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IFNs Are Critical Regulators of IL-1 Receptor Antagonist and IL-1 Expression in Human Microglia

Judy S. H. Liu, Terry D. Amaral, Celia F. Brosnan, and Sunhee C. Lee

Because IL-1 is implicated in the pathogenesis of multiple sclerosis, and IFNs are known to alter disease course, we sought to determine whether IFNs can regulate the expression of IL-1 and IL-1R antagonist (IL-1Ra) in primary cultures of human microglia and astrocytes. We found that IL-1 and IL-1Ra are products of microglia but not astrocytes, and IFN-β and IFN-γ differentially modulate LPS- and cytokine-induced IL-1 and IL-1Ra. IFN-β induces IL-1Ra and augments LPS- and IL-4-induced IL-1Ra, but suppresses LPS- and IL-1-induced IL-1, shifting the balance toward the expression of the IL-1Ra. Like IFN-β, IFN-γ suppresses the expression of both LPS and IL-1-induced IL-1β. However, IFN-γ also suppresses the expression of IL-1β and IL-4-induced IL-1Ra so that IFN-γ may enhance or suppress IL-1 activity depending on the other cytokines present. IL-4 has similar effects to IFN-β; however, other anti-inflammatory cytokines, did not regulate IL-1 or IL-1Ra in human microglia. Our data demonstrate a novel suppressive effect of IFN-β and IL-4 on IL-1 activity in human microglia, suggesting that IFN-β, a therapeutic agent used for multiple sclerosis, could have wider applications in the treatment of other central nervous system disorders in which IL-1 activity has been implicated in the pathogenesis. The Journal of Immunology, 1998, 161: 1989–1996.

Interleukin-1 is a pluripotent cytokine involved in the activation of immunologic responses and inflammation that has been implicated as a key factor in the initiation of tissue damage in a number of different disorders. The IL-1 gene family contains at least two IL-1 agonists, IL-1α and IL-1β, which require post-translational processing by IL-1-converting enzyme for biologic activity (1). Following processing, IL-1α is expressed on the cell surface, whereas IL-1β is secreted. The activity of IL-1 is blocked by a naturally occurring receptor antagonist (IL-1Ra)3 that binds to the type I IL-1R, but does not initiate signal transduction. Two receptors have been characterized. In addition to the type I receptor, which mediates the effect of IL-1 binding, the type II receptor is thought to function as a decoy receptor, capturing IL-1 and thus inhibiting its binding to the type I receptor (2). Regulation of this gene family has been shown to be complex and to involve multiple steps in the processing and release pathways as well as the differential induction of agonist and antagonist activity by other pro- or anti-inflammatory cytokines (for review, see Ref. 3).

In the central nervous system (CNS), IL-1 has been implicated as a central mediator of tissue damage and destruction in a number of diseases. It has been shown to be expressed in activated microglia and macrophages in acute and chronic active lesions of multiple sclerosis (MS) and also in HIV encephalitis (4–6). In Alzheimer’s disease, IL-1 is expressed in activated microglial cells in senile plaques (7, 8). In human stroke, we find that IL-1 expression in microglia is one of the earliest events occurring in ischemic brain tissue. Furthermore, IL-1β, but not IL-1Ra, is induced in human microglia rendered hypoxic in vitro (M. Downen and S. C. Lee, unpublished observations). In animal models of focal cerebral ischemia, administration of IL-1Ra reduces the infarct volume, suggesting that IL-1 is a pivotal cytokine in lesion formation (9).

