Granulocyte Colony-Stimulating Factor Attenuates Neuronal Death and Promotes Functional Recovery After Spinal Cord Injury in Mice

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INTRODUCTION

The pathologic sequelae that follow acute spinal cord injury (SCI) are divided into 2 broad chronologic events: the primary injury and the secondary injury (1). The primary injury encompasses the focal destruction of neural tissue caused by direct mechanical trauma. This initial insult then instigates a progressive wave of secondary injury, which exacerbates the injury to the spinal cord via the activation of pathophysiologic mechanisms. Because this wave of secondary injury leads to the apoptotic death of neuronal and glial cells left intact by the initial trauma, it is a major impediment to functional recovery after SCI (1). Thus, apoptosis of neurons and glial cells after acute SCI is one of the main therapeutic targets for various kinds of drug therapies.

Granulocyte colony-stimulating factor (G-CSF) is a 19.6-kDa glycoprotein that was identified initially as a serum component that induces differentiation of the murine myelomonocytic leukemic cell line and is capable of inducing the survival, proliferation, and differentiation of cells of neutrophil granulocyte precursors (2, 3). In addition, G-CSF inhibits apoptosis of postmitotic neutrophil lineage cells. This effect results in increased numbers of circulating neutrophils, which is used for neutropenia in clinical situations. In addition to its antiapoptotic effect for neutrophil, it was recently reported that G-CSF has the potential to inhibit apoptosis of postmitotic cells of nonhematopoietic lineage. For instance, G-CSF suppresses apoptosis of cardiomyocytes in an acute myocardial infarction model (4). It is supposed that G-CSF activates common antiapoptotic machinery, which is conserved among the different lineage cells. In the CNS, apoptosis of neurons can also be suppressed by G-CSF. Recent reports showed that G-CSF attenuates glutamate-induced neuronal death in vitro and protects neurons after stroke in vivo (5–7). These lines of evidence show the antiapoptotic potential of G-CSF in the CNS, raising the possibility that G-CSF may also act as a neuroprotectant in acute SCI.

In the current study, we tested the hypothesis that G-CSF could attenuate neuronal apoptosis and promote functional recovery after SCI.

Abstract

Granulocyte colony-stimulating factor (G-CSF) is a protein that stimulates differentiation, proliferation, and survival of granulocytic lineage cells. Recently, a neuroprotective effect of G-CSF was reported in a model of cerebral infarction. The aim of the present study was to elucidate the potential therapeutic effect of G-CSF for spinal cord injury (SCI) in mice. We found that G-CSF is neuroprotective against glutamate-induced cell death of cerebellar granule neurons in vitro. Moreover, we used a mouse model of compressive SCI to examine the neuroprotective potential of G-CSF in vivo. Histologic assessment with cresyl violet staining revealed that the number of surviving neurons in the injured spinal cord was significantly increased in G-CSF-treated mice. Immunohistochemistry for neuronal apoptosis revealed that G-CSF suppressed neuronal apoptosis after SCI. Moreover, administration of G-CSF promoted hindlimb functional recovery. Examination of signaling pathways downstream of the G-CSF receptor suggests that G-CSF might promote functional recovery by inhibiting neuronal apoptosis after SCI. G-CSF is currently used in the clinic for hematopoietic stimulation, and its ongoing clinical trial for brain injury encompasses the potential for functional recovery after SCI (1). Thus, apoptosis of neurons and glial cells after acute SCI is one of the main therapeutic targets for various kinds of drug therapies.

