Spinal Cord (2011) 49, 967–973
 1362-4393/11 $32.00
www.nature.com/sc

ORIGINAL ARTICLE

Intrathecal transplantation of stem cells by lumbar puncture for thoracic spinal cord injury in the rat

AJ Mothe[1], G Bozkurt[1], J Catapano[1], J Zabojova[1], X Wang[2], A Keating[2] and CH Tator[1]

[1]*Toronto Western Research Institute and Krembil Neuroscience Centre, Toronto Western Hospital, Toronto, Ontario, Canada and* [2]*Cell Therapy Program, Princess Margaret Hospital, Ontario Cancer Institute, University Avenue, Toronto, Ontario, Canada*

Study design: Experimental investigation of intrathecal transplantation of stem cells by lumbar puncture (LP) in a rat model that simulates human thoracic spinal cord injury (SCI).
Objectives: To examine the distribution and phenotype of spinal cord-derived neural stem/progenitor cells (NSPCs) and bone marrow-derived mesenchymal stromal cells (BMSCs) following LP transplantation in SCI rats.
Setting: Toronto Western Research Institute, Toronto, Ontario, Canada.
Methods: NSPCs or BMSCs were transplanted via LP at level L3–5 1 week after compression SCI at T8. Rats were killed at 3, 17 and 27 days after LP transplantation and the relative distribution of cells at C4, T8 and L3–5 was quantitated. The phenotype of the NSPC and BMSC was assessed with immunocytochemistry *in vitro* and following LP transplantation.
Results: By 4 weeks, more NSPC migrated to the lesion site relative to BMSC and uninjured animals. However, there was no preferential homing of either of these types of cells into the parenchyma of the injury site, and most of the transplanted cells remained in the intrathecal space. *In vitro*, spinal cord-derived NSPC proliferated and expressed nestin, but after LP transplantation, NSPC became post-mitotic and primarily expressed oligodendrocyte markers. In contrast, BMSC did not express any neural antigens *in vivo*.
Conclusion: LP is a minimally invasive method of cell transplantation that produces wide dissemination of cells in the subarachnoid space of the spinal cord. This is the first study to report and quantify the phenotype and spatial distribution of LP transplanted NSPC and BMSC in the intact and injured spinal cord.
Spinal Cord (2011) **49**, 967–973; doi:10.1038/sc.2011.46; published online 24 May 2011

Keywords: neural stem/progenitor cells; bone marrow-derived mesenchymal stromal cells; lumbar puncture transplantation; spinal cord injury

Introduction

Stem cell transplantation is a promising therapy for traumatic spinal cord injury (SCI) and degenerative conditions of the CNS. In most experimental studies, stem cells have been injected directly into the lesion site. However, the additional trauma from intramedullary transplantation can further compromise injured tissue, and impede clinical translation. A potential alternative is intrathecal transplantation via lumbar puncture (LP), a method that is frequently used in humans[1] but it has received minimal experimental investigation.[2–4] The intrathecal technique of cell injection has also been used in other animal models.[5–7] However, the phenotype of LP transplanted neural stem/progenitor cells (NSPCs) and bone marrow-derived mesenchymal stromal cells (BMSCs) and their spatial distribution along the intact and injured spinal cord has not been reported or quantitated. LP is a minimally invasive method of cell delivery, and stem cells may be well suited for LP transplantation because of their responsiveness to signals from the injured CNS.[2,4,8] Also, cell transplantation via LP may be relevant for conditions such as multiple sclerosis with widely disseminated lesions making intramedullary transplantation impractical.

NSPC reside in the adult mammalian CNS, including the spinal cord.[9] These cells have the ability to self-renew and are multipotential for both neurons and glia. NSPC derived from the periventricular region of the adult spinal cord can be cultured as multipotent, self-renewing neurospheres.[10,11] We have previously demonstrated that adult rat spinal cord NSPCs have a propensity for oligodendrocytic differentiation both *in vitro* and *in vivo*, and can myelinate axons in the

Correspondence: Dr AJ Mothe, Toronto Western Research Institute and Krembil Neuroscience Centre, McL 12-423, 399 Bathurst St., Toronto, Ontario, Canada M5T 2S8.
E-mail: amothe@uhnres.utoronto.ca
Received 11 January 2011; revised 5 April 2011; accepted 9 April 2011; published online 24 May 2011

demyelinated and myelin-deficient cord.[12] We have also shown a neuroprotective function of spinal cord NSPC after subacute transplantation directly into the injured rat spinal cord.[13] The purpose of the present study was to examine the distribution and phenotype of adult rat spinal cord-derived NSPC following LP transplantation into the injured rat spinal cord. We also examined LP transplantation of rat BMSC, which have been shown to home to sites of injury.[2,14]