Inappropriate expression of IL-1 in the CNS may contribute to CNS dysfunction in a number of ways. IL-1 can activate the endothelium, up-regulating a variety of factors, such as adhesion molecules (10) and eicosanoids (11), and resulting in alterations in blood-brain barrier permeability. In addition to its direct effect on the endothelium, IL-1 induces a number of factors in glial cells that can exacerbate blood-brain barrier disruption. In both microglia and astrocytes, it has been shown to induce β-chemokine expression (12, 13). IL-1 also induces astrocyte production of the type II nitric oxide synthase, resulting in the generation of high levels of nitric oxide (13), a potent vasodilator. IL-1 may contribute to the establishment of chronic inflammatory states through its induction of cytokines such as TNF-α and IL-6 in astrocytes as well as inducing its own gene expression in microglia. Furthermore, astrocyte production of TNF-α (14, 15) and of type II nitric oxide (iNOS) may result in the apoptosis/necrosis of neurons and oligodendrocytes (16, 17). Thus, iNOS and TNF-α expression may be implicated in the loss of myelin evident in MS and HIV encephalitis. IL-1 has also been shown to induce astrocyte production of factors implicated in the development of Alzheimer’s dementia, including amyloid precursor protein (18, 19), α1-antichymotrypsin (20), and S-100 protein (8), all of which potentiate neuronal degeneration.

It is believed that IL-1 is a key activator of astrocytes. In addition to the induction of a variety of inflammatory and cytotoxic mediators in astrocytes, IL-1 has other profound effects on astrocyte biology. In rodents, Guilian et al. have demonstrated that IL-1 is a mitogen for astrocytes in vitro and that intracerebral injection of IL-1 induces a reactive gliosis (21). Although IL-1 is not a mitogen in human astrocytes, it induces a dramatic reorganization of the cytoskeleton, resembling a stress response (22). These findings support the idea that unlike other cytokines and LPS, IL-1...
may be a key regulator of astrocyte activation (21, 23). Furthermore, IL-1Ra, a physiologically occurring IL-1 antagonist, could function as an important anti-inflammatory cytokine, inhibiting the activation of astrocytes. In human astrocyte cultures, IL-1Ra can suppress the expression of iNOS and TNF-α, while many of the inhibitory cytokines, including TGF-β, IL-4, and IL-10, have no direct effect on astrocyte activation (13).

These results suggest that the relative levels of IL-1 and IL-1Ra may determine the extent of tissue injury in the CNS. As noted above, regulation of the IL-1 family is complex and shows both cell-type and species-specific regulatory pathways. IL-1 is expressed by cells of the monocyte/macrophage lineage, and IL-1Ra is expressed by macrophages and neutrophils (3, 24). In the peripheral, IL-1β processing appears to be more tightly regulated in tissue macrophages than in blood monocytes, indicating that even within cells belonging to the same lineage, the regulation of IL-1 activity may differ (25). In the rodent CNS, cultures of both microglia and astrocytes are capable of expressing IL-1α and IL-1β after LPS stimulation (26, 27), and constitutive expression of both IL-1 and IL-1Ra has been reported in human glioma cells (28).

In this study we have examined the regulation of IL-1 and IL-1Ra expression in highly purified cultures of human fetal microglia and astrocytes in response to pro- and anti-inflammatory cytokines. Since our studies have been directed toward an understanding of the role of CNS-derived cytokines in the regulation of tissue injury in MS, we have focused on the effect of IFN-γ and IFN-β. IFN-γ is a Th1-type cytokine involved in the initiation of inflammatory events and is known to exacerbate MS, whereas IFN-β has been shown to ameliorate disease progression and is one of only two accepted therapeutic agents for this disorder. Comparisons were made with other known regulators of the IL-1 system. The results show that the production of IL-1 and IL-1Ra in human fetal glial cells differs from that found in rodents, being restricted to microglia, and that IFN-β differentially regulates IL-1 and IL-1Ra, suggesting that this cytokine may have therapeutic potential in a wide range of CNS disorders in which IL-1 has been implicated in the initiation of tissue damage.

Materials and Methods

Reagents

Recombinant human cytokines were purchased from Genzyme (Cambridge, MA; TNF-α, IFN-γ, IL-6, granulocyte-macrophage CSF, and IL-1α) or from R&D Systems (Minneapolis, MN; IL-4, IL-10, and TGF-β). IFN-β was purchased from Promega (Madison, WI). Recombinant human IL-1β was provided by Dr. C. Reynolds at National Cancer Institute. LPS (Escherichia coli) was obtained from Sigma (St. Louis, MO). FCS was purchased from Whittaker (Walkersville, MD). Culture medium was obtained from Whittaker or Cellgro (by Mediatech, Herndon, VA).

