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Potentiation of angiogenesis and regeneration by G-CSF after sciatic nerve crush injury

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ABSTRACT

Granulocyte colony-stimulating factor (G-CSF) demonstrates neuroprotective effects through different mechanisms, including mobilization of bone marrow cells. However, the influence of G-CSF-mediated mobilization of bone marrow-derived cells on injured sciatic nerves remains to be elucidated. The administration of G-CSF promoted a short-term functional recovery 7 days after crush injury in sciatic nerves. A double-immunofluorescence study using green fluorescent protein-chimeric mice revealed that bone marrow-derived CD34+ cells were predominantly mobilized and migrated into injured nerves after G-CSF treatment. G-CSF-mediated beneficial effects against sciatic nerve injury were associated with increased CD34+ cell deposition, vascular endothelial growth factor (VEGF) expression, and vascularization/angiogenesis as well as decreased CD68+ cell accumulation. However, cell differentiation and VEGF expression were not demonstrated in deposited cells. The results suggest that the promotion of short-term functional recovery in sciatic nerve crush injury by G-CSF involves a paracrine modulatory effect and a bone marrow-derived CD34+ cell mobilizing effect.

Introduction

The peripheral nerve system (PNS) possesses intrinsic potential for functional regeneration after injury. However, in spite of the endogenous regenerative capacity of the PNS, in many cases the regeneration is insufficient and recovery of function is incomplete. Recently, cell transplantation has become the focus of clinical research. Cell therapy has been shown to exert beneficial effects on peripheral nerve regeneration. Cell supplement, trophic factor secretion, extracellular matrix molecule synthesis and guidance, remyelination, microenvironment stabilization, and immune response modulation have recently been proposed as beneficial mechanisms after cell transplantation [1–7]. Among the applied cell types, bone marrow-derived cells, which contain hematopoietic, mesenchymal, and other types of cells, are candidates for cell therapy against peripheral nerve injury. Indeed, transplantation of whole bone marrow cells, bone marrow-derived hematopoietic stem cells, bone marrow-derived mesenchymal stem cells, or bone marrow-derived endothelial progenitor cells has been demonstrated to promote functional recovery in injured peripheral nerves [4–7]. These lines of evidence show the potential of bone marrow-derived cell transplantation to restore injured peripheral nerves and to promote functional recovery.

Granulocyte colony-stimulating factor (G-CSF) is widely known as a cytokine that induces survival, proliferation and differentiation of cells of hematopoietic lineage [8]. Furthermore, G-CSF can mobilize bone marrow cells into peripheral blood circulation, an action used clinically for patients with leukocytopenia and for donors of peripheral blood-derived hematopoietic stem cells for transplantation [9]. However, growing evidence has suggested that G-CSF also has important non-hematopoietic functions in other tissues including nerve tissues. Recently, a series of clinical and experimental studies have demonstrated the beneficial effects of G-CSF in several neurological diseases, including cerebral ischemia, spinal cord injury, Parkinson's disease, and peripheral nerve injury. It is proposed that G-CSF exerts beneficial effects through different mechanisms, including mobilization of bone marrow cells, anti-apoptosis, anti-inflammation, neuronal differentiation, and angiogenesis [2,3,10–15].

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Recently, we demonstrated that treatment with G-CSF promoted functional recovery in spinal cord injury and peripheral nerve injury, and concomitant treatment with G-CSF and stem cells augmented regeneration involving several beneficial actions including anti-apoptosis, anti-inflammation, and promotion of cell proliferation [2,3]. Bone marrow-derived cells mobilized by G-CSF may have the potential to migrate into and repair various injured tissues. The strategies of mobilized bone marrow-derived cells were applied and beneficial effects were demonstrated in ischemic myocardium, ischemic brain, and spinal cord injury [13,15,16]. However, the influence of G-CSF-mediated mobilization of bone marrow-derived cells on injured sciatic nerves remains to be elucidated. In green fluorescent protein (GFP) chimeric mice that underwent crush injury to the sciatic nerves, the results showed that G-CSF promoted the mobilization and migration of bone marrow cells into the injured nerves and the beneficial effects were associated with CD34+ cell deposition and increased vascularization/angiogenesis.

Material and methods

Bone marrow transplantation. Bone marrow cells were collected from 8- to 12-week-old male GFP transgenic mice (GFP-Tg) euthanized with pentobarbital. A total of 6×10^6 bone marrow cells derived from GFP-Tg mice were transplanted intravenously via the tail vein of lethally irradiated (800 cGy) male FVB/N mice [13]. In total, 24 animals received transplantation. Four animals were sacrificed 4 weeks after bone marrow transplantation and were used to evaluate the chimerism. The remaining 20 animals were subjected to further surgery.

