

IFN- β_{1b} Induces Kynurenine Pathway Metabolism in Human Macrophages: Potential Implications for Multiple Sclerosis Treatment

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ABSTRACT

Interferon- β_{1b} (IFN- β_{1b}) has limited efficacy in the treatment of relapsing-remitting multiple sclerosis (RRMS). The kynurenine pathway (KP) is chiefly activated by IFN- γ and IFN- α , leading to the production of a variety of neurotoxins. We sought to determine whether IFN- β_{1b} induces the KP in human monocyte-derived macrophages, as one explanation for its limited efficacy. Serial dilutions of IFN- β_{1b} (at concentrations comparable to those found in the sera of IFN- β_{1b} -treated patients) were added to human macrophage cultures. Supernatants were collected at various time points and assayed for the KP end product, quinolinic acid (QUIN). The effect of IFN- β_{1b} on the KP enzymes indoleamine 2,3-dioxygenase (IDO), 3-hydroxyanthranilate dioxygenase (3HAO), and quinolinate phosphoribosyltransferase (QPRTase) mRNA expression was assessed by semiquantitative RT-PCR. IFN- β_{1b} (≥ 10 IU/ml) led to increased mRNA expression of both IDO and QUIN production (7901 ± 715 nM) after 72 h at 50 IU/ml IFN- β_{1b} ($p < 0.0001$). This study demonstrates that IFN- β_{1b} , in pharmacologically relevant concentrations, induces KP metabolism in human macrophages and may be a limiting factor in its efficacy in the treatment of MS. Inhibitors of the KP may be able to augment the efficacy of IFN- β in MS.

THE KYNURENINE PATHWAY (KP) of tryptophan metabolism (Fig. 1) is induced in a number of inflammatory neurologic diseases, including experimental allergic encephalomyelitis,⁽¹⁾ and is characterized by the presence of infiltrating macrophages and activated microglia.^(2,3) These cells express all KP enzymes⁽⁴⁾ leading to tryptophan degradation after induction of the first and rate-determining enzyme, indoleamine 2,3-dioxygenase (IDO). Cytokines, such as interferon- γ (IFN- γ) and IFN- α , have been shown to upregulate IDO expression and initiate the KP,⁽⁵⁻⁷⁾ leading to the production of a variety of neuroactive metabolites.⁽⁸⁾ Of these, the N-methyl D aspartate (NMDR) receptor agonist and neurotoxin quinolinic acid (QUIN) is the most important.

IFN- β also has been shown to induce the KP,⁽⁹⁾ but published studies have not clarified which isoform of IFN- β does this.⁽⁷⁾ Moreover, it has not been clear if the concentrations of IFN- β used have been similar to those found in patients with multiple sclerosis (MS) treated with IFN- β . IFN- β is widely used to treat relapsing-remitting MS (RRMS), where it has been shown to decrease the frequency of attacks.^(10,11) However, the

benefits are modest, and there are a number of side effects associated with its use, including flu-like symptoms and possibly depression. QUIN has been associated with similar disturbances^(12,13) and is neurotoxic. Consequently, QUIN may be involved in these side effects, and its toxicity may dampen the efficiency of IFN- β_{1b} .

This present study sought to determine if IFN- β_{1b} induces the KP in human monocyte-derived macrophages (Mdm) at concentrations equivalent to those in the sera of patients being treated with this agent. If so, there could be a number of consequences, including tryptophan depletion leading to reduced availability for synthesis of serotonin and protein synthesis in general, as well as production of neuroactive KP metabolites, such as QUIN, which could disrupt normal glutamatergic neurotransmission.