Materials and methods

NSPC culture

NSPCs were isolated from the spinal cords of adult transgenic Wistar rats expressing green fluorescent protein (GFP; Wistar-TgN(CAG-GFP)184ys; YS Institute Inc., Utsunomiya, Tochigi, Japan), as described previously.[10,12] Briefly, the rat cervical and thoracic spinal cord were excised under sterile conditions, and the overlying meninges, blood vessels and white matter were removed. The periventricular region including the ependyma and some gray matter tissue immediately surrounding the central canal were harvested, enzymatically dissociated and seeded in free-floating culture in chemically defined serum-free media containing 20 ng ml^{-1} of epidermal growth factor and fibroblast growth factor-2. Neurospheres were passaged every 7 days. Cells were assessed for self-renewal and multipotentiality as described previously.[10,12] Neurospheres were transplanted at passages 3 or 4 at 4–6 days *in vitro* after passage.

BMSC culture

BMSCs were cultured as initially described[15] and as we previously reported.[16] Briefly, bone marrow was collected from the femur and tibia of adult transgenic GFP Wistar rats described above and resuspended in long-term bone marrow culture medium. BMSCs were initially passaged after 2 weeks, and then in every 5–7 days. BMSCs were dissociated by trituration and transplanted at passages 4 or 5. BMSCs were characterized by the methods recommended by Dominici *et al*.,[17] including adherence to plastic, specific surface antigen expression and multipotentiality. Flow cytometry was used to ensure that the BMSCs were CD11 and CD45 negative.

Animals

Twenty-seven adult female wild-type Wistar rats (Charles River, St. Constant, Quebec, Canada; 200–300 g) were the recipients in this study. The animal protocols were approved by the Animal Care Committee of the Research Institute of the University Health Network in accordance with policies established by the Canadian Council on Animal Care. Rats with SCI (described below) were transplanted at 1 week post-SCI with NSPC or BMSC and killed at 3- ($n=6$), 17- ($n=4$) and 27-day($n=5$) post-transplantation. In the 17 and 27 days groups, a total of three animals were excluded because of poor placement of the LP needle in the subarachnoid space. Uninjured rats were similarly transplanted with NSPC or BMSC and killed at 3- ($n=6$) and 27-day ($n=6$) post-transplantation. Thus, half the rats in each group received either NSPC or BMSC transplants.

Compression SCI and LP transplantation

Rats were anesthetized by inhalation of 5% halothane, which was reduced to 2% during surgery, in combination with a mixture of nitrous oxide and oxygen (1:2, v/v). The spinal cord was exposed by laminectomy at the T8-T9 vertebral level. A clip compression injury was made with a 26 g-force (moderate injury) for 1 min according to the method of Rivlin and Tator,[18] a clinically relevant SCI model. The overlying muscle and skin were sutured with 3-0 Vicryl (Johnson and Johnson, Peterborough, Ontario, Canada). One week after SCI, rats were anesthetized as described above and placed prone, and transplanted intrathecally with NSPC or BMSC via LP. A small longitudinal incision was made over the L3–5 spinous processes and the skin was retracted, as described previously.[3,19] A neonatal 25-gauge LP needle was advanced into the spinal canal at L3–4 or L4–5. Proper placement of the needle was determined by the presence of cerebrospinal fluid (CSF) in the hub of the needle. The CSF present in the needle hub was aspirated with a micropipette, and 2×10^6 BMSCs or NSPCs diluted in 40 μl of culture medium followed by 10 μl of culture medium to flush the contents of the needle, was slowly injected into the intrathecal space. The LP needle was then left in place for 2 min to prevent backflow of injected contents, the stylet was replaced and the needle was slowly withdrawn, after which the skin was sutured with 3-0 Vicryl sutures. The uninjured rats received LP transplants in the same way. To aid transplant survival and to be consistent with our previous work,[20] all animals received cyclosporine (15 mg kg^{-1}, Sandimmune, Novartis, Dorval, Quebec, Canada) injected subcutaneously daily from the day of transplant until sacrifice.

