

Treatment of Complete Spinal Cord Injury Patients by Autologous Bone Marrow Cell Transplantation and Administration of Granulocyte-Macrophage Colony Stimulating Factor

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ABSTRACT

Transplantation of bone marrow cells into the injured spinal cord has been found to improve neurologic functions in experimental animal studies. However, it is unclear whether bone marrow cells can similarly improve the neurologic functions of complete spinal cord injury (SCI) in human patients. To address this issue, we evaluated the therapeutic effects of autologous bone marrow cell transplantation (BMT) in conjunction with the administration of granulocyte macrophage-colony stimulating factor (GM-CSF) in six complete SCI patients. BMT in the injury site (1.1×10^6 cells/ μ L in a total of 1.8 mL) and subcutaneous GM-CSF administration were performed on five patients. One patient was treated with GM-CSF only. The follow-up periods were from 6 to 18 months, depending on the patients. Sensory improvements were noted immediately after the operations. Sensory recovery in the sacral segment was noted mainly 3 weeks to 7 months postoperatively. Significant motor improvements were noted 3 to 7 months postoperatively. Four patients showed neurologic improvements in their American Spinal Injury Association Impairment Scale (AIS) grades (from A to C). One patient improved to AIS grade B from A and the last patient remained in AIS grade A. No immediate worsening of neurologic symptoms was found. Side effects of GM-CSF treatment such as a fever ($>38^\circ\text{C}$) and myalgia were noted. Serious complications increasing mortality and morbidity were not found. The follow-up study with magnetic resonance imaging 4–6 months after injury showed slight enhancement within the zone of BMT. Syrinx formation was not definitely found. BMT and GM-CSF administration represent a safe protocol to efficiently manage SCI patients, especially those with acute complete injury. To demonstrate the full therapeutic value of this protocol, long-term and more comprehensive case-control clinical studies are required.

INTRODUCTION

TRAUMATIC SPINAL CORD INJURY (SCI) affects many people and can result in severe neurological damage. Recovery from central nervous system (CNS) injury is

difficult because of the limited ability of the injured CNS to regenerate lost cells, replace disrupted myelin, and reestablish functional neural connections. One strategy to increase axonal regeneration involves the transplantation of stem cells into the injured spinal cord.¹

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Human bone marrow cells have a long history in the treatment of hematologic diseases.² Moreover, non-hematopoietic stem cells, such as mesenchymal stem cells (MSCs), can differentiate into mature osteocytes, chondrocytes, and adipocytes.³ Some have found that bone marrow cells (BMCs) differentiate into mature neurons or glial cells^{4–6} when induced under experimental conditions. These findings raised the possibility of applying BMCs therapeutically in patients with neurological diseases, which would also obviate ethical problems in the use of embryonic stem cells.

The engraftment of BMCs into animal models of SCI has been actively studied. Transplanted BMCs were found to improve neurologic deficits in CNS injury models by generating neural cells or myelin-producing cells.^{7,8} The results in animal models suggest that BMCs could be used as a potential therapy for SCI patients. However, it has not been demonstrated clearly whether BMCs have therapeutic advantages in SCI patients.

Granulocyte-macrophage colony stimulating factor (GM-CSF) is a hematopoiesis-stimulating factor that induces proliferation and differentiation of BMCs. GM-CSF has been widely used to activate and mobilize BMCs in hematopoietic stem cell transplantation. A role for GM-CSF in the neural system has been suggested. GM-CSF activates macrophages in CNS to remove myelin debris that inhibits axonal regeneration. Furthermore, GM-CSF could increase neural stem cell proliferation and inhibits neuronal apoptosis, resulting in the improvement of neurologic functions in animal models of SCI.^{9,10} These findings suggest that GM-CSF could be used as an effective therapeutic tool in neuronal disease.

In this study, we evaluated the effect of BMCs and GM-CSF in the treatment of SCI patients, in terms of efficacy and safety. We transplanted autologous BMCs to

the injury site of SCI patients and then stimulated their bone marrow with GM-CSF. The results of the clinical follow-up are described.

MATERIALS AND METHODS

Patient selection

Six patients with complete cervical SCI (Frankel grade A or American Spinal Injury Association [ASIA] Impairment Scale [AIS] grade A 72 h after admission) were included in this study. The inclusion and exclusion criteria of the study are summarized in Table 1. Transplantation protocols were approved by institutional review board and all procedures were performed after obtaining written informed consent. The treatment schedule and follow-up results are briefly summarized in Table 2. BMC transplantation (BMT) with GM-CSF administration was performed on five patients. In case 3, the patient received only GM-CSF administration without BMT.

