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Suppression of Cell Proliferation by Interferon-Alpha through Interleukin-1 Production in Adult Rat Dentate Gyrus

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The therapeutic use of interferon-alpha (IFN-α), a proinflammatory cytokine, is known to cause various neuropsychiatric adverse effects. In particular, depression occurs in 30–45% of patients, frequently interrupting treatment. IFN-α-treated animals also show depression-like behaviors. However, mechanisms underlying the depression caused by IFN-α remain to be defined. Recently, a decrease in adult hippocampal neurogenesis was revealed as a possible neuropathological mechanism of depression. Therefore, we investigated the effect of subchronic IFN-α treatment on neurogenesis in the adult rat dentate gyrus (DG). Immediately after the administration of IFN-α for 1 week, a decrease in the number of 5-bromo-deoxyuridine-labeled proliferating cells was observed in the DG; however, no effect was detected on the expression of mature neuronal phenotype in the newly formed cells 3 weeks later. Also, an increase in the level of interleukin-1beta (IL-1β), a major proinflammatory cytokine, was observed in the hippocampus following the administration of IFN-α. Furthermore, coadministration of an IL-1 receptor antagonist completely blocked the IFN-α-induced suppression of the cell-proliferative activity in the DG. Our results indicate that IFN-α suppresses neurogenesis in the DG, and that IL-1β plays an essential role in the suppression. The decreased cell proliferation caused by IFN-α-induced IL-1β may be responsible, at least in part, for IFN-α-induced depression.

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INTRODUCTION

Interferon-alpha (IFN-α) is a leukocyte-derived cytokine that has been widely used for the treatment of chronic viral hepatitis and malignancy, because of its immune-activating, antiviral, and antiproliferative properties. In addition to its clinically beneficial effects, long-term and high-dose administration of IFN-α has been noted to be frequently associated with various neuropsychiatric adverse effects, such as insomnia, agitation, cognitive dysfunction, depression, and memory disturbance (Capuron *et al*, 2002; Dieperink *et al*, 2000; Horikawa *et al*, 2003; Malek-Ahmadi 2001). In particular, depression, frequently necessitating treatment discontinuation, has been reported in up to 30–45% of patients receiving IFN-α treatment.

The behavioral and neurochemical effects of IFN-α have been investigated in animals. Increase in the immobility time in the forced swimming test was reported in animals following IFN-α administration (Makino *et al*, 1998, 2000); prolonged immobility is considered to a behavioral despair (Porsolt, 1979, 1977). Thus, IFN-α induces depression-like behavior in animals as well as humans. In fact, IFN-α has been reported to modulate monoaminergic neurotransmission (Kamata *et al*, 2000; Morikawa *et al*, 1998; Shuto *et al*, 1997) and the activity of the hypothalamic-pituitary-adrenal (HPA) axis (Corssmit *et al*, 1996; Gisslinger *et al*, 1993; Menzies *et al*, 1996), both of which are closely associated with the depressive state. However, the precise mechanisms underlying depressive symptoms induced by IFN-α still remain to be elucidated.

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Previous studies have revealed various alterations in the immune functions of depressive patients. For example, depressed patients have been reported to show increased serum levels of proinflammatory cytokines, such as interleukin (IL)-1, IL-6, and tumor necrosis factor-alpha (TNF-α) (Anisman *et al*, 1999; Capuron *et al*, 2001; Dantzer *et al*, 1999; Wichers and Maes, 2002), and decreased IL-2 receptor sensitivity (Kanba *et al*, 1998). IFN-α, a proinflammatory cytokine, activates the peripheral immune system and induces the production of these cytokines (Haq and Maca, 1986; Sissolak *et al*, 1992; Taylor and Grossberg, 1998). Similar to IFN-α, these cytokines also cause depressive state/behavior in humans and animals (Avitsur and Yirmiya, 1999; Capuron *et al*, 2001; Dantzer, 2001; Minor *et al*, 2003; Wichers and Maes, 2002). The precise functions of proinflammatory cytokines in the CNS have not yet been defined; however, IL-1β and/or TNF-α have been shown to play a primary role in the development of the state (Avitsur and Yirmiya, 1999; Bluthe *et al*, 2000; Minor *et al*, 2003).

