, and lyophilized overnight. The powdered cell residue was resuspended in 0.5 ml of phosphate-buffered saline and, after centrifugation, the supernatants were assayed for IDO activity by the method of Takikawa *et al.* (10) with some modifications. Cell extracts (100 μl) were mixed in Eppendorf tubes with an equal volume of 2× reaction buffer (2× reaction buffer is 100 mM potassium phosphate buffer, pH 6.5/40 mM ascorbate/20 μM methylene blue/catalase at 200 μg/ml/800 μM L-Trp). The mixtures were incubated at 37°C for 30 min to permit IDO to convert L-Trp to *N*-formylkynurenine, and then 40 μl of 30% (wt/vol) trichloroacetic acid was added to stop the reaction. The tubes were incubated at 50°C for 30 min to hydrolyze the *N*-formylkynurenine produced to kynurenine. After centrifugation at 10,000 rpm in an Eppendorf centrifuge for 10 min, the supernatant (100 μl) was mixed with an equal volume of Ehrlich reagent (0.4% *p*-dimethylaminobenzaldehyde/acetic acid) in a 96-well plate. Absorbance at 490 nm was read with a microplate reader. One unit of IDO activity is defined as the amount of enzyme required to produce 1 nmol of kynurenine per hr. Protein concentration of the cell extracts was measured by a dye-binding assay (18) with Bio-Rad protein assay solution.

RNA Extraction and Northern (RNA) Blot Analysis. Total cellular RNA was isolated by the method described by Chomczynski and Sacchi (19). RNA preparations (15 μg) were electrophoresed in a formaldehyde/1% agarose denaturing gel and transferred to Hybond-N membrane (Amersham). The hybridization was done according to Ausubel *et al.* (20) by using probes radiolabeled with ^{32}P by the oligonucleotide random-priming method (21).

Antiviral Assay. Cells were seeded in 6-well plates and treated with IFN-γ for 24 hr, by which time the cells had formed confluent monolayers. IFN was removed, and the cells were infected with herpes simplex virus at multiplicity of infection of 1. After 36 hr samples were removed and titers were determined by plaque assay on monkey Vero cells (22).

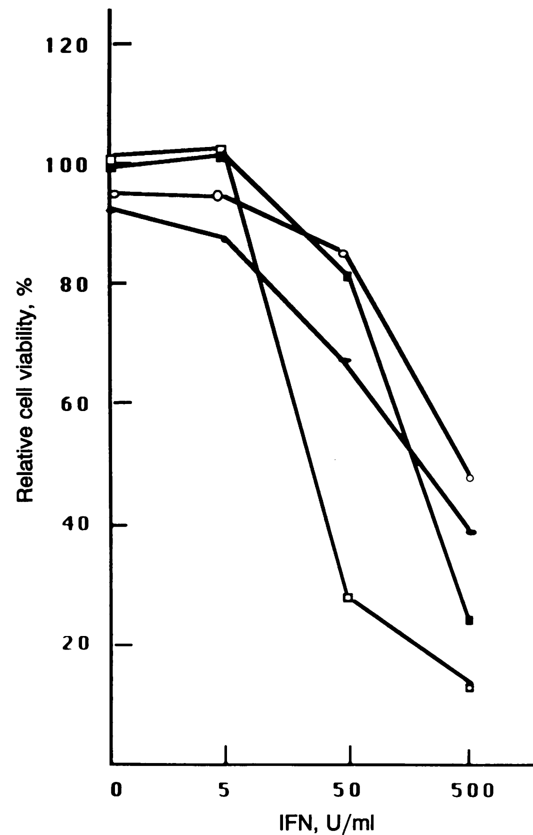

FIG. 1. Reversibility by L-Trp of the anticellular activity of IFN-γ on ME-180. Cells were incubated with various concentrations of IFN-γ in McCoy's 5A medium (containing 0.015 mM L-Trp) (□) supplemented with 0.1 (■), 0.5 (○), and 1.0 mM (●) L-Trp for 72 hr. Cell viability was determined as described in text. Each treatment group had at least four replicates, and the SD was within 10% of the mean.

