Chapter 44
Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) Prevents Apoptosis and Improves Functional Outcome in Experimental Spinal Cord Contusion Injury

Yoon Ha, M.D., Ph.D., Hyune Seon Park, M.D., Chong Woon Park, M.D., Seung Hwan Yoon, M.D., Ph.D., So Ra Park, M.D., Ph.D., Dong Keun Hyun, M.D., Ph.D., Eun Young Kim, M.D., Ph.D., and Hyung Chun Park M.D., Ph.D.

ABSTRACT

Objective: Granulocyte macrophage colony stimulating factor (GM-CSF) is a potent hematopoietic cytokine, which stimulates stem cell proliferation in the bone marrow and inhibits apoptotic cell death in leukocytes. However, the effects of GM-CSF in the central nervous system are still unclear. The present study was undertaken to determine if GM-CSF can rescue neuronal cells from apoptosis and improve neurologic function in a spinal cord injury (SCI) model.

Methods: To study the effect of GM-CSF on apoptotic neuronal death, we used a staurosporine-induced neuronal death model in a Neuro 2A (N2A) cell line (in vitro) and in a rat SCI model (in vivo). N2A cells were preincubated with GM-CSF for 60 minutes before being exposed to staurosporine for 24 hours. To inhibit GM-CSF, we pretreated N2A cells with antibodies of the GM-CSF receptor for 60 minutes. SCI was made by clip compression. Rats were treated with daily GM-CSF (20 ƒÝg/d) for 5 days. The number of apoptotic cells in the spinal cord and neurologic improvements were checked.

Results: GM-CSF pretreatment was found to significantly protect N2A cells from apoptosis, and neutralizing antibodies for the GM-CSF receptors inhibited the rescuing effect of GM-CSF on apoptosis. In the rat SCI model, neurologic functions improved significantly in the GM-CSF¡Vadministered group versus the phosphate buffered saline (PBS)-treated control. TUNEL (terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeled) staining showed that GM-CSF administration reduced apoptosis in the injured spinal cord.

Conclusion: Treatment of SCI with GM-CSF showed some beneficial effects. Neuronal protection against apoptosis is viewed as a likely mechanism underlying the therapeutic effect of GM-CSF in SCI.

Traumatic SCI affects many people and can result in severe neurological damage. After SCI, the central region of the spinal cord may be critically damaged by the primary mechanical injury and show hemorrhagic necrosis, which expands with time because of the activation of secondary injury processes (23). Moreover, apoptosis may play an important role in these secondary injury processes after SCI (25). Apoptosis was noted around the injury site in a histological study of the spinal cords of patients that died after traumatic SCI (5). These findings are the basis of the therapeutic principle of neuronal apoptosis inhibition after SCI.

Hematopoietic and neural stem cells have many similarities at the transcriptional level during differentiation (20). Furthermore, some investigators suggest that stem cells in adult tissue have the ability to transdifferentiate into other cells (10). Vescovi et al.(24) reported that nestin-positive cells from adult subventricular zone neural stem cells can proliferate extensively in vitro to form clonal progeny that are capable of differentiating into neurons or blood cells

(24). Hematopoietic differentiation was achieved by grafting nestin-positive cells into the tail veins of partially irradiated mice. However, hematopoietic cells can adopt a neuronal fate (17, 18). These studies suggest that neuronal and hematopoietic systems use associated differentiation mechanisms.

In view of these similarities, it is perhaps not surprising that similar cytokines regulate the developments of both neuronal and hematopoietic systems. In addition, hematopoietic cytokines have been reported to control the activation, proliferation, differentiation, and survival of neural stem cells. The proliferation of early rat oligodendroglial progenitors in vitro is promoted by interleukin (IL)2 and IL4 and negatively regulated by transforming growth factor-b superfamily factors (3, 21). In terms of the neuronal lineage, IL5, IL7, IL9, and IL11 have been shown to enhance the elaboration of neuroblasts from a conditionally immortalized cell line derived from embryonic murine hippocampal progenitor cells (16). Thus, hematopoietic cytokines may participate in the differentiation of all three major cell types (neurons, oligodendrocytes, and astrocytes) in the developing brain.