Granulocyte colony-stimulating factor (G-CSF) is a 19.6-kDa glycoprotein that was identified initially as a serum component that induces differentiation of the murine myelomonocytic leukemic cell line and is capable of inducing the survival, proliferation, and differentiation of cells of neutrophil granulocyte precursors (2, 3). In addition, G-CSF inhibits apoptosis of postmitotic neutrophil lineage cells. This effect results in increased numbers of circulating neutrophils, which is used for neutropenia in clinical situations. In addition to its antiapoptotic effect for neutrophil, it was recently reported that G-CSF has the potential to inhibit apoptosis of postmitotic cells of nonhematopoietic lineage. For instance, G-CSF suppresses apoptosis of cardiomyocytes in an acute myocardial infarction model (4). It is supposed that G-CSF activates common antiapoptotic machinery, which is conserved among the different lineage cells. In the CNS, apoptosis of neurons can also be suppressed by G-CSF. Recent reports showed that G-CSF attenuates glutamate-induced neuronal death in vitro and protects neurons after stroke in vivo (5–7). These lines of evidence show the antiapoptotic potential of G-CSF in the CNS, raising the possibility that G-CSF may also act as a neuroprotectant in acute SCI.

In the current study, we tested the hypothesis that G-CSF could attenuate neuronal apoptosis and promote functional recovery after SCI.
MATERIALS AND METHODS

Cell Culture

Cerebellar granule neurons (CGNs) were prepared from postnatal day 7 mice. Fresh cerebella were dissected, and the tissue was dissociated with trypsin (2.5 mg/mL; Invitrogen, Carlsbad, CA) and DNase I (0.3 mg/mL; Roche Applied Science, Indianapolis, IN). Cells were plated on poly-l-lysine-coated chamber slides (Lab-Tek Chamber Slides Permanox; Nalge Nunc International, Rochester, NY) or 6-cm dishes at a density of 7.0 × 10^4 cells/cm^2 in Dulbecco's modified Eagle's medium Gibco BRL (Grand Island, NY), supplemented with 10% fetal bovine serum, penicillin-streptomycin (100 units/mL; penicillin G sodium, 100 µg/mL streptomycin sulfate; Invitrogen), and 0.02 M HEPES. After 16 hours in culture, the medium was replaced with Dulbecco's modified Eagle's medium supplemented with penicillin-streptomycin, 20 mM HEPES, N2 supplement (0.01%; Invitrogen), KCl (20 mM), fibronectin (10 µg/mL), and cytosine arabinoside (1.0 µM). Cells were maintained in a humidified atmosphere containing 5% CO₂ at 37°C. Experiments on CGNs were performed after 7 days in culture.

Immunocytochemistry and Immunohistochemistry

To detect the G-CSF receptor (G-CSFR), immunocytochemical staining on cultured CGNs and immunohistochemistry on intact mouse spinal cord sections were performed. For histologic sections, animals were perfused through the heart by immersion in 4% paraformaldehyde in PBS and vibratome sectioned (30 µm thick) on a cryostat. Axial sections (12 µm thick) were mounted on poly-i-lysine-coated glass slides (Matsunami, Tokyo, Japan) and dried for 48 hours at room temperature. The slides were then washed 3 times with PBS and analyzed by confocal laser scanning microscopy (LSM5 PASCAL; Carl Zeiss, Germany, Oberkochen).

Reverse Transcriptase-Polymerase Chain Reaction

To detect expression of G-CSFR mRNA in CGNs and spinal cord tissue, we performed reverse transcriptase (RT)-polymerase chain reaction (PCR). Total RNA was extracted from cultured CGNs or intact spinal cord tissue using TRIzol reagent (Gibco Life Technologies, Rockville, MD) according to the manufacturer's protocol. cDNAs were prepared by reverse transcription from 2.5 µg of total RNA using the Superscript II RT Preamplification System (Gibco Life Technologies) with an oligo (dT)_{12-18} primer. PCR was performed with 2.0 µL of cDNA in a 50-µL reaction mixture containing 200 nM dNTP, 0.5 U of Extra Taq DNA polymerase (Takara, Tokyo, Japan), and forward and reverse PCR primers. Sequences of both primers were 5'-GTACTCGTGTCCACCTCGTG-3' and 5'-CAAGATA-CAAGGCACCCCAA-3' for G-CSFR (accession number M58288). The following conditions were used for PCR amplification: after denaturation at 94°C for 5 minutes, the reaction was carried out at 94°C for 1 minute, 58°C for 1 minute, and 72°C for 1 minute (40 cycles). The resulting 567-base pair product was analyzed on a 1% agarose gel.