RESULTS

Effect of L-Trp on Cytocidal Activity of IFN-γ. That IFN-γ inhibits the growth of, or even kills, many transformed cell lines *in vitro* is well known. In some cell lines the inhibitory effect of IFN-γ can be partially or completely reversed by adding L-Trp to the medium (9, 10). ME-180 cells, when incubated in McCoy's 5A medium containing only 0.015 mM L-Trp are highly sensitive to the cytotoxic effect of IFN-γ (refs. 17 and 23; Fig. 1). Treatment of the cells with IFN-γ at 50 units/ml for 3 days killed 72% of cells, and all cells died within 10 days when maintained in IFN-γ-containing medium (data not shown). Supplementing the McCoy's 5A medium with 0.1 mM of L-Trp decreased the killing efficiency of IFN-γ at 50 units/ml from 72% to 21% but exerted no significant effect on IFN-γ at 500 units/ml (from 84% to 75%). Addition of 0.5 mM of L-Trp did not reduce the effect of IFN-γ at 50 units/ml further but impaired killing by IFN-γ at 500 units/ml to 53%. A further increase of L-Trp to 1 mM did not increase the reversibility of IFN-γ activity when compared with 0.5 mM, which may be due to the toxicity of high concentrations of L-Trp (see below). Both previous reports (9) and our experiments indicate that the addition of L-Trp does not completely reverse the cytotoxicity of IFN-γ. But this blockage of IFN-γ activity is specific to L-Trp, because other amino acids, such as L-lysine, L-methionine, and L-asparagine, did not block the antiproliferative activity of IFN-γ (16). We also noticed that ME-180 cells maintained in the presence of IFN-γ even in L-Trp-enriched medium ultimately died by 10 days. There are several possible explanations for this result: (*i*) Several independent mechanisms are involved in IFN-γ cytocidal activity. (*ii*) The low levels of L-Trp added to the medium may not be enough to replenish the degradation of L-Trp mediated by IFN-γ-induced IDO, whereas higher concentrations of L-Trp in the medium are toxic to the cells. (*iii*) Accumulation of one or more products from the IDO-initiated pathway of L-Trp degradation could eventually be toxic to the cells.

Isolation of IFN-γ-Resistant Cell Variants. To understand the molecular mechanism(s) leading to the cytocidal activity of IFN-γ, we attempted to isolate IFN-γ-resistant mutants either by exposing the normal sensitive cells to increased concentrations of IFN-γ or by selecting resistant variants after mutagenesis. These initial attempts were unsuccessful due to the slow death of surviving clones maintained in the IFN-γ-selective medium. After we observed that IFN-γ killed the ME-180 cells by depleting the medium of L-Trp, we changed the selection strategy. Low amounts of IFN-γ were added to the medium and then removed from the plates after most cells had died. The cells were fed fresh medium, and the "sick" surviving cells recovered. Selection in IFN-γ was repeated to eliminate any sensitive cells and select resistant ones. In this way we could select clones resistant to different levels of IFN-γ. Two of these clones, ME-IR3b and ME-IR6g, were chosen for further analysis. Fig. 2 shows that after treatment with IFN-γ at 50 units/ml for 72 hr, the relative cell viability of ME-180 was only 28%, whereas the ME-IR3b and ME-IR6g had relative cell viabilities of 91% and 57%, respectively. Even with IFN-γ at 500 units/ml, the relative cell viabilities of ME-IR3b and ME-IR6g were 44% and 43%,

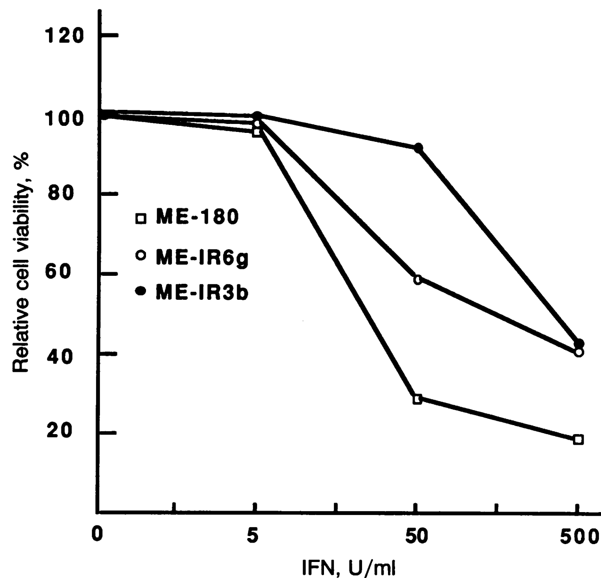

FIG. 2. Cytotoxic activity of IFN-γ on wild-type and mutant tumor cell lines. ME-180, ME-IR3b, and ME-IR6g cells were cultured in a 96-well plate (3×10^4 cells per well) with different concentrations of IFN-γ for 72 hr. Four to eight replicates were included for each treatment, and the SD was <10%.

higher than ME-180 at 16%. Thus, these mutants resist the cytotoxic effect of IFN-γ.