Serial dilutions of IFN- β_{1b} (kindly donated by Schering-Plough, Sydney, Australia) were added to Mdm cultures. Supernatants were collected at various times and assayed for QUIN. The effect of IFN- β_{1b} on the KP enzymes IDO, 3-hy-

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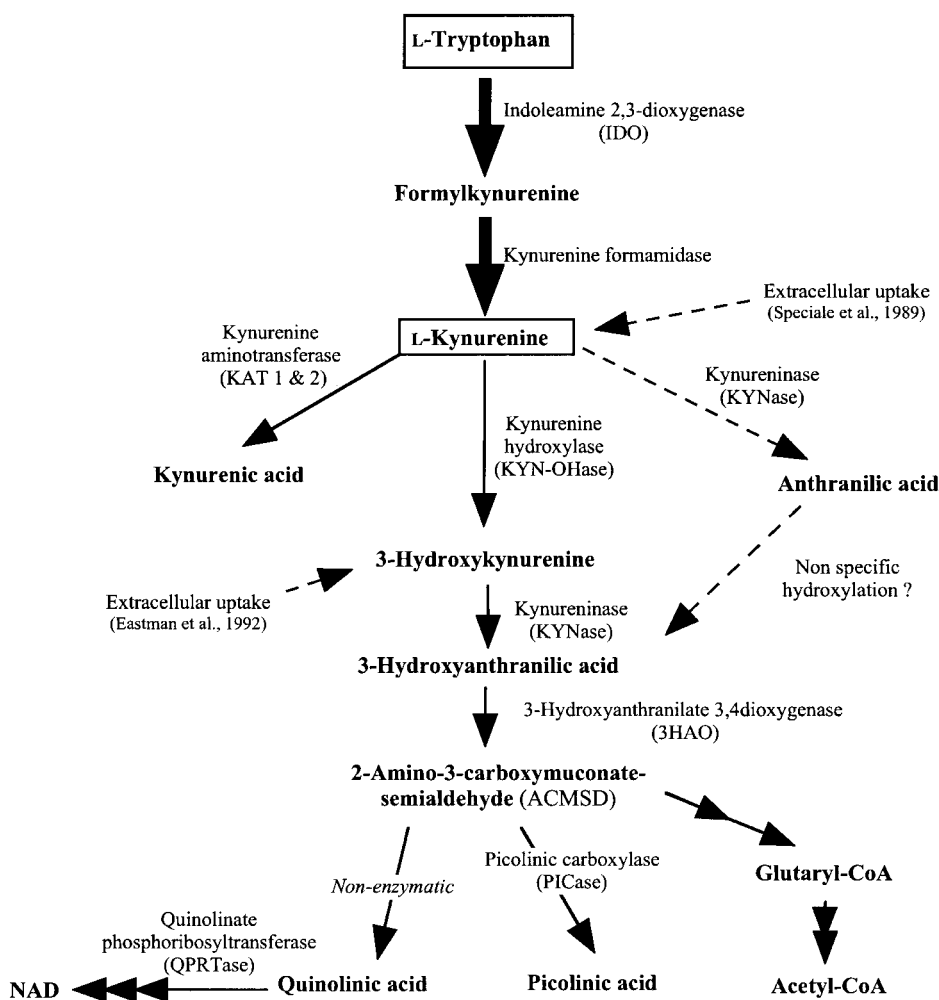


FIG. 1. Simplified kynurenine pathway (KP).

droxyanthranilate dioxygenase (3HAO), and quinolate phosphoribosyltransferase (QPRTase) mRNA expression was assessed by semiquantitative RT-PCR.

Human peripheral blood mononuclear cells (PBMC) were isolated from 100 ml blood of healthy volunteers using a standard Ficoll-Paque (Pharmacia, Uppsala, Sweden) density separation method.⁽¹⁴⁾ After 8 days *in vitro*, the serum component of the medium was eliminated completely, and cells were maintained in AIM-V, a serum-free medium containing no detectable QUIN (Life Technologies, Gaithersburg, MD). After 11 days *in vitro*, 98% of the adherent cells expressed macrophage markers, including CD68⁺⁺⁺, CD11c⁺⁺, and CD16⁺ (only on macrophages, not monocytes). Replicate cultures of primary human macrophages 10 or 11 days *in vitro* were stimulated for 24, 48, and 72 h with IFN- β_{1b} in AIM-V at concentrations of 0.1, 1, 10, and 50 IU/ml. These concentrations were chosen because they corresponded with those reported in the sera of patients after subcutaneous injection of this agent for treatment of RRMS.⁽¹⁵⁾ IFN- γ 100 IU/ml (Boehringer Mannheim, Mannheim, Germany) was used as a positive control, and AIM-V medium alone was used as a negative control. Additional controls included IFN- β_{1b} , heat inactivated at 55°C

