Depletion of Tryptophan Is Not Involved in Expression of Tryptophanyl-tRNA Synthetase Mediated by Interferon

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Gamma interferon (IFN-γ) affects tryptophan metabolism by mediating the expression of indoleamine 2,3-dioxygenase and tryptophanyl-tRNA synthetase. In the present study, we investigated the role of indoleamine 2,3-dioxygenase-mediated tryptophan depletion in the induction of tryptophanyl-tRNA synthetase by IFN-γ. The addition of excess tryptophan to the culture medium did not affect the induction of tryptophanyl-tRNA synthetase by IFN-γ, indicating that tryptophan degradation is not directly involved in the IFN-γ-mediated expression of tryptophanyl-tRNA synthetase.

Gamma interferon (IFN-γ), a cytokine produced primarily by T lymphocytes and natural killer cells, plays an important role in immunological responses, regulation of cell growth, and host cell defense mechanisms against intracellular parasites (for a review, see references 10, 11, 22, and 31). In particular, IFN-γ has potent growth-inhibitory effects on *Toxoplasma gondii* and *Chlamydia* spp., such as *C. psittaci* and *C. trachomatis*. The growth-inhibitory effect of IFN-γ on *T. gondii* and *Chlamydia* spp. has been attributed to the intracellular depletion of tryptophan by the IFN-γ-induced expression of indoleamine 2,3-dioxygenase (IDO) (4, 6, 23–25). IDO is a hemoprotein that decyclizes tryptophan to *N*-formylkynurenine by catalyzing the incorporation of superoxide anion or molecular oxygen into the pyrrole ring of tryptophan, a step that results in the rapid depletion of intracellular tryptophan (29). The growth-inhibitory effect of IFN-γ on intracellular parasites can be largely overcome by the addition of excess tryptophan to the growth medium (4, 6, 23–25). Similarly, the antiproliferative effect of IFN-γ on a number of tumor cells also appears to be due in part to the enhanced catabolism of tryptophan by IFN-γ-induced IDO expression (9, 12, 21, 30).

We and others recently described the cloning of a novel interferon-induced protein (3, 13, 27), termed IFP53 on the basis of its molecular weight. IFP53 exhibits high sequence homology to rabbit peptide chain release factor (18) and mammalian tryptophanyl-tRNA synthetases (14, 15). Functional analysis of a cloned fusion protein revealed that IFP53 represents the human tryptophanyl-tRNA synthetase (1). IDO expression and IFP53 expression are induced in many cell types, including human HeLa cells, strongly by IFN-γ but rather weakly by IFN-α or IFN-β (3, 5, 7, 9, 13, 27, 30; unpublished data).

The regulation of the biosynthesis of aminoacyl-tRNA synthetases in prokaryotes has been extensively studied (19, 20). Early experiments indicated the involvement of the cognate amino acid in the control of aminoacyl-tRNA synthetase expression; i.e., upon amino acid restriction, the cognate aminoacyl-tRNA synthetase exhibits derepression. Concerning the regulation of mammalian tRNA synthetases,

selective derepression upon restriction of the cognate amino acid has been described (16, 17).

The intracellular depletion of L-tryptophan by the IFN-γ-mediated expression of IDO, the simultaneous induction of IFP53/tryptophanyl-tRNA synthetase and the reported involvement of the cognate amino acid in the control of aminoacyl-tRNA synthetases prompted us to investigate the effect of exogenously added tryptophan on IFN-γ-mediated IFP53/tryptophanyl-tRNA synthetase expression.

HeLa cells were grown as monolayer cultures in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum. Treatment of human HeLa cells with IFN-γ (Polyferon at 2 × 10⁷ U/mg of protein; Rentschler, Laupheim, Germany) results in the increased expression of IDO (5, 9, 30; see Fig. 1 and 2). To determine the effect of exogenous tryptophan (Sigma, Deisenhofen, Germany) on the IFN-γ-mediated expression of IFP53, we added the two components simultaneously to cells grown to subconfluence at concentrations of 50, 100, and 500 U of IFN-γ per ml and 50 and 250 μg of tryptophan per ml. These doses of tryptophan have been shown to reverse completely the growth-inhibitory action of IFN-γ on intracellular parasites (4, 6, 23, 24) as well as to rescue human tumor cells from the antiproliferative effect of IFN-γ (9, 21, 30).

