

Autologous Bone Marrow Transplantation in Patients With Subacute and Chronic Spinal Cord Injury

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Stem cell transplants into spinal cord lesions may help to improve regeneration and spinal cord function. Clinical studies are necessary for transferring preclinical findings from animal experiments to humans. We investigated the transplantation of unmanipulated autologous bone marrow in patients with transversal spinal cord injury (SCI) with respect to safety, therapeutic time window, implantation strategy, method of administration, and functional improvement. We report data from 20 patients with complete SCI who received transplants 10 to 467 days postinjury. The follow-up examinations were done at 3, 6, and 12 months after implantation by two independent neurologists using standard neurological classification of SCI, including the ASIA protocol, the Frankel score, the recording of motor and somatosensory evoked potentials, and MRI evaluation of lesion size. We compared intra-arterial (via catheterization of a. vertebralis) versus intravenous administration of all mononuclear cells in groups of acute (10–30 days post-SCI, $n = 7$) and chronic patients (2–17 months postinjury, $n = 13$). Improvement in motor and/or sensory functions was observed within 3 months in 5 of 6 patients with intra-arterial application, in 5 of 7 acute, and in 1 of 13 chronic patients. Our case study shows that the implantation of autologous bone marrow cells appears to be safe, as there have been no complications following implantation to date (11 patients followed up for more than 2 years), but longer follow-ups are required to determine that implantation is definitively safe. Also, we cannot yet confirm that the observed beneficial effects were due to the cell therapy. However, the outcomes following transplantation in acute patients, and in one chronic patient who was in stable condition for several months prior to cell implantation, are promising. It is evident that transplantation within a therapeutic window of 3–4 weeks following injury will play an important role in any type of stem cell SCI treatment. Trials involving a larger population of patients and different cell types are needed before further conclusions can be drawn.

Key words: Evoked potentials; Magnetic resonance imaging; Mesenchymal stem cells; Regeneration; Spinal cord injury; Stem cells

INTRODUCTION

In the last decade, not only embryonic but also adult stem cells have been the subject of widespread investigation due to their plasticity and possible differentiation into numerous cell types, including neural cells. These

investigations have led to the idea that adult stem cells might have significant tissue regenerative potential as well as new therapeutic potential. Presently, bone marrow-derived stem cells are regularly used to treat hematological diseases. However, it has now been demonstrated that the plasticity (ability of the cell to change its

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default fate) and tissue regenerative potential of bone marrow-derived stem cells may far exceed their use in hematopoietic diseases. Hematopoietic as well as non-hematopoietic bone marrow-derived stem cells, such as mesenchymal stem cells (MSCs), have been shown to be multipotent and to differentiate into chondrocytes, osteocytes, muscle cells, adipocytes, or even neurons and glia (10,22,32,36,38). These studies revealed that besides their differentiation, which in vivo can be more limited than in vitro, bone marrow-derived stem cells can produce growth factors and cytokines, provide structural support, and suppress inflammation and the immune reaction and in this way enhance tissue regeneration.

In particular, it is spinal cord injury that often leads to severe neurological deficit and permanent invalidity and as such urgently requires new therapeutic approaches. The affected group of patients includes otherwise healthy children and young adults who suffered traumatic spinal cord injury (SCI). Autologous bone marrow-derived stem cells are ideal candidates for treating SCI in emerging clinical studies, because there are no ethical obstacles to their use and the health risk for patients with SCI is rather small. Numerous electrophysiological and histological preclinical studies have revealed that the implantation of stem cells from bone marrow or umbilical cord blood in animal models of SCI results in spared white and gray matter, neuronal and axonal regeneration, astrocyte proliferation, myelination, neovascularization, and functional improvement (1,2,9,16,17,20,21,26,39–43,45,51). These studies have also shown that the optimal therapeutic window for implantation in rat models of SCI is 7–21 days after injury. Moreover, our preclinical experiments in rats with SCI demonstrated that intravenously implanted human bone marrow-derived stem cells, labeled in vitro with iron oxide nanoparticles and followed in vivo by magnetic resonance imaging (MRI), migrate, survive, and home only to the lesion site (20,41). The cells were found to home to a lesion as early as 3–7 days postimplantation and were still present 2 months after SCI. We also found a significant improvement in behavioral scores (BBB test and plantar test), not only after the intravenous (IV) injection of in vitro expanded MSCs, but also after the injection of all mononuclear cells from bone marrow blood (BMMCs) (42,45).

This led us to initiate a nonrandomized phase I/II clinical study, which began in August 2003. In this study, we implanted autologous bone marrow, either intra-arterially via a. vertebralis (i.e., close to the lesion site) or intravenously, into 20 patients with SCI at the cervical or thoracic level; the results of the 12 month clinical follow-up are described.

MATERIAL AND METHODS

Patients and Selection Criteria

Ethical approval for this study was obtained from the Ministry of Health of the Czech Republic and the Ethical Committee of Motol Hospital in Prague. Patients with traumatic SCI and complete motor and sensory disorder were enrolled in this study, and informed consent was obtained from each patient. These patients were healthy prior to injury and had no other major injury besides SCI. All patients underwent neurosurgical stabilization, standard therapy, and rehabilitation. Prior to implantation, they underwent MRI, an electrophysiological examination of motor and somatosensory evoked potentials, and a neurological examination by two independent neurologists who used the Frankel scale and the American Spinal Injury Association (ASIA) Impairment Scale.

The patients ($n = 20$) who received autologous BMMCs between 10 days and 18 months after SCI were divided into two groups. The first group ($n = 6$) received BMMCs via transfemoral catheterization of a. vertebralis, while the second group ($n = 14$) received BMMCs intravenously. Patient characteristics are shown in Tables 1 and 2.

Procurement of Autologous Bone Marrow Cells and Implantation

Bone marrow blood (BM) was harvested under general anesthesia from the posterior superior iliac crest by multiple aspirations. As an anticoagulant, a saline solution (Saline Viaflo, Baxter, UK) containing heparin (Heparinum natricum, Spofa, Czech Republic) at a concentration of 75 IU/ml was used. Each BM aspiration, using a prefilled syringe containing 1 ml of anticoagulant, harvested 3 ml of BM. The final concentration of heparin in the harvested BM was 18.75 IU/ml. Aspirates were collected in a Bone Marrow Collection Kit with Pre-Filter and Inline Filters (Baxter R4R2107, USA) and further processed in a closed blood bag system.