There is vast evidence suggesting that the production of neurons mediated by proliferating neural progenitor cells (NPCs) continues throughout an animal's lifetime in the dentate gyrus (DG) of the hippocampus (Altman and Das, 1965; Eriksson *et al*, 1998; Gould *et al*, 1999; Kaplan and Hinds, 1977). Recent studies suggest a possible link between decreased neurogenesis and depression. Chronic psychosocial stress, which is used to create animal models of depression, has been shown to suppress cell proliferation in the DG (Czeh *et al*, 2001, 2002; Tanapat *et al*, 2001) On the other hand, administration of several antidepressive agents has been shown to increase the cell proliferation (Czeh *et al*, 2001, 2002; Madsen *et al*, 2000; Malberg *et al*, 2000), and an increase in hippocampal neurogenesis has been shown to be essential for manifestation of the behavioral effects of antidepressants (Santarelli *et al*, 2003). Although the precise mechanisms remain unclear, several studies indicate that proinflammatory cytokines may affect hippocampal neurogenesis (Monje *et al*, 2003; Vallieres *et al*, 2002). As hippocampal neurons and astrocytes normally show marked IL-1β protein and IL-1 receptor expression (Friedman, 2001; Schobitz *et al*, 1994), it is conceivable that IL-1β may regulate hippocampal neurogenesis. The aim of this study was to clarify whether subchronic IFN-α administration at clinical doses might modify the regulation of neurogenesis in the adult rat DG, directly or through its effect of induction of IL-1β.

MATERIALS AND METHODS

All animal experiments were approved by the University of Yamanashi Animal Care and Use Committee. Male Wistar rats (SLC, Japan, 7–9 weeks old, 12-h-light/dark cycle), housed individually with free access to food and water, were used for all experiments.

IFN Treatment

Rats were deeply anesthetized with diethyl ether and a chronic intravenous (i.v.) cannula was inserted into the left subclavian vein. Three days after the operation, human IFN-α (Sumiferon, Sumitomo Pharmaceutical, Osaka, Japan) was administered through the cannula at 5000 international units (IU)/kg/day (IFN5000), 20 000 IU/kg/day (IFN20 000), or 50 000 IU/kg/day (IFN50 000), or saline as a control (Saline) once a day in the early morning for 7 consecutive days ($n = 5$ or 6 for each group). An open-field test was performed in the first experiment 15 min after the last administration of INF-α, in which the total movement distance and time, and the number of rearings were measured, followed by blood sampling for measurement of the plasma corticosterone level.

Measurement of Plasma Corticosterone

Blood samples were collected into EDTA-treated tubes, centrifuged (3000 r.p.m., 4°C, 15 min), and stored at −20°C, then analyzed by a commercial laboratory (SRL Inc., Tachikawa, Tokyo, Japan).

5-Bromo-Deoxyuridine (BrdU) Labeling

In all experiments, BrdU (Sigma, St Louis, MO, USA, 50 mg/kg, i.p.), a marker of proliferating cells, was injected 24 h before the last administration of IFN-α, followed by three more BrdU injections every 6 h. To evaluate cell proliferation activity, the rats were killed 2 h after the 1-week IFN-α treatment. To investigate the differentiation of newly generated cells, the rats were killed 3 weeks after the BrdU labeling.

IL-1 Receptor Antagonist (IL-1Ra) Treatment

In the third experiment, rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and a stainless-steel guide cannula was stereotaxically implanted for intracerebroventricular (i.c.v.) injection into the left lateral ventricle (AP = −0.8 from the bregma, ML = 1.5, DV = −3.5). The rats were allowed to recover for 2 weeks, after which an i.v. cannula was inserted for IFN administration. One hour prior to each daily injection of IFN-α (50 000 IU/kg/day) for 7 days, IL-1Ra (anakinra, Amgen Inc., Thousand Oaks, CA, USA) or saline was injected through the chronic guide cannula. There were four treatment groups ($n = 6$ for each group): (1) a group treated with saline (i.c.v. and i.v.) as a control (Sal/Sal), and three IFN-α (i.v.)-treated groups that were given an i.c.v. injection of (2) saline (Sal/IFN), (3) 10 μg/rat IL-1Ra (IL1Ra10/IFN), or (4) 50 μg/rat IL-1Ra (IL1Ra50/IFN).

IL-1β Western Blot Analysis

Immediately after the final administration of IFN-α, the brains of the rats were rapidly dissected, and the hippocampal formation was isolated and frozen in liquid nitrogen. The frozen samples were homogenized in ice-cold buffer (0.05 M Tris, pH 7.4, 0.5% sodium dodecyl sulfate, 1 mM dithiothreitol). The homogenates were spun for 10 min, at 200 *g*, at 4°C. The supernatants were collected, and the protein concentrations were measured by the Lowry method. Aliquots were incubated in Tris-Glycine sodium dodecyl sulfate buffer containing 5% β-mercaptoethanol for 5 min at 100°C and stored at −20°C. Proteins were separated by 10% Tris-Glycine gel electrophoresis and