Cell culture

Human fetal brain cell cultures were established from second trimester human fetal abortuses as previously described (29), with minor modifications. Cerebral tissues were triturated and then incubated with gentle rocking for 45 min at 37°C in HBSS containing 0.05% trypsin/0.53 mM EDTA and DNase. Cells were resuspended in complete medium (DMEM with 4.5 g/l glucose and 2 ml/L L-glutamine, 5% heat-inactivated FCS, 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml fungizone) and filtered successively through 230- and 130-μm pore size nylon meshes. Cells were plated at 4 × 10⁶ cells/10 ml medium in Petri dishes for RNA preparation or at 2 to 4 × 10⁵ cells/well in flat-bottom 96-well plates for protein analysis (ELISA). Microglial cultures were washed with fresh medium at 1 to 6 h after plating to remove nonadherent contaminating cells. Enriched astrocyte cultures were prepared by subculturing mixed brain cell cultures once or twice into 75-cm² flasks, then into 96-well plates. Culture purity was determined by immunostaining for glial fibrillary acidic protein (astrocytes), mitogen-activated protein-2 (neurons), and CD68 (microglia), as previously described (29).

Cell stimulation and cytokine ELISA

Triple well plates of either microglia or astrocytes at 2 to 4 × 10⁶ cells/well in 96-well plates were treated by cytokines at the concentrations indicated in the figure legends. Culture medium was completely changed at 0 h. After the times indicated in the figure legends, culture supernatants were collected, and cell lysates were prepared by repeated freezing and thawing in 0.1 ml fresh medium (14). Initial determination of cytokine levels in the two compartments revealed that in microglia, >80% of IL-1α is secreted, while >80% of IL-1β is cell associated. All data presented are IL-1α levels determined in the supernatants and IL-1β levels of the same well. ELISAs for IL-1β, TNF-α, and IL-1Ra were performed using commercial ELISA kits following the manufacturers’ instructions. ELISA kits (or Ab pairs) purchased from either R&D Systems or Immunotech (Westbrook, ME) were used to determine the levels of TNF-α and IL-1β. The sensitivity for TNF-α was approximately 20 pg/ml, and those for IL-1β were 5 to 10 pg/ml for R&D kits and 20 pg/ml for Immunotech kits. Samples for IL-1Ra were tested either directly or after 1/10 or 1/100 dilution by ELISA kits from R&D Systems with a detection range of 20 to 1000 pg/ml.

Northern blot analysis

Microglia or astrocytes were plated in 100-mm petri dish or in 75-cm² tissue culture flasks and were treated with LPS or cytokines at concentrations described for the ELISA. At the indicated times, cells were washed twice with sterile PBS, and total RNA was extracted with Trizol (Life Technologies, Grand Island, NY) according to the instructions provided by the company. Twenty micrograms of total RNA from astrocyte samples or all RNA extracted from microglia samples were separated in a 1% formaldehyde-agarose gel by electrophoresis. RNA was transferred to a Hybond membrane (Amer sham, Arlington Heights, IL) and was cross-linked by UV and vacuum baking at −80°C for 2 h. The blots were hybridized with 32P-labeled random primed cDNA probes specific to human TNF-α (both provided by Genentech, South San Francisco, CA), human IL-1α (provided by Immunex, Seattle, WA), or 18S RNA (gift from Dr. N. Amheim, State University of New York, Stony Brook, NY). Hybridization was performed in a buffer containing 5× sodium chloride sodium phosphate EDTA (SSPE), 5× Denhardt’s, 0.5% (w/v) SDS, and 20 μg/ml ssDNA for 4 h at 65°C following prehybridization for 2 h at 65°C. Blots were washed to a final stringency of 0.1× SSPE and 0.1% SDS. Blots were exposed to x-ray film (Eastman Kodak, Rochester, NY). Densitometry was performed using the Molecular Dynamics densitometer and ImageQuant software (Sunnyvale, CA).