For the group of catheterized patients (intra-arterial BMMC graft), erythrocyte depletion was performed using Gelofusin (Braun Melsungen, Germany) sedimentation in a closed bag system. The volume of the added Gelofusin was 25% of the total BM volume, including anticoagulant solution. Following the formation of distinct layers of erythrocytes as sediment and leukocyte-rich plasma as supernatant (30–40 min at $1 \times g$), the last two layers were separated using a plasma extractor (Plasma Extractor, Baxter, UK). Superfluous plasma was separated by centrifugation, subsequently pressed in the plasma extractor, and returned to the erythrocytes for a second sedimentation. The entire process was repeated twice, with the final volume of the leukocyte-rich prod-

uct adjusted by centrifugation and the removal of a portion of the plasma. The volume of the final graft for intra-arterial administration was approximately 30 ml.

From the harvested BM, the numbers of leukocytes and erythrocytes were determined using a Micros 60 cell counter (Trigon Plus, Horiba ABX Diagnostics, France). From the final graft, the numbers of leukocytes and erythrocytes were again determined using the Micros 60, the percentage of CD34⁺ cells was determined using flow cytometry, and the colony-forming activity of the graft was evaluated by cultivation for 14 days (at 37°C and 5% CO₂) in MethoCult GF H4434 medium (Stem Cell Technologies Inc., Canada). The numbers of all mononuclear cells and CD34⁺ cells injected into each patient were $104.0 \pm 55.3 \times 10^8$ and $89.7 \pm 70.7 \times 10^6$, respectively. Previous studies demonstrated that the frequency of pluripotent stem cells in bone marrow lies somewhere between 1 in 10,000 and 1 in 100,000 cells, and it is therefore reasonable to assume that only a relatively low number of true pluripotent stem cells were transplanted into the patients in our study. For the group of patients receiving an IV BMMC graft, the harvested BM was not manipulated, only the plasma volume was reduced by centrifugation. The final grafts were evaluated in the same manner as the final grafts for intra-arterial administration.

Patients received BMMCs within 5 h of harvesting, either intra-arterially or by IV. Intra-arterial administration was performed by catheterization of a. vertebralis by inserting a catheter through the femoral artery in the right inguinal flexure; BMMCs were therefore infused over a period of 15 min via spinal arteries close to the injured cervical spinal cord. Intravenous administration was performed by cannulation of the cubital vein with subsequent infusion of BMMCs over a period of about 30 min.

Neurological Evaluation

For the initial examination and also for the follow-up examinations at 3, 6, and 12 months after BMMC implantation, two independent neurologists determined a standard neurological classification of SCI utilizing the American ASIA protocol, which provides a standardized assessment of neurological deficits in patients with SCI, as well as the Frankel score. These neurologists were not directly involved in the recruitment of patients for this study. The ASIA protocol evaluates motor and sensory functions on both sides of the body and uses an impairment scale of A to E, as follows. A = complete lesion: no motor or sensory function is preserved in the sacral segments S4–S5; B = incomplete lesion: sensory but no motor function is preserved below the neurological level and includes the sacral segments S4–S5; C =

incomplete lesion: motor function is preserved below the neurological level, and more than half of the key muscles below the neurological level have a muscle grade less than 3; D = incomplete lesion: motor function is preserved below the neurological level, and at least half of the key muscles below the neurological level have a muscle grade of 3 or more; E = normal function. To test motor function, muscle strength is examined in 10 key muscle groups. Five points are assigned to the normal function of each muscle group (maximal total motor score is 100). The sensory examination is completed through the testing of sensitivity to light touch and pinprick in 28 dermatomes. Normal function is scored as two points in each dermatome (maximal total light touch and pinprick score is 112). The major inclusion criterion for the study was severe SCI (ASIA impairment grade A or B) without any serious accompanying disease or trauma.

For the Frankel score, a five subdivision scale was used: A = complete loss of motor and sensory function; B = complete motor and incomplete sensory function disorder; C = incomplete motor and sensory function disorder; D = useful motor function with or without auxiliary means; E = normal function.

MRI Evaluation

When T2-weighted MR images were available prior to stabilization, the location of the damage to the spinal cord was quantified by locating the longitudinal boundary of the spinal cord hemorrhage and edema, as described by Flanders and colleagues (15). The quantification of spinal cord damage was based on measurements made on midsagittal MR images. The location was named for the nearest vertebral segment. Each segment was subdivided into three parts: the upper half of the vertebral body was named segment 1 (e.g., C4.1), the lower part of the vertebral body segment 2 (e.g., C4.2), and the intervertebral disk below the body segment 3 (C4.3). The number of segments between the upper and lower limits represented the length of edema.

Electrophysiology

To assess the functional integrity of the corticospinal tract and the dorsal columns, different electrophysiological parameters, including motor evoked potentials (MEPs) and somatosensory evoked potentials (SEPs), were examined prior to and at 3, 6, and 12 months after BMMC implantation.

MEPs were elicited by transcranial and spinal magnetic stimulation (TMS). TMS was performed using MAGSTIM 200 equipment (Magstim Company Ltd., UK) with a circular 9-cm-diameter coil for the upper limbs and with a double cone coil for the lower limbs.

Motor evoked responses were elicited by motor cortex or spinal root stimulation with maximal output, and the evoked responses were registered with a conventional electromyograph (Medelec Synergy-Oxford Instruments, UK). The surface recording electrodes were placed over the biceps brachii and abductor digiti quinti muscles for the upper limbs and over the tibial anterior and medial vastus muscles for the lower limbs. A total of six responses from each muscle were recorded for further analysis. We calculated the total conduction time (cortical latency – time taken between cortical stimulation and the response registered in the target muscle) and the peripheral conduction time (spinal latency – time taken between spinal root stimulation and the response registered in the target muscle). By subtracting the peripheral conduction time from the total conduction time, we obtained the central motor conduction time. To evaluate the condition of the peripheral nerves and limb muscles, conventional electrical stimulation of the peripheral nerves was performed in one session with magnetic stimulation of the brain cortex and spinal roots.

The tibial and median SEPs were elicited by electrical stimulation of the tibial and median nerves. The stimuli were defined as square wave pulses of 0.2 ms duration applied at a frequency of 3.1 Hz. The surface stimulating electrodes were placed over the tibial nerve at the medial ankle and over the median nerve at the wrist, with the cathode proximal to the anode. The stimulus intensity was set to produce a visible muscle contraction (with a maximum of 25 mA). The surface recording electrodes were placed over the spinous processes of Th 12 and L1 for tibial SEPs and over Erb's point and the spinous process of C6 for median SEPs. The cortical evoked responses were registered by surface electrodes placed at Cz'-Fz for the tibial nerve and at C3'-Fz or C4'-Fz for the median nerve in the "ten-twenty" international registration system. Two sets of more than 1,000 sweeps for each nerve were averaged and superimposed. We evaluated the presence of cortical evoked responses (latency of the primary cortical complex and the amplitudes of the cortical responses) and central conduction time (CCT—the time taken between the cortical response and the evoked response obtained from the C6 electrode).

