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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 IFN-β 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) 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 affect 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 periphery, 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 cell cultures 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). IL-1β was purchased from Peprotech (Rocky Hill, NJ). Recombinant human IFN-β (provided by 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 shaking for 45 min at 37°C in HBSS containing 0.05% trypsin/0.33 mM EDTA and DNase. Cells were resuspended in complete medium (DMEM with 4.5 g/l glucose and 2 mM L-glutamine, 5% heat-inactivated FCS, 100 μg/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml fungazone) and filtered successively through 230- and 130-μm pore size nylon meshes. Cells were plated at 4 × 10⁶ cells/ml in various cell culture flasks. Culture medium was completely replaced once at 7 days in vitro. At 14 days in vitro, floating cells were collected by pooling the culture medium and centrifuging. Enriched microglial cultures were prepared at 1.5 to 2 × 10⁶ cells/100-mm plastic petri dishes for RNA preparation or at 2 to 4 × 10⁶ 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

Triplicate wells 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-1Ra is secreted, while >80% of IL-1β is cell associated. All data presented are IL-1β levels determined in the supernatants and IL-1Ra 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 by 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 microglial samples were separated in a 1% formaldehyde-agarose gel by electrophoresis. RNA was transferred to a Hybond membrane (Amersham, 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-1Ra (provided by Immunex, Seattle, WA), or 18S RNA (gift from Dr. N. Anheim, 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, and IFN-γ and IFN-β were potent down-regulators of IL-1β mRNA expression induced by IL-1 itself. Densitometric ratios of IL-1Ra to 18S showed that among the three cytokines tested, only IFN-β was effective in inducing IL-1Ra mRNA, and that IFN-γ down-regulated IFN-β-induced IL-1Ra mRNA. IL-1α itself had essentially no effect on IL-1Ra expression in microglia. Results shown are from one of two separate experiments with identical results.
IL-1Ra mRNA to that induced by LPS alone. In contrast to the down-regulation of IL-1Ra by IFN-β, IL-4 down-regulated LPS-induced IL-1Ra. While IFN-β down-regulated IL-1Ra mRNA expression in human microglia, consistent with the results obtained by ELISA, IFN-γ or IFN-β, when used alone, 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 mRNA expression induced by LPS-mediated TNF-α expression.

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.

**Modulation of IL-1β and IL-1Ra expression in human microglia by IL-4**

In Figure 1, we showed by ELISA that IL-4 also significantly down-regulated IL-1 production induced by LPS and IL-1 and potentiated up-regulated IL-1Ra expression when given alone and in combination with LPS. Therefore, we sought to determine the effects of IL-4 on the expression of mRNA for IL-1β and IL-1Ra in human microglia (Fig. 4). The results showed low constitutive levels of IL-1Ra mRNA expression in human microglia, consistent with the results obtained by ELISA. Following exposure to LPS or IL-4, a marked increase in IL-1Ra mRNA was observed (LPS > IL-4), while IL-1 had little stimulatory effect. Costimulation with LPS and IL-4 resulted in a further increase in the level of IL-1Ra mRNA (Fig. 4B). In contrast, IL-1β mRNA was not expressed in unstimulated microglia or in microglia stimulated with IL-4 alone. Both IL-1 and LPS induced high levels of IL-1β mRNA in microglia (LPS > IL-1), and IL-4 reduced the amounts of IL-1β mRNA induced by both IL-1 and LPS (Fig. 4, A and B, respectively). Thus, IL-4 negatively affects the expression of the IL-1β mRNA.
system in microglia by enhancing the basal and LPS-induced IL-1Ra and also by suppressing the levels of IL-1β induced by LPS or by IL-1 itself.

**IFN-γ also suppresses IL-4-induced IL-1Ra in microglia**

Since IL-4 itself markedly up-regulated IL-1Ra expression in microglia, and IFN-γ had a down-regulatory effect on IL-1Ra expression, we determined whether IFN-γ also down-regulated the amounts of IL-1Ra induced by IL-4. In three separate experiments, the levels of IL-1Ra were determined by ELISA in microglia cultures that were untreated, treated with IL-4 (10 ng/ml) alone, or treated with IL-4 plus IFN-γ (200 U/ml). In at least three different experiments, the results showed that IL-4 up-regulated the amounts of IL-1Ra to 180 to 520% that in control cultures and that IFN-γ down-regulated IL-4 induced IL-1Ra to between 7 to 78% of IL-4-induced levels (data not shown).