GM-CSF is a well-known hematopoietic cytokine. GM-CSF was originally identified because of its ability to stimulate the differentiation and function of hematopoietic cells (1, 9). GM-CSF stimulates bone marrow stem cell proliferation and reduces leukocyte apoptosis, increasing the white blood cell number in the peripheral blood. Because of these hematopoietic stimulating effects, GM-CSF has been used as a therapeutic cytokine in patients suffering from diseases related to bone marrow suppression. However, it remains unclear whether GM-CSF stimulates differentiation and prevents apoptosis in the neuronal system.

To study the role of GM-CSF in neuronal protection, we hypothesized that GM-CSF would be able to rescue N2A cells from staurosporine-induced apoptosis and that it would improve neurologic functioning after SCI in a rat model.

MATERIALS AND METHODS

In Vitro Assay

Cell Culture and GM-CSF Treatment

The mouse neuroblastoma N2A cell line was originally obtained from the American Tissue Culture Collection. Cells were seeded in 24-well plates at a density of 3 ¡Ñ 104 cells/cm2 and grown in Dulbecco modified Eagle¡¦s medium (DMEM; Gibco Brl., Grand Island, NY) supplemented with 2 mM of L-glutamine, penicillin (20 units/mL), streptomycin (20 mg/mL), and 10% fetal bovine serum (FBS; Gibco Brl). Cells were maintained in a humidified atmosphere containing 5% CO2 at 37oC.

To induce apoptosis, staurosporine (Sigma, St Louis, MO) was treated for 24 hours, and to determine the optimal concentration of staurosporine required to induce apoptosis, we tested a range of concentrations (0, 0.5, 1, 1.5, and 2 ƒÝM) of staurosporine and compared apoptotic numbers in the different groups.

GM-CSF Treatment

Recombinant human GM-CSF was purchased from LG Chemical Co (Seoul, Korea). To identify the preventive effects of GM-CSF on neuronal apoptosis, we pretreated GM-CSF before administering staurosporine (1 ƒÝM) at

different concentrations (0, 50, 500, and 5000 ng/mL) for 6 hours.

Treatment with Neutralizing Antibodies Against the GM-CSF Receptor

After confirming the effects of GM-CSF on neuronal apoptosis, we treated N2A cells with neutralizing antibodies (Chemicon, Temecula, CA) to block GM-CSF interaction with its receptor. Neutralizing antibodies were administered at a concentration of 1 ƒÝg/mL for 6 hours before treating with GM-CSF (50 ng/mL for 6 h) and then staurosporine (1 ƒÝM for 24 h).

TUNEL Staining and Apoptosis Counting

The presence of apoptotic cells after staurosporine treatment was evaluated by TUNEL assay. Cells were fixed for 10 minutes with 4% paraformaldehyde at 4 ¢XC and permeabilized with 0.1% Triton X-100 (Sigma) and 0.1% sodium citrate (Sigma). Fluorescein-labeled UTP was added with TdT (Roche, Deutschland, Germany), and cells were incubated for 1 hour at 37 ¢XC, washed, and then analyzed by laser scanning confocal microscopy (LSCM) equipped with a Bio-Rad MRC 1024 (argon and krypton) laser scanning confocal imaging system mounted on a NIKON microscope (Bio-Rad, Hercules, CA).

Animal Model of Spinal Cord Injury

A contusion injury was induced by applying vascular clip to the spinal cord for 10 minutes. Adult male Sprague-Dawley rats (300¡V350 g of body weight) were anesthetized intraperitoneally (i.p.) with ketamine, and a laminectomy was performed at the T8-10 level. To study the effect of GM-CSF on apoptosis after spinal cord injury, three groups (n = 5 sham control, n = 5 PBS control, and n = 5 GM-CSF) were divided. To study the behavior changes after GM-CSF treatment, three groups (n = 5 sham control, n = 25 PBS control, and n = 20 GM-CSF) were divided.

An opening was made that was large enough to accommodate the clip head. Immediate after the laminectomy, the exposed spinal cord was compressed with a vascular clip (a pressure of 50g; Aesculap, Melsungen, Germany) for 10 minutes. After surgery, the muscles and skin were sutured. The sham-operated control group was made by laminectomy alone without making spinal cord injury.

GM-CSF Administration and Behavioral Testing

The GM-CSF group (n = 20) received recombinant human GM-CSF (20 ƒÝg/d i.p.) daily for 5 days from 1 day after injury. The PBS control group (n = 25) received subcutaneous injections of PBS (pH 7.4) daily for 5 days from 1 day after injury.

Behavioral testing was performed on each hind limb weekly using the Basso-Beattie-Bresnahan (BBB) locomotor rating system. Behavioral studies were performed for 5 weeks by two independent observers in a double blind manner.