for 1 h, and human albumin at the same concentration as present in the IFN- β_{1b} formulation. Each treatment was performed in triplicate or quadruplicate using macrophages derived from four different donors. Statistical comparisons were made with Statview 4.5 (Abacus Concepts, Berkeley, CA) using an ANOVA. QUIN was measured by gas chromatography electron capture negative ion mass spectrometry.⁽¹⁴⁾

In a parallel set of experiments, total RNA was extracted at 24 h using a Trizol protocol (Life Technologies), and mRNA expression of IDO, 3HAO, QPRTase, and GAPDH was assessed by RT-PCR.⁽¹⁶⁾

We showed that IFN- β_{1b} induces biosynthesis of QUIN. QUIN concentrations were significantly higher in cultures treated with IFN- β_{1b} at 1 IU/ml ($p < 0.05$), 10 IU/ml ($p < 0.0001$), and 50 IU/ml ($p < 0.0001$) than in unstimulated controls. IFN- β_{1b} 50 IU/ml was not significantly different from cultures stimulated with IFN- γ 100 IU/ml (Fig. 2). After 72 h, mean concentrations of QUIN (\pm SE) in cultures treated with IFN- β_{1b} were 0.1 IU/ml, 87 ± 38 nM; 1 IU/ml, 303 ± 69 nM; 10 IU/ml, 4922 ± 284 nM, 50 IU/ml, 7901 ± 715 nM; IFN- γ 100 IU/ml, 9618 ± 162 nM; unstimulated controls, 57 ± 34 nM.

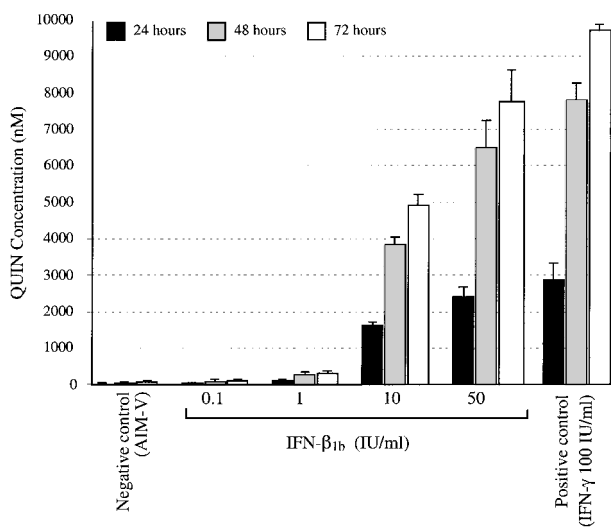


FIG. 2. Representative histogram showing mean cumulative QUIN concentrations present in tissue culture supernatants 24, 48, and 72 h after stimulation of single donor human macrophages (10 days *in vitro*) with IFN-β_{1b} or IFN-γ (n = 4).

IFN-β_{1b} induces mRNA expression of IDO but not 3HAO and QPRTase. No IDO mRNA expression was detected after stimulation of macrophages with 0.1 or 1 IU/ml IFN-β_{1b}. However, 1 IU/ml IFN-β_{1b} did lead to significant QUIN production (303 nM). IFN-β_{1b} at 10 and 50 IU/ml induced concentration-related increases in IDO mRNA expression (Fig. 3 and Table 1). IFN-γ induced both the higher level of IDO mRNA expression and QUIN synthesis. 3HAO mRNA was expressed in the presence or absence of IFN-β_{1b} or IFN-γ. QPRTase mRNA was consistently expressed in very low amounts regardless of the presence of cytokines. However, because of the low level of QPRTase mRNA expression, accurate quantitation relative to the reporter gene GAPDH was not possible. mRNA expression for GAPDH was positive and homogeneous in all cultures (Fig. 3).