Induction of IDO by IFN-γ in Parental and Mutant Cells. Protection of ME-180 from IFN-γ cytotoxic activity by addition of L-Trp suggests that induction of IDO may occur in ME-180 cells upon IFN-γ treatment. Using a modified colorimetric assay, we measured the IDO activity in both parental and mutant cells (Fig. 3). Without IFN-γ, almost no IDO activity was detectable. However, after growth in IFN-γ (50 units/ml) for 2 days, ME-180 cells produced 180 units/mg of protein, ME-IR3b produced 12 units/mg of protein, and ME-IR6g produced 50 units/mg of protein. After treatment with IFN-γ at 500 units/ml, ME-180 produced 305 units/mg of protein, ME-IR3b produced 143 units/mg of protein, and ME-IR6g produced 203 units/mg of protein. Thus, these mutants, especially ME-IR3b, showed decreased induction of IDO activity by IFN-γ: IFN-γ at 50 units in ME-IR3b produced only background IDO activity, and IFN-γ at 500 units/ml in ME-IR3b had about half of the IDO activity of ME-180. Correlating this data to the sensitivity of ME-IR3b

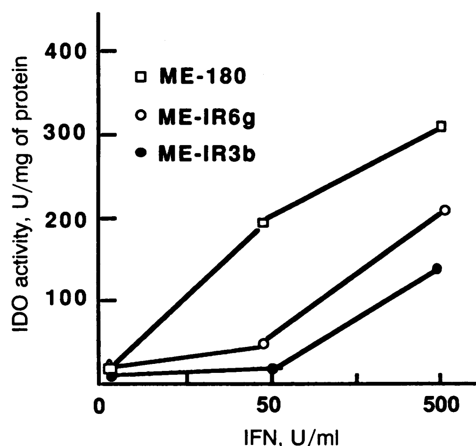

FIG. 3. IDO activity of the cell lines induced with IFN-γ. Cells were incubated in plastic 60-nm dishes (2×10^6 cells per dish) with IFN-γ at 50 and 500 units/ml for 48 hr. IDO assay was done as described in text. All values are the means of duplicate dishes.

Table 1. Reduction of herpes simplex virus yields by IFN-γ in wild-type and mutant cells

Supernatant source	IFN-γ treatment, pfu/ml		
	0 U/ml	50 U/ml	500 U/ml
ME-180	1.61×10^7	1.38×10^4	2.65×10^4
ME-IR3b	0.80×10^7	1.05×10^4	1.53×10^4
ME-IR6g	0.73×10^7	3.85×10^4	1.10×10^4

Data, averaged from duplicated samples, are expressed as plaque-forming unit (pfu)/ml on monkey Vero cell line. U, units.

to the cytotoxicity of IFN-γ in Fig. 2, we see that at 50 units/ml of IFN-γ, ME-IR3b was almost totally resistant to the cytocidal effect of IFN-γ, and at 500 units/ml of IFN-γ, ME-IR3b significantly decreased sensitivity to IFN-γ. Therefore, induction of this enzyme activity is inversely correlated with resistance to IFN-γ. A few clones of ME-IR3b resistant to higher concentrations of IFN-γ have been isolated after a second round of mutagenesis with ICR-191 (Polysciences). Preliminary results in immunologic blotting analysis also indicate that all these first- and second-round mutants exhibited lower or no production of IDO protein with IFN-γ (unpublished work).