Sciatic nerve crush injury and G-CSF treatment. Male FVB/N mice (25–30 g) were used in this study; permission was obtained from the Ethics Committee of Taichung Veterans General Hospital. Four weeks after transplantation, surgery and G-CSF treatment were performed. The mice were anesthetized with 2% isoflurane in induction followed by a maintenance dose (0.5–1%). The left sciatic nerve was exposed under a microscope using the gluteal muscle splitting method. A vessel clamp was applied 10 mm from the internal obturator canal for 20 min [3]. The animals were categorized into two groups: group I (crush, $n = 10$), the mice received one intra-peritoneal injection of normal saline per day for 5 consecutive days; group II (crush + G-CSF, $n = 10$), the mice were concomitantly injected with G-CSF (50 μg/kg) intra-peritoneally for 5 consecutive days. Normal mice which did not receive bone marrow transplantation were also subjected to the same surgery and G-CSF treatment ($n = 5$). All animals were sacrificed for examination 7 days after surgery.

Assessment of functional recovery. To evaluate sciatic nerve function, several measurements were taken from the red ink footprint [3]: (i) distance from the heel to the third toe, the print length; (ii) distance from the first to the fifth toe, the toe spread (TS); and (iii) distance from the second to the fourth toe, the intermediary toe spread (ITS). All three measurements were taken from the experimental (E) and normal (N) sides. The sciatic functional index (SFI) was calculated according to the equation: $SFI = -38.3(EPL - NPL/NPL) + 109.5(ETS - NTS/NTS) + 13.3(EIT - NIT/NIT) - 8.8$. The SFI oscillates around 0 for normal nerve function, whereas SFI around −100 represents total dysfunction.

Electrophysiological study. The sciatic nerves from individual groups were exposed. Electric stimulation was applied to the proximal side of the injured site; the conduction latency, and the compound muscle action potential (CMAP) were recorded with an active electrode needle 5 mm below the tibia tubercle and a reference needle 15 mm from the active electrode. The stimulation intensity and filtration ranges were 5 mA and 20–2000 Hz, respectively. The CMAP data and conduction latency were converted to ratios of the injured side divided by the normal side to adjust for the effect of anesthesia [3].

Immunohistochemistry. Serial 8 μm-thick sections of sciatic nerve were cut on a cryostat and mounted on superfrost/plus slides and were subjected to immunohistochemistry with antibodies against CD68, CD34, von-Willebrand factor (vWF), vascular endothelial growth factor (VEGF), and vimentin. The cell nuclei were stained with Dapi. The immunoreactive signals were observed by goat anti-mouse IgG (FITC) or anti-mouse IgG (Rhodamine). Among longitudinal consecutive resections, five consecutive resections contiguous to a maximum diameter were chosen to be measured. Of 100 squares with a surface area of 0.01 mm^2 each, 20 were randomly selected in an ocular grid and used to count the number of immunoreactive cells. The quantitative results are expressed as cell counts/0.05 mm^2. Immunohistochemical staining for isolectin B4 as an endothelial cell marker was visualized with fluorescence. For the determination of vWF, VEGF, and isolectin B4 reactivity, areas of activities (0.2 mm^2) from five consecutive resections appeared as density against the background and were measured by a computer image analysis system [3].

Statistical analysis. Data are expressed as means ± standard deviation. The statistical significance of differences between groups was determined by one way analysis of variance (ANOVA) followed by Dunnett's test. In SFI, the results were analyzed by repeated-measurement of ANOVA followed by the multiple comparison method of Bonferroni. A p value less than 0.05 was considered significance.

Results and discussion

Twenty-four mice tolerated the irradiation and bone marrow transplantation. There was no mortality associated with irradiation or transplantation. The FACS analysis showed that 87.1 ± 8.3% of the whole bone marrow cells were positive for GFP 4 weeks after bone marrow transplantation, indicating that GFP-Tg-derived bone marrow cells survived and reconstituted hematopoiesis in grafted mice. Further analysis revealed that CD34+ cells, an endothelial/hematopoietic progenitor-enriched cell population [1], constituted 9.1 ± 1.3% of GFP+ cells. After treatment with G-CSF for 5 days, white blood cell count was elevated significantly (8525 ± 1278 counts/mm^3) in the treated group when compared to the non-treated group (4750 ± 1500 counts/mm^3). These results suggest that G-CSF has a mobilization effect in bone marrow-transplanted mice.