Tissue preparation

Rats were deeply anesthetized with intraperitoneal sodium pentobarbitol and perfused with 4% paraformaldehyde in 0.1 M phosphate buffered saline as described previously.[12] Segments of spinal cord 6 mm in length were excised encompassing the lesion site at T8, the cervical region at C4 and the injection site between L3 and L5. The spinal cord segments were cryosectioned transversely into 20 μm serial sections to examine the distribution of transplanted cells following LP. The brain was also examined in sagittal orientation and in cross-section at the level of the lateral and fourth ventricles to examine whether transplanted cells would be carried along with the CSF flow into the subarachnoid space around the brain or into the ventricles of the brain.

Immunofluorescent staining

Tissue sections for fluorescence immunohistochemistry were rehydrated in 0.1 M phosphate buffered saline, blocked for 1 h, and incubated with primary antibodies overnight at 4 °C. For immunocytochemistry on cells *in vitro*, NSPCs or BMSCs were plated on Matrigel coated wells for a minimum of 2 h, and then fixed for 20 min with 4% paraformaldehyde in

0.1M phosphate buffered saline. The following primary antibodies were used: nestin (1:100; BD Biosciences Pharmingen, Mississauga, Ontario, Canada) for neural precursor cells, Ki67/MM1 (1:100; Novocastra Laboratories, Newcastle, UK) for proliferating cells, GFAP (1:200; Chemicon, Temecula, CA, USA) for astrocytes, NeuN (1:500; Chemicon) and mouse anti-βIII tubulin (1:500; Covance Research Products, Berkeley, CA, USA) for neurons, CC1/APC (1:1000; Calbiochem, San Diego, CA, USA) for oligodendrocytes, Olig2 (1:500; R&D Systems, Minneapolis, MN, USA) for oligodendrocyte lineage cells, fibronectin (1:800; Sigma, Oakville, Ontario, Canada) and vimentin (1:500; Sigma) for stromal cells, and GFP (1:200; Chemicon) for detecting the transplanted cells. Tissue sections or cells were then washed and incubated with fluorescent-conjugated secondary antibodies for 1 h at room temperature, washed with phosphate buffered saline and then coverslipped with Vectashield mounting medium containing DAPI (Vector Laboratories, Burlington, Ontario, Canada) nuclear counterstain, or incubated in Hoechst (Sigma). Species-specific non-immune immunoglobulin G and omission of primary antibody was used as negative controls. Immunofluorescent staining was examined using a Nikon Eclipse TE 300 microscope (Nikon, Mississauga, ON, Canada) and a Zeiss LSM 510 confocal microscope (Zeiss, Toronto, ON, Canada).

Quantitative and statistical analysis

For quantitative analysis of GFP^+ transplanted cells in the spinal cord, 30 transverse sections, 140 μm apart, were examined for GFP^+ cells containing a $DAPI^+$ nucleus throughout the length of each excised spinal cord segment from C4, T8 and L3–5 for each animal. As described previously,[12] cell counts were adjusted to compensate for the sampling frequency. Data for the 17 and 27 days time points were similar and were combined because there were lower numbers of animals in these groups because of improper needle placement as described above. To quantify the phenotype of the transplanted cells, we used confocal microscopy to count the number of GFP^+ double-labeled cells as a proportion of GFP^+ transplanted cells, as described previously.[20] All data are presented as mean ± s.e. Data were analyzed using SigmaStat v.3.11 software (Systat, Point Richmond, CA, USA). The relative proportion of GFP^+ cells at each site was analyzed using one-way analysis of variance and a pairwise multiple *post-hoc* comparison using the Bonferroni *t*-test. Statistical significance was determined at the $P<0.05$ level.

Results

LP transplantation

The rat spinal cord was injured by clip compression at T8. Figure 1 illustrates the absence of the T8 lamina after laminectomy and clip compression, and a grey area depicting the lesion cavity. One week later, GFP^+ were transplanted via LP with the needle between L3–5, as shown in Figure 1. The transplanted cells were found in the intrathecal space migrating towards the site of SCI. In some rats, transplanted cells also migrated into the spinal cord tissue surrounding the lesion cavity.