transferred to a PVDF membrane (Invitrogen Corp., Carlsbad, CA, USA). The membrane was treated with a blocking solution composed of 0.1% Tween-TBS (TBS-T) and 5% skim milk for 1 h and with anti-IL-1β (1:1500, Endogen, Woburn, MA, USA) at 4°C overnight. After being thoroughly washed with TBS-T, the membrane was incubated with the secondary antibody (HRP-conjugated anti-rabbit IgG, Amersham Biosciences, Piscataway, NJ, USA) for 1 h at room temperature. Immunoreaction was detected using an enhanced chemiluminescence system (ECL plus, Amersham) and quantified by densitometry using the Scion Image analysis program (Scion Corporation). The amount of protein blotted onto each lane was compared with the amount of the constitutive protein, β-actin (1:5000, Cytoskeleton, Denver, CO, USA).

Tissue Preparation

Rats were deeply anesthetized with sodium pentobarbital (50 mg/kg), and transcardially perfused with 4% paraformaldehyde. The brains were dissected and stored overnight in 4% paraformaldehyde, and then transferred into 20% sucrose in PBS. The dehydrated blocks were rapidly frozen in dry ice, then cut into coronal sections (40 μm thick) on a microtome (Leica Microsystems, Wetzlar, Germany) and stored at −20°C in cryoprotectant.

Immunohistochemistry

For BrdU staining, 12 free-floating sections cut at 240-μm intervals were incubated in 2 N HCl for 30 min at 55°C, and then in anti-BrdU antibody (1:5000, Exalpha Biologicals, Watertown, MA, USA) for 24 h at 4°C. The sections were incubated in biotinylated anti-sheep IgG (Vector Laboratories, Burlingame, CA, USA) for 2 h followed by signal amplification with the avidin–biotin complex (ABC kit, Vector Laboratories, Burlingame, CA, USA) and color development by diaminobenzidine. For IL-1β and TNF-α immunostaining, six free-floating sections cut at 480-μm intervals were incubated in anti-IL-1β antibody (1:1000, Endogen, Woburn, MA, USA) or anti-TNF-α antibody (1:200, R&D Systems, McKinley Place, Minneapolis, MN, USA) for 24 h at 4°C, then in biotinylated secondary antibody (biotinylated anti-rabbit IgG, 1:2000, biotinylated anti-goat IgG, 1:2000, Vector Laboratories, Burlingame, CA, USA, respectively) for 2 h followed by signal amplification with the avidin–biotin complex, and color development by diaminobenzidine.

Immunofluorescence

Following incubation in 2 N HCl for 30 min at 55°C, the sections were incubated in anti-BrdU antibody (1:100, Quanta Biotech Ltd, Lightwater, Surrey, UK) and anti-Neuronal Nuclei (anti-NeuN, 1:200, Chemicon International, Temecula, CA, USA) for 24 h at 4°C, and then in biotinylated anti-rat IgG (1:200, Vector) and FITC-conjugated anti-mouse IgG (1:50, Amersham) for 2 h at room temperature. Following signal amplification with the avidin–biotin complex, the sections were incubated in streptavidin–TexasRed (1:300, Amersham) for 2 h.

Quantification

BrdU-labeled cells in the subgranular zone (SGZ), the border between the granule cell layer (GCL) and the hilus, and the inner two-cell layer in the GCL were counted at $\times 400$ under a light microscope (BX50, Olympus, Tokyo, Japan), and the total number of BrdU-labeled cells was estimated by multiplying the number of cells counted in every sixth section (200 μm apart) by six. The immunohistochemistry results for IL-1β and TNF-α were expressed as the ratio of the immunopositive area to the total area, separately for the hippocampus, neocortex, and hypothalamus. Images of each area were obtained by light microscopy ($\times 40$), and processed and measured with Adobe Photoshop (Adobe Systems). To confirm double-labeling of the cells, imaging was performed by laser-scanning confocal microscopy (TCS4D, Leica Microsystems, Wetzlar, Germany). The total number of BrdU-labeled cells in the GCL and SGZ was estimated by multiplying the number of cells counted in every sixth section (200 μm apart) by six, and the ratio of the number of BrdU/NeuN double-labeled (BrdU + NeuN +) cells to the total number of BrdU + cells in the DG was determined.

Statistics

The data were expressed as means ± SEM. Differences between means were determined by one-way ANOVA, followed by the Bonferroni *post hoc* multiple comparison test. Data from the open-field test were analyzed by the Kruskal–Wallis test (nonparametric ANOVA). Differences were regarded as statistically significant when $p < 0.05$.