For TUNEL staining, rats from the experimental (n = 5), PBS control (n = 5), and sham control (n = 5) groups were killed under urethane anesthesia 5 days after the injury, and the spinal cords were removed. Portions of the spinal

cords were processed for paraffin embedding after being fixed in 4% paraformaldehyde in PBS.

Immunohistochemical Staining for the GM-CSF Receptor

For immunologic studies, deparaffinized spinal cord sections were boiled in citrate buffer (pH 6) for 10 minutes in a microwave oven. After blocking in normal serum, sections were incubated with monoclonal antibodies against the GM-CSF receptor (1:100 in PBS; Chemicon, Temecula, CA) for 1 hour at room temperature. The slides were then washed extensively in 20 mM of PBS. Biotinylated secondary antibodies were incubated on slides for 10 min at 37oC. After extensive washing in 20 mM of PBS, the slides were incubated in streptavidin conjugated to horseradish peroxidase in Tris-HCl buffer for 10 minutes. Prepared substrate-chromogen solution using diaminobenzidine substrate kit (Vector Laboratories, Burlingame, CA) was applied for 5 minutes, and the slides were counterstained in Meyer's hematoxylin for 1 minute. All slides were examined under a light microscope (Olympus, Tokyo, Japan).

To identify cells co-expressing GM-CSF receptors with neuronal marker and glial fibrillary acidic protein (neurofilament and GFAP), we used fluorescence immunohistochemical staining. After blocking in 10% fetal bovine serum in PBS, sections were placed in a medium containing antineurofilament (mAb; dilution, 1:100; Dako, Carpenteria, CA) or GFAP (mAb; concentration, 1:100; Sigma) antibodies overnight at 4¢XC. On the following day, the sections were rinsed and incubated in a solution containing secondary antibody to mouse antibodies conjugated with Texas red (anti-mouse poly-Ab; 1:200; Vector). Subsequently, sections were treated with GM-CSF antibody labeled with fluorescein isothiocyanate (FITC; Vector) for 1 hour at room temperature. Sections were then washed, mounted, and examined under a LSCM equipped with a Bio-Rad MRC 1024 (argon and krypton) laser scanning confocal imaging system mounted on a NIKON microscope (Bio-Rad). For immunofluorescence, double-labeled green (FITC for anti¡VGM-CSF receptors) and red (Texas red for neurofilament or GFAP) fluorochromes on the sections were excited using laser beams at 488 and 647 nm; emissions were sequentially acquired using two separate photomultiplier tubes through 520- and 680-nm emission filters, respectively.

TUNEL Staining for SCI

The presence of apoptotic cells after SCI was evaluated by the TUNEL method, as described above. All nuclei were stained blue using 4'6' diamidino-2-phenylindole (DAPI; 1 ƒÝg/mL; Sigma). Blue (nuclei) and green (TUNEL) fluorescences were analyzed by LSCM using a Bio-Rad MRC 1024 laser scanning confocal imaging system mounted onto a NIKON microscope.

For immunofluorescence, double-labeled green (TUNEL) and blue (nuclei) fluorochromes on sections were excited using laser beams at 488 and 405 nm; emissions were sequentially acquired using two separate photomultiplier tubes using 520- and 460-nm emission filters, respectively.

Statistical Analysis

Inter-group comparisons of the effect of staurosporine on N2A cell apoptosis, the neuronal protective effect of GM-CSF on N2A cells, the effects of adding blocking antibodies against the GM-CSF receptor, and the number of apoptotic cells in the spinal cord were made using the one-way analysis of variance. Differences in hind limb function, based on the BBB scores, were assessed by Kruskal-Wallis nonparametric test. Significance was accepted for P

values < 0.05. Values are presented as mean ¡Ó SE or SD.

RESULTS

When N2A cells were cultured in the presence of various concentrations of staurosporine, the number of apoptotic cells increased significantly when staurosporine was added in the range of 1.0 to 2.0 ƒÝM versus 0.5 ƒÝM. Maximal responses were observed at 1.0 and 1.5 ƒÝM (Fig. 44.1).

Before inducing apoptosis with staurosporine (1 ƒÝM), N2A cells were pretreated with GM-CSF at various concentrations for 6 hours. The number of apoptotic cells reduced significantly when the GM-CSF was added at 5, 50, 500, and 5000 ng/mL, respectively (Fig. 44.2).