RESULTS

Summary of Clinical Course, Electrophysiology, and MRI Findings

The clinical characterization of the patients participating in the study ($n = 20$) is presented in Tables 1 and 2. Fifteen patients were diagnosed with a complete transversal spinal cord lesion (ASIA grade A) and five patients with an incomplete spinal cord lesion (ASIA grade B or C).

Table 1 and Figure 1 summarize the results of the first group of patients ($n = 6$) who received BMMCs via catheterization of a. vertebralis. In this group all 4 subacute patients (cases 1–4), who received autologous BMMCs between 11 days and 30 days after injury, significantly improved their ASIA score or Frankel score, and the electrophysiological testing showed a recovery of EPs when first tested (3 or 6 months after implantation); for details, see cases 1, 2, 3, and 4. Two patients in this group were chronic (cases 5 and 6). The first chronic patient, who had an ASIA grade of A and who was implanted at 2 months after injury, has not improved (see case 5). The second patient, with an ASIA grade of C, was implanted at 17.5 months after injury and has shown an improved ASIA motor score as well as MEPs and SEPs (see case 6).

The second group of patients ($n = 14$) received BMMCs intravenously. Patient characteristics (cases 7–20) are shown in Table 2. In this group, only one patient, who received BMMCs relatively early after injury (10 days after injury), showed an improved ASIA score as well as electrophysiology results (see case 7, Fig. 1). The other subacute patients, who were implanted later at 21, 30, or 33 days after injury ($n = 3$), have not improved (see cases 7, 8, and 9, Fig. 1). The remainder of the patients ($n = 10$) were chronic, and none of these significantly improved their ASIA or Frankel score after the IV administration of BMMCs. Table 3 shows the amplitude and latency of MEPs and SEPs in those patients in whom we found some change.

Case 1

A 25-year-old male sustained a C6 vertebral body fracture in a motorcycle accident, with fragments dislocated into the vertebral canal. Neurological examination revealed a complete SCI at C6 with no motor functions preserved below the level of injury; no tactile or painful stimuli were recognized by the patient below the Th4 dermatome (ASIA impairment grade A, Frankel grade B). MRI showed a contusion at the C6/7 level, edema/gliosis from C6.1 to C7.2. SEPs were elicited only from both median nerves, and MEPs registered only in both biceps brachii muscles. The patient underwent C6 corpectomy with Harms cage replacement and C5–7 fixation. BMMC implantation was performed intra-arterially 11 days after SCI. Three months after implantation, SEPs were also elicited from the right tibial nerve. Sensory functions were continually improving during follow-up, and at 12 months the patient reported almost completely normal tactile sensation in all dermatomes (ASIA impairment grade B, Frankel grade B). SEPs were elicited from both the left and right tibial nerves, while the MEPs remained unchanged. MRI performed 58 days postimplantation of BMMCs showed gliosis of

Table 1. Characteristics of Patients With Intra-Arterial BMMC

Case No.	Age (Gender)	Level of SCI	Location of Edema (MRI Evaluation)	Time of BMMC After SCI	ASIA Classif. Before BMMC		Time of 1st Control After BMMC	Improvement of SEP/MEP	ASIA Classif. 1st Control After BMMC			ASIA Classif. 2nd Control After BMMC			ASIA Classif. 3rd Control After BMMC		
					(MS/PPS/ LTS)	(MS/PPS/ LTS)			(MS/PPS/ LTS)	(MS/PPS/ LTS)	(MS/PPS/ LTS)	(MS/PPS/ LTS)	(MS/PPS/ LTS)	(MS/PPS/ LTS)	(MS/PPS/ LTS)	(MS/PPS/ LTS)	(MS/PPS/ LTS)
1	25 years (M)	C6	C6.1–C7.2	11 days	A 24/30/30	56 days	y/n		A 26/48/48	6 months	y/n	B 34/86/84	12 months	y/n	B 28/69/111		
2	21 years (M)	C4	C2.1–C7.2	18 days	A 0/19/16	48 days	n/y			6 months	n/y	A 8/29/38	12 months	n/y	A 3/24/35		
3	29 years (F)	C5		22 days	A 8/10/10	3 months	y/n		A 12/16/15/	6 months	y/n	A 12/15/16	12 months	n/n	A 9/16/16		
4	41 years (M)	C6	C6.1–C7.1	30 days	B 35/64/64	48 days	y/n		D 63/78/78	6 months	y/n	D 93/78/78	12 months	y/n	D 93/78/78		
5	38 years (M)	Th9	Th8.1–Th8.3	2 months	A 50/67/67	3 months	n/n		A 52/68/58	6 months	n/n	A 54/64/64	12 months	n/n	A 51/64/64		
6	39 years (F)	C5	C5.1–C5.3	17 months	C 22/64/64	3 months	n/n		C 41/64/64	6 months	y/y	C 35/64/64	12 months	y/y	C 36/64/64		

A summary of the neurological and electrophysiological assessments of patients who received BMMCs intra-arterially via catheterization of a vertebral. The time of BMMC implantation ranged from 11 days to 17 months after SCI. All patients, except for case 5, improved in sensory and/or motor function. A marked increase in the light touch score (LTS) and pin prick score (PPS) was seen in patient 1, who also improved in the ASIA impairment scale from A to B. Motor score (MS) markedly increased in patient 4, with an improvement in the ASIA impairment scale from B to D. ASIA scores that showed improvement compared to pretransplantation scores are indicated in bold.

17 mm; at 6 and 12 months the gliosis was only 14 mm. However, motor functions did not markedly improve.

Case 2

A 21-year-old male suffered an undislocated fracture of the C4 vertebral body after he dove into shallow water. MRI showed a spinal cord contusion at C3–5, and neurological examination confirmed a complete SCI at the C4 level with a large edema/gliosis from C2.1 to C7.2., with no motor or sensory functions preserved below the level of injury (ASIA impairment grade A, Frankel grade A).