Separation of human bone marrow cells

Bone marrow blood (100–150 mL) was aspirated from the iliac bone and diluted in Hanks' balanced salt solution (HBSS) at a ratio of 1:1. After centrifugation of samples at $1000 \times g$ for 30 min through a density gradient (Ficoll-Paque Plus, 1.077 g/L; Amersham Biosciences, Piscataway, NJ), the mononuclear cell layer was recovered from the gradient interface and washed with HBSS. The cells were centrifuged at $900 \times g$ for 15 min and resuspended in 1.8 mL of phosphate-buffered saline (PBS) at a density of 1.1×10^6 cells/ μ L. The concentrated BMCs were then transferred to the operation room.

TABLE 1. INCLUSION AND EXCLUSION CRITERIA FOR AUTOLOGOUS BONE MARROW CELL TRANSPLANTATION WITH GM-CSF TREATMENT OF SPINAL CORD INJURY PATIENTS

Inclusion criteria	
	Traumatic spinal cord injury (within 14 days)
	Age between 16 and 65 years
	Complete spinal cord injury confirmed 72 h after injury (AIS grade A)
	A single spinal cord lesion with last fully preserved neurological level from C3 to T11
	Informed consent obtained and consent form signed
Exclusion criteria	
	More than 14 days have elapsed since the injury
	Women who are pregnant or lactating
	Penetrating trauma
	Fever (above 39°C)
	Ventilator assistance
	Anatomical transection of the cord visualized by C-MRI
	Serious preexisting medical diseases

TABLE 2. SUMMARY OF CASES

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Sex	Male	Male	Male	Male	Male	Male
Age (years)	42	28	51	41	17	36
Level of injury	C6/7	C6/7	C6/7	C6/7	C4/5/6	T11
BMT date (days after trauma)	7	7	None	11	14	8
GM-CSF administration cycle (times)	5	5	5	5	5	5
Associated injury	None	Aortic dissection	Malleolar fracture (left)	None	None	None
Frankel grade (72 h after admission)	A	B	A	A	A	A
AIS grade (72 h after admission)	A	A	A	A	A	A
Time to AIS grade B (weeks after trauma)	3	2 (13 days)	8	—	20	4
Time to AIS grade C (weeks after trauma)	3	3	4	—	—	2
Follow-up duration (months)	12	15	18	15	6	7
Last follow-up AIS grade MRI	C Enhancement	C Enhancement	C Increased T2 signal	A —	B —	C —
Complication	Fever, leukocytosis	Fever, leukocytosis	Fever, leukocytosis	Fever, skin rash, leukocytosis chilling	Dizziness, skin rash, itching	Fever

Abbreviations: AIS, American Spinal Injury Association Impairment Scale; BMT, bone marrow transplantation; GM-CSF, granulocyte-macrophage colony stimulating factor; MRI, magnetic resonance imaging.

Operation

Transplantation was done 7 days after injury. Neurologic examination was performed immediately before operation to confirm complete SCI. Complete laminectomy was performed from one vertebra above to one below in order to provide sufficient access to the transplantation site. The dura was then incised, sparing the arachnoid, which was subsequently opened separately with a microscissors. The dorsal surface of the contusion site was located under high-power microscopic magnification. After exposure of sufficient surface in the contusion site, 300- μ L aliquots of cell paste (total volume, 1.8 mL) were injected into six separate points surrounding the margin of the contusion site. To avoid direct cord injury, $2 \times$

10^8 cells were delivered at a rate of 30 μ L/min, using a 21-gauge needle attached to a 1-mL syringe. The depth of the injection site was 5 mm from the dorsal surface. To prevent cell leakage through the injection track, the injection needle was left in position for 5 min after completing the injection, after which the dura and arachnoid were closed. The muscle and skin were closed layer by layer.

GM-CSF injection schedule

After surgery, a total of five cycles (daily for the first 5 days of each month over 5 months) of GM-CSF (Leucogen; LG Life Sciences, Seoul, Korea) was injected subcutaneously ($250 \mu\text{g}/\text{m}^2$ of body surface area).