RESULTS

Effects of IFN-α on Cell Proliferation and IL-1β and TNF-α Productions in the Hippocampus

During the week of IFN-α administration, there were no significant changes in the body weights of the rats or their intakes of food and water (data not shown). IFN-α did not affect the total movement distance, total movement time, or the number of rearings in the open-field test conducted on the last day of administration, suggesting that it may have no effect on the voluntary activities of rats (Figure 1d). Plasma corticosterone levels were not influenced by IFN-α treatment, which showed a minimal correlation with the number of BrdU-positive proliferating cells in the DG (Figure 1e, correlation coefficient = 0.256). In contrast, in all of the IFN-α-treated groups, even in the group receiving the lowest dose (IFN5000), the number of BrdU-positive proliferating cells in the DG at the end of the treatment was significantly decreased, by about 20–35% (Saline: 2087.0 ± 122.9; IFN5000: 1521.6 ± 188.4; IFN20 000: 1352.4 ± 81.2; IFN50 000: 1430.4 ± 79.5, respectively), as shown in Figure 1(a–c). Three weeks after the BrdU injection, the number of BrdU-positive cells that were double-labeled with a marker for mature neurons, NeuN, confirmed by laser-scanning confocal microscopy, also tended to be lower in the IFN-α-treated groups (Figure 1d and e, Saline: 288.0 ± 43.9, IFN5000: 230.0 ± 18.6, IFN50 000: 263.0 ± 18.8), although the difference did not reach statistical significance.

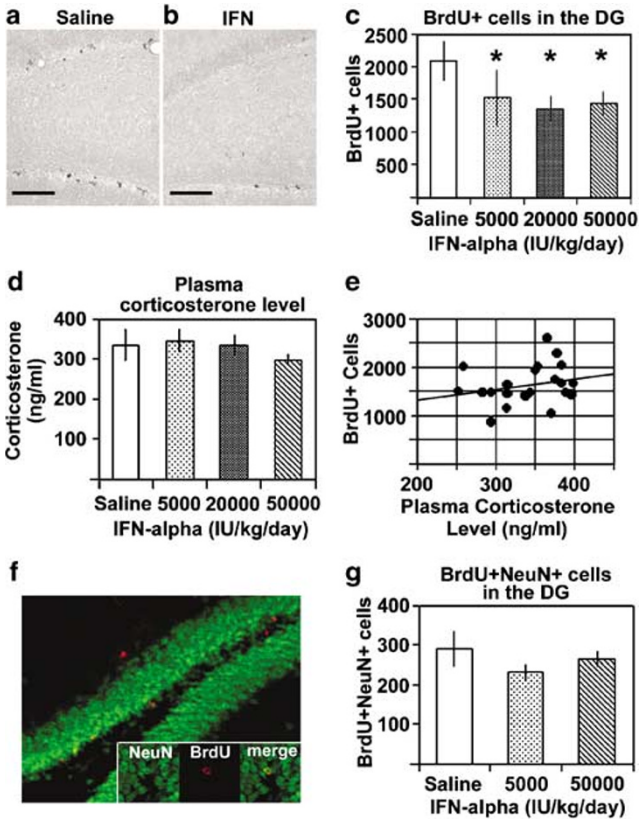

Figure 1 Detection of BrdU-labeled cells in the DG of rats treated with IFN-α for 1 week. BrdU was injected 24 h before the last administration of IFN-α, followed by three more BrdU injections every 6 h. To assess the proliferation of cells in the DG, the rats were killed 2 h after the last IFN-α treatment. Then, the numbers of BrdU-labeled proliferating cells in the DG in all IFN-α-treated groups were found to be significantly lower than that in the control group (Saline) (a, Saline; b, IFN-α, 50 000 IU/kg/day, scale bars: 50 μm; c, $n=5$ or 6 per group, $F_{(3,\ 17)}=7.553$, $^{*}p<0.05$ *vs* Saline; error bar: ±SE). There were no significant differences in the plasma corticosterone level at the end of the 1-week-treatment period among the groups (d, $n=5$ or 6 per group, error bar: ±SE), which showed only a weak correlation with the number of BrdU-labeled cells (e, correlation coefficient = 0.256). To detect the neuronal differentiation of the BrdU-labeled cells, the rats were allowed to survive for 3 weeks after the treatment. A laser-scanning confocal image of BrdU (red) and a mature neuron marker, NeuN (green) (f). The numbers of BrdU/NeuN double-labeled cells in the DG in the IFN-α-treated groups (5000 and 50 000) appeared to be lower than that in the control group (Saline), but the differences were not statistically significant (g, $n=5$ or 6 per group, $F_{(2,\ 14)}=1.099$, error bar: ±SE).