During the course of tryptophan addition in conjunction with IFN-γ treatment, the specific activity of tryptophanyl-tRNA synthetase as well as IFP53 mRNA expression was determined. Total cellular RNA was prepared by the guanidinium isothiocyanate procedure (28) and purified by centrifugation through cesium chloride. RNA (7.5 μg) was electrophoresed on formaldehyde gels and transferred to nitrocellulose, and Northern (RNA) blotting was performed by standard procedures (28). Quantitative loading of RNA was determined by staining with ethidium bromide and by hybridization to a pyruvate kinase probe (2). A 1.3-kb *Eco*RI fragment from clone 9.2 (3) was used as a hybridization probe for IFP53; for 9.27 (see below), a 260-bp *Eco*RI cDNA fragment was used (26). The probe for IDO was generated by amplification of a 760-bp fragment by use of specific oligonucleotides (8). Treatment with 50, 100, and 500 U of IFN-γ per ml resulted in a significant increase in IFP53, 9.27, and IDO mRNA expression (Fig. 1 and 2). The increased expression of IDO mRNA induced by IFN-γ was strictly dependent on the dose of IFN-γ used, whereas 9.27 and IFP53 mRNA
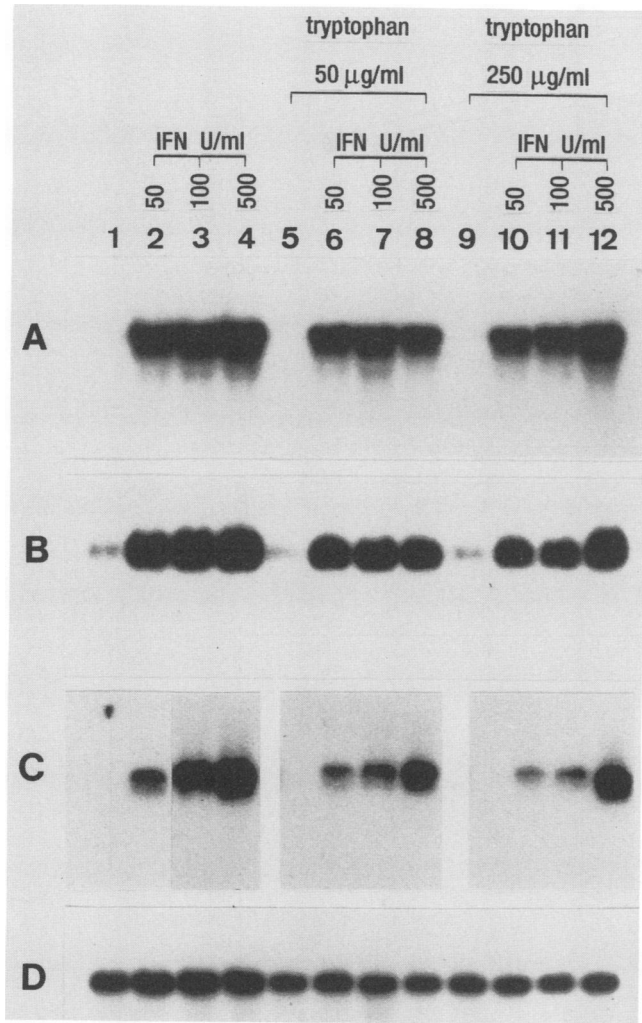
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FIG. 1. Northern blot analysis of IFP53 mRNA expression in HeLa cells. RNA samples (7.5 μg) were resolved on an 0.8% agarose gel and hybridized to IFP53 (A), 9.27 (B), IDO (C), and pyruvate kinase (D). Total cellular RNA was extracted from HeLa cells after a 24-h treatment with the indicated amounts of IFN-γ. In addition, samples 5 to 8 received 50 μg of tryptophan per ml, and samples 9 to 12 received 250 μg of tryptophan per ml.
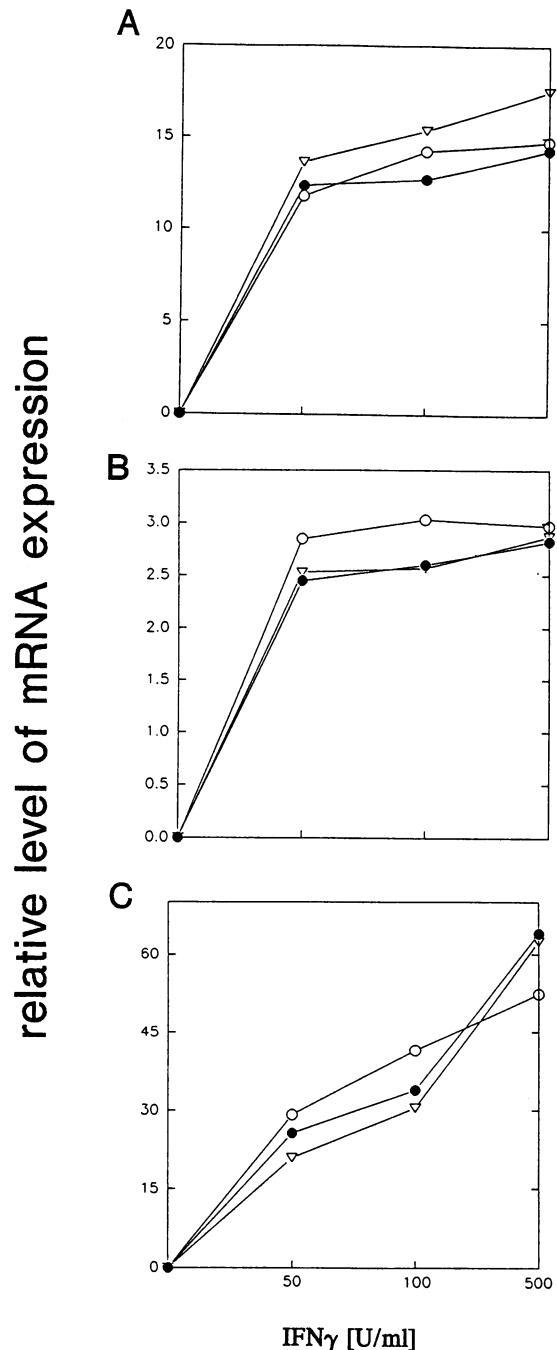