Statistics

Data analysis was performed using SigmaStat (Jandel Scientific, San Rafael, CA). Cytokine concentrations in experimental groups were compared with the values in control cultures or in LPS-treated cultures using one-way analysis of variance and post-hoc analysis by Bonferroni’s method. p < 0.05 was considered significant. For pooling data from multiple experiments and comparing differences between groups, Wilcoxon signed rank test was performed using StatView for Windows, version 4.57 (Abacus Concepts, Berkeley, CA).

Results

IFNs and IL-4 regulate IL-1 and IL-1Ra expression in human microglia

In the first set of experiments, we tested whether type I and type II IFNs as well as the prototypical Th2 cytokine IL-4 could modulate the expression of IL-1 and IL-1Ra in primary cultures of human fetal microglia. We used LPS and IL-1α to activate the microglial cultures, since previous studies have shown that both these factors potently induce IL-1 mRNA and protein expression in cells of the monocyte/macrophage series. The response was measured using ELISAs specific for IL-1β and IL-1Ra (Fig. 1). Consistent with previous reports (14), no appreciable levels of IL-1β were detected in supernatants from untreated cells, whereas exposure to LPS and IL-1β led to the induction of nanogram amounts of IL-1β (Fig. 1A). When tested alone, IFN-γ, IFN-β, and IL-4 did not induce
IL-1, but each of these cytokines markedly suppressed IL-1β induction by LPS and IL-1α (p < 0.05 in all).

Levels of IL-1Ra were then determined in these same supernatants (Fig. 1B). In contrast to the results for IL-1, supernatants from untreated microglial cultures contained nanogram amounts of IL-1Ra, suggesting a predisposition toward antagonist expression in the normal CNS. The addition of IFN-γ to the culture medium did not affect this constitutive expression of IL-1Ra, but the addition of IFN-β and IL-4 led to a marked increase in the levels of IL-1Ra comparable to that noted following treatment with LPS (p < 0.05 in both). Experiments to determine the concentrations of IFN-β effective in enhancing IL-1Ra production revealed that IFN-β at 5 ng/ml or higher was necessary for IL-1Ra induction, and the levels of IL-1Ra kept increasing even at 0.5 to 1 μg/ml of IFN-β. Values from a representative experiment expressed as nanograms per milliliter of IL-1Ra (mean ± SD; n = 3) for 0, 0.5, 5, 50, and 500 ng/ml of IFN-β were 3.5 ± 0.3, 2.7 ± 0.8, 9.3 ± 1.0, 27.3 ± 5.4, and 101.6 ± 9.4. The addition of both IFN-β and IL-4 resulted in an enhancement of LPS-induced IL-1Ra expression (p < 0.05), whereas IFN-γ down-regulated LPS induction of IL-1Ra (p < 0.05). IL-1α did not induce IL-1Ra in human microglial cells (0–30% increase, but not significantly different from controls), nor did it modulate IL-1Ra production by IFN-β and IL-4.