This study confirms and extends existing results,^(7,9) showing that concentrations of IFN-β_{1b}, equivalent to those occurring in MS patients treated with IFN-β, induce expression of IDO, the first and rate-determining enzymatic step of the KP and thus activate the KP in human macrophages. 3HAO and QPRTase were constitutively expressed by macrophages regardless of IFN-β_{1b}. These findings may have relevance to the limited efficacy of IFN-β_{1b} in addition to its side effect profile.

Before considering the potential significance of these findings, there are some issues that might mitigate their importance. First, it is possible that KP induction *in vivo* secondary to IFN-β therapy would be a transient phenomenon, although this is unlikely. A recent study has documented elevated serum neopterin concentrations in IFN-β-treated patients after 52 weeks of therapy.⁽¹⁷⁾ Neopterin, a product of activated macrophages, is elevated when the KP is activated. Indeed, we and others have shown previously that neopterin concentrations are significantly associated with QUIN concentrations.^(18,19) Second, the concentrations of IFN-β_{1b} used in this study may not truly correspond to those in MS patients. This too is unlikely.

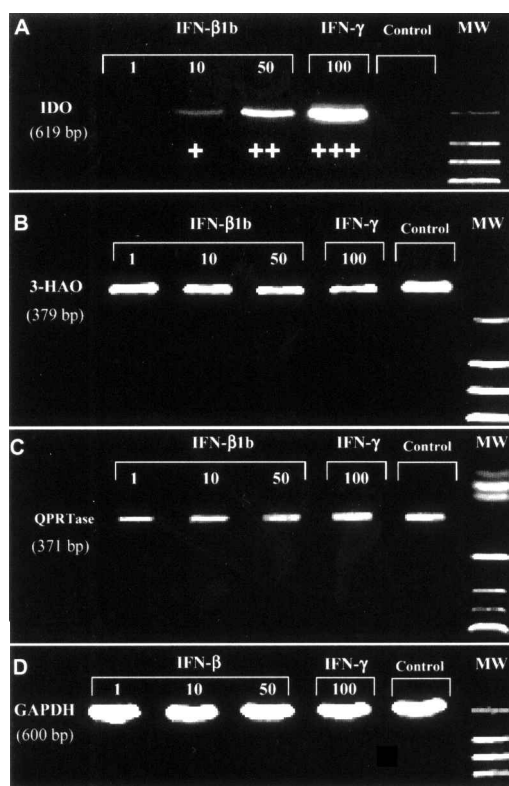


FIG. 3. Ethidium bromide-stained gel showing PCR products of macrophages 24 h after stimulation with IFN-β_{1b} or IFN-γ.

The concentrations of IFN-β_{1b} leading to IDO induction were less than the concentrations reported by Khan et al.⁽¹⁵⁾ in the serum of patients after subcutaneous injection of this agent for treatment of RRMS. In this study, 11 of 15 patients treated with 8 × 10⁶ IU/ml IFN-β_{1b} had detectable serum levels ranging from 120 to 475 IU/ml when samples were drawn between 12 and 36 h after subcutaneous injection. Third, systemic activation of the KP might not be relevant to the brain. This too is unlikely. Although IFN-β_{1b} does not readily cross the blood-brain barrier, systemic KP induction, by a poorly understood mechanism, can activate this pathway in the central nervous system (CNS).⁽²⁰⁾ Moreover, KP intermediates, such as kynurenine (Fig. 1), which are synthesized peripherally after IDO induction, readily cross the blood-brain barrier^(18,21) and are also

TABLE 1. EFFECT OF IFN-β_{1b} AND IFN-γ ON IDO, 3HAO, AND QPRTASE EXPRESSION IN MACROPHAGES AFTER 24 H^a

Treatment	IDO	3HAO	QPRTase
IFN-β _{1b} , 1 IU/ml	0	0.58	0.20
IFN-β _{1b} , 10 IU/ml	0.02	0.53	0.18
IFN-β _{1b} , 50 IU/ml	0.26	0.43	0.19
IFN-γ, 100 IU/ml	0.95	0.41	0.28
Control	0	0.76	0.31

^aImage analysis ratios of KP mRNA are expressed relative to GAPDH mRNA.

synthesized at high level by astrocytes within the brain,⁽⁴⁾ where they can provide extra substrate into the KP of infiltrating macrophages and microglia, leading to QUIN production.

