

# Activation of Central Nervous System Inflammatory Pathways by Interferon-Alpha: Relationship to Monoamines and Depression

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**Background:** Interferon (IFN)-alpha has been used to study the effects of innate immune cytokines on the brain and behavior in humans. The degree to which peripheral administration of IFN-alpha accesses the brain and is associated with a central nervous system (CNS) inflammatory response is unknown. Moreover, the relationship among IFN-alpha-associated CNS inflammatory responses, neurotransmitter metabolism, and behavior has yet to be established.

**Methods:** Twenty-four patients with hepatitis C underwent lumbar puncture and blood sampling after ~12 weeks of either no treatment ( $n = 12$ ) or treatment with pegylated IFN-alpha 2b ( $n = 12$ ). Cerebrospinal fluid (CSF) and blood samples were analyzed for proinflammatory cytokines and their receptors as well as the chemokine, monocyte chemoattractant protein-1 (MCP-1), and IFN-alpha. Cerebrospinal fluid samples were additionally analyzed for monoamine metabolites and corticotropin releasing hormone. Depressive symptoms were assessed using the Montgomery Asberg Depression Rating Scale.

**Results:** Interferon-alpha was detected in the CSF of all IFN-alpha-treated patients and only one control subject. Despite no increases in plasma IL-6, IFN-alpha-treated patients exhibited significant elevations in CSF IL-6 and MCP-1, both of which were highly correlated with CSF IFN-alpha concentrations. Of the immunologic and neurotransmitter variables, log-transformed CSF concentrations of the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA), were the strongest predictor of depressive symptoms. Log-transformed CSF concentrations of IL-6, but not IFN-alpha or MCP-1, were negatively correlated with log-transformed CSF 5-HIAA ( $r^2 = -.25, p < .05$ ).

**Conclusions:** These data indicate that a peripherally administered cytokine can activate a CNS inflammatory response in humans that interacts with monoamine (serotonin) metabolism, which is associated with depression.

**Key Words:** Cerebrospinal fluid, cytokines, depression, inflammation, monoamines

There has been increasing interest in the role of innate immune cytokines in behavioral disorders including depression in medically ill and medically healthy individuals (1–3). For example, elevations in the innate immune cytokines, interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)-alpha as well as their soluble receptors, have been found in peripheral blood and cerebrospinal fluid (CSF) of patients with major depression (1). In addition, innate immune cytokines have been shown to influence virtually every pathophysiologic domain relevant to depression including neurotransmitter metabolism, neuroendocrine function, synaptic plasticity, and regional brain activity (1,2).

Because cytokines are relatively large molecules (~15–25 kd) that do not freely pass through the blood-brain barrier (BBB) (4), much attention has been paid to the pathways by which peripheral cytokine signals reach the brain. Data in laboratory animals indicate that cytokines elaborated or administered peripherally can access the brain through several routes including 1) passage through leaky regions in the BBB; 2) active transport via saturable transport molecules; 3) activation of endothelial cells (as well as other cells lining the cerebral vasculature), which then release inflammatory mediators within the brain parenchyma; and 4)

binding to cytokine receptors associated with peripheral afferent nerve fibers (e.g., the vagus nerve), which, in turn, relay signals to relevant brain nuclei (2,5–7). It should be noted, however, that it remains to be established whether peripherally administered cytokines can access the brain and activate central inflammatory pathways in humans. Moreover, it has yet to be determined whether cytokine-induced changes in behavior in humans are related to effects of central cytokines on the metabolism of behaviorally relevant neurotransmitter systems.

One opportunity to explore the access and action of peripheral blood cytokines on the brain in humans is to study patients undergoing treatment with interferon (IFN)-alpha. Interferon-alpha is an innate immune cytokine that has both antiviral and antiproliferative activities and is therefore used to treat infectious diseases and cancer (8,9). Although an effective therapy, IFN-alpha induces high rates of behavioral disturbance, including depression, which develops to a clinically significant degree in 30% to 50% of IFN-alpha-treated patients (10–15). Prior studies have linked development of depression during IFN-alpha therapy to changes in peripheral blood inflammatory markers as well as changes in peripheral availability of monoamine neurotransmitter precursors including tryptophan, the primary precursor for serotonin (16–20). Moreover, exaggerated hypothalamic-pituitary-adrenal (HPA) axis responses have been associated with depression during IFN-alpha therapy, suggesting that sensitized neuroendocrine pathways, including corticotropin-releasing hormone (CRH), may represent a vulnerability to IFN-alpha-induced behavioral changes (21).

To date, however, no studies have examined central nervous system (CNS) immune, monoamine, or neuropeptide effects of IFN-alpha that might be relevant to depression. Therefore, we sought to determine 1) whether IFN-alpha enters the brain when

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administered peripherally; 2) whether peripherally administered IFN- $\alpha$  is associated with activation of CNS inflammatory pathways; 3) whether activation of these inflammatory pathways affects metabolism of relevant monoamines or CRH; and 4) whether IFN- $\alpha$ -associated changes in CNS inflammatory pathways, monoamine metabolism, or CRH are associated with depression.