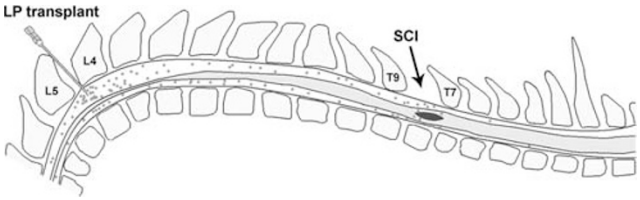

Figure 1 Schematic diagram of LP transplantation in a rat. The spinal cord shown in grey was injured at T8 indicated by the arrow and excised lamina. One week following SCI, GFP fluorescent cells indicated in green were transplanted into the intrathecal space at L4–5 via LP using a neonatal LP needle. Transplanted cells were distributed along the length of the spinal cord in the intrathecal space migrating towards the site of injury. Some cells also migrated into the parenchyma of the injured spinal cord. Very few cells were found rostral to the site of injury. A full color version of this figure is available at the *Spinal Cord* journal online.

Phenotype of NSPC and BMSC in culture

Spinal cord-derived NSPCs were cultured as free-floating neurospheres in proliferative growth medium supplemented with epidermal growth factor and fibroblast growth factor-2 (Figure 2a). Undifferentiated NSPC expressed high levels of nestin, a marker for neural precursor cells (Figure 2c). We have previously characterized spinal cord-derived NSPC and determined that undifferentiated NSPC express nestin (83%), and low levels of GFAP (0.4%), RIP (9%) and βIII-tubulin (0.5%).[10,12] Both NSPCs and BMSCs were generated from the same GFP transgenic Wistar rat strain and showed native GFP fluorescence (Figure 2b NSPC and Figure 2e BMSC). Nearly all BMSCs expressed high levels of fibronectin (Figure 2f) and vimentin (Figure 2g). Fewer BMSCs expressed nestin (Figure 2h), and neuronal expression was not detected (NeuN shown in Figure 2i). BMSC did not express other neural markers such as GFAP and CC1 (data not shown).

LP transplanted NSPC primarily express glial markers

Ki67 immunostaining was present in only 2.1% (± 0.9%) and 0.9% (± 0.4%) of the transplanted NSPC in either the injured or uninjured transplanted spinal cord at 3- and 17-day post-transplantation, respectively (Figure 3a). Nestin expression was downregulated to 11.9% (± 4.3%) by 27 days post-transplantation (Figure 3b). The majority of transplanted NSPCs (62.1 ± 2.9%) expressed CC1, a marker of mature oligodendrocytes (Figure 3f). NSPC also expressed Olig2, a transcription factor expressed by oligodendrocyte lineage cells (Figure 3e). Also, 31.2% (± 3.7%) NSPC differentiated into astrocytes (GFAP, Figure 3c), and neuronal differentiation was not evident (NeuN, Figure 3d).

BMSC do not express neural antigens in vivo

In comparison with NSPC, there was more proliferation of BMSC as shown by Ki67 immunostaining at 3-day post-transplantation in both the injured and uninjured spinal cords (Figure 3g, sham shown). At 3-day post-transplantation, 7.6% (± 3.8%) of the transplanted BMSCs showed Ki67 immunostaining. However, by 27 days post-transplantation,