RESULTS

Summary of clinical course and MRI findings

Case 1. Initial neurologic examination showed paraplegia below the C7 level, and the complete loss of tactile and pain sensation below the T2 level (Frankel grade A, AIS grade A). An initial cervical MRI showed a cervical cord contusion at the C6/7 level. On day 7 after admission, cervical anterior interbody fusion with autologous iliac bone, posterior C5/6/7 fixation, and autologous BMC transplantation were performed. After surgery, a total of five cycles of GM-CSF was injected. The patient showed no immediate symptom changes (aggravation or improvement) after transplantation. Perianal sensation improved steadily during the first 3 weeks postoperatively (AIS grade B). Low-extremity spasticities developed after 14 days and voluntary low-extremity movements started 3 months postoperatively (Frankel grade C, AIS grade C). The last follow-up at 12 months showed that the hip and knee joints were able to flex against gravity (Fig. 2). The 5-month magnetic resonance image revealed a small enhanced area within the transplantation zone in the cervical cord (Fig. 1A). No discernable syrinx formation was found.

Case 2. Complete paralysis below C7 was noticed on admission. However, sensory impairments were not complete. The zone of partial preservation of sensation was wide. Analgesia and anesthesia were checked only in the sacral segment (Frankel grade B, ASA grade A). The initial cervical MRI showed a cord contusion at C6/7. Posterior C6/7–T1 fixation and autologous BMC transplantation were performed 7 days after injury. After surgery, five cycles of GM-CSF were injected. The neurologic status of the patient immediately after transplantation remained unchanged (Frankel grade B, AIS grade A). Sensory improvement started 3 days postoperatively. Perianal sensation was recovered (Frankel grade B) by 13 days. The flaccid-type paralysis was converted to the spastic type at 1 week. Trivial voluntary muscle control was observed at 3 months. Voluntary hip joint flexion (muscle power grade 4) was checked at the follow-up of 4 months; however, ankle movement was not checked (Fig. 2). The follow-up MRI showed a small enhancing lesion in the area of transplantation (Fig. 1B).

Case 3. Complete paralysis below C7, and complete loss of tactile and pain sensation below T2, were initially noticed (Frankel grade A, AIS grade A). The cervical MRI showed a cervical cord contusion at C6/7. On day 3 after admission, cervical anterior interbody fusion with autologous iliac bone and posterior C5/6/7 fixation were performed. After the operation, a total of five cycles of GM-CSF was injected intramuscularly without BMC

transplantation, because of the patient's refusal. Sensory improvement was noticed 2 months postoperatively (Frankel grade B). Motor paralysis did not improve before 4 months, when trivial voluntary hip and knee joint movements were checked. Voluntary ankle and toe movements were observed at 5 months. The last (18-month) follow-up showed the possibility of self-control of all hip, knee, and ankle joint flexions (Frankel grade C, AIS grade C; Fig. 2). The MRI at 6-month follow-up showed spinal cord atrophy and an increased signal at the injury site; however, no enhanced area within the cord injury zone was evident (Fig. 1C).

Case 4. The patient was a 41-year-old man who experienced C6/7 vertebral body fractures and SCI. Cervical anterior interbody fusion (C6/7) with plate had been performed at another hospital, and then he was transferred to our hospital for BMC transplantation. On admission, he showed paraplegia below the C7 level and anesthesia below the C8 dermatome (Frankel grade A, AIS grade A). BMC transplantation was performed 11 days after SCI. A total of five cycles of GM-CSF was injected. A remarkable improvement of motor function has not been found in this patient, except for a slight progression of sensory improvement for light touch and pin prick to the L3 level (Fig. 2).

Case 5. The patient was a 17-year-old boy who had SCI from a motor accident. The initial neurologic examination showed paraplegia below the C5 level and anesthesia below the T3 dermatome (Frankel grade A, AIS grade A). Cervical anterior interbody fusion (C4/5/6) with BMC transplantation was performed 14 days later, and a total of five cycles of GM-CSF was injected subcutaneously. No remarkable neurologic improvement was shown until 6 weeks after BMC transplantation; rather, the patient complained of a worsening of lower extremity rigidity (both limbs) 3 weeks later. Improvement in right wrist extension (C6 level) was noticed 6 weeks after the operation, and he felt a dull sensation near the perianal area that had not been present previously. After 10 weeks, voiding sensation was recovered and the indwelling Foley catheter was removed. A subtle sensation in both lower extremities was noticed 5 months later, and the patient showed improvement in the motor function of both elbows from grade 0 to grade 2 (Frankel grade A, AIS grade B; Fig. 2).