The patient underwent fusion of C3–5 with anterior fixation. No SEPs or MEPs were elicited. BMMC implantation was performed intra-arterially 18 days after SCI. Ten weeks after implantation, active movement was noticed (gravity eliminated) in the right elbow flexors, and MEPs were registered in the right biceps brachii muscle. During further follow-up, an improvement in sensory functions was observed: the patient was able to feel tactile and painful stimuli in the Th2–Th6 dermatomes (ASIA impairment grade A, Frankel grade B). MRI performed 8 days postimplanta-

Table 2. Characteristics of Patients With IV BMMC

Case No.	Age (Gender)	Level of SCI	Location of Edema (MRI Evaluation)	Time of BMMC After SCI	ASIA Classif. Before BMMC		Time of 1st Control After BMMC	Improvement of SEP/MEP	ASIA Classif. 1st Control After BMMC			ASIA Classif. 2nd Control After BMMC			ASIA Classif. 3rd Control After BMMC		
					(MS/PPS/ LTS)	(MS/PPS/ LTS)			(MS/PPS/ LTS)	(MS/PPS/ LTS)	(MS/PPS/ LTS)	(MS/PPS/ LTS)	(MS/PPS/ LTS)	(MS/PPS/ LTS)	(MS/PPS/ LTS)	(MS/PPS/ LTS)	(MS/PPS/ LTS)
7	36 years (M)	C6	C2.3–Th1.2	10 days	A 10/32/34	3 months			no control	6 months	n/n	A 17/77/77	12 months	y/n	A 19/78/78		
8	40 years (M)	Th11	Th11.1	21 days	A 50/70/70	3 months	n/n		A 50/70/70	6 months	n/n	A 50/70/70	12 months	n/n	A 50/70/70		
9	21 years (M)	Th9	Th7.1–Th9.2	30 days	A 50/63/63	3 months	n/n		A 50/62/63	6 months	n/n	A 50/64/64	12 months	n/n	A 50/64/64		
10	41 years (M)	Th8	Th8.2–Th9.2	33 days	A 50/64/64	3 months	n/n		A 50/64/65	6 months	n/n	A 50/64/64	12 months	n/n	A 50/64/64		
11	24 years (F)	Th4	Th3.1–Th3.2	43 days	A 50/44/44	3 months	n/n		A 50/44/44	6 months	n/n	A 50/44/44	12 months	n/n	A 50/44/44		
12	37 years (M)	Th6		22 months	A 50/56/56	3 months	n/n		A 50/52/52	6 months	n/n	A 50/52/52	12 months	n/n	A 50/52/52		
13	30 years (M)	Th4		2 months	A 50/42/42	3 months	n/n		A 50/42/42			died (suicide)					
14	26 years (M)	C5	C4.2–C5.1	2 months	B 3/62/62	3 months			B 3/62/62	6 months	n/n	B 8/62/62					
15	23 years (M)	C6		4 months	B 20/66/66	3 months	n/n		B 24/68/68	6 months	n/n	B 22/64/64					
16	34 years (M)	C6		6 months	A 26/28/32	3 months	n/n		A 26/28/32	6 months	n/n	A 25/23/23	12 months	n/y	A 25/23/23		
17	27 years (M)	C5		8 months	A 9/28/28	3 months	n/n		A 8/34/34	6 months	y/n	A 9/28/28	12 months	n/n	A 9/28/28		
18	19 years (M)	Th8		9 months	A 50/64/64	3 months	n/n		A 50/64/64	6 months	n/n	A 50/64/64	12 months	n/n	A 50/64/64		
19	26 years (M)	C5		9 months	A 8/30/30	3 months	n/n		A 12/30/30	6 months	n/n	A 8/28/32	12 months	n/n	A 8/22/22		
20	24 years (F)	C6	C4.1–C5.2	15 months	B 11/64/65	3 months	n/n		B 11/64/64	6 months	n/n	B 8/64/64	12 months	n/n	B 9/64/64		

A summary of the neurological and electrophysiological assessments of patients who received BMMCs intravenously. The time of BMMC implantation ranged from 10 days to 15 months after SCI. Only patient 7 improved in sensory and motor functions; the ASIA impairment grade, however, remained unchanged. Other patients, during follow-up, did not improve their motor or sensory score. ASIA scores that showed improvement compared to pretransplantation scores are indicated in bold.