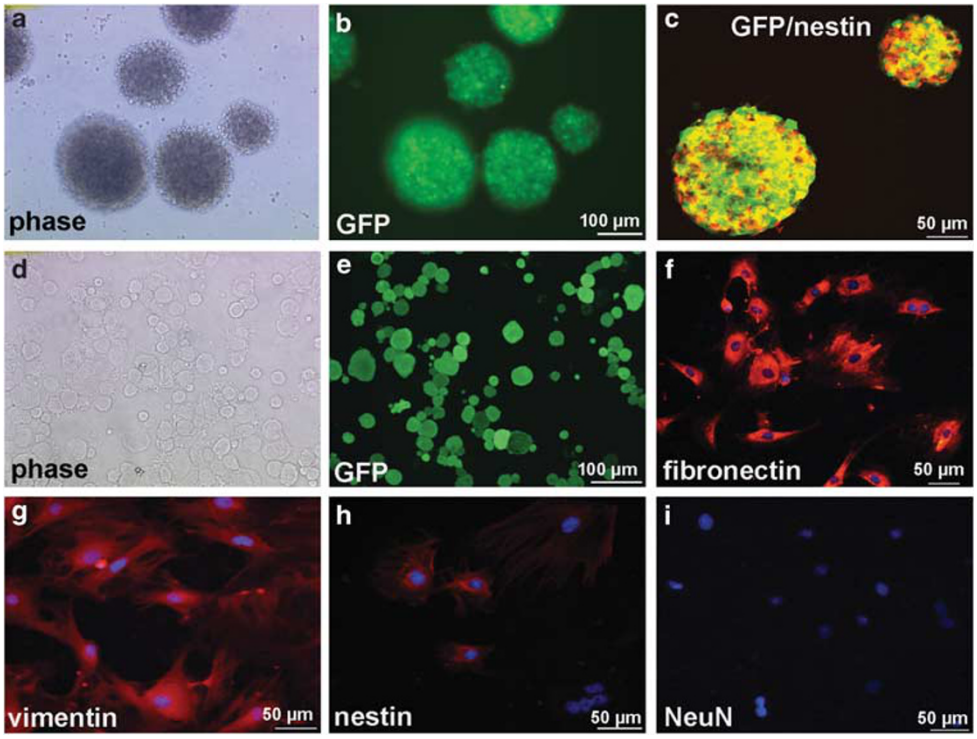

Figure 2 Immunofluorescent photomicrographs showing phenotypes of NSPC and BMSC in culture before transplantation. Spinal cord-derived NPSCs were cultured as free-floating neurospheres (**a**, phase contrast; **b**, corresponding GFP fluorescence in green), which express high levels of nestin (red) as shown with immunocytochemistry (**c**, GFP/nestin co-localization in yellow). An aliquot of BMSC was placed onto a slide before transplantation, phase contrast is shown in **d**, and the corresponding GFP fluorescence in **e**. Nearly all cultured BMSC expressed fibronectin (**f**, red) and vimentin (**g**), whereas nestin (**h**) was detected in a minority of BMSC, and NeuN (**i**) expression was completely absent. The blue signal in panels **f**–**i** indicates Hoechst labeling of cell nuclei.

only 0.6% (±0.3%) of them were proliferating (Figure 3g, lower inset and Figure 3h). Both transplanted NSPCs and BMSCs were often found surrounding the spinal arteries in the subarachnoid space (BMSC shown in Figures 3i and l). BMSC did not express neural antigens, such as nestin (Figure 3i), GFAP (Figure 3j), CC1 (not shown), or neuronal markers such as NeuN (Figure 3k) and BIII-tubulin (not shown). However, BMSC continued to express high levels of fibronectin (Figure 3l) after LP transplantation.

Relative distribution of NSPC and BMSC following LP transplantation

The majority of LP transplanted NSPCs or BMSCs were found in the intrathecal space (Figure 3), distributed along the length of the spinal cord. In some rats, a small number of transplanted cells migrated towards the lesion cavity. We compared the relative distribution of LP transplanted NSPC and BMSC in the brain and at the following levels of the spinal cord: C4 (cervical region), T8 (site of injury in SCI rats) and L3–5 (injection site; Figure 4). At 3-day post-transplantation, the majority of NSPCs were located at the injection site in SCI and control rats (63 ± 4.1% for SCI and 68.9 ± 13.7% for control). By 17/27 days post-transplantation, significantly more NSPCs were found at T8 at the site of injury (72.6 ± 8.3%), relative to the injection site (24.1 ± 9.9%) or cervical region (3.3 ± 1.8%) in SCI rats, and relative to T8 in uninjured controls (27.9 ± 5.8%; $P = 0.043$; Figure 4a). In contrast, the majority of BMSCs were found at the injection site at 3-day (97.9 ± 0.6%) and 17/27-day (70.5 ± 10.2%) post-SCI compared with the other regions examined. For both NSPC and BMSC, fewer cells were detected in the cervical region in SCI rats than uninjured controls. Transplanted cells were not detected in the brain in any of the rats.

Results and Discussion

We showed that spinal cord-derived NSPC and BMSC (generated from the same adult strain of GFP rats) can reach the injured rat thoracic spinal cord using the minimally invasive method of LP injection of cells into the lumbar intrathecal space. In uninjured animals there was no preferential localization to the thoracic or cervical region. In SCI rats by 17/27 days post-transplantation, the majority of NSPCs were found at the site of injury. In contrast, this relative homing to the site of injury was not seen with BMSC transplanted SCI rats or uninjured controls. These data suggest that NSPC migrate preferentially to the injury site, a feature not seen with BMSC. This may be due to the differential response of these cells to chemokines that are released at the site of injury. Previous reports of lumbar injection of cells have indicated that embryonic neural precursor cells transplanted in rats with a hemisection SCI[4]