To accomplish these aims, CSF and plasma concentrations of several inflammatory mediators, monoamine neurotransmitter metabolites, and CRH were assessed in patients undergoing treatment with pegylated IFN- $\alpha$  plus ribavirin for hepatitis C virus (HCV) infection. Hepatitis C virus infected patients awaiting IFN- $\alpha$  therapy served as control subjects. Inflammatory mediators included IL-6 and its soluble receptor (sIL-6R), TNF- $\alpha$  and its type 2 soluble receptor (sTNF-R2), IL-1- $\beta$ , and monocyte chemoattractant protein-1 (MCP-1). Administration of IFN- $\alpha$  has been shown to induce IL-6 and TNF- $\alpha$  and their soluble receptors as well as IL-1- $\beta$  in the peripheral blood of relevant patient populations and/or peripheral blood mononuclear cells and a variety of cell lines (21,22). In addition, peripheral blood concentrations of IL-6 and TNF and their soluble receptors, both at baseline and during IFN- $\alpha$  therapy, have been found to predict depression during IFN- $\alpha$  therapy (16,20,23,24). Monocyte chemoattractant protein-1 has been shown to be elevated in CSF of patients with human immunodeficiency virus (HIV) infection (25–27), a condition associated with increased CSF concentrations of IFN- $\alpha$  (28,29). Interferon- $\alpha$  is also known to induce inflammatory responses through induction of MCP-1 (30), and MCP-1 has been found to prime microglia in the brain following CNS administration of inflammatory stimuli (e.g., lipopolysaccharide [LPS]) (31).

To evaluate the effect of IFN- $\alpha$  on CNS neurotransmission, CSF concentrations of the primary metabolites for serotonin (5-hydroxyindoleacetic acid [5-HIAA]), dopamine (homovanillic acid [HVA]), and norepinephrine (3-methoxy-4-hydroxyphenylglycol [MHPG]) were examined. These metabolites were selected based on data that IFN- $\alpha$  alters peripheral availability of the serotonin precursor, tryptophan, and on a large literature linking these monoamines and their metabolites to depression and other behavioral disturbances (32–34). Finally, CSF and plasma concentrations of IFN- $\alpha$  as well as the neuroregulatory peptide, CRH, were determined.

## Methods and Materials

### Subjects

Twenty-four HCV-positive subjects (15 male subjects, 9 female subjects) were enrolled in the study. Subjects were required to be serum positive for anti-HCV antibodies or HCV-RNA positive by reverse transcription-polymerase chain reaction. Exclusion criteria included decompensated liver disease; liver disease from any cause other than HCV; infection with HIV (as reported by the subjects' treating physician); unstable cardiovascular, endocrinologic, hematologic, renal, or neurologic disease (as determined by physical examination and laboratory testing); history of schizophrenia or bipolar disorder or a diagnosis of major depression (MD) or substance abuse/dependence within 6 months of study entry (as determined by the Structured Clinical Interview for DSM-IV [SCID]) (35), and/or a score <24 on the Mini-Mental State Exam, indicating more than mild cognitive impairment (36). Patients were required to be off all antidepressant, antipsychotic, or mood stabilizer medications for at least 4 weeks prior to study entry (8 weeks for fluoxetine). Subjects

were also required to discontinue other agents that might affect study results (i.e., narcotic analgesics, benzodiazepines, and anti-inflammatory agents) at least 2 weeks prior to undergoing lumbar puncture (LP) or blood collection. The subjects reported on herein represent a subsample of subjects included in previous studies on effects of IFN- $\alpha$  on cognitive performance and neuroendocrine function (20,37). All subjects provided written informed consent, and study procedures received a priori approval by the Emory University Institutional Review Board.

### Study Design

Study participants were enrolled in a longitudinal study examining immune and other neurobiological variables at baseline and after ~12 weeks of either no treatment or treatment with IFN- $\alpha$ /ribavirin (see above). Cross-sectional assessments of immune and neurotransmitter variables in CSF and peripheral blood of HCV patients awaiting IFN- $\alpha$ /ribavirin therapy (control subjects,  $n = 12$ ) versus HCV patients treated with IFN- $\alpha$  plus ribavirin for ~12 weeks (treatment group,  $n = 12$ ) were evaluated for the purposes of this study. All subjects who underwent IFN- $\alpha$  treatment received pegylated IFN- $\alpha$ -2b (Pegintron, Schering Plough, Kenilworth, New Jersey) 1.5  $\mu$ g/kg weekly (administered subcutaneously) and ribavirin (800–1400 mg/day). Participation in the treatment versus control group was determined by patients and their physician based on scheduling constraints and personal preferences and was not based on standardized criteria or controlled by study protocol.