Given the observations above, we hypothesized that N2A cells express GM-CSF receptors, through which cytokines stimulate the signaling pathways that mediate their trophic effects. To test this hypothesis, we pretreated cells with neutralizing antibodies for the GM-CSF receptor £\ subunit to determine whether the GM-CSF receptor is involved in cell survival. The number of apoptotic cells increased significantly after neutralizing antibody treatment for 6 hours (Fig. 44.3).

Before we examined the effect of GM-CSF on SCI, an immunocytochemical study was conducted to determine whether GM-CSF receptors are present in spinal cord tissue. GM-CSF receptor expressing cells were found to be distributed throughout the spinal cord, especially in gray matter (Fig. 44.4). Figures 4, B to D show that the cells bearing GM-CSF receptors were mainly neurons. However, no discernable GM-CSF receptor expression was found on glial cells (Fig. 44.4D).

The distribution of apoptotic cells 5 days after injury at three spinal cord regions (caudally and rostrally 5 mm from injury epicenter and injury epicenter) in the PBS controls, the GM-CSF, and the sham-control groups are shown (Fig. 44.5).<44.5> Apoptotic cells were hardly detected in the sham controls at any of the three regions, and this group showed no significant differences in the incidences of apoptotic cells at the three locations. At 5 days after injury, the number of apoptotic cells increased at all three locations in the GM-CSF group and in the PBS controls versus the sham controls. Significant reductions in the numbers of apoptotic cells by GM-CSF were observed at the injury epicenter ($P < 0.01$) and 5 mm caudally of the injury center ($P < 0.05$) versus the PBS controls. Figure 44.5A shows a typical example of TUNEL staining in the SCI PBS controls at 5 days after SCI.

Fifty rats (25 PBS controls, 20 GM-CSF, and 5 sham control rats) were tested using the BBB scoring system at Weeks 1, 2, 3, 4, and 5. The BBB locomotor rating scores of these two groups at Weeks 1, 2, 3, 4, and 5 are shown (Fig. 44.6). The maximum possible BBB normal score was 21, and all PBS control and GM-CSF treated rats were allocated a maximum score before spinal cord injury and a score of 0, i.e., complete paralysis of the hind limb after contusion. In the PBS control rats, scores improved from 5.7 at 1 week to 12.3 at 3 weeks and 15.9 at 5 weeks. Rats in the GM-CSF group showed a significant score improvement to 9.3 at 1 week to 16.3 at 3 weeks and to 17.9 at 5 weeks ($P < 0.01$ for overall treatment effect).

DISCUSSION

The results of the present study demonstrate that GM-CSF is neuroprotective in vitro and that GM-CSF administration showed a significant functional improvement effect after SCI. Moreover, neurons at the periphery of the injury and at the epicenter exhibited specific anti-GM-CSF¡Vreceptor binding.

The dose of the i.p. delivered GM-CSF (20 ƒÝg/d for 5 d) used in the present study was based on the results of our in vitro experiments and was comparable to the approved doses used to treat of human hematologic diseases.

Recombinant human GM-CSF is one of the few cytokines used clinically. GM-CSF plays a vital role in hematopoiesis by inducing the growth of several different cell lineages. It also enhances the numerous functional activities of mature effector cells involved in antigen presentation and cell-mediated immunity, including neutrophils, monocytes, macrophages, and dendritic cells (9). However, the effects of GM-CSF on neuronal systems have not been well studied. Moreover, there are no therapeutic applications for GM-CSF in neurologic diseases.

We found that when N2A cells were cultured in the presence of physiological concentrations of GM-CSF for 6 hours, the number of apoptotic cells was reduced. This finding supports the idea that GM-CSF has a preventive role in neuronal death. Some researchers have found that hematopoietic cytokines, including GM-CSF, directly influence neuronal cells and enhance cell survival (3, 15, 16).

GM-CSF also increased neurite outgrowth in undifferentiated murine sympathetic ganglion cells (8). Furthermore, IL3 and GM¡VCSF support functional maturation by increasing the expressions of glutamic acid decarboxylase and gamma-aminobutyric acid in embryonic murine septal neuroblasts (6, 7, 22). These findings issue the studies for effect of GM-CSF on differentiation and neurite outgrowing of immature neural stem cells and their significance to the repair of injured central nervous system.