To detect neuronal differentiation of BrdU-labeled cells, the ratio of the number of BrdU/NeuN-double-labeled cells to the total number of BrdU-positive cells was evaluated, there was no significant difference in these ratios between the control group and any of the IFN-α-administered groups (Saline, 86.6±3.2%; IFN5000, 85.9±2.6%; IFN50 000, 83.7±2.9%). Thus, the IFN-α treatment suppressed cell-proliferative activity in the DG, but showed no effect on the subsequent differentiation of newly generated cells into neurons.

IL-1β immunohistochemistry revealed a 1.6- to 1.7-fold increase in the total IL-1β-immunopositive area in the hippocampus in the groups given IFN-α (Figure 2a, b, and f, left). The IL-1β-immunoreactive cells were morphologically identified as astrocytes residing in the dentate hilus (Figure 2a and b). In the group receiving the highest dose of IFN-α, a number of large intensely stained cells was found to be distributed from the corpus callosum to the adjacent neocortex (Figure 2c–e and f, middle). However, there were no significant differences in the immunopositivity rate in the hypothalamus between the control group and the IFN-α-administered groups (Figure 2f, right). In contrast to this region-specific increase in IL-1β immunoreactivity, an increase in the total TNF-α-immunopositive area and large TNF-α-positive cells were also found in all of these brain regions, but only in the animals receiving the highest dose of IFN-α (Figure 3). Western blot analysis revealed a dose-dependent increase in the amount of activated IL-1β protein (17 kDa) in the hippocampus in the animals given IFN-α (IFN5000, 1.85-fold; IFN50 000, 2.85-fold, *vs* Saline), as shown in Figure 4.

Effect of an IL-1 Receptor Antagonist on the IFN-α Induced Decrease in Neurogenesis

As shown in Figure 5, coadministration of IL-1Ra (i.c.v.) with IFN-α (i.v.) once a day for a week completely reversed the IFN-α-induced decrease in the number of BrdU-labeled proliferating cells in the DG (Sal/Sal, 1472.4±62.6; IL1Ra10/IFN, 1402.8±110.3; IL1Ra50/IFN, 1532.4±134.0 *vs* Sal/IFN, 985.2±99.0). There were no significant differences in the number of BrdU-labeled proliferating cells between the animals receiving the lower and higher doses of IL-1Ra (IL1Ra10/IFN and IL1Ra50/IFN).

DISCUSSION

IFN-α treatment induces depressive states in humans and depression-like behaviors in animals, but the precise mechanisms underlying its development remain unknown. In this study, we focused on the effect of IFN-α on neurogenesis in the DG, which has been demonstrated to be decreased in animals exposed to chronic psychosocial stress (animal models of depression) (Czeh *et al*, 2001, 2002; Tanapat *et al*, 2001), and found that subchronic and peripheral administration of IFN-α at clinical doses decreased the number of BrdU-labeled proliferating cells in the DG of adult rats. It is noteworthy that similar doses of IFN-α were reported to cause learned helplessness, an animal (rodent) model of depression (Makino *et al*, 1998, 2000), without affecting the motor activities or the nutritional status of the animals. Taking into consideration recent reports suggesting that decreased neuronal proliferative activity in the DG may play important roles in the neuropathology of depression (Czeh *et al*, 2001; Malberg *et al*, 2000; Santarelli *et al*, 2003; Tanapat *et al*, 2001), it might be reasonable to propose that the IFN-α-induced reduction of cell-proliferative activity may underlie the development of depression associated with IFN-α administration. We further investigated the fate of newly born cells 3 weeks after IFN-α treatment. Following the decrease in cell proliferation during IFN-α treatment, there seemed to be fewer BrdU/NeuN double-labeled cells in the DG in animals treated with IFN-α, but the differences were no longer statistically significant. A previous study showed that