Case 6. The patient was a 36-year-old man who fell from a 2-m height, injured his spinal cord (T11/12 vertebral fracture and dislocation), and was transferred to our hospital after emergency steroid megadose therapy. He showed paraplegia below the T12 level and anesthesia below the T12 dermatome on admission. Posterior interbody fusion (T9–12) with BMC transplantation was

performed on day 8 after initial trauma. A total of five cycles of GM-CSF was injected subcutaneously after BMC transplantation. On postoperative day 5, the patient showed improvement in sensory function above the L5 dermatome. However, perianal sensation was not noticed. After 8 weeks, a slight hip joint flexion (motor grade 3) was possible on exertion and perianal sensation was restored (AIS grade C; Fig. 2). Ten weeks after BMC transplantation, he could stand and step with braces that support the lumbar and pelvic bone.

DISCUSSION

In this study, we showed that patients with a complete acute SCI (AIS grade A) were treated by BMC transplantation and GM-CSF administration without serious complications such as increased mortality and morbidity. In particular, no immediate worsening of neurologic status was observed after transplantation. All patients showed fever ($>38^{\circ}\text{C}$), myalgic pain, and leukocytosis during GM-CSF administration. Despite these transient

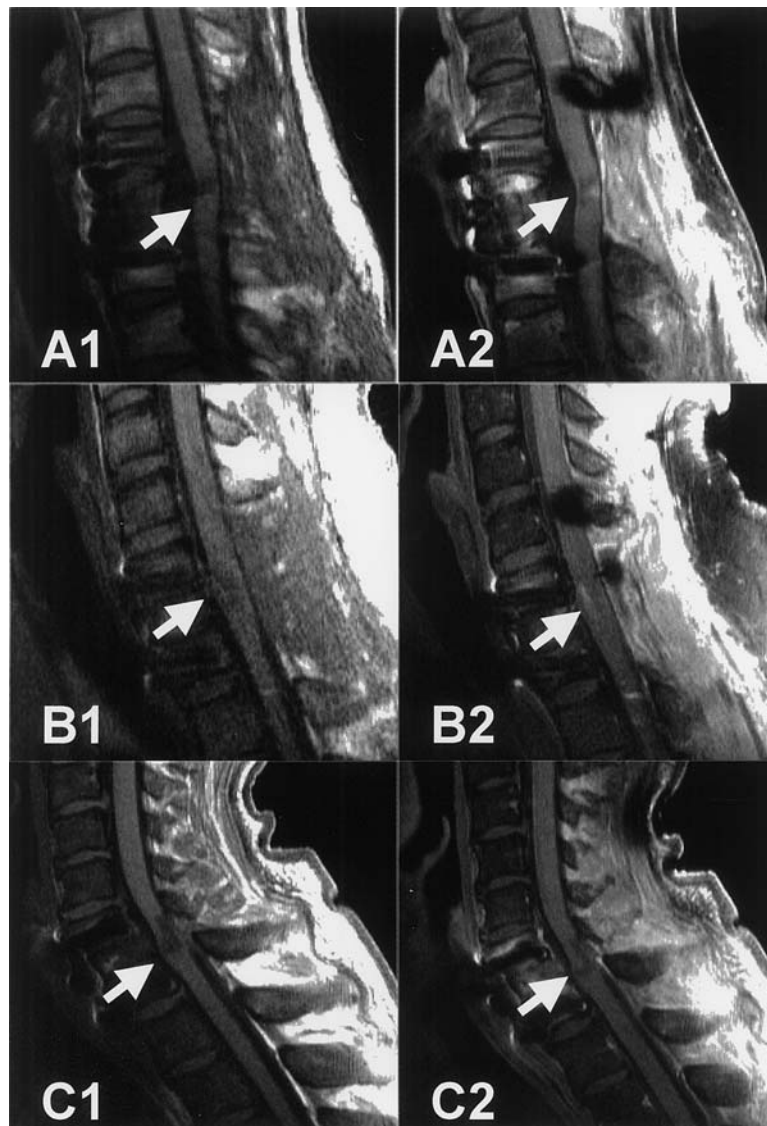


FIG. 1. Follow-up MRI findings. (A1) Initial sagittal T1W magnetic resonance image (MRI) and (A2) corresponding post-contrast image 5 months postoperatively in patient 1, showing a small patch enhancement in the BMC transplantation area (arrows). (B1) Initial sagittal T1W MRI and (B2) corresponding postcontrast image 4 months postoperatively in case 2, showing a small linear enhancement in the BMC transplantation area (arrows). (C1) Initial sagittal T1W MRI and (C2) corresponding post-contrast image in case 3, showing a small nonenhanced and well-defined cyst in the cord (arrows), which is associated with a slight cord expansion and adjacent myelomalacia. No distinct enhancement in injury area is found.