For LP, subjects were admitted to the Emory University General Clinical Research Center (GCRC) at 1:00 PM. Lumbar puncture was performed between 4:00 PM and 5:00 PM by a trained physician. For each subject, ~10 cc of CSF was withdrawn, after discarding the initial 1 cc to avoid blood contamination. Samples were collected into chilled tubes, aliquoted into 1 cc vials, and immediately frozen at  $-80^{\circ}\text{C}$  until assay. Subjects were then discharged after an overnight stay. To limit the impact of the stress of CSF sampling on peripheral blood immune parameters and assessments of depression, blood sampling and depression assessments were conducted 6 to 7 days after LP. For blood sampling, subjects were admitted to the Emory GCRC in the evening with lights out at 10:00 PM. The following morning, subjects were awakened at 7:15 AM and served breakfast, and neuropsychiatric assessments were conducted. Following lunch at 12:00 PM, blood was withdrawn from an indwelling catheter into chilled ethylenediaminetetraacetic acid (EDTA)-coated tubes at 4:00 PM, corresponding to the time of the LP. During blood sampling, subjects were asked to rest quietly for 30 minutes prior to blood withdrawal. Following sampling, blood was immediately centrifuged at 1000Xg for 10 minutes at  $4^{\circ}\text{C}$ . Plasma was then removed and frozen at  $-80^{\circ}\text{C}$  until assay. Because behavioral effects of pegylated IFN- $\alpha$  tend to be most pronounced immediately following the weekly injection, both LP and blood sampling were scheduled 4 to 5 days following each subject's last injection. Urine drug screens were conducted at all visits to rule out substance abuse. Control subjects participated in all study procedures in parallel with IFN- $\alpha$ /ribavirin-treated patients.

### Behavioral Assessments

Depression was evaluated using the mood disorders module of the SCID and the Montgomery-Asberg Depression Rating Scale (MADRS) (38). The MADRS is a 10-item, clinician-administered scale that assesses the severity of depressive symptoms.

### Assessment of Immune Parameters and CSF Monoamine Metabolites

Tumor necrosis factor- $\alpha$ , IL-1- $\beta$ , and IL-6 were measured in duplicate by high sensitivity quantitative enzyme-linked immunosorbent assays (ELISA) (R&D Systems, Minneapolis, Minnesota). Cytokine soluble receptors (sTNF-R2, IL6-sR) and MCP-1 were determined by R&D Quantikine ELISA kits (R&D Systems). Plasma IFN- $\alpha$  was measured by a high sensitivity quantitative ELISA (Amersham Biosciences, Piscataway, New Jersey), which was found to identify both recombinant human IFN- $\alpha$  as well as the pegylated IFN- $\alpha$  preparation. Interassay and intra-assay variability were reliably <12% for TNF- $\alpha$ , IL-1- $\beta$ , and IL-6 and <10% for sTNF-R2, sIL-6R, MCP-1, and IFN- $\alpha$ . Assay sensitivities were as follows: TNF- $\alpha$ : .11 pg/mL; IL-1- $\beta$ : .06 pg/mL; IL-6: .04 pg/mL; sTNF-R2: 1.0 pg/mL; sIL-6R: 6.5 pg/mL; MCP-1: 5.0 pg/mL; and IFN- $\alpha$ : .1 pg/mL.

C-reactive protein (CRP) was measured using the Beckman Coulter High Sensitivity CRP assay (Beckman Coulter Diagnostics, Brea, California) on the Synchron LX-20 analyzer (Beckman Coulter, Fullerton, California).

5-Hydroxyindoleacetic acid, HVA, and MHPG were measured in duplicate 20  $\mu$ L CSF aliquots using high-performance liquid chromatography as described (39). Electrochemical detection was performed using a dual electrode system (ESA Incorporated, Chelmsford, Massachusetts), and peak areas were computed using Turbochrom software (PE-Nelson, San Jose, California). Sensitivities for all analytes were  $\sim$ 1 pg on column.

Corticotropin-releasing hormone was measured in quadruplicate 50  $\mu$ L CSF aliquots by nonequilibrium radioimmunoassay using a high-affinity antibody (S-2021, raised in rabbit against human CRH) (Bachem Americas Inc., Torrance, California). Antibody (25  $\mu$ L) was added to CSF, and the mixture was incubated at 2°C for 24 hours. Trace  $^{125}$ I-tyr<sup>0</sup>-CRH (25  $\mu$ L) was then added, and the mixture was incubated for an additional 16 hours. The CRH-antibody complex was precipitated using goat anti-rabbit immunoglobulin G (IgG). Assay sensitivity was 2.8 pg/mL, and the coefficient of variation between quadruplicates was 7.5%.

All biological samples were analyzed by research staff blinded to the clinical status of study participants.