Cell Culture Experiment

After 7 days in culture, CGNs were treated with glutamate (100 µM) for 6 hours to induce cell death. Recombinant human G-CSF was provided by KIRIN Brewery (Tokyo, Japan). Neuronal death was detected by double staining with propidium iodide and calcein using a Live/Dead Double Staining Kit (MBL, Nagoya, Japan). The ratio of dead cells to total cells was calculated (number of dead cells / total cells) and compared between each group. G-CSF was added to the culture medium of glutamate-treated CGN cells in different concentrations (i.e. 0, 10, and 100 ng/mL) to assess the dose dependency of its neuroprotective effects. G-CSF activation was blocked by addition of anti G-CSFR antibody (30 minutes before glutamate treatment; 2 µg/mL of antibodies SC 9173 and SC694; Santa Cruz Biotechnology) to examine the specificity of G-CSF effects on attenuation of CGN cell death.

Signaling pathways downstream of G-CSFR activation were blocked with specific inhibitors as follows: AG490 (100 nM, a specific Janus kinase 2 [JAK2] signal transducer and activator of transcription [STAT] inhibitor; Calbiochem, San Diego, CA), wortmannin (50 nM, a specific phosphatidylinositol 3-kinase [PI3K] inhibitor; Calbiochem), and PD98059 (0.2–20 µM), a specific inhibitor of Ras/mitogen-activated protein kinase. The inhibitors were added to the culture medium 30 minutes before glutamate treatment. After 6 hours of incubation, cell death was determined as described above.

Western Blot Analysis

To determine the precise mechanism of the neuroprotective action of G-CSF, Western blot analysis was performed with CGNs in different concentrations (i.e. 0, 10, and 100 ng/mL) to examine the specificity of G-CSF effects on...
performed as described previously (9). Briefly, after washing with PBS, cultured CGNs were lysed in homogenization buffer (0.05 M Tris-HCl, pH 7.6, 2% Triton X, and 0.5% protease inhibitor cocktail [Sigma, St. Louis, MO]) at 37°C for 15 minutes. Homogenates were centrifuged at 10,000 x g for 5 minutes at 4°C. Samples were stored at −80°C until use. The protein concentration of the samples was determined by the Bradford method. Samples containing 50 μg of protein were electrophoresed on 8% or 12% sodium dodecyl sulfate-polyacrylamide gels, followed by transfer to polyvinylidene difluoride membranes (Hybond-P; Amersham, Piscataway, NJ). Phosphorylated STAT3 (pSTAT3) and Bcl-2 were detected with the anti-pSTAT3 antibody (1:100; Santa Cruz Biotechnology) and anti-actin antibody (1:500, Cell Signaling) respectively. The signal was developed with chemiluminescence (ECL Plus Kit; Amersham). After signal detection, the blots were stripped and rebotted with anti-STAT 3 antibody (1:100; Cell Signaling Technology, Inc., Danvers, MA) and anti-mouse IgG antibody (1:2,500; Amersham), followed by a peroxidase-conjugated anti-rabbit IgG antibody (1:100; Cell Signaling). The animals sweep 1 or both feet while walking (an obvious friction noise can be heard).

8 Normal movements except for reduced speed of walking.
9 Normal movements, ability to walk on a 2-cm-wide bar.
10 Normal movements, ability to walk on a 1.5-cm-wide bar.
11 Normal movements, ability to walk on a 1-cm-wide bar.
12 Normal movements, ability to walk on a 0.7-cm-wide bar.
13 Normal movements, ability to walk on a 5-mm-wide bar.

### Surgery and Drug Treatment

Tissue samples were obtained from 9- to 10-week-old female BALB/cCr mice (18–21 g, average weight 19.5 g; SLC, Hamamatsu, Japan). Animals were anesthetized with 1% to 1.2% halothane in 0.5 L/min oxygen. After laminectomy (T7–8 level), the animals were placed in a stereotaxic apparatus and their spines were fixed with forceps. Compressive SCI was produced at the T7–8 level using a compression rod. The tip of the weight was a 1-mm × 2-mm rectangular plastic plate (10), and the static load (20 g) was applied for 5 minutes. Food and water were given ad libitum. All animals were treated and cared for in accordance with the Chiba University School of Medicine guidelines pertaining to the treatment of experimental animals.