Evaluation of Other IFN-γ-Inducible Activities in These Mutants. The inhibitory effect of virus replication and induction of HLA-DRα mRNA by IFN-γ were assessed in these mutants to clarify whether the IDO deficiency is due to a mutation specific for IDO induction or due to a global decrease of cellular response to IFN-γ. Table 1 shows that, IFN-γ at the level of 50 and 500 units/ml significantly inhibited the multiplication of herpes simplex virus type 1 in both the parental ME-180 cells and the two mutants; the viral yields decreased from 10^7 plaque-forming units per ml to 10^4 plaque-forming units per ml in the plaque assay on monkey Vero cells. Likewise, Northern blotting analysis showed that the induction of HLA-DRα mRNA by IFN-γ was not decreased in these mutants (Fig. 4). Without IFN-γ induction ME-180 and its mutants generally did not express this major histocompatibility complex class II gene as in Hela cells (24). Upon IFN-γ treatment for 24 hr, ME-IR3b and ME-IR6g, as

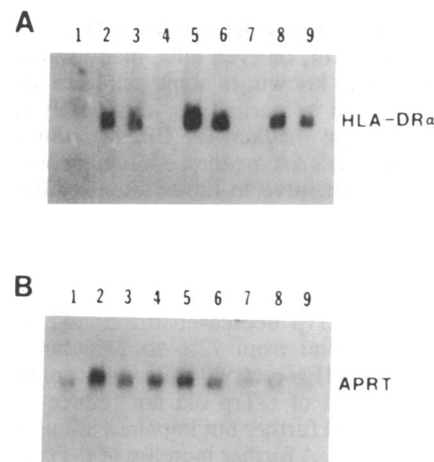

FIG. 4. Induction of HLA-DRα mRNA by IFN-γ. RNA extraction and Northern analysis were done according to refs. 19–21. (*A*) The membrane was hybridized to a ^{32}P-labeled 1.3-kilobase (kb) cDNA fragment for the *HLA-DR1A* gene isolated by BamHI cut from pCDV1-pL2 (obtained from ATCC catalog 57392). (*B*) The membrane was thoroughly washed by boiling in distilled water for 10 min and reprobed with ^{32}P-labeled cDNA (0.54 kb) for the human adenine phosphoribosyltransferase (*APRT*) gene, cleaved by BamHI from pcHuap (supplied by J. A. Tischfield, Indiana University Medical School). (Lanes 1, 2, and 3) ME-180. (Lanes 4–6) ME-IR3b. (Lanes 7–9) ME-IR6g. Control lanes are lanes 1, 4, and 7; IFN-γ treatment at 50 units/ml (lanes 2, 5, and 8) and 500 units/ml (lanes 3, 6, and 9).

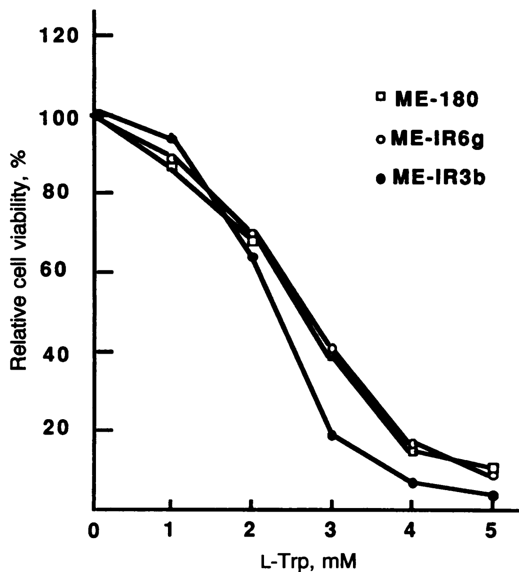

FIG. 5. Sensitivity of cell growth to L-Trp. Normal McCoy's 5A medium (containing 0.015 mM L-Trp) was supplemented with 1–5 mM L-Trp and used to incubate the ME-180, ME-IR3b, and ME-IR6g cells for 72 hr. All values represent the mean of four to eight duplicates; SD was <10%.

well as ME-180, synthesized the HLA-DRα mRNA at high levels. These results suggest that mutation in ME-IR3b and ME-IR6g does not occur at the initial step of IFN-γ effect, which may have caused decreased sensitivity of the cells to IFN-γ, but rather in the specific pathway leading to expression of IDO. That the IDO-deficient mutants responded normally to the antiviral and immunoregulatory effects of IFN-γ also indicates that separate pathways for these physiological functions of IFN-γ exist and that IDO does not contribute to the antiviral activity.