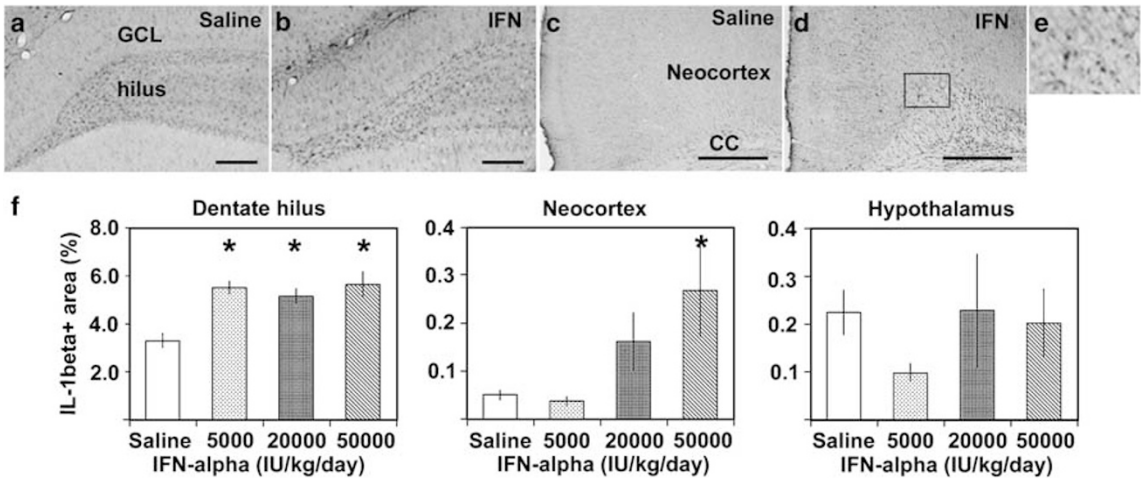

Figure 2 IL-1β immunohistochemistry in rats treated with IFN-α for 1 week. Treatment with IFN-α increased the immunopositivity of cells in the dentate hilus of the hippocampus (a, Saline; b, IFN-α, 50 000 IU/kg/day, scale bars: 50 μm), and resulted in the appearance of immunopositive cells in the neocortex (c, Saline; d, IFN-α, 50 000 IU/kg/day, scale bars: 200 μm; e, a higher magnification of d). The graphs show the size of the IL-1β-immunopositive area (%) in the dentate hilus (f, left, $F_{(3, 17)} = 10.764$), neocortex (f, middle, $F_{(3, 17)} = 29.203$), and hypothalamus (f, right, $F_{(3, 17)} = 0.709$) ($n = 5$ or 6 per group, $^*p < 0.05$ *vs* Saline; error bar, $\pm$SE). GCL, granule cell layer; CC, corpus callosum

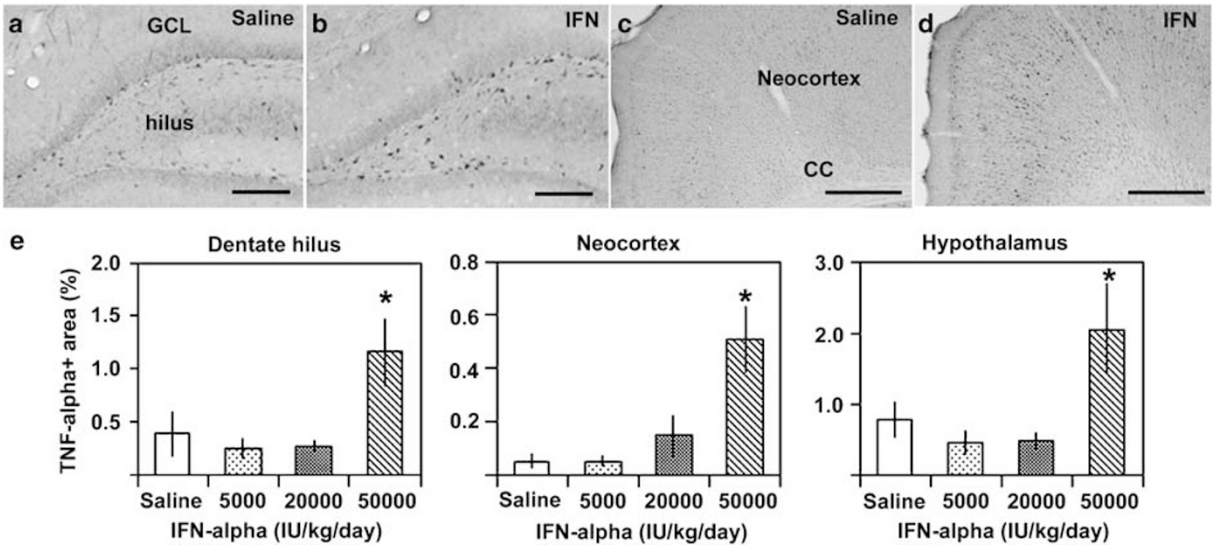

Figure 3 TNF-α immunohistochemistry in rats treated with either saline or IFN-α for 1 week revealed large TNF-α-immunopositive cells in the dentate hilus (a, Saline; b, IFN-α, 50 000 IU/kg/day, scale bars: 100 μm) and neocortex (c, Saline; d, IFN-α 50 000 IU/kg/day, scale bars: 200 μm), and an increase in the sizes of immunopositive areas in the dentate hilus (e, left, $F_{(3, 17)} = 4.684$), neocortex (e, middle, $F_{(3, 17)} = 9.652$), and hypothalamus (e, right, $F_{(3, 17)} = 4.581$). ($n = 5$ or 6; $^*p < 0.05$ *vs* Saline; error bar, $\pm$SE). GCL, granule cell layer, CC, corpus callosum.