The mice were randomly divided into 2 groups (G-CSF group and control group). The G-CSF group mice were subcutaneously injected with recombinant human G-CSF (200 μg/kg/day) for 5 days. The control group was injected with vehicle (1% bovine serum albumin in PBS) only.

### Tissue Preparation and Immunohistochemical and Histologic Assessments

Animals were killed at 1 day, 3 days, and 6 weeks after SCI. After transcardiac perfusion, spinal cords were dissected out and 12-μm-thick serial sections were made as described above. Every fifth sections (60 μm apart) were used for histologic (6 weeks after injury) and immunohistochemical (1 day and 3 days after injury) examinations.

**FIGURE 1.** Granulocyte colony-stimulating factor receptor (G-CSFR) expression in mouse intact spinal cord. Immunofluorescence staining for (A) anti-neuronal nuclei mouse monoclonal antibody, a marker for neuron (green), (B) G-CSFR, and (C) merged view. G-CSFR expression was predominantly neuronal in mouse spinal cord. (D) Reverse transcriptase-polymerase chain reaction for mouse G-CSFR. Expression of G-CSFR mRNA was detected in mouse intact spinal cord with an expected size of 567 base pairs. Scale bar = (A–C) 50 μm.
Then, 10 sections were picked up from both the rostral and caudal segments to the lesion epicenter and were used for histologic and immunohistochemical examinations. Sections near the lesion epicenter (from 300 km rostral to 300 km caudal) were excluded from immunohistochemical and histologic assessment because the tissue destruction was too severe to count neurons precisely in that area. Therefore, the analyses covered the area from either 300 to 900 km rostral or 300 to 900 km caudal to the lesion epicenter.

Immunohistochemistry for apoptotic cells was performed as described above to quantify neuronal apoptosis in the injured spinal cord. Mouse Neu-N antibody (1:400 dilution) was used as a neuronal specific marker, and rabbit anti-cleaved caspase-3 antibody (1:800 dilution; Genzyme/Techne, Minneapolis, MN) was used as a marker for apoptotic cells. Secondary antibodies were as follows: goat anti-mouse Alexa Fluor 488 and goat anti-rabbit Alexa Fluor 594 (1:800 dilution). The number of apoptotic neurons was determined by counting cells that were double positive for Neu-N and cleaved caspase-3. Neuronal survival was assessed 6 weeks after injury using cresyl violet staining.

**Behavioral Testing for Recovery of Hindlimb Motor Function**

The functional recovery of hindlimb was determined by measuring the hindlimb motor function score as described previously by Farooque et al (10). Mice were allowed to move freely in an open field with a rough surface for 5 minutes at each time tested. The hindlimb movements of mice were videotaped and scored by 2 independent observers who were unaware of the treatment group. Measurement of motor function was performed before surgery and 1 and 3 days and 1 to 6 weeks (once a week) after SCI. The scale ranged from 0 to 13, and scores are shown in the Table. In brief, a score of 0 indicates complete paralysis, a score of 1 to 3 indicates movement of hindlimbs without rhythmic stepping, a score of 4 to 5 indicates rhythmic motion of hindlimbs without weight-bearing ability, a score of 6 to 7 indicates weight-bearing ability, a score of 8 to 12 indicates walking ability with an increase in the hindlimb gait width, and a score of 13 indicates full recovery.

**Statistical Analysis**

Motor function scores were subjected to repeated measures analysis of variance followed by post hoc test using the Scheffe F-test. The final motor function score was statistically analyzed using the Mann-Whitney U-test. The neuron survival counts and apoptotic neuron counts were subjected to the Student t-test. Data are presented as mean ± SE. *, significant difference compared with control (p < 0.05); **, significant difference compared with control (p < 0.01).