Our results have potential clinical significance. Whetsell and Schwarcz⁽²²⁾ showed time-dependent damage to rat neurons by QUIN concentrations as low as 100 nM. We have also reported that exposure of human neurons to QUIN concentrations of 350 nM produces a number of neuronal changes, including segmented dendritic beading and microtubular fragmentation.⁽²³⁾ This concentration is close to the one induced by IFN- β_{1b} after 72 h (303 nM). Therefore, QUIN may cause neurologic and behavioral changes by disturbing the ability of neuronal dendrites to integrate incoming signals. Moreover, a recent study demonstrated that QUIN can kill oligodendrocytes.⁽²⁴⁾

Our results are in accord with the existing literature and extend such data.^(7,9) A number of studies have compared the effects of IFN- β and IFN- γ on tryptophan degradation by monocytes. However, their relevance to IFN- β as used in MS has been obscured by the lack of information as to which subtype, 1 α or 1 β , was used⁽⁷⁾ or by the use of monocytes that may not have matured sufficiently to mimic tissue macrophages, making studies difficult to compare. Peripheral blood monocytes *in vitro* are known to differentiate over time into culture-derived macrophages, which share many of the same attributes as resident tissue macrophages.^(25,26) Although some of these characteristics are present after 1–2 days *in vitro*, others, including changes in enzyme and functional activity, cytokine production, and changes in membrane markers, occur between 5 and 10 days *in vitro*.^(25–27) Our study used macrophages at 10–11 days *in vitro*, when differentiation is complete (CD16⁺). Werner-Felmayer et al.⁽²⁸⁾ reported that human MDM cultures stimulated (3 days after they reached confluence) for 48 h with 1000 IU/ml IFN- β catabolized approximately 50% less of tryptophan than after stimulation with 100 IU/ml IFN- γ . Jansen and Reinhard,⁽⁷⁾ using human MDM cultures after 7 days *in vitro*, showed that IFN- γ was the most potent stimulus for QUIN synthesis rather than IFN- β , and IFN- α was more often least potent. Murray et al.⁽²⁹⁾ studied IFN and the relationship between tryptophan degradation and the antimicrobial activity of macrophages after 5 days *in vitro*. They reported that 500 IU IFN- β or IFN- γ readily depleted the culture medium of tryptophan secondary to IDO activation.⁽²⁹⁾ It has also been reported that concentrations of IFN- β ranging from 20 to 200 IU induced expression of IDO in macrophages between 10 and 14 days *in vitro*.⁽³⁰⁾

We also report here that the QUIN-degrading enzyme, QPRTase, is constitutively expressed at very low levels by macrophages.⁽⁴⁾ QPRTase activity has been observed in cortical and subcortical regions of the human brain,⁽³¹⁾ and the enzyme is localized to both glial cells and neurons.^(4,32) These observations, together with QUIN accumulations in these experiments and CNS accumulations of QUIN in inflammatory brain disease,⁽²⁾ suggest that the activity of QPRTase may be easily saturated, thereby leaving neurotoxic concentrations of QUIN unchecked.

Clinical trials of IFN- β_{1b} in MS demonstrate a decrease in the frequency of RRMS attacks and a small delay in development of disability. However, the mechanism of the beneficial effects of this agent in MS are uncertain. IFN- β has a variety of proinflammatory and anti-inflammatory effects on different immunologic cells, which are implicated in MS pathogenesis. It has been suggested that the complexity and, at times, contradictory effects of IFN- β on T lymphocytes, endothelial cells, and microglia may

explain the modest clinical effects of this agent.⁽³³⁾ KP induction by IFN- β_{1b} introduces another factor into this scenario. Yet another variable is suggested by our previous report that macrophages from different donors produce vastly differing amounts of QUIN in response to the same stimulus.⁽³⁴⁾ Jansen et Reinhard⁽⁷⁾ subsequently demonstrated an intersubject variability for QUIN production after exposure to the IFN. In some subjects, IFN- α was more potent than IFN- β , and conversely.