To elicit the effect of G-CSF on peripheral nerve regeneration, irradiated/bone marrow-transplanted mice were subjected to crush injury in the sciatic nerve. Crush injury in the sciatic nerve caused deficits in neurobehavior and nerve electrophysiology. The escalation of the sciatic nerve functional index (SFI) in the G-CSF-treated group primarily implicated the improvement of peripheral nerve regeneration (Fig. 1A). Studies suggest that the amplitude of CMAP reflects the number of axons reinnervating the muscle and is related to the amount of acetylcholine release, and the nerve conduction latency is reciprocal to motor function improvement [17,18]. Electrophysiological recordings demonstrated an improvement in nerve conduction latency (Fig. 1B) and CMAP (Fig. 1C), indicating an increased recovery of nerve function after G-CSF treatment. The results suggest that G-CSF administration provokes the improvement of neurobehavior in animals with crush injury in sciatic nerves.

To elicit the potential beneficial mechanisms of G-CSF against crush injury in the sciatic nerve involved, the mobilization and deposition of bone marrow cells was analyzed (Fig. 2). No fluorescent signal was detected in sciatic nerves obtained from normal

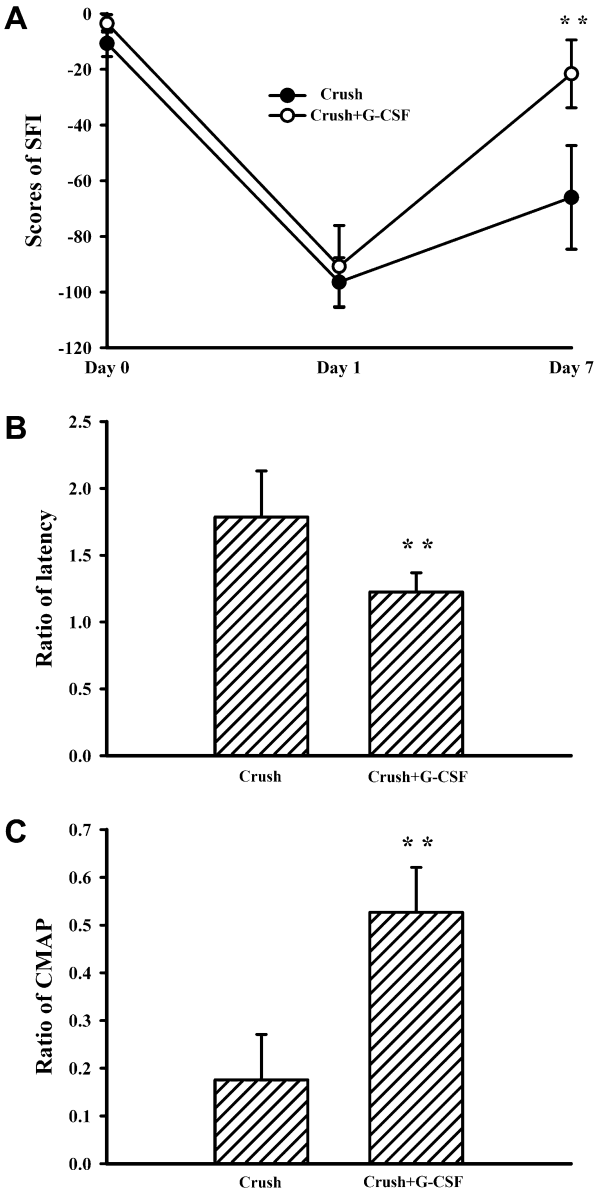

Fig. 1. Evaluation of neurobehavior. The score of SFI was recorded before injury (Day 0), and 1 day (Day 1) and 7 days (Day 7) after injury (A). The ratio of nerve conduction latency (B) and CMAP (C) was measured 7 days after injury. $^{**}p < 0.01$ vs. crush, $n = 10$.

mice. A scattering of GFP+ cells was detected in the non-injured nerves (0.7 ± 0.5). However, the deposition of GFP+ cells into non-injured nerves was not significantly altered by G-CSF (1.1 ± 0.6). Crush injury caused the deposition of GFP+ cells into the injured sites (15.4 ± 4.1). The elevation of GFP+ cells deposition was detected in the G-CSF-treated group (38.3 ± 5.2). That is, the improvement in peripheral nerve regeneration by G-CSF was accompanied by increased bone marrow cell deposition.