![FIGURE 2. Granulocyte colony-stimulating factor (G-CSF) attenuates glutamate-induced neuronal death of cultured cerebellar granule neurons (CGNs). CGNs were exposed to glutamate (100 μM), and G-CSF was added to the culture medium. Viability of CGNs was evaluated with a Live/Dead Double Staining Kit. Granulocyte colony-stimulating factor attenuated glutamate-induced cell death of CGNs in a dose-dependent manner (A). At the G-CSF concentration of 100 ng/mL, the neuroprotective effect was most apparent. Pretreatment of CGNs with anti-G-CSFR antibody abolished G-CSF-mediated neuroprotection against glutamate-induced neuronal death (B). Both the JAK2 inhibitor AG490 and the PI3K inhibitor wortmannin partially abolished G-CSF-mediated neuroprotection (C, D). Data are expressed as means ± SE. *, significant difference compared with control (p < 0.05); **, significant difference compared with control (p < 0.01).](http://jnen.oxfordjournals.org/)

![FIGURE 3. Western blot analysis for signaling molecules downstream of granulocyte colony-stimulating factor (G-CSF) receptor in cultured cerebellar granule neurons (CGNs). Cultured CGNs were exposed to G-CSF (100 ng/mL) and expression of phosphorylated signal transducer and activator of transcription 3 (pSTAT3) and Bcl-2 was detected by Western blot. The level of pSTAT3 increased by 15 minutes after G-CSF exposure, peaked 1 or 2 hours after exposure and then decreased after 6 hours. The level of Bcl-2 increased by 30 minutes after G-CSF exposure and remained elevated for 6 hours. There was no change in the expression levels of total STAT3 and actin, showing equal protein sample loading.](http://jnen.oxfordjournals.org/)
RESULTS

Granulocyte Colony-Stimulating Factor Receptor Expression in Vitro and in Vivo

In cultured CGNs, G-CSFR was detected by immunocytochemistry (not shown) and G-CSFR mRNA was detected by RT-PCR (not shown). Similarly, immunohistochemistry on intact mouse spinal cord sections revealed G-CSFR expression on Neu-N-positive neurons (Fig. 1A–C), and RT-PCR on intact mouse spinal cord showed G-CSFR mRNA expression (Fig. 1D). These data indicate that G-CSFR is expressed by neurons in vitro and in vivo (in spinal cord).

Cell Culture Experiment

To examine neuroprotection against glutamate-induced neuronal death, G-CSF was administered to cultured CGNs simultaneously with glutamate. The results showed that G-CSF significantly decreased glutamate-induced neuronal death in a dose-dependent manner, with a concentration of 100 ng/mL exhibiting the optimal neuroprotective effect (Fig. 2A).

To examine the specificity of G-CSF-mediated neuroprotection, functional blocking of G-CSFR was performed. Pretreatment of CGNs with anti G-CSFR antibody 30 minutes before glutamate treatment abolished G-CSF-mediated neuroprotection against glutamate-induced neuronal death (Fig. 2B).

Next, signaling pathways downstream of G-CSFR were blocked with specific inhibitors to clarify the contribution of each signaling pathway to the neuroprotective effects of G-CSF. Both the JAK2/STAT inhibitor AG490 and the PI3K inhibitor wortmannin partially abolished G-CSF-mediated neuroprotection against glutamate-induced neuronal death (Fig. 2C, D), whereas the mitogen-activated protein kinase inhibitor PD98059 had no influence on the neuroprotective effects of G-CSF.

Western blot analysis was performed to examine signaling events after G-CSF treatment. In cultured CGNs,
the expression level of pSTAT3 increased by 15 minutes after G-CSF exposure, peaked 1 to 2 hours after exposure, and then decreased 6 hours after G-CSF treatment (Fig. 3). Levels of Bcl-2, an antiapoptotic target of STAT3, were elevated within 30 minutes of G-CSF exposure and remained elevated at 6 hours (Fig. 3). The expression level of total STAT3 was unchanged during observation (Fig. 3). There was also no change in the expression level of actin, indicating equal protein loading (Fig. 3).