FIG. 2. Densitometric analysis of the Northern blot results from a representative experiment. Cells were treated for 24 h with the indicated amounts of IFN-γ. IFP53 (A), 9.27 (B), and IDO (C) mRNA values were corrected by hybridization to a pyruvate kinase probe to ensure equal loading of mRNA. Symbols: ○, cells to which no exogenous tryptophan was added; ●, cells to which 50 μg of tryptophan per ml was added; ∇, cells to which 250 μg of tryptophan per ml was added.

expression showed a less pronounced dose-response relationship. The addition of exogenous tryptophan to the culture medium at the time of stimulation with IFN-γ did not affect cell viability after 24 h of simultaneous IFN-γ and tryptophan treatment. To control for nonspecific effects, we hybridized the mRNAs to 9.27, an interferon-inducible gene of unknown function (26). The expression of 9.27 was not influenced by exogenous tryptophan. More importantly, the expression of IDO mRNA was not impaired by exogenous tryptophan. With respect to the expression of IFP53 mRNA, the addition of exogenous tryptophan to the culture medium did not significantly affect IFN-γ-mediated induction.

The influence of L-tryptophan on the expression of tryptophanyl-tRNA synthetase activity was investigated with extracts of untreated cells or cells treated for 24 h with IFN-γ (500 U/ml) alone or IFN-γ (500 U/ml) and tryptophan (250 μg/ml). Tryptophanyl-tRNA synthetase activity in crude HeLa cell extracts was assayed as the aminoacylation