To determine whether the regulation of IL-1 and IL-1Ra by IFNs was modulated at the level of transcription, we performed a Northern blot analysis of total RNA extracted from microglial cultures that had been stimulated with IFN-β and IFN-γ and tested the effects of these cytokines on IL-1-stimulated IL-1 production. The results are shown in Figure 2. Significant induction of IL-1β mRNA was observed only in cultures treated with IL-1β. The addition of IFN-γ or IFN-β potently suppressed the IL-1 mRNA induced by IL-1 (Fig. 2, A and B). When this same blot was stripped and reprobed for IL-1Ra, the results clearly showed that only IFN-β led to significant induction of IL-1Ra. Interestingly, cotreatment with IFN-γ reduced the level of IL-1Ra expression induced by IFN-β. IL-1β had little or no effect on the level of
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IL-1β, IL-1Ra, and TNF-α mRNA expression by IFNs. Microglia were treated for 18 h with LPS (100 ng/ml), IFN-γ (200 U/ml), and IFN-β (10 ng/ml), alone or in the combinations shown. Northern analysis was performed on total RNA, and the blot was probed for IL-1β, IL-1Ra, and TNF-α mRNA expression. Densitometric ratios to 18S RNA were determined. LPS induced both IL-1β and IL-1Ra. Cotreatment with IFN-γ down-regulated both IL-1β and IL-1Ra while IFN-β down-regulated IL-1β, but up-regulated IL-1Ra. Administration of both IFN-γ and IFN-β down-regulated LPS-induced IL-1β. IFN-γ down-regulated IFN-β-induced IL-1Ra, reducing the level of IL-1Ra mRNA to that induced by LPS alone. In contrast to the down-regulatory effect of IFN-γ on IL-1β and IL-1Ra expression, IFN-β enhanced LPS-induced TNF-α mRNA expression in microglia. IFN-β also had a mild stimulatory effect on LPS-mediated TNF-α expression.

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IL-1Ra mRNA expression in either the control or the IFN-treated microglial cultures. Thus, these data are in accord with the results obtained by ELISA.

From these results we conclude that IFN-β acts as a negative regulator of IL-1 in two ways: blocking the production of IL-1 and promoting the induction of IL-1Ra. In contrast, the effect of IFN-γ was more complex, with reduction of both IL-1 and IL-1Ra. All these effects occurred at the level of transcription.

We then tested the effects of IFN-β and IFN-γ on LPS-induced IL-1β and IL-1Ra mRNA expression (Fig. 3). In these experiments, both IFN-β and IFN-γ down-regulated IL-1β expression, but only IFN-β up-regulated LPS-induced IL-1Ra expression, again consistent with the results obtained by ELISA shown in Figure 1. Interestingly, when both IFN-γ and IFN-β were added to the LPS-treated cultures, the levels of IL-1Ra mRNA remained suppressed, indicating that IFN-γ can overcome the effect of IFN-β. These results with IFNs in LPS-stimulated cultures are similar to those in IL-1-stimulated microglia shown in Figure 2.

Since the results with IFN-γ demonstrated suppression of both IL-1 and IL-1Ra, we sought to determine whether IFN-γ exerted a global transcriptional down-regulation in microglia. To determine this, the same blot was stripped and reprobed for TNF-α mRNA expression (Fig. 3). The results demonstrated that in contrast to IL-1/IL-1Ra, the levels of TNF-α in LPS-treated microglia slightly increased after co-stimulation with IFN-γ. In addition, IFN-β also enhanced the level of LPS-induced TNF-α mRNA in microglia. The relatively minor increment in TNF-α mRNA induced by LPS treatment alone reflects the delayed time point chosen (18 h) for this experiment. When the protein levels were determined in three separate experiments, both IFN-γ and IFN-β up-regulated the levels of LPS-stimulated TNF-α in microglia by an average of 36 and 27%, respectively (data not shown). IFN-γ or IFN-β, when used without LPS, did not induce TNF-α. Thus, the results with TNF-α support the conclusion that the effects of IFN-γ on microglial cytokine expression do not represent a global down-regulation of transcription.