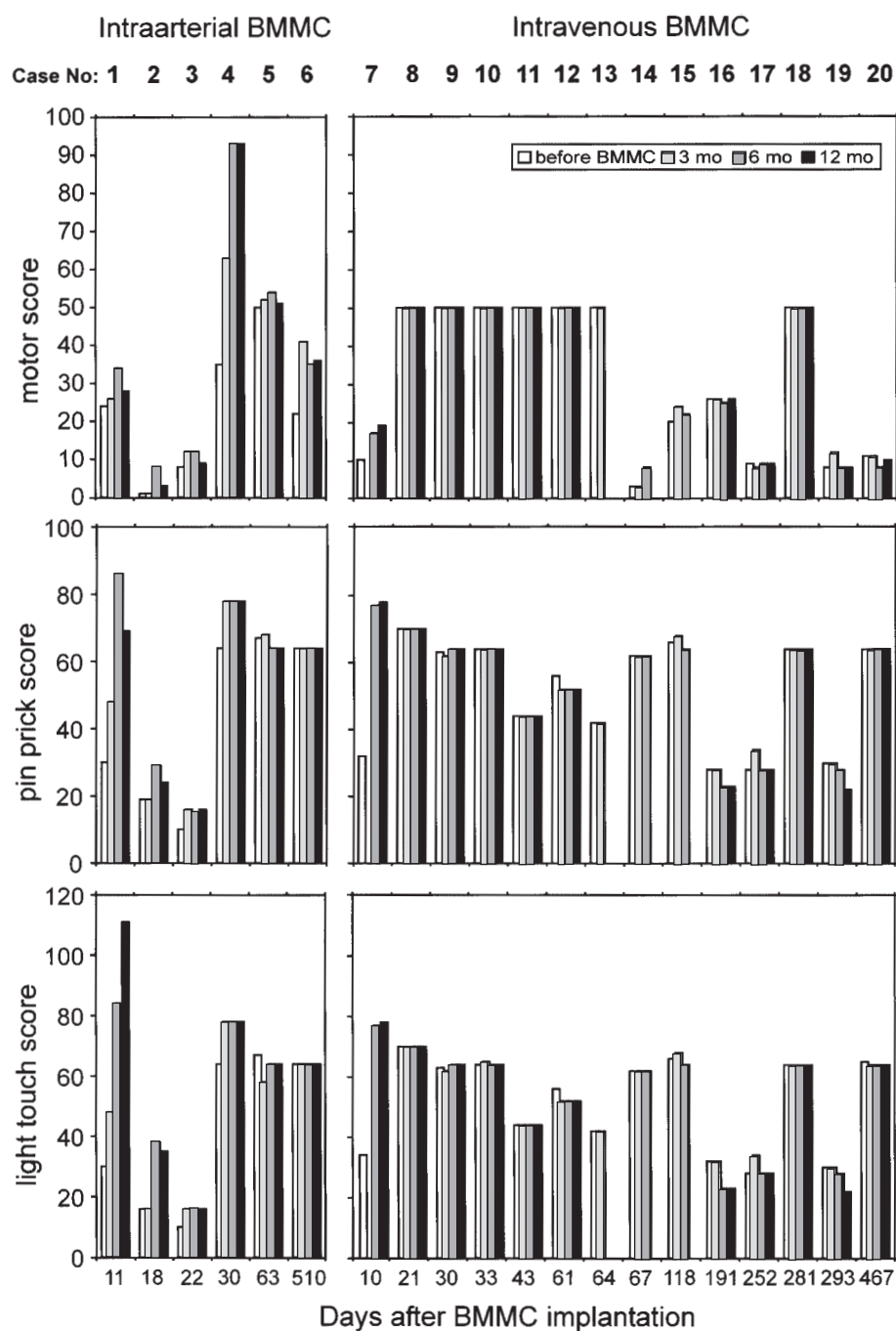


Figure 1. Neurological evaluation of 20 patients according to the ASIA protocol before and 3, 6, and 12 months after BMMC implantation. Individual patients, shown as case numbers 1–20, were divided into two groups based on the route of BMMC administration, then ordered within each group according to the time between SCI and BMMC implantation. Motor score improved in all acute patients and in one chronic patient who received BMMCs via a. vertebralis (cases 1, 2, 3, 4, and 6). Motor score was also improved in one acute patient who received BMMCs intravenously (case 7). Sensory functions were markedly improved in one patient with intra-arterial (case 1) and one with IV (case 7) BMMC implantation.

Table 3. Electrophysiological Characteristics of Patients With Changes in MEPs or SEPs During Follow-up

Case No.	SEP	MEP	Before BMMC				1st Control After BMMC				2nd Control After BMMC				3rd Control After BMMC			
			Latency		Amplitude		Latency		Amplitude		Latency		Amplitude		Latency		Amplitude	
			Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
1	TBN		-	-	-	-	36.8	-	0.4	-	47.2	-	0.5	-	48.0	45.2	0.82	0.23
	MDN		20.3	20.5	1.7	0.9	21.5	21.6	0.6	2.7	20.3	20.3	1.04	2.6	21.6	21.1	2.7	4.1
	BB		13.3	13.3	4.1	0.9	13.0	12.3	6.8	7.0	12.1	12.9	5.8	3.8	12.4	11.3	12.2	8.1
	ADQ		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	VM		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	TA		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	TBN		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	MDN		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	BB		-	-	-	-	-	13.2	-	0.4	-	12.3	-	0.3	-	16.9	-	1.0
	ADQ		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	VM		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	TA		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	TBN		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	MDN		22.8	-	1.4	-	22.6	21.4	1.4	0.6	25.0	25.9	0.16	0.6	-	-	-	-
	BB		11.3	13.3	0.3	0.2	12.0	11.9	1.6	1.3	11.4	11.1	2.0	1.9	11.2	12.3	5.0	5.4
	ADQ		-	-	-	-	-	16.7	-	0.2	27.7	22.7	0.2	0.2	20.1	-	0.2	-
	VM		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	TA		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	TBN		-	-	-	-	-	50.1	-	0.3	45.3	45.3	0.5	0.7	47.3	45.2	0.3	0.5
	MDN		22.0	21.0	0.9	1.3	21.9	20.0	0.5	0.8	20.3	21.5	0.5	1.5	21.0	21.9	1.2	1.9
	BB		12.2	12.6	4.2	2.1	13.8	10.6	3.8	2.6	11.4	13.8	1.6	3.6	11.5	14.2	11.5	4.4
	ADQ		20.6	21.0	0.3	0.4	-	19.9	-	1.0	21.6	23.1	1.9	1.8	21.1	22.7	2.5	4.8
	VM		-	25.0	-	0.6	32.0	25.0	0.4	0.3	-	-	-	-	-	-	-	-
	TA		-	40.6	-	0.8	-	38.4	-	1.0	-	32.2	-	0.7	-	-	-	-
6	TBN		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	MDN		-	-	-	-	-	-	-	-	-	19.8	-	1.0	26.7	23.3	2.4	0.9
	BB						10.5	9.7	2.2	6.2	11.8	10.8	6.5	6.9	12.7	10.6	2.2	5.7
	ADQ						27.5	-	4.1	-	23.8	22.1	4.2	0.4	25.8	28.6	1.0	0.1
	VM						-	-	-	-	-	-	-	-	-	-	-	-
	TA						-	-	-	-	-	-	-	-	-	-	-	-
7	TBN		-	51.2	-	0.5					-	51.4	-	0.4	44.9	51.4	0.3	0.4
	MDN		20.9		20.8	0.5	0.8				20.9	-	1.2	-	21.6	-	1.2	-
	BB		13.6	12.3	5.8	6.1					10.6	11.1	10.3	18.8	11.1	11.0	11.0	12.3
	ADQ		-	-	-	-					-	-	-	-	-	-	-	-
	VM		-	-	-	-					-	-	-	-	-	-	-	-
	TA		-	-	-	-					-	-	-	-	-	-	-	-
16	TBN		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	MDN		19.9	20.4	0.8	1.3	20.1	20.3	1.5	1.9	19.9	20.6	1.4	1.4	20.5	20.1	0.5	1.4
	BB		11.9	10.7	4.2	4.2	13.7	16.3	6.1	4.2	11.4	13.5	9.2	7.0	11.2	12.0	6.8	6.8
	ADQ		-	-	-	-	-	-	-	-	-	-	-	-	31.5	-	0.7	-
	VM		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	TA		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

A detailed summary of the latencies and amplitudes of MEPs and SEPs from patients who showed improved electrophysiological parameters during follow-up. MEPs were elicited by transcranial magnetic stimulation and responses were registered by surface electrodes placed over the biceps brachii (BB), abductor digiti quinti (ADQ), tibial anterior (TA), and medial vastus (VM) muscles. SEPs were elicited by electrical stimulation of the tibial (TBN) and median (MDN) nerves. Dashes indicate that stimulation was performed but no evoked potentials were elicited. Empty cells indicate that stimulation was not performed.

tion of BMMCs showed edema/gliosis of 56 mm; at 3 months the gliosis was only 33 mm and at 12 months only 29 mm.