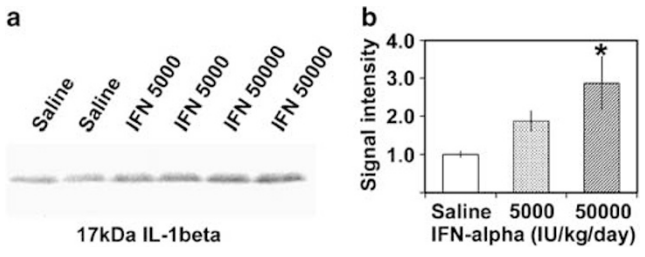

Figure 4 Western blot analysis for IL-1β in the rat hippocampus after 1-week treatment with IFN-α (a, left two lanes; Saline, middle two lanes; IFN-α 5000 IU/kg/day, right two lanes; IFN-α 50 000 IU/kg/day). The amount of active form of IL-1β protein in the hippocampus increased following IFN-α treatment in a dose-dependent manner (b, $n = 6$ per group, $F_{(2, 15)} = 4.408$, $^*p < 0.05$ *vs* Saline).

BrdU-labeled cells in the DG are drastically reduced within a month (Kempermann *et al*, 2003), which would depend on a significant proportion of BrdU-labeled cells being lost to label dilution during subsequent divisions, or simply on death of newly born cells. It is speculated that this phenomenon might reflect our failure to detect a significant decrease in newly generated neurons in animals treated with IFN-α 3 weeks after BrdU labeling.

We then investigated the possible mediators of the IFN-α-induced decrease in neurogenesis. In contrast to previous reports (Corssmit *et al*, 1996; Dunn and Crnic, 1993; Gisslinger *et al*, 1993; Menzies *et al*, 1996), there were no significant changes in food intake, motor activities, or plasma glucocorticoid levels, all of which have been

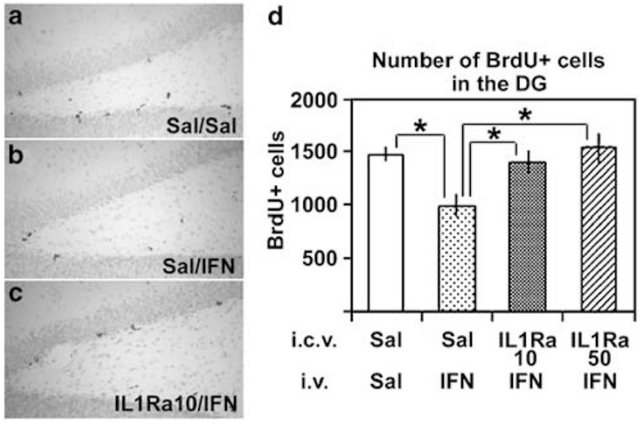

Figure 5 Detection of BrdU-labeled proliferating cells in the DG of rats treated with IL-1Ra (10 or 50 μg/rat, i.c.v.) in combination with IFN-α (50 000 IU/kg/day) for one week (a, Sal/Sal, b, Sal/IFN, c, IL1Ra10/IFN, scale bars: 100 μm). The graph shows the significant reduction in the number of BrdU-labeled cells in the DG in the group treated with IFN-α (d, $n = 6$ per group, $F_{(3,\ 20)} = 5.599$, $^*p < 0.05$ *vs* Sal/IFN; error bar, ±SE), whereas coadministration of IL-1Ra completely reversed the effect of IFN-α.

demonstrated to affect hippocampal neurogenesis (Cameron and Gould, 1994; Gould *et al*, 1997; Mattson *et al*, 2003; van Praag *et al*, 1999), in the rats given IFN-α for a week. The differences in the results may be explained by differences in the doses of IFN-α used; we used much lower doses than that used in a previous study reporting these effects (Dunn and Crnic, 1993), as these adverse events associated with IFN-α have been shown to be dose-dependent (Dunn and Crnic, 1993; Kirkwood *et al*, 1985). Consistent with the results of previous studies in which interferons were shown to stimulate the production of inflammatory cytokines in peripheral immunocytes (Haq and Maca, 1986; Sissolak *et al*, 1992; Taylor and Grossberg, 1998), IFN-α increased IL-1β immunoreactivity in hippocampal astrocytes, and the amount of the active form of IL-1β protein in the hippocampus, as shown by Western blotting. TNF-α-immunoreactivity was also found to be increased in the various brain regions examined, but only at the highest dose of IFN-α. Interestingly, blockade of IL-1β signal transduction by injection of IL-1Ra completely abolished the IFN-α-induced decrease in the number of BrdU-labeled proliferating cells. These results indicate that IFN-α may suppress the proliferation of progenitor cells in the DG via a pathway including astrocyte-derived IL-1β.