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**FIGURE 4.** IL-4 modulates LPS- and IL-1-stimulated IL-1β and IL-1Ra expression in microglia. Microglia were stimulated with IL-1β (10 ng/ml) or LPS (100 ng/ml) in the presence or the absence of IL-4 (10 ng/ml) as indicated to determine the role of IL-4 in the expression of IL-1β and IL-1Ra mRNA. The duration of stimulation was 16 h for the IL-1β experiment shown in A, and 6 h for the LPS experiment shown in B. Total RNA from 1 × 10^6 cells was extracted and subjected to Northern analysis. Densitometric ratios of IL-1β or IL-1Ra to 18S were determined. A, IL-4 inhibited IL-1β mRNA expression induced by IL-1β. IL-4 induced IL-1Ra expression, while IL-1β had little or no effect on IL-1Ra expression. B, IL-4 down-regulated LPS-induced IL-1β mRNA levels, but enhanced LPS-induced IL-1Ra.
IL-1β when tested alone. As noted previously, IL-4 dramatically down-regulated LPS-induced IL-1β production, whereas IL-10 induced a smaller, but consistent, down-regulatory effect. Neither TGF-β nor IL-6 had an inhibitory effect on LPS-induced IL-1β production in microglia. When the same microglial cultures were examined for the production of IL-1Ra, IL-4 showed the most dramatic effect on basal and LPS-induced levels of IL-1Ra, while both IL-10 and TGF-β had minor stimulatory effects (Fig. 5B). IL-6 did not affect the production of IL-1Ra in microglia. The results with IL-10 and TGF-β in microglial IL-1Ra production were variable from experiment to experiment, while IL-4 showed a consistent stimulatory effect. When results from five consecutive experiments were pooled, neither TGF-β nor IL-10 showed statistically significant differences in their effects on IL-1Ra production, alone or with LPS (Wilcoxon signed rank test; data not shown). These results demonstrate that in human microglia, IL-4 and IFN-β profoundly affect the expression of components of the IL-1 system, while TGF-β plays an insignificant role. The role of IL-10 is limited to the suppression of LPS-induced IL-1β expression, without affecting the levels of IL-1Ra or IL-1-induced IL-1β expression (data not shown).

**Human astrocytes produce neither IL-1β nor IL-1Ra**

We tested the ability of human astrocytes to produce components of the IL-1 system by exposing cells to the same set of stimuli as that used for microglia. Neither Northern blot analysis (data not shown) (14) nor ELISA demonstrated expression of IL-1β or IL-1Ra in human fetal astrocytes in experiments that included numerous stimuli and cells from many different brains. Figure 6 shows an ELISA from a representative experiment, comparing the levels of IL-1Ra and IL-1β in astrocyte cultures with those in control and LPS-stimulated microglial cultures. The presence of low levels of IL-1β in IL-1α-stimulated cultures probably represents either release from a few microglia in astrocyte cultures (<1%) and/or a cross-reactivity between IL-1α and anti-IL-1β Abs. Thus, the results indicate that in primary human glia, IL-1β and IL-1Ra are expressed exclusively in microglia.

**Discussion**

In this report we have shown that in human fetal glial cells in culture, only microglia can be activated to express IL-1β and IL-1Ra and that IFNs are potent modulators of this response. IFN-β acted as a negative modulator of IL-1 by suppressing both LPS- and IL-1-induced IL-1β, while inducing the production of IL-1Ra when used alone and in combination with LPS and IL-4. The induction of IL-1Ra was well over a 100-fold molar excess of maximal induction of IL-1β by LPS. This level of expression is sufficient to be of physiologic significance because it is much greater than the amount of IL-1Ra that was found to be necessary for complete suppression of the effect of IL-1β (13, 30). The effect of IFN-γ was more complex, with reduction of both LPS- and IL-1-induced IL-1β and reduction of LPS-, IFN-β-, and IL-4-induced IL-1Ra. Consistent with the data obtained in other cells of the monocytic/macrophage lineage, IL-4 induced IL-1Ra expression and suppressed LPS- or IL-1-induced IL-1 expression (31). IFN-β and IL-4 showed identical responses and potencies with respect to the regulation of IL-1β and IL-1Ra expression. The cytokines TGF-β, IL-10, and IL-6 had no significant effect on microglial IL-1β and IL-1Ra expression, except that IL-10 consistently suppressed LPS-induced IL-1β expression.