### Statistical Analysis

Descriptive statistics (mean, median, standard deviation, and interquartile range) were used to characterize relevant variables in the groups. Differences between groups were assessed using *t* tests and Wilcoxon rank-sum tests for continuous measures and chi-square or Fisher tests (as appropriate) for categorical variables. Generalized linear models (GLM) were employed to complement statistical comparisons of immune and neurotransmitter variables between groups, controlling for factors that may have influenced relevant CSF and plasma biomarkers including age, sex, body mass index (BMI), history of MD, and baseline inflammatory status (as reflected by plasma CRP determined at the initial GCRC visit). Despite the robustness of GLM against nonnormality, only transformed data were used for these analyses for the sake of consistency across tests and variables and to improve the homogeneity of variances as well as the normality of the residuals. In *t* tests where the Levene test indicated nonhomogeneity of variances, Welch's *t* tests were employed.

Pearson correlations were computed to evaluate associations between relevant continuous variables, following log transformation to improve normality. Based on the sample size of 24, the study had  $\sim$ 80% power to detect medium effect sizes ( $r^2 = .25$ )

using two-tailed tests of significance and an alpha level of .05. Correlations between CSF IFN- $\alpha$  and relevant continuous variables were conducted using Spearman tests due to the large number of 0 values for CSF IFN- $\alpha$  in control subjects. Where indicated, Bonferroni correction was used to control for multiple comparisons within and across tissue compartments. To explore the relative contribution of relevant immune and neurotransmitter variables to scores of depression, a stepwise regression analysis was applied using both backward and forward selection. The level of significance for independent predictors in the final model was set at  $p < .05$ .

## Results

### Sample Characteristics

As shown in Table 1, IFN- $\alpha$ /ribavirin-treated subjects and control subjects did not differ significantly on relevant clinical characteristics including age, race, gender, past history of substance abuse, BMI, and baseline CRP. Nevertheless, IFN- $\alpha$ -treated subjects were more likely to have a past history of MD. At the time of study, scores of depression were significantly higher in IFN- $\alpha$ /ribavirin-treated subjects versus control subjects. Of note, depression scores were not significantly different in IFN- $\alpha$ /ribavirin-treated patients with or without a past history of MD (data not shown). No patients met symptom criteria for MD at the time of CSF or blood sampling.

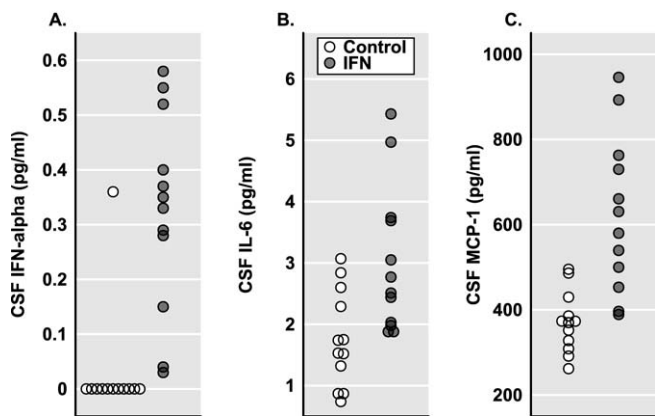
### CSF Concentrations of Relevant Immune Biomarkers

Evaluation of selected immune biomarkers in CSF of study participants revealed that IFN- $\alpha$ -treated patients exhibited significantly higher concentrations of CSF IFN- $\alpha$ , IL-6, and MCP-1 compared with control subjects (Figure 1, Table 2). Tumor necrosis factor- $\alpha$  and IL-1- $\beta$  were also assayed. However, in the majority of samples, results were below the limits of assay detection. Cerebrospinal fluid concentrations of soluble receptors for both IL-6 and TNF were slightly elevated in IFN- $\alpha$ /ribavirin-treated subjects, although the differences only reached statistical significance for sIL-6R. To confirm differences in relevant CSF immune variables between groups, analyses were repeated controlling for age, sex, BMI, baseline inflammatory status (CRP), and history of depression. Differences between groups remained significant for all variables. In addition,

**Table 1.** Characteristics of Study Participants

Characteristic	Control Subjects (n = 12)	Interferon-Alpha (n = 12)	p Value
Age (mean, SD)	48.3 (7.7)	48.3 (3.6)	1.00
Gender (n, %) Males	6 (50.0)	9 (75.0)	.40
Race (n, %)			
Caucasian	6 (50.0)	4 (33.3)	.68
Black	6 (50.0)	7 (58.3)	
Asian	0 (.0)	1 (8.3)	
Education (n, %)			
College (1 or more years)	10 (83.3)	7 (58.3)	.37
Past MD (n, %)	0 (0)	5 (41.7)	.04
Past Substance Abuse (n, %)	6 (50)	6 (50)	1.00
MADRS (mean, SD)	3.2 (3.8)	8.3 (5.8)	.02
BMI (mean, SD)	29.2 (5.6)	29.5 (4.5)	.88
Baseline CRP (mg/L)(mean, SD)	.78 (.8)	.87 (1.0)	.80

BMI, body mass index; CRP, C-reactive protein; MADRS, Montgomery-Asberg Depression Rating Scale; MD, major depression.