It has been demonstrated that G-CSF-mobilized bone marrow cells include hematopoietic stem cells, mesenchymal stem cells, and even mature immune cells [13,15,16]. To determine the involved cell population in G-CSF-mediated mobilization and deposition of bone marrow cells, immunohistochemistry was performed. Micrographs of double-immunofluorescence study for GFP and cell lineage-specific markers in injured nerves are shown in Supplementary Fig. 1. The quantitative results (Supplementary Fig. 1D) showed that G-CSF mobilized GFP+ cells into the injured nerve from 15.4 ± 4.1 to 38.3 ± 5.2 cell counts. To investigate the alteration of hematopoietic stem cells, the deposition of CD34+ and CD34+/GFP+ cells was counted. The number of CD34+ cells was 13.8 ± 4.1 in the injured group, and the number increased to 36.3 ± 4.9 after treatment with G-CSF. The number of CD34+/GFP+ cells in the crush and crush + G-CSF groups was 13.7 ± 5.2 and 36.0 ± 5.8, respectively. The deposition of mesenchymal stem cell lineage was increased by G-CSF, as evidenced by the elevation of the number of vimentin+ cells from 84 ± 21 to 298 ± 81. However, G-CSF had little effect on vimentin+/GFP+ cell deposition, which showed only a slight increase from 1.5 ± 0.6 to 1.8 ± 1.3. As in our previous study in a rat model of peripheral nerve injury [3], G-CSF decreased CD68+ monocyte/macrophage deposition in the injured nerves (from 34.3 ± 5.9 to 14.8 ± 6.5). Intriguingly, the deposition of CD68+/GFP+ cells in injured nerves was not altered by G-CSF, 3.8 ± 1.6 and 4.5 ± 2.2, respectively. These findings suggest that the most prominent bone marrow cells mobilized by G-CSF into crush-injured sciatic nerves are CD34+ hematopoietic lineage cells.

In addition to being a hematopoietic lineage marker, CD34+ cells also represent an endothelial progenitor-enriched cell population contributing to neovasculogenesis/angiogenesis [1]. Toth et al. [15] reported that G-CSF and stem cell factor increased bone marrow-derived endothelial cells and promoted angiogenesis in the ischemic brain. To determine the potential association between G-CSF treatment, CD34+ cell deposition, and angiogenesis in crushed sciatic nerves, the vessel density was measured. In non-irradiated/non-bone marrow-transplanted mice, G-CSF administration also increased CD34+ cell deposition into sciatic nerves after crush injury, 14.5 ± 5.6 to 38.5 ± 5.5 (Supplementary Fig. 2A). Crush injury in sciatic nerves disrupted the vascular integrity as evidenced by the decreased reactivity to endothelial- and vessel-related vWF (16.8 ± 3.4 to 10.5 ± 1.5) and isolectin B4 (17.8 ± 2.4 to 9.3 ± 1.2) staining (Supplementary Fig. 2B and C). G-CSF increased the reactivity to vWF (26.5 ± 4.4) and isolectin B4 (29.0 ± 5.4) in the injured nerves (Supplementary Fig. 2B and C), indicating an elevation of vessel density. These results demonstrate a potential association between vascularization/angiogenesis and deposition of CD34+ cells in the injured nerves after G-CSF treatment. The vascularization/angiogenesis provoked by G-CSF in the injured nerves was also demonstrated in irradiated/bone marrow-transplanted mice (Fig. 3). The relative density of vWF immunoreactivity increased from 21.4 ± 4.1 to 43.6 ± 6.2 after G-CSF treatment (Fig. 3A). Besides, G-CSF increased VEGF expression (53.2 ± 9.1 to 101 ± 15) in the injured nerves (Fig. 3B). However, the double-immunofluorescence study revealed that the co-localization of GFP and vWF or GFP and VEGF was rare (Fig. 3).

The G-CSF-mediated mobilization and migration of bone marrow cells include bone marrow-derived neutrophils, monocytes, mesenchymal, and endothelial precursors that might have distinct and additive therapeutic potentials [13,15,16]. Many studies that investigated a possible therapeutic role of these cells used a specific subpopulation. However, no consensus has been reached regarding the type of bone marrow cell subpopulation which is most effective for cell therapy [4–7]. The current study showed that nerve injury attracted bone marrow cell deposition and this deposition was augmented by G-CSF. In contrast, the integration of bone marrow-derived cells into non-injured sciatic nerves was scattered and the deposition was not altered by G-CSF (Fig. 2). Although the mobilization and migration of different bone marrow cell subpopulations by G-CSF were reported, our double-immunofluorescence study showed that CD34+ cells were the most prominent bone marrow-derived cells mobilized and deposited into the crush-injured sciatic nerves after G-CSF treatment (Supplementary Fig. 1). G-CSF attenuated injury-induced CD68+ cells but increased vimentin+ cell deposition at the injured nerves. Intriguingly, the