IFN-β is a type I IFN originally identified by its role as an antiviral factor in host defense (32). It is up-regulated in many cells

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**FIGURE 5.** Effect of anti-inflammatory cytokines on IL-1β and IL-1Ra expression. Microglia were plated as described in Figure 1 and were treated for 24 h. TGF-β, IL-10, and IL-6 were used at 10 ng/ml. A. Cell lysates were harvested for analysis by ELISA for IL-1β. None of the cytokines tested above induced IL-1β. LPS-induced IL-1β was down-regulated by IL-4 and to a lesser extent by IL-10. B. Cell culture supernatants were analyzed for IL-1Ra expression by ELISA. IL-1Ra was induced by IL-4 and to lesser degrees by TGF-β and IL-10. IL-4 enhanced LPS-induced expression of IL-1Ra, while IL-10, TGF-β, and IL-6 had minimal effects. Data are the mean ± SD from triplicate wells and are from one representative experiment of five with similar results.
Magnetic resonance imaging studies show enhancing lesions, indicating an effect of this cytokine on events treated as described in the text. ELISA was performed to determine the with cytokines and LPS as described in Figure 1. Microglia were plated and effect of IFN-\(\beta\) on the blood-brain barrier, tissue culture studies have implicated IL-1 as a unique factor responsible for human astrocyte activation. A reactive astrogliosis is a common response to injury in the CNS and is believed to be a barrier to regenerative activity (35). In acute MS lesions, hypertropic astrocytes are most abundant at the lesion edge, but are also present in the lesion center and in the surrounding white matter, and more chronic lesions are composed almost exclusively of gliotic astrocytes (35). Hypertropic astrocytes have been implicated in the production of a number of toxic factors, including TNF-\(\alpha\) (36), reactive nitrogen intermediates (5), and components of the complement system (37, 38), as well as factors that could contribute to the development of chronic inflammatory events. Thus, the effect of IFN-\(\beta\) on microglial or macrophage-derived IL-1 activity may function to suppress astrocyte activation and subsequent astrogliosis.

IFN-\(\gamma\), the prototypical Th1 cytokine and type II IFN, is a product of T cells and NK cells and is essential in the development of cell-mediated immune responses. IFN-\(\gamma\) has specific effects on monocytes and macrophages such as induction of MHC class II and priming of macrophages for phagocytic and antimicrobial activities. The role of IFN-\(\gamma\) in the expression of cytokines is less well defined. We and others found IFN-\(\gamma\) primes astrocytes to produce TNF-\(\alpha\) (14, 15, 23). In human microglia, we found that IFN-\(\gamma\) down-regulated mRNA and protein expression of both IL-1\(\beta\) and IL-1Ra, but had no effect on processing of IL-1\(\beta\) (data not shown). The fact that IFN-\(\gamma\) may act as a negative regulator of IL-1 in peripheral macrophages and adult rat microglia has been observed previously. Interestingly, however, differences were noted in that for peripheral macrophages this effect was observed when IL-1 was used as the stimulus (39), but in rat microglia, down-regulation was noted when LPS was used as the stimulus (40). The results reported here show that in human microglia, IFN-\(\gamma\) down-regulates both LPS- and IL-1-induced IL-1\(\beta\) production. It has been noted in several experimental model systems that IFN-\(\gamma\) down-regulates inflammation, including the animal model for MS, experimental autoimmune encephalomyelitis (41). Since IL-1 levels are dramatically increased at the onset of experimental autoimmune encephalomyelitis in the mouse, these results may explain the exacerbating effects of deletion of the gene for IFN-\(\gamma\) on disease expression in this model. IFN-\(\gamma\) has also been shown to down-regulate other effects mediated by IL-1, such as expression of IL-6 (42), IL-8 (43), and IL-10 (44); however, the mechanism by which IFN-\(\gamma\) mediates these inhibitory effects has not yet been determined.