**Figure 1.** CSF immunologic biomarkers in control subjects versus IFN-alpha/ribavirin-treated patients with HCV. Cerebrospinal fluid (CSF) samples for the assessment of relevant innate immune cytokines, including interferon (IFN)-alpha (A) and interleukin (IL)-6 (B) as well as the chemokine, monocyte chemoattractant protein-1 (MCP-1) (C), were compared in control subjects (*n* = 12) and interferon (IFN)-alpha/ribavirin-treated subjects (*n* = 12) patients with hepatitis C virus (HCV) infection. IFN-alpha/ribavirin-treated subjects were studied after ~12 weeks on IFN-alpha/ribavirin therapy. CSF concentrations of IFN-alpha, IL-6, and MCP-1 were significantly elevated in IFN-alpha-treated subjects compared with control subjects (*p* < .01). CSF, cerebrospinal fluid; HCV, hepatitis C virus; IFN, interferon; IL, interleukin; MCP-1, monocyte chemoattractant protein-1.

tion, after adding the noted covariates into the model, the difference in CSF sTNF-R2 between groups was found to be significant (*p* < .01).

Correlational analyses among immune biomarkers that were significantly elevated in CSF revealed high correlations between IFN-alpha and log-transformed (ln)IL-6 and lnMCP-1 (*r*<sup>2</sup> = .52, *df* = 22, *p* < .0001, and *r*<sup>2</sup> = .53, *df* = 22, *p* < .0001, respectively), as well as a significant correlation between lnIL-6 and lnMCP-1 (*r*<sup>2</sup> = .23, *df* = 22, *p* = .018).

**Plasma Concentrations of Relevant Immune Biomarkers**

Examination of immune measures in peripheral blood revealed significant elevations in plasma IFN-alpha and MCP-1 in

**Table 2.** CSF Concentrations of Immune Biomarkers

Immune Measure (Median, IQR)	Control Subjects ( <i>n</i> = 12)	Interferon-alpha ( <i>n</i> = 12)	<i>p</i> Value <sup>a</sup>
<b>Cytokines (pg/mL)</b>			
IFN-alpha	0 (0)	.34 (.25)	.0001 <sup>b</sup>
IL-6	1.64 (1.35)	2.64 (1.71)	.005 <sup>b</sup>
TNF-alpha <sup>c</sup>	-	-	
IL-1-beta <sup>c</sup>	-	-	
<b>Chemokines (ng/mL)</b>			
MCP-1	371.55 (89.6)	605.3 (296.8)	.0001 <sup>b</sup>
<b>Soluble Receptors (ng/mL)</b>			
sIL-6R	.76 (.12)	.98 (.44)	.03
sTNF-R2	.26 (.07)	.36 (.19)	.07 <sup>d</sup>

CSF, cerebrospinal fluid; IFN, interferon; IL, interleukin; IQR, interquartile range; MCP-1, monocyte chemoattractant protein-1; sIL-6R, soluble IL-6 receptor; sTNF-R2, soluble TNF-alpha receptor 2; TNF, tumor necrosis factor.

<sup>a</sup>Exact Wilcoxon Rank Sum test.

<sup>b</sup>Indicates significance after adjustment for multiple comparisons as well as controlling for relevant covariates (see text).

<sup>c</sup>TNF-alpha and IL-1-beta were not detectable in the majority of subjects.

<sup>d</sup>sTNF-R2 exhibited a significant difference between groups (*p* < .01) after controlling for relevant covariates as well as controlling for multiple comparisons.

**Table 3.** Plasma Concentrations of Immune Biomarkers

Immune Measure (Median, IQR)	Control Subjects ( <i>n</i> = 12)	Interferon-Alpha ( <i>n</i> = 12)	<i>p</i> Value <sup>a</sup>
<b>Cytokines (pg/mL)</b>			
IFN-alpha	4.65 (8.35)	37.85 (40.16)	.0001 <sup>b</sup>
IL-6	3.67 (2.1)	3.17 (1.5)	.48
TNF-alpha	1.46 (1.22)	1.77 (1.90)	.75
IL-1-beta <sup>c</sup>	-	-	
<b>Chemokines (ng/mL)</b>			
MCP-1	225.0 (142.5)	365.5 (132.0)	.005 <sup>b</sup>
<b>Soluble Receptors (ng/mL)</b>			
sIL-6R	33.38 (9.7)	28.95 (10.5)	.51
sTNF-R2	2.05 (1.89)	2.84 (0.96)	.09

IFN, interferon; IL, interleukin; IQR, interquartile range; MCP-1, monocyte chemoattractant protein-1; sIL-6R, soluble IL-6 receptor; sTNF-R2, soluble TNF-alpha receptor 2; TNF, tumor necrosis factor.

<sup>a</sup>Exact Wilcoxon Rank Sum test.