Our present results demonstrate that macrophages stimulated with IFN- β_{1b} at concentrations equivalent to those in the serum of MS patients treated with this agent produced significantly higher levels of QUIN than did unstimulated macrophages. The QUIN accumulation and KP activation occurred secondary to induction of IDO. Individual donor differences in distribution and elimination of IFN- β_{1b} ⁽¹⁵⁾ and individual variations in susceptibility to KP induction^(7,34) may identify patients who would be poor responders or those at risk for adverse effects. MS patients who demonstrate a proinflammatory response to IFN- β_{1b} may be less likely to benefit from this therapy.⁽⁷⁾ In these patients, concomitant blockade of the KP pathway with one of the KP inhibitors that are under development⁽³⁵⁾ may offer a way to reduce the severity of some side effects of IFN- β_{1b} and may improve the neurologic efficacy of this agent.

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REFERENCES

1. FLANAGAN, E.M., ERICKSON, J.B., VIVEROS, O.H., CHANG, S.Y., and REINHARD, J.F., Jr. (1995). Neurotoxin quinolinic acid is selectively elevated in spinal cords of rats with experimental allergic encephalomyelitis. *J. Neurochem.* **64**, 1192–1196.
2. HEYES, M.P., SAITO, K., CROWLEY, J.S., DAVIS, L.E., DEMITRACK, M.A., DER, M., DILLING, L.A., ELIA, J., KRUESI, M.J.P., LACKNER, A., LARSEN, S.A., LEE, K., LEONARD, H.L., MARKEY, S.P., MARTIN, A., MILSTEIN, S., MOURADIAN, M.M., PRANZATELLI, M.R., QUEARRY, B.J., SALAZAR, A., SMITH, M., STRAUSS, S.E., SUNDERLAND, T., SWEDO, S.W., and TOURTELLOTTE, W.W. (1992). Quinolinic acid and kynurenine pathway metabolism in inflammatory and non-inflammatory neurological disease. *Brain* **115**, 1249–1273.
3. SANI, L.A., THOMAS, S.R., TATTAM, B.N., MOORE, D.E., CHAUDHRI, G., STOCKER, R., and HUNT, N.H. (1998). Dramatic changes in oxidative tryptophan metabolism along the kynurenine pathway in experimental cerebral and non-cerebral malaria. *Am. J. Pathol.* **152**, 611–619.
4. GUILLEMIN, G.J., KERR, S.J., SMYTHE, G.A., SMITH, D.G., KAPOOR, V., ARMATI, P.J., CROITORU, J., and BREW, B.J. (2001). Kynurenine pathway metabolism in human astrocytes: a paradox for neuronal protection. *J. Neurochem.* **78**, 1–13.
5. TAKIKAWA, O., KUROIWA, T., YAMAZAKI, F., and KIDO, R. (1988). Mechanism of interferon- γ action. Characterization of