of tRNA with L-[^{14}C]tryptophan and unfractionated brewer's yeast tRNA (Boehringer, Mannheim, Germany) as substrates (1). tRNA aminoacylation was carried out at 30°C with 100 mM Tris-HCl (pH 8.0)–1 mM EDTA–15 mM magnesium acetate–0.05 mg of bovine serum albumin per
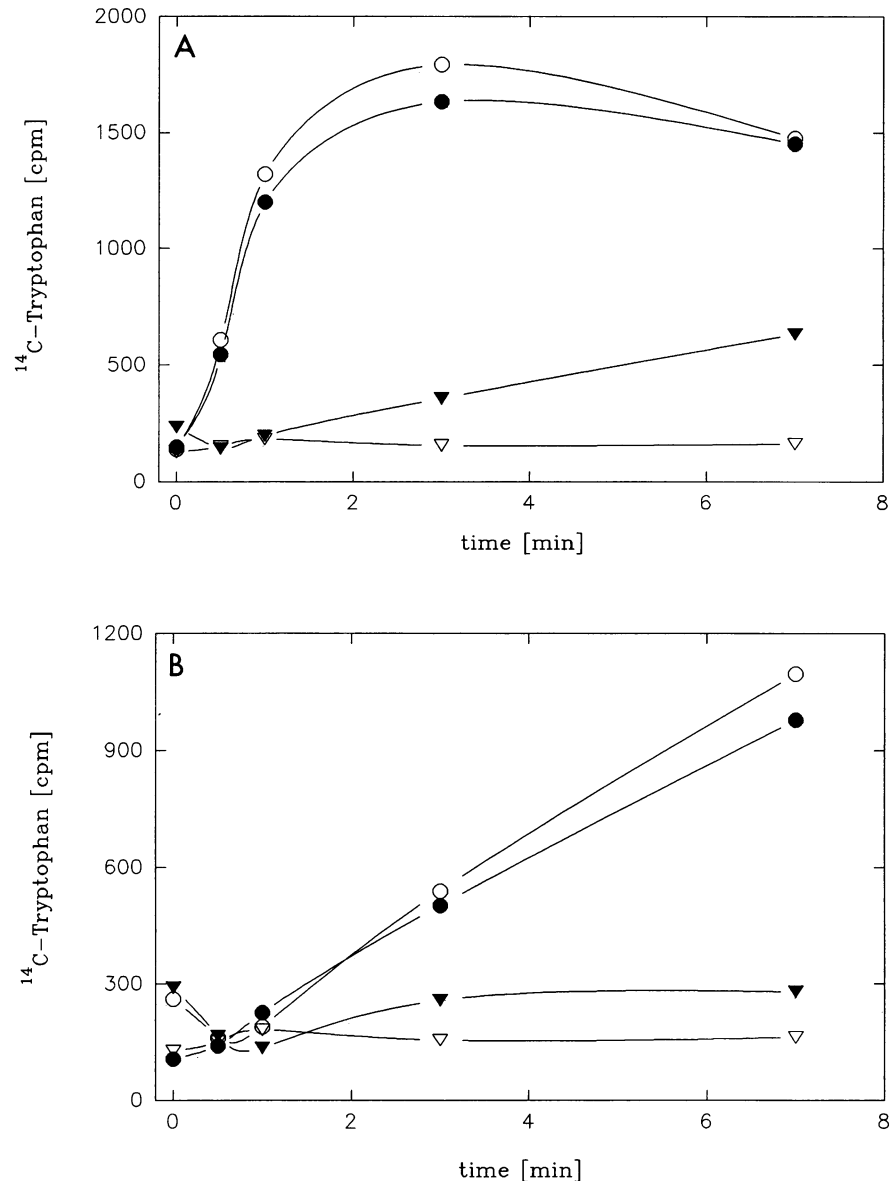
FIG. 3. Determination of the aminoacylation activity of HeLa cell extracts with L-[^{14}C]tryptophan. Cells received no treatment (▼), were treated for 24 h with 500 U of IFN-γ per ml (○), or were treated for 24 h with 500 U of IFN-γ per ml and 250 μg of tryptophan per ml (●). The background activity represents that for heat-treated (10 min, 95°C) extracts from IFN-γ-treated HeLa cells (▽). The values shown correspond to specific enzymatic activities determined with 28 μg (A) or 3.7 μg (B) of crude HeLa cell extract and are expressed as counts per minute of radioactivity retained on the filters when L-[^{14}C]tryptophan was used.

ml–7 mg of total yeast tRNA per ml–8 mM ATP–0.1 mM L-[^{14}C]tryptophan (54 mCi/mmol; Amersham). At various times, 9-μl aliquots from a 60-μl reaction mixture were removed, spotted on a Whatman GFC disk, and immediately precipitated with trichloroacetic acid, and the radioactivity retained on the filters was counted. The levels of IFN-γ-induced tryptophanyl-tRNA synthetase activity were not affected by exogenous tryptophan (Fig. 3).