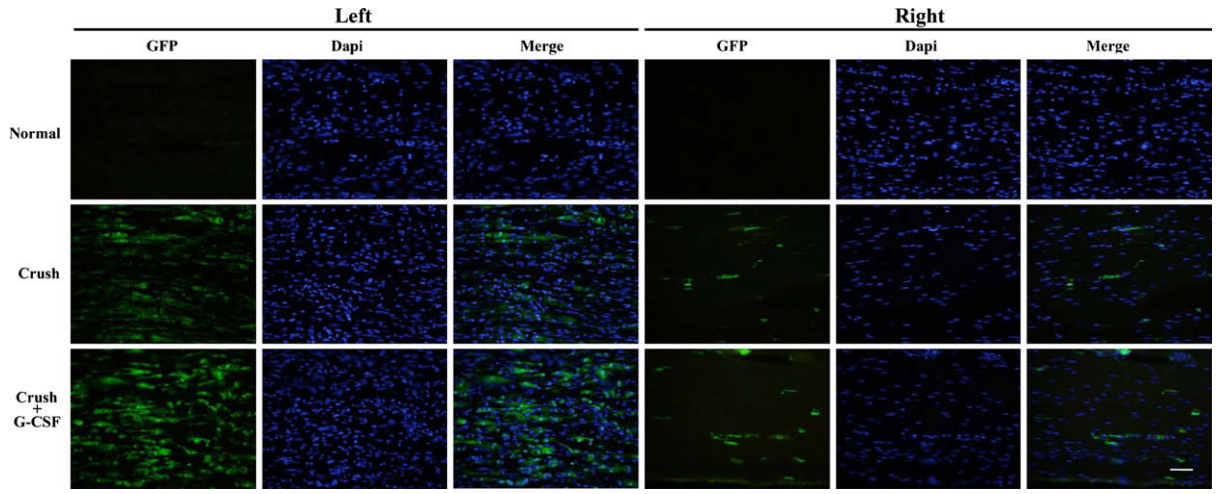

Fig. 2. Examination of GFP positive cells in sciatic nerves. After sacrifice, the left and right sciatic nerves were isolated and the longitudinal sections were visualized with fluorescence under a fluorescent microscope. The left and right sciatic nerves obtained from normal mice (Normal) were examined as a control. A representative micrograph is shown (n = 5). Bar length = 50 μm.

changes in CD68+/GFP+ monocytes and vimentin+/GFP+ mesenchymal cells after injury were hardly detectable, indicating a reduction of CD68+/GFP− cells and an elevation of vimentin+/GFP− cells (Supplementary Fig. 1). These observations suggest that G-CSF might have modulatory effects on long-lasting circulating and parenchymal resident CD68+ and vimentin+ cells. The reduction of CD68+/GFP− cell deposition by G-CSF reflected its anti-inflammatory effect and implied its modulatory effect is exerted mainly on pre-existing monocytes/macrophages rather than on acute bone marrow mobilization. On the other hand, the elevation of vimentin+/GFP− but not vimentin+/GFP+ cell deposition by G-CSF suggested its preferential activity in the recruitment of peripheral and/or the activation of localized vimentin+ cells. However, these proposed modulatory effects of G-CSF in crush-injured sciatic nerves merit further investigation.

Which signaling molecules attract bone marrow-derived CD34+ cells and direct their migration into injured sites? Peripheral nerve injury causes an increase in stromal-derived factor-1