<sup>b</sup>Indicates significance after adjustment for multiple comparisons.

<sup>c</sup>IL-1-beta was not detectable in the majority of subjects.

IFN-alpha-treated subjects versus control subjects but no differences between groups in IL-1-beta, IL-6, and TNF-alpha or their soluble receptors (Table 3). There was a trend for an increase in plasma sTNF-R2 in subjects receiving IFN-alpha. Differences between groups in plasma IFN-alpha and MCP-1 remained significant after controlling for relevant clinical and biological variables. Of note, there was no significant correlation between plasma lnIFN-alpha and plasma lnMCP-1 (*r*<sup>2</sup> = .06, *df* = 22, *p* = .23) in the study sample.

Regarding the relationship between plasma and CSF immune variables, plasma lnIFN-alpha was correlated with CSF IFN-alpha and CSF lnIL-6 (*r*<sup>2</sup> = .26, *df* = 22, *p* = .011 and *r*<sup>2</sup> = .20, *df* = 22, *p* = .03, respectively). Nevertheless, these correlations were not significant after controlling for the multiple (6) comparisons of relevant variables across these tissue compartments (*p* corrected = .066 and *p* corrected = .18, respectively). The correlation between plasma lnIFN-alpha and CSF lnMCP-1 (*r*<sup>2</sup> = .13, *p* = .08) as well as correlations between plasma and CSF concentrations of lnIL-6 (*r*<sup>2</sup> = .001, *p* = .97) and lnMCP-1 (*r*<sup>2</sup> = .12, *p* = .10) also did not reach statistical significance, nor did the correlation between plasma lnMCP-1 and CSF lnIL-6 (*r*<sup>2</sup> = .12, *p* = .10).

**CSF Monoamine Metabolites**

No differences were found between groups for the monoamine metabolites, 5-HIAA, HVA, and MHPG (Table 4). Cerebrospinal fluid lnHVA was highly correlated with CSF ln5-HIAA (*r*<sup>2</sup> = .74, *p* < .001) as well as CSF lnMHPG (*r*<sup>2</sup> = .24, *p* = .014). No significant correlation was found between CSF ln5-HIAA and CSF lnMHPG (*r*<sup>2</sup> = .13, *p* < .08).

**CSF CRH**

No differences were found in CSF CRH concentrations between control subjects versus IFN-alpha/ribavirin-treated patients (40.1 SD ± 16.9 versus 33.3 SD ± 6.5 pg/mL, respectively, *t* = 1.3, *df* = 14.5, *p* = .22). Correlational analyses revealed significant correlations between CSF lnCRH and CSF lnHVA (*r*<sup>2</sup> = .23, *p* = .02) and CSF lnMHPG (*r*<sup>2</sup> = .21, *p* = .03), but these correlations were not significant after controlling for the multiple (6) comparisons made between CSF CRH and relevant CSF monoamine and immune variables (*p* corrected = .12 and *p* corrected = .18, respectively). No significant correlations were found between CSF lnCRH and ln5-HIAA or any of the immune

**Table 4.** CSF Concentrations of Neurotransmitters

Monoamine Metabolite (Median, IQR)	Control Subjects (n = 12)	Interferon-Alpha (n = 12)	p Value <sup>a</sup>
HVA (ng/mL)	104.7 (60.15)	76.8 (63.20)	.27
5-HIAA (ng/mL)	33.45 (21.55)	29.60 (21.70)	.16
MHPG (ng/mL)	16.80 (5.55)	15.65 (5.00)	.45

CSF, cerebrospinal fluid; 5-HIAA, 5-hydroxyindoleacetic acid; HVA, homovanillic acid; IQR, interquartile range; MHPG, 3-methoxy-4-hydroxyphenylglycol.

<sup>a</sup>Exact Wilcoxon Rank Sum test.

variables that were found to be elevated in the CSF (i.e., IFN-alpha, IL-6, or MCP-1).

### Stepwise Regression Analysis: Immune, Monoamine, and Neuropeptide Predictors of Depression

To explore the relative contribution of immune and neurotransmitter variables to depressive symptoms, a stepwise regression analysis using both forward and backward selection was conducted including immune variables that were significantly increased in IFN-alpha-treated subjects versus control subjects as well as mood relevant monoamine metabolites and CRH. Cerebrospinal fluid IFN-alpha, plasma lnIFN-alpha, plasma MCP-1, CSF lnMCP-1, CSF lnIL-6, CSF ln5-HIAA, CSF lnHVA, CSF lnMHPG, and CSF lnCRH were entered into the model. The final, reduced model using forward or backward selection included only CSF ln5-HIAA [ $F(1,23) = 6.05, p = .02$ ] ( $R^2 = .21$ ). Of note, plasma lnIFN-alpha approached significance ( $p = .07$ ) but was not included in the final model because the criteria for independent contribution was preset at an alpha of .05 (see Methods and Materials). Of the immune variables that were found to be elevated in the CSF, only CSF lnIL-6 was negatively correlated with CSF ln5-HIAA ( $r^2 = -.25, df = 22, p = .013$ ) (Figure 2). No correlations were found between CSF ln5-HIAA and CSF IFN-alpha or lnMCP-1 ( $r^2 = -.05, df = 22, p = .31$  and  $r^2 = -.01, df = 22, p = .66$ , respectively).