Previous studies have revealed a relationship between depression and proinflammatory cytokines, including IL-1β. Exposure to these cytokines has been shown to induce depressive symptoms in humans and depression-like behaviors in animals (Avitsur and Yirmiya, 1999; Bluthe *et al*, 2000; Dantzer, 2001; Kent *et al*, 1992; Pugh *et al*, 1998); on the other hand, depressed patients show increased serum levels of proinflammatory cytokines, with normalization of these levels following antidepressive treatment (Anisman *et al*, 1999; Capuron *et al*, 2001; Dantzer *et al*, 1999; Wichers and Maes, 2002). The precise roles of proinflammatory cytokines in the onset of depression have not yet been defined; however, these findings indicate that neuronal cytokines may be involved in the development of depression.

Considering that peripherally circulating cytokines, including IFN-α, only minimally penetrate the blood–brain barrier (BBB) (Smith *et al*, 1985), the mechanism by which IFN-α promotes cytokine production in the brain and affects CNS functions remains unclear, although several pathways have been suggested. The vagus nerve may be one possible link between the two. As another possible mechanism, several peripheral proinflammatory mediators have been demonstrated to increase BBB permeability (Abbott, 2000; Didier *et al*, 2003; Huber *et al*, 2001), implying that IFN-α and/or IFN-α-induced inflammatory agents may facilitate the ability of IFN-α itself and of other circulating cytokines to enter the CNS. In this context, it may be noteworthy that even a relatively low dose of IFN-α increased IL-1β immunoreactivity, mainly in hippocampal astrocytes, in our study. The hippocampus is one of the brain regions showing the most intense expression of IL-1 and its receptor, even in the normal, intact brain (French *et al*, 1999; Friedman, 2001; Schobitz *et al*, 1994). Thus, this region might be constitutively sensitive to inflammatory stimuli, including IL-1β, which has been shown to upregulate IL-1β expression in this region. IL-1β protein expression is rapidly induced within a few hours after brain injury, and acts to amplify the inflammatory process, implying that IL-1β in the CNS may trigger sequential neuropathological processes (Abbott, 2000; Huber *et al*, 2001; Rothwell, 1999; Turrin *et al*, 2001), causing depressive symptoms.

Recent studies have shown that proinflammatory cytokines regulate the proliferation, differentiation and bioactivities of NPCs (Monje *et al*, 2003; Storch *et al*, 2001; Vallieres *et al*, 2002; Vela *et al*, 2002). Although expression of IL-1β mRNA was reported in hippocampal progenitor cells in the adult rat (Klassen *et al*, 2003), there have been no reports on the direct effects of IL-1β on NPCs. However, it has been demonstrated that increased IL-1β expression downregulates the expression of BDNF (Lapchak *et al*, 1993), a neurotrophic factor that promotes cell proliferation and neurogenesis (Linnarsson *et al*, 2000; Scharfman *et al*, 2005) in the hippocampus, suggesting indirect inhibition of the proliferation of NPCs. In fact, increased IL-1β production impairs hippocampus-dependent learning, which has been shown to be correlated with the amount of neurogenesis (Palin *et al*, 2004; Pugh *et al*, 1998). Taking these observations together, overexpression of IL-1β in hippocampal astrocytes either directly or indirectly inhibits the proliferation of NPCs in the DG, which further indicates that astrocytes may control the proliferation of precursor cells in the DG via IL-1β production.

In this study, we demonstrated that IFN-α suppressed neuronal proliferation via IL-1β in the adult DG. Further studies are needed to verify the link between this finding and the onset of depressive symptoms associated with IFN-α administration. However, studies designed to demonstrate the neuropathology of psychiatric diseases are extremely difficult to perform and fraught with limitations, as the developments of these diseases are highly dependent on profoundly complex brain functions specific to humans. Nevertheless, considering that IFN-α-induced depression is sometimes refractory to conventional anti-depressive treatments, the findings of our study may provide further insight into our understanding of the mechanism of IFN-α-induced depression and the inter-relationships between immune

responses and depressive symptoms, which, in turn, may lead to the discovery of new pharmacological interventions for depression.

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