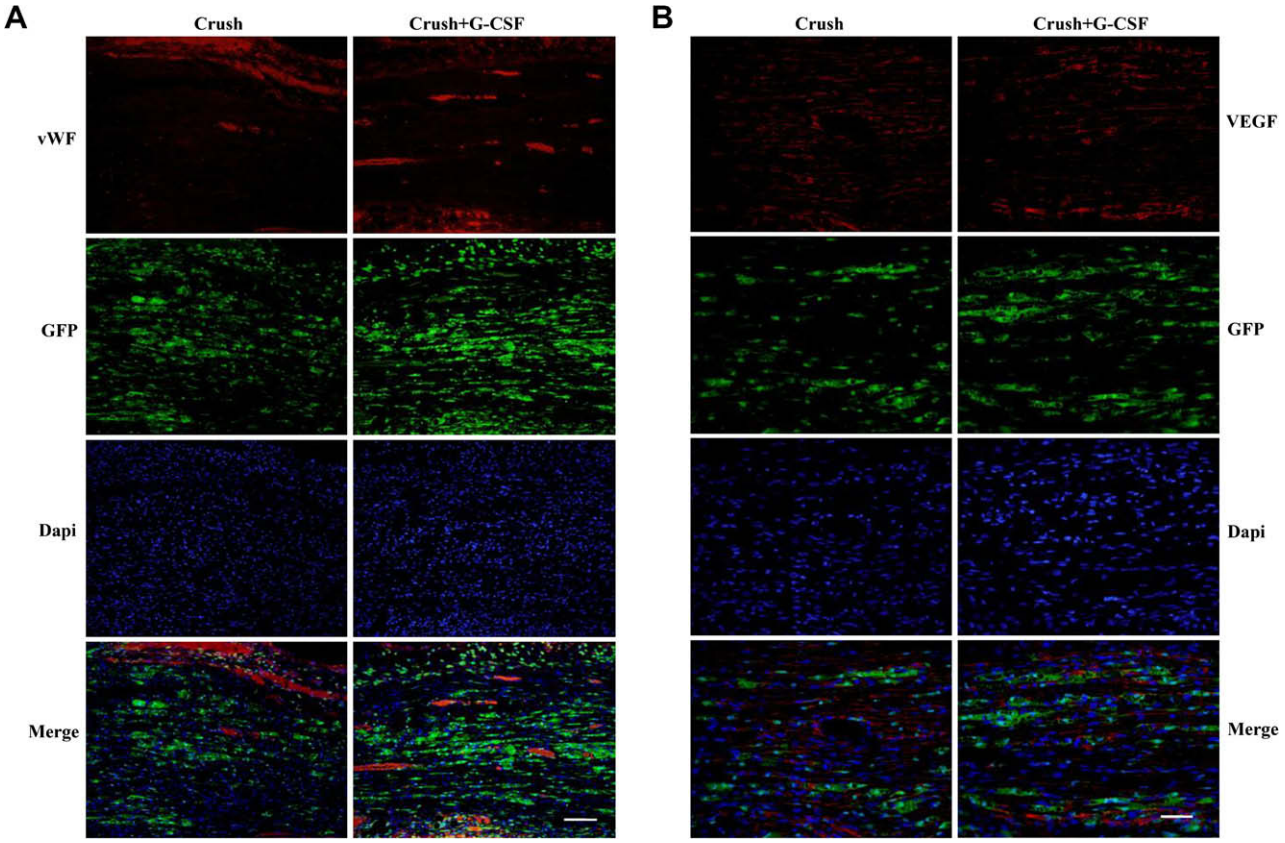

Fig. 3. Double-immunofluorescence study of GFP and vWF or VEGF. After sacrifice, the left sciatic nerves were isolated and the longitudinal sections were subjected to immunohistochemistry with antibodies against vWF (A) and VEGF (B). The signals of GFP, Dapi, and immunoreactivity were visualized with fluorescence under a fluorescent microscope. A representative micrograph is shown (n = 5). Bar length = 100 μm (A) and 50 μm (B).

(SDF-1) and its receptor CXCR4 [19]. Although we did not determine SDF-1 and CXCR4 expression, related studies suggest that hematopoietic CD34+ cells undergo directional migration to injured sites through the SDF-1/CXCR4 signaling cascade. What are the roles of CD34+ cells in the beneficial effects of G-CSF against sciatic nerve crush injury? One possibility is that CD34+ cells integrate into the tissue, replace damaged cells, and reconstruct nerve integrity. Another reasonable hypothesis is that the CD34+ cells interact with the host parenchymal cells to produce trophic factors that contribute to the functional recovery. Schwann cells play important roles in the regeneration of injured peripheral nerves [5]. There was no evidence of co-localization between GFP+ and GFAP+ cells in our study (data not shown). Thus, at this stage, the differentiation of CD34+ cells into Schwann or glial cells is unlikely. In non-irradiated/non-bone marrow-transplanted mice, G-CSF caused an increased deposition of CD34+ cells (Supplementary Fig. 2A) and promoted vascularization/angiogenesis (Supplementary Fig. 2B and C) in the injured sciatic nerves. The presence of bone marrow cells increases vessel density during sciatic nerve regeneration [6]. Increasing evidence demonstrates that CD34+ cells are mobilized into peripheral blood and recruited into damaged sites, contributing to neovascularization [1,7]. Local transplantation of bone marrow-derived endothelial progenitor cells shows angiogenic and neurotrophic effects against peripheral nerve injury [7]. Although the vascularization/angiogenesis was improved by G-CSF, the co-localization of GFP+ and vWF+ signals was rarely observed (Fig. 3A). The result of the double-immunofluorescence study argues against transdifferentiation and integration of bone marrow-derived CD34+ cells into vasculature [7]. This discrepancy might be due to the differences in the CD34+ cell deposition (local administration vs. G-CSF mobilization) and the treatment course (8 weeks vs. 1 week). VEGF is an angiogenic factor critical to vascularization/angiogenesis and its expression is elevated in injured sciatic nerves [20]. Studies show that G-CSF stimulates VEGF expression, augments angiogenesis, and increases vessel density *in vivo* after injury [14,21,22]. G-CSF also activates endothelial cell proliferation and shows angiogenic activity *in vitro* [23]. Bone marrow-derived CD34+ cells express VEGF and their administration stimulates endothelial cell proliferation and increases angiogenesis in damaged tissues [5,7]. Although the expression of VEGF in the injured nerves was up-regulated by G-CSF, the double-immunofluorescence study revealed rare co-localization of GFP+ and VEGF+ signals, indicating CD34+ cells might not be the cells responsible for VEGF expression. G-CSF also exhibits several neuroprotective functions, including anti-inflammatory, anti-apoptotic, and neurotrophic activity [2,3,14]. The administration of bone marrow-derived CD34+ cells also stimulates anti-apoptotic and neurotrophic molecule expression [7]. That is, the beneficial mechanisms of G-CSF against crush injury in sciatic nerves might be multifactorial, involving G-CSF and G-CSF-mobilized bone marrow-derived CD34+ cells.