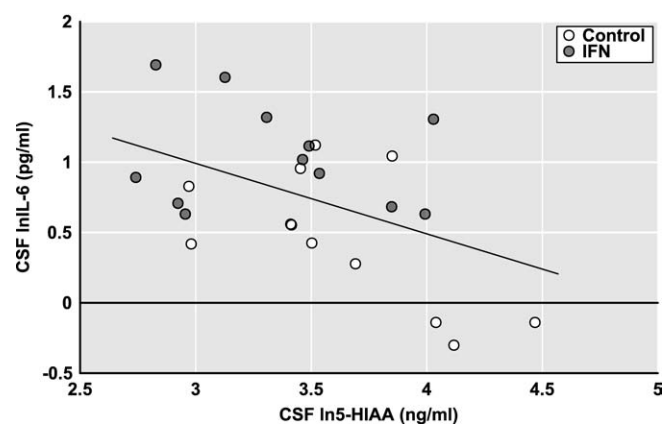
### Discussion

The data indicate that peripherally administered IFN-alpha is capable of accessing the brain in humans and is associated with an inflammatory response in the CNS as reflected by elevations in CSF IL-6 and MCP-1. In addition, increases in IL-6 were associated with decreases in the serotonin metabolite, 5-HIAA, which, in turn, were correlated with depression.

The current study provides the first demonstration that pegylated IFN-alpha administered peripherally leads to increases in CSF IFN-alpha in humans. Although it remains to be established whether the IFN-alpha in the CSF is reflective of the pegylated preparation administered peripherally, these data are consistent with studies in rhesus monkeys, where increased CSF IFN-alpha concentrations have been found after acute and chronic (4 weeks) administration of nonpegylated preparations of IFN-alpha (40,41). In addition, studies in mice have found profound CNS induction of IFN-alpha inducible genes following intraperitoneal injection of recombinant mouse IFN-alpha. These studies in laboratory animals demonstrate that IFN-alpha is capable of accessing (and possibly entering) the brain (41,42). Nevertheless, to date, no saturable transport system for IFN-alpha has been described (43). Thus, the appearance of IFN-alpha in the CSF of IFN-alpha-treated patients suggests that IFN-alpha either enters the brain via passage through leaky regions in the BBB or alternatively activates cells at the BBB to induce local IFN-alpha production. Regarding the latter possibility, IFN-alpha has been

shown to upregulate multiple immune genes in human endothelial cells including genes for toll-like receptor 3 and interferon regulatory factor 7, both of which play major roles in IFN-alpha production in response to relevant immunologic stimuli (e.g., viruses) (44–46). In addition, recombinant human IFN-alpha has been shown to induce IFN-alpha production from human peripheral blood mononuclear cells (47). It should also be noted that BBB permeability during IFN-alpha therapy may be facilitated by MCP-1, which was found to be elevated in the peripheral blood and CSF of IFN-alpha-treated patients. Previous *in vitro* and *in vivo* studies have indicated that prolonged exposure to MCP-1 can significantly increase BBB permeability through effects of MCP-1 on the receptor for C-C motif chemokine receptor-2 (48). In the context of viral infection, IFN-alpha has been shown to induce the production of MCP-1, which, in turn, plays a key role in recruiting relevant cell types including monocytes to the site of infection (30). Thus, IFN-alpha-induced production of MCP-1 in the periphery may increase BBB permeability to IFN-alpha as well as to MCP-1 itself.

In association with IFN-alpha, CSF IL-6 was also found to be elevated in IFN-alpha-treated patients. However, in contrast to IFN-alpha, plasma IL-6 concentrations were not elevated in IFN-alpha-treated subjects and did not correlate with CSF concentrations of IL-6. These data suggest that IL-6 is being synthesized *de novo* in the brain as a function of IFN-alpha penetration. Relevant in this regard is that MCP-1 has been shown to play an important role in priming cells within the CNS (e.g., microglia) to produce local proinflammatory cytokines. For example, MCP-1 knockout mice produce significantly less IL-1-beta and TNF-alpha following intraparenchymal injection of LPS despite morphological evidence of activation of microglia and astrocytes (31). Moreover, IFN-beta (which binds to the identical receptor as IFN-alpha) has been shown to induce MCP-1 messenger RNA (mRNA) and protein in human fetal microglia (49). Thus, IFN-alpha induction of MCP-1 within the CNS may contribute to increases in IL-6 through priming of local glial elements, which,