After observing the effects of GM-CSF on N2A cells, we hypothesized that the biologic effects of GM-CSF are mediated by its binding to receptors expressed on the surfaces of target cells. Neutralizing antibodies that inhibit interactions between GM-CSF and its receptor were thus treated to test this hypothesis. The number of apoptotic cells increased significantly after this treatment with neutralizing antibodies for 6 hours. This result shows that the activity of GM-CSF on the inhibition of apoptosis can be blocked by its corresponding neutralizing antibodies. Interestingly, we found that GM-CSF receptors were expressed in spinal cord tissue. These findings advocate the use of GM-CSF in neurologic diseases involving the spinal cord.

The main means used by hematologic cells to avoid apoptosis is believed to be mediated by binding to the high-affinity GM-CSF receptors (1). Interaction with this receptor activates the Janus family kinases (JAKs) and STATs. The JAKs are nonreceptor type tyrosine protein kinases that become activated on ligand-induced receptor dimerization. The GM-CSF¡Vinduced activation of JAK phosphorylates STAT on a conserved tyrosine residue, which induces STAT dimerization. Furthermore, STAT translocates to the nucleus and subsequently regulates gene expression. However, the signal transduction pathways of neuronal systems are still under evaluation. Kannan et al. (8) showed that the IL3 and GM-CSF receptor subunits are present on almost all neuronal cell bodies and on some neurites of nerve growth factor-differentiated sympathetic ganglion neurons. They suggested that IL3 and GM-CSF exert neurotrophic effects on sympathetic neurons, via specific receptors and through the activation of the mean arterial blood pressure kinase pathway, which then mediates neurite outgrowth and differentiation. However, relatively few have studied the signaling pathways related to neuronal survival and differentiation. Further studies are

required to determine whether the induction of c-myc and the activation of DNA replication are associated with neuronal proliferation and differentiation.

In the present study, we examined the role of GM-CSF in SCI. However, our in vitro study shows that GM-CSF has a protective effect on immature neural cells, and expression of GM-CSFR on mature neural cells in the spinal cord suggest that GM-CSF could protect the spinal cord neuron from trauma and improve neurologic functions. GM-CSF treatment administered i.p. for 5 days after a contusion injury was found to significantly improve functional outcomes, as determined by the BBB test. Moreover, this functional improvement persisted for up to 4 weeks. However, the precise role of GM-CSF in functional improvement remains unclear. To test the hypothesis that GM-CSF enhances survival from apoptotic cell death, as we had found in vitro, we compared the number of apoptotic cells in a GM-CSF¡Vtreated rat spinal cord with numbers in the PBS control group and found that the number of apoptotic cells in the spinal cord tissue of the GM-CSF¡Vtreated rats was much reduced. Although the number of apoptotic cells was found to have reduced at three locations (injury epicenter and 5 mm rostral and caudal from the epicenter), we were not able to identify the cell types sensitive to the apoptotic-rescuing effect of GM-CSF, because inflammatory cell infiltration and astrocyte migration increased the cell number and altered the cell type distribution at the injury area. In this study, we could not differentiate hematologic cell apoptosis from neuroglial cell apoptosis, especially in the epicenter. Further studies identifying the distributions of apoptotic cell populations and changes in their profiles by cell type are required after GM-CSF treatment.

Bone marrow stem cell research on neuronal injury models is providing evidence that some transplanted bone marrow cells differentiate to mature neurons and that these improve neurologic functions (11). Some reports show that the intravenous administration of bone marrow cells improves outcomes and that bone marrow cells migrate into the injury site (12¡V14). These findings raise the idea that bone marrow mobilization therapy by using GM-CSF or G-CSF could improve neurologic outcomes. Corti et al. (4) showed the expansion and mobilization of circulating bone marrow stem cells by in vivo treatment with G-CSF and that stem cell factor increases the number of bone marrow¡Vderived neuronal cells in the mouse brain. These findings support the hypothesis that in vivo mobilization therapy could improve neurologic functions in SCI. However, in the present experiment, we could not examine the fate of mobilized bone marrow stem cells in the injury site. Although, the mobilized bone marrow stem cell effect was not identified in the present study, we conclude that GM-CSF treatment has a direct neuroprotective effect, and that this may explain the neurologic improvement in SCI after treatment.