- indoleamine 2,3-dioxygenase in cultured human cells induced by interferon- γ and evaluation of the enzyme mediated tryptophan degradation in its anticellular activity. *J. Biol Chem.* **263**, 2041–2048.
6. PEMBERTON, L.A., KERR, S.J., SMYTHE, G., and BREW, B.J. (1997). Quinolinic acid production by macrophages stimulated with IFN- γ , TNF- α and IFN- α . *J. Interferon Cytokine Res.* **17**, 589–595.
 7. JANSEN, M., and REINHARD, J.F., JR. (1999). Interferon response heterogeneity: activation of a proinflammatory response by interferon alpha and beta. A possible basis for diverse responses to interferon beta in MS. *J. Leukocyte Biol.* **65**, 439–443.
 8. BOTTING, N.P. (1995). Chemistry and neurochemistry of the kynurenine pathway of tryptophan metabolism. *Chem. Soc. Rev.* **24**, 401–412.
 9. KERR, S.J., PEMBERTON, L.A., GUILLEMIN, G.J., ARMATI, P.J., SMYTHE, G., and BREW, B.J. (1998). Interferon- β induces kynurenine pathway metabolism in human macrophage [Abstract]. *Neurology* **50**, P01.074.
 10. IFNB MULTIPLE SCLEROSIS STUDY GROUP. (1993). Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. I. Clinical results of a multicenter, randomized, double-blind, placebo controlled trial. *Neurology* **43**, 655–661.
 11. IFNB MULTIPLE SCLEROSIS STUDY GROUP AND THE UNIVERSITY OF BRITISH COLUMBIA MS/MRI ANALYSIS GROUP. (1995). Interferon beta-1b in the treatment of multiple sclerosis: final outcome of the randomized controlled trial. *Neurology* **45**, 1277–1285.
 12. MILASIUŠ, A.M., GRINEVICIUS, K.K., and LAPIN, I.P. (1990). Effect of quinolinic acid on wakefulness and sleep in the rabbit. *J. Neural Transm. Gen. Sect.* **82**, 67–73.
 13. POPOLI, P., PEZZOLA, A., DOMENICI, M.R., SAGRATELLA, S., DIANA, G., CAPORALI, M.G., BRONZETTI, E., VEGA, J., and SCOTTI, d.C.A. (1994). Behavioral and electrophysiological correlates of the quinolinic acid rat model of Huntington's disease in rats. *Brain Res. Bull.* **35**, 329–335.
 14. KERR, S.J., ARMATI, P.J., PEMBERTON, L.A., SMYTHE, G., TATTAM, B., and BREW, B.J. (1997). Kynurenine pathway inhibition reduces toxicity of HIV-infected macrophages. *Neurology* **49**, 1671–1681.
 15. KHAN, O.A., XIA, Q., BEVER, C.T., JOHNSON, K.P., PANTICH, H.S., and DHIB-JALBUT, S.S. (1996). Interferon beta-1b serum levels in multiple sclerosis patients following subcutaneous administration. *Neurology* **46**, 1639–1643.
 16. GUILLEMIN, G.J., KERR, S.J., SMYTHE, G.A., ARMATI, P.J., and BREW, B.J. (1999). Kynurenine pathway metabolism in human astrocytes. *Adv. Exp. Med. Biol.* **467**, 125–131.
 17. RUDICK, R.A., SIMONIAN, N.A., ALAM, J.A., CAMPION, M., SCARAMUCCI, J.O., JONES, W., COATS, M.E., GOODKIN, D.E., WEINSTOCK-GUTTMAN, B., HERNDON, R.M., MASS, M.K., RICHERT, J.R., SALAZAR, A.M., MUNSCHAUER, F.E., COOKFAIR, D.L., SIMON, J.H., and JACOBS, L.D. (1998). Incidence and significance of neutralizing antibodies to interferon beta-1a in multiple sclerosis. Multiple Sclerosis Collaborative Research Group (MSCRG). *Neurology* **50**, 1266–1272.
 18. HEYES, M.P., BREW, B.J., SAITO, K., QUEARRY, B.J., PRICE, R.W., LEE, K., BHALLA, R.B., DER, M., and MARKEY, S.P. (1992). Inter-relationships between quinolinic acid, neuroactive kynurenines, neopterin and β_2 -microglobulin in cerebrospinal fluid and serum of HIV-1-infected patients. *J. Neuroimmunol.* **40**, 71–80.
 19. BREW, B.J., DUNBAR, N., PEMBERTON, L., and KALDOR, J. (1996). Predictive markers of AIDS dementia complex: CD4 cell count and cerebrospinal fluid concentrations of beta $_2$ -microglobulin and neopterin. *J. Infect. Dis.* **174**, 294–298.