In conclusion, the administration of G-CSF promotes functional recovery in sciatic nerves after crush injury. The current study identifies bone marrow-derived CD34+ cells as a dominant cell subpopulation involved in G-CSF-mediated mobilization and deposition into injured sciatic nerves. G-CSF-mediated beneficial effects are associated with increased CD34+ cell deposition, VEGF expression, and vascularization/angiogenesis. On the other hand, G-CSF also exerts an immunomodulatory effect by suppressing the accumulation of macrophages at the injured nerve. Thus, the administration of G-CSF in short-term functional recovery in sciatic nerve crush injury involves a paracrine modulatory effect and a bone marrow-derived CD34+ cell mobilizing effect.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2009.03.003.

References

[1] K. Tei, T. Matsumoto, Y. Mifune, K. Ishida, K. Sasaki, T. Shoji, S. Kubo, A. Kawamoto, T. Asahara, M. Kurosaka, R. Kuroda, Administration of peripheral blood CD34-positive cells contribute to medial collateral ligament healing via vasculogenesis, Stem Cells 26 (2008) 819–830.
[2] H.C. Pan, F.C. Cheng, S.Z. Lai, D.Y. Yang, Y.C. Wang, M.S. Lee, Enhanced regeneration in spinal cord injury by concomitant treatment with granulocyte colony-stimulating factor and neuronal stem cells, J. Clin. Neurosci. 15 (2008) 656–664.
[3] H.C. Pan, C.J. Chen, F.C. Cheng, S.P. Ho, M.J. Liu, S.M. Hwang, M.H. Chang, Y.C. Wang, Combination of G-CSF administration and human amniotic fluid mesenchymal stem cell transplantation promotes peripheral nerve regeneration, Neurochem. Res. (2008), doi:10.1007/s11064-008-9815-5.
[4] T. Hasegawa, A. Kosaki, K. Shimizu, H. Matsubara, Y. Mori, H. Masaki, N. Toyoda, M. Inoue-Shibata, M. Nishikawa, T. Iwasaka, Amelioration of diabetic peripheral neuropathy by implantation of hematopoietic mononuclear cells in streptozotocin-induced diabetic rats, Exp. Neurol. 199 (2006) 274–280.
[5] C.J. Chen, Y.C. Ou, S.L. Liao, W.Y. Chen, S.Y. Chen, C.W. Wu, C.C. Wang, W.Y. Wang, Y.S. Huang, S.H. Hsu, Transplantation of bone marrow stromal cells for peripheral nerve repair, Exp. Neurol. 204 (2007) 443–453.
[6] M. Fernandes, S.G. Valente, M.J. Fernandes, E.P. Félix, M. Mazzacoratti, D.A. Scerni, J.B. Dos Santos, V.M. Leite, F. Faloppa, Bone marrow cells are able to increase vessels number during repair of sciatic nerve lesion, J. Neurosci. Methods 170 (2008) 16–24.
[7] J.O. Jeong, M.O. Kim, H. Kim, M.Y. Lee, S.W. Kim, M. Ii, J.U. Lee, J. Lee, Y.J. Choi, H.J. Cho, N. Lee, M. Silver, A. Wecker, D.W. Kim, Y.S. Yoon, Dual angiogenic and neurotrophic effects of bone marrow-derived endothelial progenitor cells on diabetic neuropathy, Circulation 119 (2009) 699–708.
[8] N.A. Nicola, D. Metcalf, M. Matsumoto, G.R. Johnson, Purification of a factor inducing differentiation in murine myelomonocytic leukemia cells: identification as granulocyte colony-stimulating factor, J. Biol. Chem. 258 (1983) 9017–9023.
[9] J. Jansen, S. Hanks, J.M. Thompson, M.J. Dugan, L.P. Akard, Transplantation of hematopoietic stem cells from the peripheral blood, J. Cell. Mol. Med. 9 (2005) 37–50.