The fact that IFN-\(\gamma\) also down-regulated LPS-induced IL-1Ra expression indicates, however, that the regulation of the IL-1 system in microglia by IFN-\(\gamma\) is complex. It is important to note that this result did not reflect a global down-regulation of transcriptional events by IFN-\(\gamma\) in microglia, since LPS-induced TNF-\(\alpha\) expression was not similarly affected, indicating a level of specificity for this response. A similar inhibition of LPS-induced IL-1Ra expression by IFN-\(\gamma\) was observed by Sone et al. (45) in peripheral blood monocytes; however, in that study IFN-\(\gamma\)-potenti-ated LPS-induced IL-1 production, leading to a net proinflammatory bias in the LPS-induced response. The overall bias toward general down-regulation of the IL-1 system demonstrated in this report suggests a tissue-specific effect. The fact that tissue macrophages (as opposed to circulating monocytes) are generally con-stitutively biased toward an IL-1Ra response has been noted in the lung and skin (45, 46). Our data indicate that CNS microglia show a similar bias and add further support to the observations that IFN-\(\gamma\) may act as a negative regulatory signal for inflammatory events in some circumstances.

The effect of IFN-\(\beta\) on the IL-1 system closely resembled that found with IL-4, a Th2 cytokine. Negative regulation of the IL-1 system by IL-4 in cells of the monocyte/macrophage lineage is well documented (31), and our data show a similar effect on human microglia. In addition to IL-4, granulocyte-macrophage CSF is also recognized as an inducer of IL-1Ra in the periphery (47, 48) and has the same effect on human fetal microglia (data not shown). In contrast to the findings with IL-4 and with peripheral macrophages, other cytokines with known negative regulatory roles in inflammatory responses, such as TGF-\(\beta\), IL-10, and IL-6, had slight and inconsistent effects on IL-1Ra production by human microglia. While TGF-\(\beta\) had no effect on IL-1 expression, IL-10 inhibited LPS-induced IL-1 expression (but not IL-1-induced IL-1 expression) in microglia, similar to that found in macrophages.
(49) Ongoing studies in our laboratory clearly indicate that this is a species-specific effect, at least for TGF-β, since this cytokine has been found to consistently down-regulate type II NOS in rodent glia (50). These data emphasize, therefore, the importance of studying both cell type- and species-specific effects of these regulators of inflammation.

Because our cultures are derived from fetal brain, the relevance of these studies to adult microglia and astrocytes may be questioned. However, previous work in this and other laboratories has shown that microglia cultured from second trimester fetal brain have a phenotype similar to that of adult microglia. Unlike cells derived from first trimester cultures (51, 52), second trimester microglia are capable of cytokine production, phagocytosis, MHC class II expression, and productive infection by HIV-1 (14, 29, 53). Human fetal astrocytes, like adult astrocytes, express glial fibrillary acidic protein (35), are coupled by gap junctions, express neurotransmitter receptors, and support the growth of neurons (J. S. H. Liu and S. C. Lee, unpublished results). Hence, human fetal microglia and astrocytes have similarities to adult cells and have proven to be a reliable model system for the study of inflammation in the human CNS.

In summary, we have demonstrated that in human fetal glial cells in culture, only microglia are a source of both IL-1β and IL-1Ra, and that both type I and type II IFNβ regulate components of the IL-1 system following activation by LPS or IL-1 itself. These data are the first to demonstrate that IFN-β is a potent negative regulator of IL-1 activity in microglia, down-regulating the production of IL-1 and up-regulating the production of IL-1Ra at the mRNA level. IFN-β has anti-inflammatory effects on other glial cells, as well. We have demonstrated selective down-regulation of iNOS in human fetal astrocytes by IFN-β (54). Recently, IFN-β has also been shown to down-regulate MHC class II expression on CNS cells (55), T cell-matrix protein interactions (56), and adhesion molecule expression (57). In contrast, it up-regulates monocyte-derived IL-10 (58) and astrocyte-derived nerve growth factor (59). The data presented here add substantially to the known regulatory activities of IFN-β and suggest that IFN-β may have wider implications in diseases of the CNS in which IL-1 has been implicated.

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