 20. SAITO, K., CROWLEY, J.S., MARKEY, S.P., and HEYES, M.P. (1993). A mechanism for increased quinolinic acid formation following acute systemic immune stimulation. *J. Biol. Chem.* **268**, 15496–15503.
 21. FUKUI, S., SCHWARCZ, R., RAPOPRT, S.I., TAKADA, Y., and SMITH, Q.R. (1991). Blood-brain barrier transport of kynurenines: implications for brain synthesis and metabolism. *J. Neurochem.* **56**, 2007–2017.
 22. WHETSELL, W.O., Jr., and SCHWARCZ, R. (1989). Prolonged exposure to submicromolar concentrations of quinolinic acid causes excitotoxic damage in organotypic cultures of rat corticostriatal system. *Neurosci. Lett.* **97**, 271–275.
 23. KERR, S.J., ARMATI, P.J., GUILLEMIN, G.J., and BREW, B.J. (1998). Chronic exposure of human neurons to quinolinic acid results in neuronal changes consistent with AIDS dementia complex. *AIDS* **12**, 355–363.
 24. CAMMER, W. (2001). Oligodendrocyte killing by quinolinic acid *in vitro*. *Brain Res.* **896**, 157–160.
 25. ANDREESSEN, R., BRUGGER, W., SCHEIBENBOGEN, C., KREUTZ, M., LESER, H.-G., REHM, A., and LOHR, G.W. (1990). Surface phenotype analysis of human monocyte to macrophage maturation. *J. Leukocyte Biol.* **47**, 490–497.
 26. JOHNSON, W.D., MEI, B., and COHN, Z.A. (1977). The separation, long-term cultivation, and maturation of the human monocyte. *J. Exp. Med.* **146**, 1613–1626.
 27. TRIGLIA, T., BURNS, G.F., and WERKMEISTER, J.A. (1985). Rapid changes in surface antigen expression by blood monocytes cultured in suspension or adherent to plastic. *Blood* **65**, 921–928.
 28. WERNER-FELMAYER, G., WERNER, E.R., FUCHS, D., HAUSEN, A., REIBNEGGER, G., and WACHTER, H. (1989). Characteristics of interferon induced tryptophan metabolism in human cells *in vitro*. *Biochim. Biophys. Acta* **1012**, 140–147.
 29. MURRAY, H.W., SZURO-SUDOL, A., WELLNER, D., OCA, M.J., GRANGER, A.M., LIBBY, D.M., ROTHERMEL, C.D., and RUBIN, B.Y. (1989). Role of tryptophan degradation in respiratory burst-independent antimicrobial activity of gamma interferon-stimulated human macrophages. *Infect. Immun.* **57**, 845–849.
 30. HISSONG, B.D., and CARLIN, J.M. (1977). Potentiation of interferon-induced indoleamine 2,3-dioxygenase mRNA in human mononuclear phagocytes by lipopolysaccharide and interleukin-1. *J. Interferon Cytokine Res.* **17**, 387–393.
 31. FOSTER, A.C., WHETSELL, W.O., Jr., BIRD, E.D., and SCHWARCZ, R. (1985). Quinolinic acid phosphoribosyltransferase in human and rat brain: activity in Huntington's disease and in quinolinate-lesioned rat striatum. *Brain Res.* **336**, 207–214.
 32. DU, F., OKUNO, E., WHETSELL, W.O., KÖHLER, C., and SCHWARCZ, R. (1991). Immunohistochemical localization of quinolinic acid phosphoribosyltransferase in the human neostriatum. *Neuroscience* **42**, 397–406.
 33. HALL, G.L., COMPSTON, A., and SCOLDING, N.J. (1977). Beta-interferon and multiple sclerosis. *Trends Neurosci.* **20**, 63–67.
 34. CUNNINGHAM, A., NAIF, H., SAKSENA, N., LYNCH, G., RASO, V., LI, S., CHANG, J., ALALI, M., SOZWIAK, R., WANG, B., SLOANE, A., PEMBERTON, L., and BREW, B. (1997). HIV infection of macrophages and the pathogenesis of the AIDS dementia complex: interaction of the host cell and viral genotype. *J. Leukocyte Biol.* **62**, 117–125.
 35. STONE, T.W. (2000). Inhibitors of the kynurenine pathway. *Eur. J. Med. Chem.* **35**, 179–186.

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