**Figure 2.** Correlation between CSF IL-6 and 5-HIAA in control subjects and IFN-alpha/ribavirin-treated patients with HCV. Cerebrospinal fluid (CSF) samples for the assessment of interleukin (IL)-6 and the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA), were obtained from control subjects ( $n = 12$ ) and interferon (IFN)-alpha/ribavirin-treated ( $n = 12$ ) patients with hepatitis C virus (HCV) infection. IFN-alpha/ribavirin-treated subjects were studied after ~12 weeks on IFN-alpha/ribavirin therapy. CSF immune and monoamine measures were log-transformed (ln) to achieve normality. CSF lnIL-6 was found to significantly correlate with CSF ln5-HIAA ( $r = -.50, df = 24, p = .013$ ). CSF, cerebrospinal fluid; 5-HIAA, 5-hydroxyindoleacetic acid; HCV, hepatitis C virus; IFN, interferon; IL, interleukin; ln, log-transformed.

in turn, produce IL-6 within the brain. Given microglial expression of IFN- $\alpha$  receptors (50), direct effects of IFN- $\alpha$  on microglial cytokine production including IL-6 may also be involved.

Interferon- $\alpha$  has been detected in CSF of patients with a number of infectious diseases, including viral and bacterial meningitis as well as systemic lupus erythematosus and Aicardi-Goutieres syndrome, where high concentrations of blood and CSF IFN- $\alpha$  are associated with diffuse neuropathology (29,51–55). In HIV infection, CSF IFN- $\alpha$  was found to be significantly higher in subjects with HIV dementia compared with patients without dementia and control subjects (28,29). These data indicate that increased CSF IFN- $\alpha$  in the context of a number of disorders (as well as IFN- $\alpha$  therapy) may contribute to behavioral changes.

Stepwise linear regression analysis found that 5-HIAA was the primary predictor of depression among the relevant immune and neurotransmitter variables entered into the model. These data suggest that IFN- $\alpha$ -induced depressive symptoms may be mediated by effects on serotonin metabolism. The ability of serotonin reuptake inhibitors to prevent or reverse depressive symptoms in patients treated with IFN- $\alpha$  supports this notion (10,56–59). Moreover, these data are consistent with a recent study indicating that polymorphisms in the serotonin transporter gene interact with IL-6 gene polymorphisms to influence depressive symptoms in IFN- $\alpha$ -treated HCV patients (60). There are several mechanisms whereby IFN- $\alpha$  may influence serotonin metabolism. For example, IFN- $\alpha$  is a potent inducer of p38 mitogen-activated protein kinase (MAPK), which has been shown to upregulate expression and function of membrane transporters for serotonin (61,62). Indeed, p38 MAPK activation in peripheral blood mononuclear cells has been associated with decreased CSF 5-HIAA in rhesus monkeys exposed to maternal abuse/neglect in infancy (63). In addition, IFN- $\alpha$  and p38 MAPK pathways have been shown to activate the enzyme indoleamine 2,3 dioxygenase (64), which breaks down tryptophan into kynurenine and quinolinic acid. Decreased peripheral blood tryptophan has been found to correlate with IFN- $\alpha$ -induced depressive symptoms, and increased peripheral blood kynurenine has been found in IFN- $\alpha$ -treated patients with MD (17,18,65). Interferon- $\alpha$  also has been found to down-regulate serotonin 1A receptors in relevant cell lines (66). Of note, IFN- $\alpha$ -associated increases in CSF IL-6 may further contribute to alterations in serotonin metabolism. Previous studies have shown that IL-6 administration to laboratory animals can have a profound influence on serotonin metabolism in a number of brain regions (67,68). It should also be mentioned that IL-6 has been associated with impaired growth of neuronal progenitor cells, suggesting that IL-6 may have direct effects on synaptic plasticity and behavior (69).

There are several limitations of the current study that warrant consideration. Because of the relatively small sample of subjects, we only had adequate statistical power (80% or greater) to detect medium effect sizes ( $r^2 = .25$ ), accounting for ~25% or more of the variance in observed relationships. A larger sample size may have allowed detection of more subtle relationships among variables, notably the relationship between plasma and CSF MCP-1. Another limitation is that blood samples and depression assessments were obtained 6 to 7 days after LP, and CSF samples were only obtained once during the study. This design strategy was employed to limit the impact of the stress and anxiety of CSF sampling on peripheral blood and behavioral measures and to limit the risk of dropout due to repeated LP. However, because

blood and behavioral assessments were not obtained concurrently with CSF samples, relationships among these variables may have been less robust. Longitudinal assessments would also have considerably strengthened the study design and allowed greater control of individual differences and assessment of cause and effect relationships. It also should be noted that because the analyses were restricted to depressive symptoms as measured by the MADRS, relationships among immune and neurotransmitter variables and other symptom domains remain to be determined. Finally, because group assignment was not randomized, the IFN- $\alpha$ /ribavirin treatment group had a higher rate of past history of MD. Nevertheless, controlling for a past history of depression did not significantly affect observed differences between groups.

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