Our data indicate that the depletion of intracellular tryptophan is not directly involved in the IFN-γ-mediated expression of tryptophanyl-tRNA synthetase. It is, however, unlikely that the IFN-γ-induced degradation of tryptophan and the induction of tryptophanyl-tRNA synthetase by IFN-γ are unrelated events. Linking of these two events

is probably advantageous for host cells with respect to defense mechanisms against intracellular parasites. The induction of IDO by IFN-γ leads to starvation of the cells for an essential amino acid. *T. gondii* and *Chlamydia* spp., like host cells, are incapable of tryptophan synthesis (23). As free tryptophan but not tryptophanylated tRNA is accessible to intracellular parasites, the induction of tryptophanyl-tRNA synthetase may represent a mechanism for rendering residual tryptophan accessible for host cell protein synthesis to the exclusion of the parasite.

REFERENCES

1. **Bange, F.-C., T. Flohr, U. Buwitt, and E. C. Böttger.** 1992. An interferon induced protein with release factor activity is a tryptophanyl-tRNA synthetase. FEBS Lett. **300:**162–166.
2. **Böttger, E. C., M. A. Blanar, and R. A. Flavell.** 1988. Cycloheximide, an inhibitor of protein synthesis, prevents γ-interferon-induced expression of class II mRNA in a macrophage cell line. Immunogenetics **28:**215–220.
3. **Buwitt, U., T. Flohr, and E. C. Böttger.** 1992. Molecular cloning and characterization of an interferon induced human cDNA with sequence homology to a mammalian peptide chain release factor. EMBO J. **11:**489–496.
4. **Byrne, G. I., L. K. Lehmann, and G. J. Landry.** 1986. Induction of tryptophan catabolism is the mechanism for gamma-interferon-mediated inhibition of intracellular *Chlamydia psittaci* replication in T24 cells. Infect. Immun. **53:**347–351.
5. **Capten, H. S., and S. L. Gupta.** 1988. Differential regulation of a cellular gene by human interferon-γ and interferon-α. J. Biol. Chem. **263:**332–339.
6. **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.
7. **Dai, W., and S. L. Gupta.** 1990. Regulation of indoleamine 2,3-dioxygenase gene expression in human fibroblasts by interferon-γ. J. Biol. Chem. **265:**19871–19877.
8. **Dai, W., and S. L. Gupta.** 1990. Molecular cloning, sequencing and expression of human interferon γ-inducible indoleamine 2,3-dioxygenase cDNA. Biochem. Biophys. Res. Commun. **168:**1–8.
9. **de la Maza, L. M., and E. M. Peterson.** 1988. Dependence of the in vitro antiproliferative activity of recombinant human γ-interferon on the concentration of tryptophan in culture media. Cancer Res. **48:**346–350.
10. **De Mayer, E., and J. De Mayer-Guignard.** 1988. Interferons and other regulatory cytokines, p. 91–113. J. Wiley & Sons, Inc., New York.
11. **De Mayer, E., and J. De Mayer-Guignard.** 1988. Interferons and other regulatory cytokines, p. 134–153. J. Wiley & Sons, Inc., New York.
12. **Feng, G. S., and M. W. Taylor.** 1989. Interferon γ-resistant mutants are defective in the induction of indoleamine 2,3-dioxygenase. Proc. Natl. Acad. Sci. USA **86:**7144–7148.
13. **Fleckner, J., H. H. Rasmussen, and J. Justesen.** 1991. Human interferon γ potently induces the synthesis of a 55-kDa protein (γ2) highly homologous to rabbit peptide chain release factor and bovine tryptophanyl-tRNA synthetase. Proc. Natl. Acad. Sci. USA **88:**11520–11524.