Case 3

A 29-year-old female suffered a C5 and C7 vertebral body fracture with a complete SCI at the C5 level in a car accident, with no motor or sensory functions preserved below the level of injury (ASIA impairment grade A, Frankel grade A). The patient underwent C5 and C7 corpectomy with subsequent interbody fusion using a tricortical graft and anterior C4–Th1 fixation. MRI showed a large contusion at the C4/5 level. Initial SEPS were elicited only from both median nerves, while MEPs were registered only in both biceps brachii muscles and the right adductor digiti quinti muscle. BMMC implantation was performed intra-arterially 22 days after SCI. Six months after implantation, there were newly registered MEPs in the left adductor digiti quinti muscle. Nevertheless, neurological examination showed only very slight improvement (ASIA impairment grade A, Frankel grade A).

Case 4

A 41-year-old male after a car accident was diagnosed with an incomplete SCI at the C6 level. There was lower extremity paraplegia and upper extremity paraparesis; tactile and pain sensation was decreased below C6 (ASIA impairment grade B, Frankel grade B). Initial MRI showed ventral luxation of the C6 vertebrae compressing the spinal cord and edema at C6.1–C7.2 (29 mm long). The patient was admitted to the neurosurgery department where he underwent repositioning of C6 with posterior C6–7 fixation. BMMC implantation was performed intra-arterially 30 days after SCI. SEPs were elicited only from both median nerves; MEPs were registered in all target muscles except for those in the left lower extremity. An increase in strength in all key muscles in both the upper and lower extremities was observed at 10 weeks after BMMC implantation (ASIA impairment grade D, Frankel grade D). The patient was able to walk using a walker. In SEP testing, an EP from the right tibial nerve was newly registered (Fig. 2). At 6 months after implantation there was further improvement in the motor score, and SEPS were elicited from both median and tibial nerves, suggesting the functional reintegration of the dorsal columns; the patient was able to walk without a walker. The last follow-up at 12 months was identical in terms of motor and sensory indices as that conducted at 6 months. MRI at 29 days post-implantation showed edema/gliosis only 8 mm long. The last MRI at 6 months showed a small posttraumatic pseudocyst and gliosis at the C6/7 level (Fig. 3).

Case 6

A 39-year-old female suffered an incomplete SCI at the C5 level after being assaulted. Initially, there was tactile hypesthesia and hypalgesia below C4 and lower extremity paraplegia, while on the upper extremities only contraction in the right biceps brachii muscle was observed (3/5). MRI showed C5 listesis narrowing the vertebral canal and a spinal cord contusion at the C5 level. The patient underwent repositioning with anterior C4/6 fixation and C4/5 discectomy. BMMC implantation was performed intra-arterially 17.5 months after SCI. Neurological examination prior to implantation showed active movement, gravity eliminated, in almost all the key muscles of the upper extremities and the left lower extremity. There were no SEPs elicited, and MEPs were registered only in both biceps brachii and right adductor digiti quinti muscles. There was plegia of the right lower extremity (ASIA impairment grade C, Frankel grade C). During the 12 months of follow-up, increased strength was observed in all key muscles of the upper extremities except for the right finger flexors and abductors; the motor score for the lower extremities remained unchanged. MRI performed 1 day before and 3 months and 6 months after implantation showed gliosis of 13 mm, 12 mm, and 12 mm, respectively. Twelve months after BMMC implantation, SEPs were newly elicited from the right median nerve and MEPs registered in the right adductor digiti quinti muscle.

Case 7

A 36-year-old male sustained a C5 vertebral body fracture after he accidentally fell while disembarking from a canoe. Neurological examination showed a complete SCI at the C6 level with lower extremity paraplegia and upper extremity paraparesis; no sensory functions were preserved below the Th5 dermatome (ASIA impairment grade A, Frankel grade B). MRI showed an extensive spinal cord contusion at the C4–6 levels (edema from C2.3 to Th1.2, 80 mm long). SEPs were elicited from both median nerves and the right tibial nerve; MEPs were registered only in both biceps brachii muscles. The patient underwent partial C5 corpectomy with subsequent fusion using a tricortical graft and C4–7 fixation. BMMC implantation was performed intravenously 10 days after SCI. This patient refused to be tested at 3 months after implantation. At 6 months after implantation, active movement against gravity was observed in both wrist extensors and palpable contractions in both elbow extensors. Both tactile and painful stimuli were newly recognized by the patient in the Th6–L1 dermatomes. At the 12-month follow-up examination, SEPs were elicited from both median and tibial nerves (ASIA impairment grade A, Frankel B).