**Immunohistochemical and Histologic Assessment**

Our results using a model of compressive SCI demonstrate that G-CSF also exhibits neuroprotective effects in vivo. The number of cells double positive for Neu-N and cleaved caspase-3 was significantly smaller in the G-CSF-treated group than in the control group 1 day and 3 days after injury (p < 0.05) (Fig. 4). The average number of double positive cells for Neu-N and cleaved caspase-3 in the G-CSF group was 8.8 (5–13) 1 day after injury and 7.7 (3–11) 3 days after injury, whereas the number of apoptotic neurons in the control group was 16.3 (8–19) 1 day after injury and 14.1 (9–17) 3 days after injury, respectively.

Six weeks after injury, cresyl violet-positive neurons in the anterior horn of the spinal cord were counted as surviving neurons. The number of surviving neurons in the G-CSF group was significantly larger than that in the control group (p < 0.05) (Fig. 5). The average number of surviving neurons in the G-CSF group was 24.0/slice, whereas that in the control group was 4.6/slice.

**Recovery of Hindlimb Motor Function**

We assessed the recovery of hindlimb function using the motor function scale (10), in which the maximum hindlimb motor function scale score is 13. All mice had a score of 13 before surgery, and the score dropped to 0 immediately after SCI. Significant recovery of hindlimb function was observed in mice from the G-CSF-treated group 4 weeks after injury compared with those of the vehicle-treated group (p < 0.05) (Fig. 6A). The average recovery score in the G-CSF group 6 weeks after injury was 4.0 (3–6), indicating stepping and forward propulsive movements of 1 hindlimb without weight bearing, whereas the average recovery score in the control group was 2.9 (2–5), indicating obvious movements of 1 or more joints in both hindlimbs without coordination, alternate stepping movements, or weight-bearing (Fig. 6B). The highest recovery score in the G-CSF group was 6, indicating weight-bearing ability of hindlimbs with abnormal walking (external rotation of 1 or both limbs and/or hip instability) whereas the highest recovery score in the control group was 5, indicating alternate stepping and forward propulsive movement of the hindlimbs without weight bearing.

**DISCUSSION**

Our results from this study demonstrate that G-CSF prevented glutamate-induced neuronal death. Furthermore, administration of G-CSF prevented neuronal apoptosis during the acute phase after SCI, resulting in elevated neuronal survival in the spinal cord and improved hindlimb motor function 6 weeks after injury.

G-CSF regulates survival of postmitotic neutrophils by inhibition of apoptosis in the hematopoietic system with activation of the G-CSFR (11). Known signaling cascades downstream of G-CSFR are divided mainly into 2 classes, activation of the STAT family or the PI3K/Akt pathway. In hematopoietic lineage cells, G-CSF activates intracellular signaling pathways including STAT3 (12) and Akt (13), which are both linked to suppression of apoptosis and proliferation. The antiapoptotic function of STAT3 is mediated through its regulation of Bcl-2 expression (14). Akt promotes cell survival through multiple pathways, including the phosphorylation and inactivation of the proapoptotic protein Bad and through its induction of the antiapoptotic protein Bcl-2 (15–17). Recent reports have revealed that many basic cellular pathways are highly conserved between hematopoietic and neural cells. For instance, in addition to its expression in hematopoietic cells, G-CSF is also expressed in neurons in the cerebral cortex (5), by Purkinje cells in the cerebellum and cerebellar nuclei (6), and by neurons and glial cells in the corpus callosum (5). G-CSF exerts antiapoptotic effects in these neurons through activation of pro-survival pathways similar to those in hematopoietic cells (18).