14. **Frolova, L. Y., M. A. Sudomoina, A. Y. Grigorieva, O. L. Zinovieva, and L. L. Kisselev.** 1991. Cloning and nucleotide sequence of the structural gene encoding for human tryptophanyl-tRNA synthetase. Gene **109:**291–296.
15. **Garret, M., B. Pajot, V. Trézéguet, J. Labouesse, M. Merle, J.-C. Gandar, J.-P. Benedetto, M.-L. Sallafranque, J. Alterio, M. Gueguen, C. Sarger, B. Labouesse, and J. Bonnet.** 1991. A mammalian tryptophanyl-tRNA synthetase shows little homology to prokaryotic synthetases but near identity with mammalian peptide chain release factor. Biochemistry **30:**7809–7817.
16. **Lazard, M., M. Mirande, and J.-P. Waller.** 1986. Expression of the aminoacyl-tRNA synthetase complex in cultured Chinese hamster ovary cells. J. Biol. Chem. **261:**3982–3987.
17. **Lazard, M., M. Mirande, and J.-P. Waller.** 1986. Overexpression of mammalian phenylalanyl-tRNA synthetase upon phenylalanine restriction. FEBS Lett. **216:**27–30.
18. **Lee, C. C., W. J. Craigen, D. M. Muzny, E. Harlow, and C. T. Caskey.** 1990. Cloning and expression of a mammalian peptide chain release factor with sequence similarity to tryptophanyl-tRNA synthetases. Proc. Natl. Acad. Sci. USA **87:**3508–3512.
19. **Morgen, S., and O. Söll.** 1978. Regulation of the biosynthesis of amino acid: tRNA ligases and of tRNA. Prog. Nucleic Acid Res. Mol. Biol. **21:**181–207.
20. **Neidhardt, F. C., J. Parker, and W. G. McKeever.** 1975. Function and regulation of aminoacetyl-tRNA synthetases in procaryotic and eucaryotic cells. Annu. Rev. Microbiol. **29:**215–250.
21. **Ozaki, Y., M. P. Edelstein, and D. S. Duch.** 1988. Induction of indoleamine 2,3-dioxygenase: a mechanism of the antitumor activity of interferon γ. Proc. Natl. Acad. Sci. USA **85:**1242–1246.
22. **Pestka, S., J. A. Langer, K. C. Zoon, and C. E. Samuel.** 1987. Interferons and their actions. Annu. Rev. Biochem. **56:**727–777.
23. **Pfefferkorn, E. R.** 1984. Interferon γ blocks the growth of Toxoplasma gondii in human fibroblasts by inducing the host cell to degrade tryptophan. Proc. Natl. Acad. Sci. USA **81:**908–912.
24. **Pfefferkorn, E. R., S. Rebhun, and M. Eckel.** 1986. Characterization of an indoleamine 2,3-dioxygenase induced by gamma interferon in cultured human fibroblasts. J. Interferon Res. **6:**267–279.
25. **Rapoza, P. A., S. G. Tahija, J. P. Carlin, S. L. Miller, M. C. Padilla, and G. I. Byrne.** 1991. Effect of interferon on a primary conjunctival epithelial cell model of trachoma. Invest. Ophthalmol. Visual Sci. **32:**2919–2923.
26. **Reid, L. E., A. H. Brasnett, C. S. Gilbert, A. C. Porter, D. R. Gewert, G. R. Stark, and I. M. Kerr.** 1989. A single DNA response element can confer inducibility by both alpha- and gamma-interferons. Proc. Natl. Acad. Sci. USA **86:**840–844.
27. **Rubin, B. Y., S. L. Anderson, L. Xing, R. J. Powell, and W. P. Tate.** 1991. Interferon induces tryptophanyl-tRNA synthetase expression in human fibroblasts. J. Biol. Chem. **266:**24245–24248.
28. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
29. **Shimizu, T., S. Nomiyama, F. Hirata, and O. Hayaishi.** 1978. Indoleamine 2,3-dioxygenase. Purification and some properties. J. Biol. Chem. **253:**4700–4706.
30. **Takikawa, O., T. Kuroiwa, F. Yamazaki, and R. Kido.** 1988. Mechanisms of interferon-γ action. J. Biol. Chem. **263:**2041–2048.
31. **Taylor, M. W., and G. Feng.** 1991. Relationship between interferon γ, indoleamine 2,3-dioxygenase, and tryptophan metabolism. FASEB J. **5:**2516–2522.