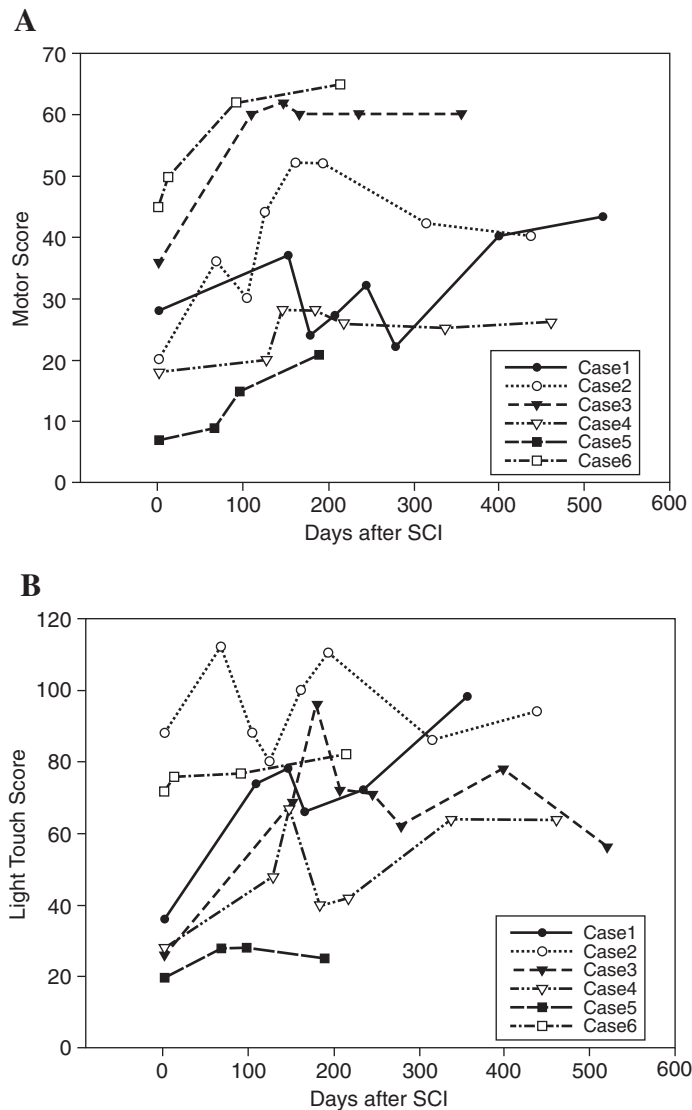


FIG. 2. Motor and sensory score changes after treatment. (A) Motor improvement was found 3 to 7 months postoperatively. Thereafter, a slight decrease in motor score was found in patients 1, 2, 3, and 4. (B) Light touch score was improved within 7 months. No significant improvements were found after 7 months. (C) Pin prick score shows that initial improvement is found in 7 months; scores then decrease.

side effects, all patients completed the protocols successfully. These results suggest that this therapeutic protocol should be investigated further in a well-designed case-control clinical study of SCI patients.

In our patients with a complete SCI (AIS grade A), slightly improved neurologic functions were shown after BMC transplantation with GM-CSF in acute phases (7–14 days). The main purpose of our study was to investigate the safety of the protocol, not to conclude on the basis of the current results whether our therapeutic protocol improved neurologic functions in patients with complete SCI. However, despite the limitations imposed by the small number of cases with short-term follow-up,

our results provide preliminary data sufficient to ensure further studies.