[10] C.Z. Liu, B.G. Xiao, G-CSF and neuroprotection: a therapeutic perspective in cerebral ischaemia, Biochem. Soc. Trans. 34 (2006) 1327–1333.
[11] K. Meuer, C. Pitzer, P. Teismann, C. Krüger, B. Göricke, R. Laage, P. Lingor, K. Peters, J.C. Schlachetzki, K. Kobayashi, G.P. Dietz, D. Weber, B. Ferger, W.R. Schäbitz, A. Bach, J.B. Schulz, M. Bähr, A. Schneider, J.H. Weishaupt, Granulocyte-colony stimulating factor is neuroprotective in a model of Parkinson's disease, J. Neurochem. 97 (2006) 675–686.
[12] W.C. Shyu, S.Z. Lin, C.C. Lee, D.D. Liu, H. Li, Granulocyte colony-stimulating factor for acute ischemic stroke: a randomized controlled trial, CMAJ 174 (2006) 927–933.
[13] M. Koda, Y. Nishio, T. Kamada, Y. Someya, A. Okawa, C. Mori, K. Yoshinaga, S. Okada, H. Moriya, M. Yamazaki, Granulocyte colony-stimulating factor (G-CSF) mobilizes bone marrow-derived cells into injured spinal cord and promotes functional recovery after compression-induced spinal cord injury in mice, Brain Res. 1149 (2007) 223–231.
[14] Y. Sehara, T. Hayashi, K. Deguchi, H. Zhang, A. Tsuchiya, T. Yamashita, V. Lukic, M. Nagai, T. Kamiya, K. Abe, Potentiation of neurogenesis and angiogenesis by G-CSF after focal cerebral ischemia in rats, Brain Res. 1151 (2007) 142–149.
[15] Z.E. Toth, R.R. Leker, T. Shahar, S. Pastorino, I. Szalayova, B. Asemenew, S. Key, A. Parmelee, B. Mayer, K. Nemeth, A. Bratincsák, E. Mezey, The combination of granulocyte colony-stimulating factor and stem cell factor significantly increases the number of bone marrow-derived endothelial cells in brains of mice following cerebral ischemia, Blood 111 (2008) 5544–5552.
[16] Z. Cheng, X. Liu, L. Ou, X. Zhou, Y. Liu, X. Jia, J. Zhang, Y. Li, D. Kong, Mobilization of mesenchymal stem cells by granulocyte colony-stimulating factor in rats with acute myocardial infarction, Cardiovasc. Drugs Ther. 22 (2008) 363–371.
[17] M.J. Aminoff, Electrodiagnosis in Clinical Neurology, fourth ed., vol. 4, Churchill Livingstone, New York, 1999, pp. 257–263.
[18] J.M. Rosen, H.N. Pham, V.R. Hentz, Fascicular tubulization: a comparison of nerve experimental nerve repair technique in the cat, Ann. Plast. Surg. 22 (1989) 467–478.
[19] F.A. White, N.M. Wilson, Chemokines as pain mediators and modulators, Curr. Opin. Anaesthesiol. 21 (2008) 580–585.

[20] R. Pola, T.R. Aprahamian, M. Bosch-Marcé, C. Curry, E. Gaetani, A. Flex, R.C. Smith, J.M. Isner, D.W. Losordo, Age-dependent VEGF expression and intraneural neovascularization during regeneration of peripheral nerves, Neurobiol. Aging 25 (2004) 1361–1368.
[21] K.H. Jung, K. Chu, S.T. Lee, S.J. Kim, D.I. Sinn, S.U. Kim, M. Kim, J.K. Roh, Granulocyte colony-stimulating factor stimulates neurogenesis via vascular endothelial growth factor with STAT activation, Brain Res. 1073–1074 (2006) 190–201.
[22] T. Yagi, K. Fukuda, J. Fujita, J. Endo, Y. Hisaka, Y. Suzuki, M. Tamura, S. Ogawa, G-CSF augments small vessel and cell density in canine myocardial infarction, Keio J. Med. 57 (2008) 139–149.
[23] M. Bussolin, M. Ziche, J.M. Wang, D. Alessi, O. Morbidelli, A. Cremona, P.C. Bosai, A. Marchisio, A. Mantovani, In vitro and in vivo activation of endothelial cells by colony-stimulating factors, J. Clin. Invest. 87 (1991) 986–995.