Toxicity of Excess L-Trp to the Parental and Mutant Cells. When different concentrations of L-Trp were added to the medium to test reversibility of the cytotoxic effects of IFN-γ, we noticed that high levels of L-Trp, such as 10 mM, were toxic to cell growth without any IFN-γ; this effect has been reported (16). To find the optimal L-Trp level that is nontoxic to cells but shows maximum retardation of IFN-γ toxicity, we incubated the parental and resistant cells in the medium supplemented with different concentrations of L-Trp for 72 hr and measured relative cell viability (Fig. 5). We found that addition of <1 mM L-Trp was not toxic to cell growth; 2 mM L-Trp inhibited cell growth, whereas 5 mM L-Trp killed all the cells. The killing effect was proportional to the increase of L-Trp concentration. Interestingly, ME-IR3b showed higher sensitivity to 2–5 mM L-Trp than either ME-180 or ME-IR6g cells. One explanation for this phenomenon is that high levels of L-Trp are toxic to cell growth and IDO functions to degrade excess L-Trp, decreasing it to normal levels. When IDO activity in ME-IR3b is impaired by mutagenesis, the cells are more sensitive to higher concentrations of L-Trp.

DISCUSSION

The biological activities of IFN-γ are mediated by its ability to activate various genes after binding to a specific cell receptor (25–27). The gene(s) induced by IFN-γ are not well characterized. This study shows that IFN-γ-resistant cells obtained by mutagenesis are defective in inducing IDO activity. These mutants are aberrant either in IDO gene or its specific regulatory factor because the cells are still normally responsive to IFN-γ initiation of the antiviral process and expression of the HLA-DRα gene. Therefore, this report directly correlates IDO induction by IFN-γ and the antiproliferative activity in a single cell line. That the cytotoxic effect of IFN-γ in the parental cell line ME-180 could be partially reversed by addition of excess L-Trp to the culture medium provides physiological evidence that IFN-γ exerts a major part of its anticellular activity on this cell line by inducing IDO and degrading L-Trp. Although supplementing culture medium with low amounts (0.1–1 mM) of L-Trp protects cells against the toxicity of IFN-γ, higher concentrations of L-Trp were toxic to cells (Fig. 5 and ref. 16). ME-IR3b cells, which have severely decreased IDO production, are more sensitive than ME-180 parental cells to higher concentrations of L-Trp (Fig. 5), suggesting that IDO may also be inducible by its substrate L-Trp and that possibly intracellular IDO acts physiologically to degrade L-Trp at a specific toxic level.

IDO is a hemoprotein and the first enzyme of the L-Trp catabolic pathway, catalyzing the conversion of L-Trp to *N*-formylkynurenine by incorporating the superoxide anion or molecular oxygen into the pyrrole ring of L-Trp (28). In 1978 Yoshida and Hayaishi reported (11) that intraperitoneal administration of bacterial lipopolysaccharide in mice caused increased IDO levels in lung tissue. Subsequently, the same group observed increased IDO activity in lung tissue after infection of mice with influenza virus (12). In an *in vitro* system with mouse lung slices, IDO was significantly induced by bacterial endotoxin and mouse IFN (13). Thus, the inducibility of IDO by endotoxin and virus infection may have been due to IFN induction. In human lung cancer patients, pulmonary IDO was also increased, and both human IFN-α and IFN-γ induced IDO production in human lung slices *in vitro*, although IFN-γ was more potent than IFN-α (14). A decreased plasma level of L-Trp has also been reported in cancer patients after i.v. bolus injection of recombinant IFN-γ (29). These observations together with the recent reports of IDO induction by IFN-γ in a variety of transformed cell lines (9, 10) and the data presented here indicate that IFN-γ-mediated IDO induction and L-Trp degradation may be important in the antiproliferative mechanism of IFN-γ both *in vivo* and *in vitro*.

The mechanism leading to IDO induction is complex and remains unknown. IFN-γ appears to induce the *de novo* synthesis of IDO because the induction is inhibited by cycloheximide or actinomycin D (ref. 15; unpublished work). Reports in which the authors claim that all types of IFNs (30, 31), tumor necrosis factor (30), and interleukin 2 (31) can induce IDO are contradicted by other reports that (9, 10, 26) show only IFN-γ can induce IDO. Although these discrepancies may be from the use of different cell lines, the method of measuring IDO, or the amounts of specific lymphokines used, these different results may also reflect a differential regulation of IDO production among these cytokines and cell types. In other cases IFN-γ failed to induce IDO (9) and inhibition of *Rickettsia prowazekii* growth by IFN-γ was not blocked by supplementing medium with L-Trp (32), indicating that other mechanisms of IFN-γ antimicrobial action may exist. Obviously, further studies on the regulation of IDO gene expression by IFN-γ and the physiological roles of the latter are required.