BMCs have long been used as donor cells for bone marrow transplantation in hematologic diseases.² It was found that stem cells derived from bone marrow differentiate into nonhematopoietic cells including muscle, skin, liver, lung, cardiac myocytes, endothelial cells, and neuronal cells.³ Interestingly, these findings are not restricted to animal studies. Male donor BMCs differentiated to form neurons in female recipient brains.¹¹

The use of BMCs for stem cell therapy in SCI patients has more advantages compared with embryonic stem cell use. First, one can avoid all problems associated with im-

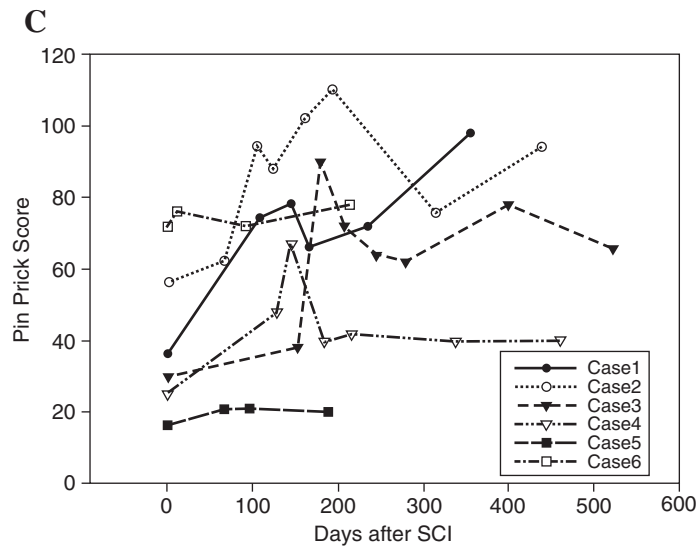


FIG. 2. (continued).

munological rejection or graft-versus-host reactions, which are frequently caused by allografts.¹² Second, bone marrow stem cell-based therapy is not associated with carcinogenesis, which sometimes occurs in embryonic stem cell therapy.¹³ Third, extensive scientific data on BMCs have been accumulated from our wide-ranging experiences in bone marrow transplantation for hematological diseases. These advantages have made cell therapy using BMCs widely applied and investigated clinically in various human diseases including cardiovascular disease and cancer therapy.

Studies of BMCs transplantation in neurological injury models provide much information about their potential to improve functional outcome.^{14,15} Moreover, several hypotheses have been proposed to explain the role of bone marrow stem cells in SCI models. First, BMCs improve neurologic deficit by generating either neural cells or myelin-producing cells.^{7,16} Second, transplanted BMCs do not differentiate into neurons; rather, they work by guiding axonal regeneration by producing extracellular matrix. Third, transplanted BMCs promote compen-

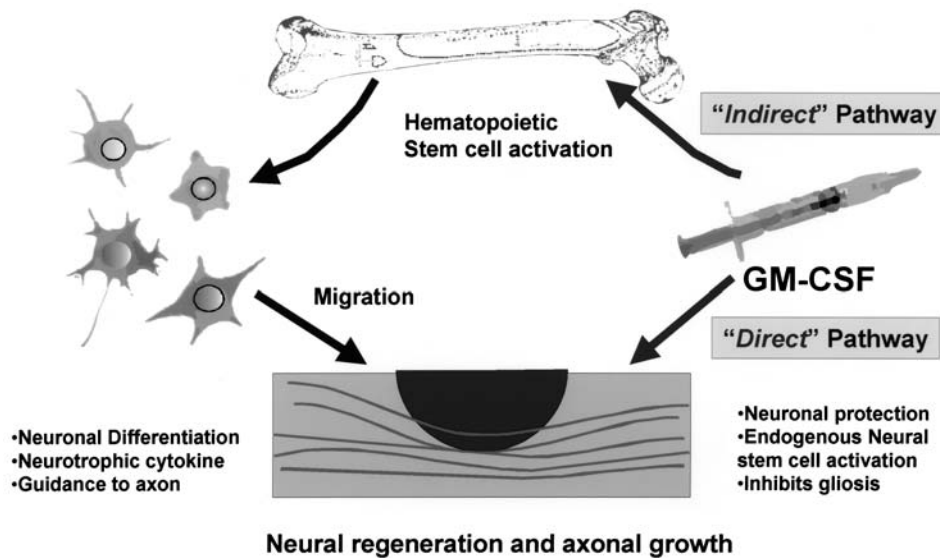


FIG. 3. Suggestive mode of action for BMC transplantation and GM-CSF treatment in SCI patients. GM-CSF increases the proliferation of bone marrow hematopoietic stem cells. Mobilized bone marrow cells migrate into the injury site and then could act on neural differentiation and neurotrophic cytokine release, and support axonal regeneration (indirect, systemic effect). GM-CSF also could directly increase neural stem cell proliferation or inhibit apoptosis and gliosis (direct, local effect).