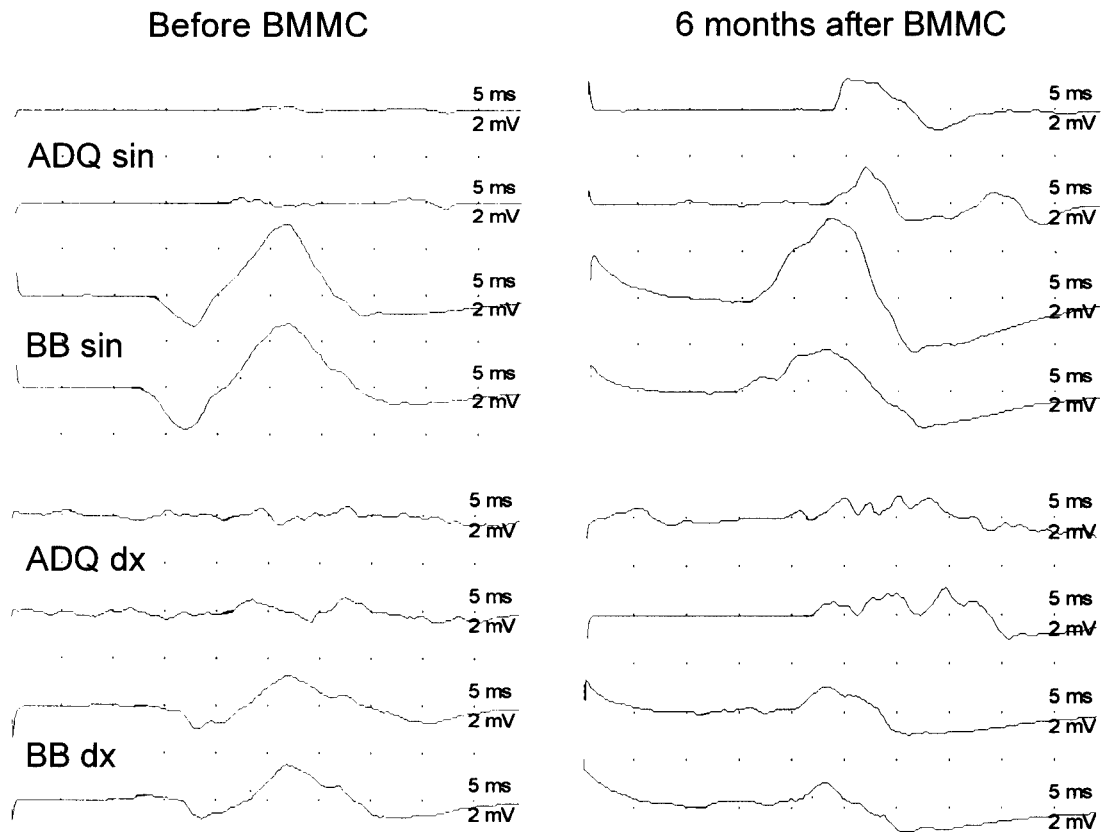


Figure 2. Motor evoked potentials from the right (dx) and left (sin) upper limb before (A) and 6 months after (B) BMMC implantation (case 4). Transcranial magnetic stimulation at the cortical level for target muscles in the upper limbs elicited responses in both the abductor digiti quinti (ADQ) and the biceps brachii (BB) muscles bilaterally before BMMC implantation and 6 months after. The recordings from ADQ muscles showed increased response amplitudes bilaterally 6 months after BMMC implantation. These findings confirm some degree of improvement in spinal cord function at the C8 level.

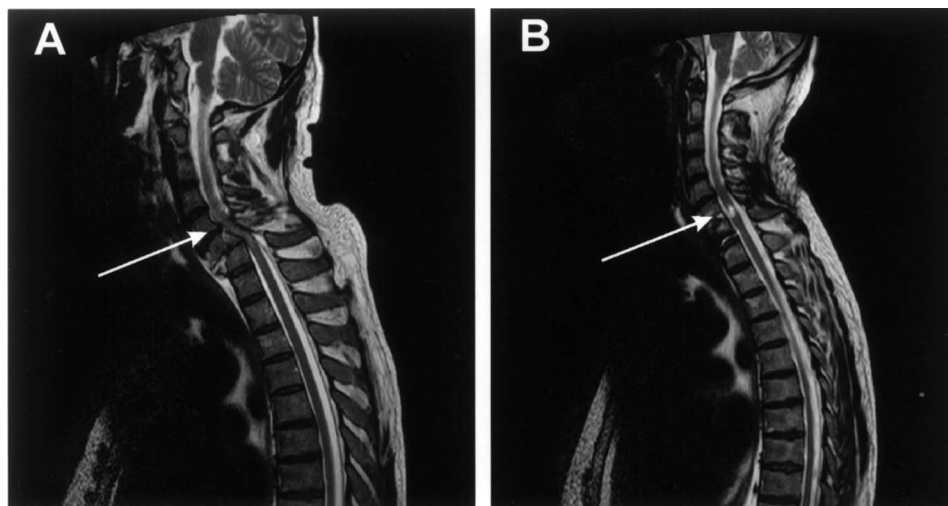


Figure 3. MR images before (A) and 6 months after (B) BMMC implantation (case 4). The image taken at the time of admission to the neurosurgery department shows a ventral shift of the C6 vertebrae compressing the spinal cord and edema at the C6–C7 level. At this level, 6 months after BMMC implantation, the MR image shows a small posttraumatic cavity and gliosis.

Case 8

A 40-year-old male was diagnosed with a complete SCI at the Th11 level after a car accident, with no motor or sensory functions preserved below the level of injury (ASIA impairment grade A, Frankel grade A). MRI showed a Th11 vertebral body fracture dislocated into the vertebral canal and a spinal cord contusion at the Th11 level. The patient underwent Th11 corpectomy with Synex cage replacement and Th9–12 fixation. No MEPs or SEPs were elicited in lower limbs. BMMC implantation was performed intravenously 21 days after SCI. No improvement was noticed during 12 months of follow-up.

Case 9

A 21-year-old male developed flaccid paraplegia of the lower extremities after a bicycle accident. Neurological examination showed a complete SCI at the Th9 level with no motor or sensory functions preserved below the level of injury (ASIA impairment grade A, Frankel grade A). MRI showed a fracture of the Th9 vertebral body dislocated into the vertebral canal and a large contusion of the spinal cord at the Th8/9 level (edema from Th7.1 to Th9.2, about 50 mm long). No MEPs or SEPs were elicited in the lower limbs. The patient underwent repositioning with posterior Th8–10 fixation. BMMC implantation was performed intravenously 30 days after SCI. MRI at 3 months showed edema/gliosis 44 mm long. No sensory or motor improvement was noticed during 12 months of follow-up.

Case 10

A 41-year-old male was diagnosed with a complete SCI at the Th8 level after a car accident, with no motor or sensory functions preserved below level of injury (ASIA impairment grade A, Frankel grade A). A CT scan showed a Th9 vertebral body fracture dislocated into the vertebral canal. The patient underwent a Th9 laminectomy and Th8–10 fixation. MRI showed a spinal cord contusion at the Th8/9 level (edema/gliosis 24 mm long). No MEPs or SEPs were elicited in the lower limbs. BMMC implantation was performed intravenously 33 days after SCI. No sensory or motor improvement was noticed during 12 months of follow-up.

DISCUSSION

Satisfactory outcomes have not been achieved to date in treating complete SCI by means of a single approach. A number of studies report a poor prognosis; only about 5–6% of patients with complete SCI (ASIA grade A) improve after 1 year (6,30). Spinal cord injury represents a complex event (44), and therefore effective therapeutic strategies will consist of a series of interventions. First, secondary tissue loss should be prevented

through early neuroprotective, anti-inflammatory, or immunomodulatory interventions. Subsequently, strategies to promote the regrowth of axons and the restoration of function will involve multiple approaches: reducing scar formation, overcoming additional inhibitory molecules, stimulating damaged nerve cells to regenerate axons, facilitating axonal growth across the site of injury, and enabling the formation of new connections.

Postnatal bone marrow has traditionally been seen as an organ composed of two main systems rooted in distinct lineages: the hematopoietic tissue proper and the associated supporting stroma—marrow stromal cells. Unlike hematopoietic stem cells, whose role in the treatment of hematopoietic diseases has been known for a long time (23), MSCs were originally examined only because of their critical role in the formation of the hematopoietic microenvironment. More recent data led to the recognition that MSCs are stem/progenitor cells of ectodermal, mesodermal, and endodermal tissues [for review, see (32)]. Their potential to differentiate into non-hematopoietic organ cells granted them membership in the family of somatic stem cells. Besides the neuronal protective role of MSCs, hematopoietic stem cells also foster neuroprotection (8). There is little doubt that bone marrow stem cells represent one of the most accessible sources of stem cells for therapeutic use. The ease with which they are harvested and the simplicity of the procedures required for their extensive growth in culture, together with easy expansion *in vitro*, may make them ideal candidates.