**Effects of IL-10, TGF-β, and IL-6 on IL-1 and IL-1Ra expression in human microglia**

Because of the striking effects of IL-4 and IFN-β on the expression of IL-1 and IL-1Ra, we screened the cytokines IL-10, TGF-β, and IL-6, which have been shown in other systems to possess anti-inflammatory activities. The results are shown in Figure 5. With respect to IL-1β production, none of these cytokines induced 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) 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 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 monocyte/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...
understood (33, 34). Magnetic resonance imaging studies show
apies for MS, but its mechanism with regard to this use is not well
inflammation and blood-brain barrier disruption, and IFN-
occurring at the blood-brain barrier. IL-1 is a potent mediator of
enhancing lesions, indicating an effect of this cytokine on events
levels of IL-1

FIGURE 6. Human astrocytes express neither IL-1β nor IL-1Ra. En-
riched astrocyte cultures were prepared at 3 × 10⁶ cells/well and treated
with cytokines and LPS as described in Figure 1. Microglia were plated and
treated as described in the text. ELISA was performed to determine the
levels of IL-1β and IL-1Ra in cell lysates and cell culture supernatants,
respectively. Astrocytes expressed neither IL-1β nor IL-1Ra in response to
the treatments shown. In contrast, microglia expressed nanogram levels of
both cytokines in response to LPS. In addition, microglia expressed IL-1Ra
constitutively. Data are the mean ± SD from triplicate wells and are from
one representative experiment of four with similar results.

types in response to a wide range of stimuli, including viral infec-
tion. As noted previously, IFN-β is one of only two accepted ther-
apies for MS, but its mechanism with regard to this use is not well
understood (33, 34). Magnetic resonance imaging studies show
that administration of IFN-β leads to a rapid reduction in contrast-
enhancing lesions, indicating an effect of this cytokine on events
occurring at the blood-brain barrier. IL-1 is a potent mediator of
inflammation and blood-brain barrier disruption, and IFN-β, by
down-regulating the activity of the IL-1 system in microglia, may
contribute to the therapeutic effect of this cytokine in patients with
acute exacerbations of MS.

In addition to the effect of IL-1 on the blood-brain barrier, tissue
culture studies have implicated IL-1 as a unique factor responsible
for human astrocyte activation. A reactive astroglialosis is a com-
mon 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 astro-
cytic processes (35). Hypertropic astrocytes have been implicated
in the production of a number of toxic factors, including TNF-α
(36), reactive nitrogen intermediates (5), and components of the
complement system (37, 38), as well as factors that could contrib-
ute to the development of chronic inflammatory events. Thus, the
effect of IFN-β on microglial or macrophage-derived IL-1 activity

may function to suppress astrocyte activation and subsequent
astroglialosis.

IFN-γ, the prototypical Th1 cytokine and type II IFN, is a prod-
uct of T cells and NK cells and is essential in the development of
cell-mediated immune responses. IFN-γ 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-γ in the expression of cytokines is less
well defined. We and others found IFN-γ primes astrocytes to
produce TNF-α (14, 15, 23). In human microglia, we found that
IFN-γ down-regulated mRNA and protein expression of both
IL-1β and IL-1Ra, but had no effect on processing of IL-1β (data
not shown). The fact that IFN-γ 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-γ down-regulates both LPS- and IL-1-induced IL-1β produc-
tion. It has been noted in several experimental model systems that
IFN-γ 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-γ
on disease expression in this model. IFN-γ 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-γ mediates these inhibitory effects has not yet been
determined.

The fact that IFN-γ also down-regulated LPS-induced IL-1Ra
expression indicates, however, that the regulation of the IL-1 sys-
tem in microglia by IFN-γ is complex. It is important to note that
this result did not reflect a global down-regulation of transcriptional
events by IFN-γ in microglia, since LPS-induced TNF-α
expression was not similarly affected, indicating a level of speci-
cificity for this response. A similar inhibition of LPS-induced IL-
1Ra expression by IFN-γ was observed by Sone et al. (45) in
peripheral blood monocytes; however, in that study IFN-γ poten-
tiated LPS-induced IL-1 production, leading to a net proinflam-
matory 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 macro-
phages (as opposed to circulating monocytes) are generally con-
stitutively biased toward an IL-1Ra response has been noted in the
lungs and skin (45, 46). Our data indicate that CNS microglia show
a similar bias and add further support to the observations that
IFN-γ may act as a negative regulatory signal for inflammatory
events in some circumstances.

The effect of IFN-β 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 macro-
phages, other cytokines with known negative regulatory roles in
inflammatory responses, such as TGF-β, IL-10, and IL-6, had
slight and inconsistent effects on IL-1Ra production by human
microglia. While TGF-β 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

[Image 77x432 to 272x733]
(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 IFNs 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 suggest that IFN-β may have wider applications in diseases of the CNS in which IL-1 has been implicated.

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References