In the present study, we have confirmed that G-CSFR is expressed by cerebellar granule neurons in vitro and are the first to show that G-CSF is also expressed by spinal cord neurons in vivo. Previous reports have shown that G-CSF treatment attenuates glutamate-induced neuronal death of CGNs (5), staurosporine-induced apoptosis of rat primary cortical neurons, and camptothecin-induced apoptosis of the human neuroblastoma cell line SHSY-5Y (6). Our results showed that simultaneous G-CSF administration had a partial neuroprotective effect against glutamate-induced neuronal death of CGNs. These results differ from those of Schäbitz et al (5), who reported that pretreatment with G-CSF completely suppressed glutamate-induced neuronal death of CGNs.
death in CGNs. This discrepancy may be due to the difference in the time of G-CSF administration. Furthermore, we showed that pretreatment with anti-G-CSFR antibody completely abolished G-CSF-mediated neuroprotection against glutamate-induced neuronal death of CGNs, suggesting that G-CSF exerts its neuroprotective effect on CGNs via binding to G-CSFR. Schneider et al (6) have demonstrated that the antiapoptotic effects of G-CSF in rat primary cortical neurons treated with staurosporine and the human neuroblastoma cell line SHSY-5Y treated with camptothecin occurs via activation of PI3K/Akt pathway and could be abolished by the specific PI3K/Akt inhibitor LY294002. They also showed that activation of STAT3 and upregulation of its antiapoptotic target Bcl-xL were induced by G-CSF treatment in rat primary cortical neurons (6). Komine-Kobayashi et al (7) reported that G-CSF-induced activation of STAT3 and its antiapoptotic target Bcl-2 resulted in inhibition of apoptotic neuronal death after transient focal cerebral ischemia in mice. In the present study, G-CSF suppression of glutamate-induced cell death of cultured CGNs occurred via the activation of both the JAK/STAT and PI3K/Akt pathways, which could be abolished by their specific inhibitors. Moreover, the present results show that G-CSF promotes the activation of STAT3 and upregulation of Bcl-2 in cultured CGNs. These lines of evidence suggest that the neuroprotective effect of G-CSF is mediated at least partially via the activation of the JAK/STAT and PI3K/Akt pathways as previously reported (18).

Our in vivo results indicate that G-CSF suppressed neuronal apoptosis during the acute phase and increased the number of surviving neurons after the chronic phase of SCI, suggesting that G-CSF is also neuroprotective in vivo. In addition to its direct effects on spinal cord neurons, G-CSF has several actions in vivo that may contribute to neuroprotection. G-CSF suppresses inflammatory cytokines in experimental allergic encephalitis in mice (19) and tumor necrosis factor-α expression in lipopolysaccharide-stimulated human monocytes in vitro (20). Therefore, G-CSF may exert neuroprotective effects by suppressing inflammatory cytokine expression during the acute phase of SCI. G-CSF also can promote mobilization of bone marrow-derived cells and their migration into injured tissues including ischemic brain tissue (7, 21). Recently, Urdzikova et al (22) reported that subacutely (7–11 days postinjury) administered G-CSF promotes functional recovery after SCI in rats via mobilization of bone marrow cells.

Moreover, G-CSF has several actions on the vascular system, and, in a model of stroke, G-CSF suppresses brain edema formation (23) and stimulates angiogenesis (24). Finally, G-CSF stimulates neurogenesis both directly (6) or via the upregulation of vascular endothelial growth factor (25). All of these mechanisms may contribute to the neuroprotective effects of G-CSF after SCI. Although this is the first report showing G-CSF-mediated neuroprotection that results in functional recovery, further exploration is needed to clarify the precise mechanism of action.

One of the major obstacles for conducting clinical trials for neuroprotective drugs is to first establish the safety and competency for use in human subjects. The complexity, size, and duration of clinical trials of novel drugs often make them quite costly to conduct and may impede the development of therapeutic agents that could have a significant impact in clinical practice. Therefore, although the efficacy of various drug therapies in models of SCI has been reported, few drugs have been practically carried into clinical trials. Thus, drugs with proven clinical exploitability have a significant advantage for clinical trials for novel therapeutic purposes. From this point of view, the correct use of G-CSF in the clinic for hematopoietic stimulation and its ongoing clinical trial for brain infarction (26) make it an appealing molecule that could be rapidly placed into trials for patients with acute SCI. Although many hurdles (e.g. optimal dosage, therapeutic time windows, and more precise mechanisms of action) still need to be resolved, the present results encourage us to pursue future clinical trials of G-CSF for patients with acute SCI.

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