In our preclinical study in rats, a balloon-induced compression SCI was treated with MSCs, and improved motor scores as well as sensory function were found when the cells were implanted at 7 days postinjury (20,41,42). Similarly, improvement was also found in the same model of SCI when all mononuclear cells were implanted, as was done in the present clinical study, or even when bone marrow was mobilized by granulocyte colony stimulating factor (Neupogen, G-CSF) (45). However, the question of which cell type is most beneficial for SCI treatment is still unresolved as are the mechanisms underlying the beneficial effect(s). One possible effect of cell therapy is “replacement,” meaning that the grafted cells integrate into the host tissue and replace damaged or lost cells. Several studies have been performed using *in vitro* expanded neural stem/progenitor cells, which were then implanted into injured rat or marmoset spinal cord. The cells survived and differentiated into neurons, astrocytes, and oligodendrocytes and had a positive effect on functional outcome (18,33,35). Similarly, MSCs can also differentiate into neuron-like cells and glia (3,5,14,19,32,34,38,50). In our preclinical study (19), we injected MSCs into rats with a cortical photochemical lesion and studied the differentiation of the

grafted cells. We found that only a few (<5%) BrdU-labeled MSCs expressed the neuronal marker NeuN, and we did not find any BrdU-labeled MSCs expressing the astrocytic marker GFAP.

Besides replacement, there are several other possible explanations why MSCs can be useful in the treatment of SCI. A number of studies have described MSCs as cells that express factors beneficial to the nervous tissue or that activate compensatory mechanisms and endogenous stem cells within the tissue following their migration into an injured environment. MSCs secrete cytokines such as colony stimulating factor (CSF), interleukins, stem cell factor (SCF) (13,29), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), hepatocyte growth factor (HGF), and vascular endothelial cell growth factor (VEGF) (4). It has also been reported that MSCs stimulate glial cells to produce neurotrophic factors such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) (28,29,46). MSCs can promote axonal regeneration by guiding nerve fibers (16). Wu and coworkers showed that transplanted MSCs promote compensatory mechanisms to reorganize neural networks and activate endogenous stem cells (51). It was also shown that MSCs improve neurologic deficits by generating either neural cells or myelin-producing cells (9,39). Understanding the actual differentiation spectrum of MSCs and the mechanism of their beneficial role in CNS injury requires further investigation. Nevertheless, studies of MSCs transplanted into different models of CNS injury (1,9,16,27,45) have provided considerable evidence about their potential to improve functional outcome.

Although these studies indicate that MSCs are more effective in the treatment of SCI, there are several good reasons supporting the use of BMMCs, which include hematopoietic stem cells, macrophages, and lymphocytes, as well as marrow stromal cells, in SCI therapies. One reason is that the identities of the subpopulations responsible for neuronal differentiation remain unknown. Second, the neuronal protective roles of not only MSCs, but also of hematopoietic stem cells, are well known (7,8). Hematopoietic stem cells secrete many cytokines, including thrombopoietin and interleukin L1 (11,31). These cytokines are known to be essential factors for the survival and differentiation of neuronal progenitor cells.

In our clinical study with BMMC implantation, we found partial functional (motor and sensory) improvement in subacute patients, which corresponds well with the results of preclinical studies in rats and nonhuman primates (2,18,39). Even when we observed an improvement in the ASIA and Frankel scores, accompanied by enhanced MEPs and SEPS during electrophysiological testing, the improvement was generally only from the

A to B grade; however, in one patient implanted intra-arterially, improvement was seen from B to D in the ASIA scale and from B to D in the Frankel scale (see case 4). Interestingly, improved function was observed in 5 out of 6 patients who received BMMCs close to the injury site (i.e., by catheterization of a. vertebralis). This is in agreement with the small recent clinical study performed by Park and colleagues (37) on 6 patients with SCI, which showed improvement after treatment with BMMCs implanted intraspinally within 7 days postinjury. Besides direct implantation to the injury site, these authors used a combination of autologous BMMC implantation and subsequent repetitive mobilization of bone marrow cells with granulocyte macrophage-colony stimulating factor (GM-CSF). This treatment resulted in improved motor and/or sensory function in 5 out of 6 patients (37).

Our phase I/II clinical study shows that the implantation of autologous BMMCs is safe, but we cannot yet confirm that the observed beneficial effects were due to the cell therapy. Currently, 6 patients have remained free of any adverse side effects for more than 30 months following implantation and a further 5 patients for more than 24 months; however, longer follow-ups of more patients are required to determine whether BMMC implantation is definitively safe. It is out of the scope of this article to discuss the contribution of spinal shock, although we are aware that its disappearance contributes to functional recovery [for review, see (12)]. However, the outcome from BMMC implantation in acute patients, and in one chronic patient who was in stable condition for several months prior to cell implantation, is promising. It is evident that the therapeutic window will play an important role in any type of SCI treatment. There seems to be a similar therapeutic window in humans as in animals, which is up to 3–4 weeks after SCI. In view of our study, we suggest that administering the cells closer to the injury site, such as through the catheterization of a. vertebralis, or into the cerebrospinal fluid (34), or even intraspinally at the lesion border (37), might be important for a better outcome. The observed partial recovery might be attributable to a “rescue effect,” a reduction in tissue loss from secondary injury processes, as well as to diminished glial scarring.

Clinical studies are necessary for transferring preclinical findings from animal experiments to humans. The therapeutic window, the implantation strategy, the method of administration, the number of cells, and the possible side effects can only be tested in human clinical trials. In our study, we included all patients who agreed to be involved in the study. Obviously, in the case of complete and large lesions, cells alone are not able to repair the tissue. It is necessary to bridge the gap left by the lost cell population in order to provide support for tissue res-

toration, reduce the glial scar and the deposition of extracellular matrix proteins, particularly chondroitin sulphate proteoglycans, and create a permissive environment for cellular ingrowth. BMMCs can partially serve as bridging material; however, their number is not sufficient to bridge larger defects. Biocompatible polymer hydrogels, based on pHEMA or pHPMA, have viscoelastic and adhesive properties that promote their rapid integration at the host–tissue boundary (24,25,47–49). Their macromolecular network provides mechanical cues that stimulate the ingrowth of cells. Water present within the network provides free space for the diffusion of host tissue extracellular fluids containing trophic and growth factors released by neighboring cells. In the future, the chemical and physical properties of hydrogels could be tailored to a specific use, and the gels could be seeded with different types of stem cells to create cell–polymer constructs.

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