We thank Dr. J. A. Tischfield for sending us the plasmid pcHuap. We are grateful to our colleagues Karen Etchberger, Howard Hershey, Raniero De Stasio, Sean Kimbro, Qing Wang, and other members of the Taylor lab for helpful discussion and advice. This work was supported by a grant from the United Cancer Campaign.

1. Wheelock, E. F. (1965) *Science* **149**, 310–311.
2. Content, J. (1986) in *The Molecular Basis of Viral Replication*, ed. Bercoff, R. P. (Plenum, New York), pp. 163–189.
3. Weigent, D. A., Langford, M. P., Fleischmann, W. R. & Stanton, G. J. (1983) *Infect. Immun.* **40**, 35–38.

4. Pace, J. L., Russell, S. W., Schreiber, R. D., Altman, A. & Katz, D. H. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 3782–3786.
5. Samuel, C. E. (1987) in *Mechanisms of Interferon Actions*, ed. Pfeffer, L. M. (CRC, Boca Raton, FL), Vol. 1, pp. 111–131.
6. Samuel, C. E. (1982) *Tex. Rep. Biol. Med.* **41**, 463–470.
7. Pfefferkorn, E. R. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 908–912.
8. Byrne, G. I., Lehmann, L. K. & Landry, G. J. (1986) *Infect. Immun.* **53**, 347–351.
9. Ozaki, Y., Edelstein, M. P. & Duch, D. S. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 1242–1246.
10. Takikawa, O., Kuroiwa, T., Yamazaki, F. & Kido, R. (1988) *J. Biol. Chem.* **263**, 2041–2048.
11. Yoshida, R. & Hayaishi, O. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3998–4000.
12. Yoshida, R., Urade, Y., Tokuda, M. & Hayaishi, O. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4084–4086.
13. Yoshida, R., Imanishi, J., Oku, T., Kishida, T. & Hayaishi, O. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 129–132.
14. Yasui, H., Takai, K., Yoshida, R. & Hayaishi, O. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 6622–6626.
15. Rubin, B. Y., Anderson, S. L., Hellermann, G. R., Richardson, N. K., Lunn, R. M. & Valinsky, J. E. (1988) *J. Interferon Res.* **8**, 691–702.
16. de la Maza, L. M. & Peterson, E. M. (1988) *Cancer Res.* **48**, 346–350.
17. Sugarman, B. J., Lewis, G. D., Eessalu, T. E., Aggarwal, B. B. & Shepard, H. M. (1987) *Cancer Res.* **47**, 780–786.
18. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254.
19. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159.
20. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (1987) *Current Protocols in Molecular Biology*, (Greene Publishing Associates and Wiley-Interscience, New York), pp. 4.9.1–4.9.8.
21. Paeratakul, U., De Stasio, P. R. & Taylor, M. W. (1988) *J. Virol.* **62**, 1132–1135.
22. Weigent, D. A., Stanton, G. J., Langford, M. P., Lloyd, R. E. & Baron, S. (1981) *Methods Enzymol.* **78**, 346–351.
23. Feng, G.-S., Gray, P. W., Shepard, H. M. & Taylor, M. W. (1988) *Science* **241**, 1501–1503.
24. Blanar, M. A., Boettger, E. C. & Flavell, R. A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 4672–4676.
25. Weil, J., Epstein, L. B., Sedmak, J. J., Sabran, J. L. & Grossberg, S. E. (1983) *Nature (London)* **301**, 437–439.
26. Aguet, M., Dembic, Z. & Merlin, G. (1988) *Cell* **55**, 273–280.
27. Caplen, H. S. & Gupta, S. L. (1988) *J. Biol. Chem.* **263**, 332–339.
28. Shimizu, T., Nomiyama, S., Hirata, F. & Hayaishi, O. (1978) *J. Biol. Chem.* **253**, 4700–4706.
29. Byrne, G. I., Lehmann, L. K., Kirschbaum, J. G., Borden, E. C. & Lee, C. M. (1986) *J. Interferon Res.* **6**, 389–396.
30. Bianchi, M., Bertini, R. & Ghezzi, P. (1988) *Biochem. Biophys. Res. Commun.* **152**, 237–242.
31. Carlin, J. M., Borden, E. C., Sondel, P. M. & Byrne, G. I. (1987) *J. Immunol.* **139**, 2414–2418.
32. Turco, J. & Winkler, H. H. (1986) *Infect. Immun.* **53**, 38–46.