The immunomodulatory effect of GM-CSF could provide a means of improving neurologic function by promoting inflammatory cell recruitment and by increasing the rate of myelin phagocytosis (2, 19). GM-CSF is transiently upregulated immediately after spinal cord and peripheral nerve injury, and its effects are believed to lead to myelin clearance. Moreover, myelin clearance and the degree and type of inflammatory response may be critical in determining not only the amount of regeneration but also the amount of cell death that occurs after injury. Whether motor improvement is because of a direct neuroprotective effect, stimulating endogenous neural precursor cells, bone marrow cell mobilization, or immunomodulation remains to be determined.

CONCLUSION

This study shows that GM-CSF enhances neuronal survival from apoptosis and reduces functional deficit after spinal cord contusion injury. Our findings suggest that GM-CSF treatment merits further investigation as a potential

therapeutic intervention after SCI.

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Fig. 44.1 The effect of staurosporine on N2A cell apoptosis. N2A cells were treated with staurosporine at different concentrations (1, 0.5, 1.0, 1.5, and 2.0 μM) for 24 hours. Cells treated at larger concentrations showed increased levels of apoptosis. However, no significant differences in apoptotic ratio was observed in groups treated with staurosporine at >1 μM. The number of N2A cells undergoing apoptosis was determined by counting cells at the centers of wells under a confocal microscope and expressing observations as percentages of apoptotic cells on total examined cell count in five high-power fields. Results represent mean ± SE. **P < 0.01.

FIG. 44.2 Effects of GM-CSF on staurosporine-induced apoptosis. N2A cells were cultured in the absence or presence of 5, 50, 500, or 5000 ng/mL of GM-CSF for 6 hours before inducing apoptosis (staurosporine 1.0 μM). N2A cell apoptotic inhibition was found to be dose-dependently related to GM-CSF. One-way analysis of variance shows significant differences between these concentration groups. *$P < 0.05$; **$P < 0.01$.

FIG. 44.3 Inhibition of the protective effect of GM-CSF on N2A cells achieved by adding antibodies against the GM-CSF receptor. N2A cells were cultured in the presence of 1 μg/mL of antibodies for 6 hours before treating GM-CSF (50 ng/mL). After adding staurosporine (1.0 μM), the numbers of apoptotic cells were expressed calculated by the average percentages of apoptotic cells in a well. Non-paired Student *t* test shows a significant difference between the groups. **$P < 0.01$.

FIG. 44.4 Detection of GM-CSF receptor á subunits in the rat spinal cord by immunohistochemical staining. *A*, GM-CSF receptor expressing cells are distributed throughout the spinal cord, especially in gray matter (*dotted lines show the border between gray and white matter*). Scale bar = 100 μm. *B*, Confocal photomicrographs showing cells immunoreactive to anti-GM-CSF receptor antibodies with a neuronal morphology (*arrows indicate GM-CSF receptor expressing cells morphologically similar to the neurons*). *C*, Cells expressing GM-CSF receptors are also immunoreactive to neuronal marker (neurofilament) (*arrow indicates cells co-expressing NF and GM-CSF receptors*). *D*, Cells expressing GM-CSF receptors but not expressing glial cell marker (GFAP) on the cytoplasm (*arrow indicates cells expressing GM-CSF receptors only; asterisk shows a GFAP expressing astrocyte*). Scale bar = 20 μm (*B–D)*.

FIG. 44.5 The number of apoptotic cells 5 days after injury. *A*, Confocal microscopic findings of the spinal cord after TUNEL staining showing the injury epicenter. *Arrow* indicates TUNEL positive cells (*bright green spots*) in the injury epicenter of PBS control rat spinal cord. All nuclei were stained blue with 4'6' diamidino-2-phenylindole. Scale bar = 40 μm). *B*, The number of apoptotic cells 5 days after injury at three locations (injury epicenter and 5 mm rostrally and caudally from the injury center). Apoptotic cells were barely detected in the sham-control group in all three regions. In addition, this group showed no significant differences in the incidences of apoptotic cells at the three locations. At 5 days after injury, in both the GM-CSF– and the PBS-treated groups, the numbers of apoptotic cells increased at all three locations versus the sham control. GM-CSF significantly reduced apoptotic cells at the injury epicenter ($P < 0.01$) and 5 mm caudally from the injury center ($P < 0.05$) versus the PBS-treated group.

FIG. 44.6 Analysis of locomotor recovery as determined by the BBB locomotor rating system. Rats treated with GM-CSF show significantly improved locomotor recovery versus rats in the PBS-control group. *$P < 0.05$; **$P < 0.01$. Data represent mean ± SD. Significant differences were analyzed on Days 3, 7, 15, 21, 28, and 35.