satory mechanisms to reorganize neural network and activate endogenous stem cells.¹⁵

In the present study, we present three reasons why we transplanted isolated whole bone marrow mononuclear cell fractions instead of MSCs. Isolated bone marrow mononuclear cell fractions include hematopoietic stem cells, macrophages, lymphocytes, as well as marrow stromal cells. One reason is that the identities of the sub-fractions responsible for neuronal differentiation remain unknown. Second, neuronal protective roles are well understood not only in MSCs but also in hematopoietic stem cells.^{17,18} In addition, mechanisms regulating lineage commitment and cellular differentiation in the neural and hematopoietic systems are similar.¹⁹ Hematopoietic stem cells excrete many types of cytokines including thrombopoietin and interleukin 11.^{19–21} These cytokines are also known as essential factors for the survival and differentiation of neuronal progenitor cells. Colony-stimulating factor 1 is one of the important hematopoietic cytokines that also acts as a growth factor in the central nervous system.²² Last, one study shows that activated microglial cells or macrophages enhance axonal regeneration by removal of myelin debris in injury site.²³ These suggestive modes of action of bone marrow cell transplantation are summarized in Fig. 3.

In the present study, we used neurological impairment scales (AIS motor, light touch, and pin prick score) and MRI after autologous BMC transplantation. Several studies have reported that complete SCI (Frankel grade A, AIS grade A) is both detrimental and has a poor prognosis. Burns *et al.* reported that overall, 5 of 81 patients (6.2%) with AIS grade A converted to motor-complete and sensory-incomplete status (AIS grade B) between the initial and >1-year follow-ups.²⁴ Marino *et al.* showed that 84% of 775 patients (Frankel grade A) did not improve after 1 year.²⁵ Only 4.9% of patients experience recovery in neurologic status from Frankel grade A to C. They also found that 84.6% of 482 patients (AIS grade A) did not improve and that 5.8% of patients showed neurologic status improvement from AIS grade A to C. Some therapeutic trials have been conducted to study megadose methylprednisolone treatment and omental transposition.^{26–28} Although comprehensive multicenter studies have been performed, it remains unclear whether the use of megadose methylprednisolone or omental transposition in SCI improves neurologic recovery.^{29,30} Furthermore, some reported an increased early mortality and morbidity possibly related to the procedures.^{31,32} No immediate worsening of neurologic status was observed after transplantation in our patients. Transient side effects to with GM-CSF, such as a fever, myalgic pain, and leukocytosis, were noticed. Our results suggest that BMC transplantation is intrinsically safer than other treatment protocols such as megadose methylprednisolone therapy and omental transposition.

A small enhancing lesion within the zone of transplantation with an accompanying increase in T2 signal was noticed in transplanted patients. However, it is unclear whether this MRI finding reflects the biological activities of transplanted bone marrow cells. Further studies are required to compare imaging changes with clinical outcomes.

GM-CSF has been used in patients who have bone marrow failure.³³ GM-CSF stimulates bone marrow stem cells. Bone marrow stem cells increase significantly in peripheral blood after the administration of GM-CSF.^{34,35} It has been reported that elevated numbers of stem cells in the peripheral blood, caused by the intravenous administration of bone marrow cells, improve functional outcomes in a stroke model.¹⁴ Furthermore, GM-CSF prevents apoptotic cell death not only in hematologic cells³⁶ but also in neuronal cells. These results encouraged the application of GM-CSF therapy in SCI patients. We expected that GM-CSF would not only activate patient bone marrow but would also have a direct effect on the transplanted BMCs, enabling the BMCs to survive in the spinal cord and to excrete neurotrophic cytokines. Interestingly, we have found that one patient (case 3) who received GM-CSF only, without BMT, showed improvement of neurologic functions from AIS grade A to C. We think that GM-CSF enhanced the activity of the patient's bone marrow to increase the number of bone marrow stem cells in the circulating peripheral blood, which subsequently prevented apoptotic neuronal death after the initial injury (Fig. 3). The efficacy of the combined treatment of GM-CSF and BMT on the recovery of SCI patients should be further supported in animal models of SCI.

CONCLUSION

We believe that autologous bone marrow transplantation with GM-CSF administration has no attendant serious complications, and recommend that the therapeutic effects of such treatments in SCI should be made the subject of a more comprehensive multicenter study.

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