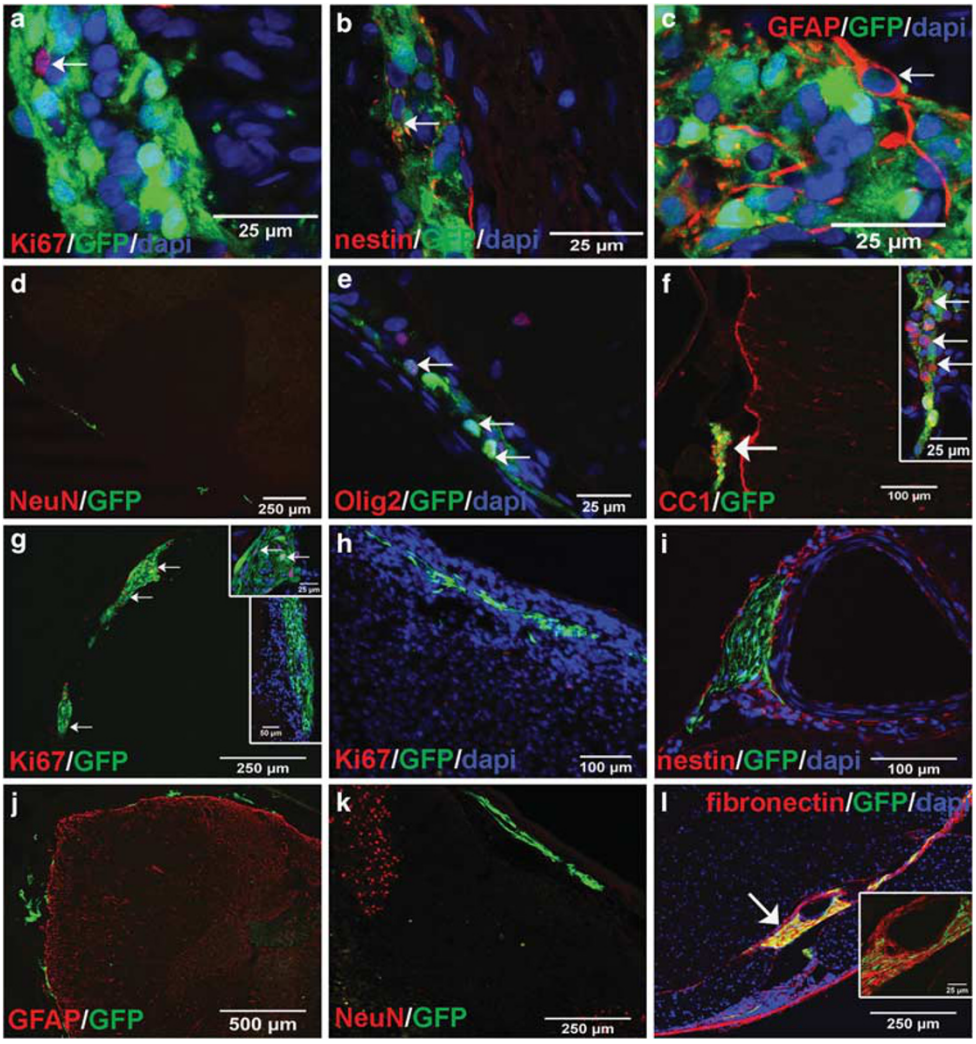

Figure 3 Confocal photomicrographs showing the phenotypes of NSPC (**a**–**f**) and BMSC (**g**–**l**) following LP transplantation. In all the panels, the GFP signal is in green, the cell type specific marker is in red, and the DAPI nuclear counterstain is in blue. Arrows denote double-labeled cells. Very few spinal cord-derived NSPCs show Ki67 (**a**, 17 days post-transplantation at the site of injury at T8) or nestin (**b**, 27 days post-transplantation at T8) immunoreactivity after LP transplantation. NSPC expressed GFAP (**c**, 27 days post-transplantation at T12), but NeuN immunoreactivity was not detected in transplanted NSPC (**d**, 17 days at T12). Many transplanted NSPCs expressed Olig2 (**e**, 17 days at T12) and CC1 (**f**, 17 days at T8). The inset in **f** is a higher magnification of the transplanted cells expressing GFP/CC1. In contrast, many BMSC were Ki67$^+$ at 3 days post-transplantation (**g**, shown 3 days in uninjured rat at T8 at low magnification and higher magnification in upper inset), although very few cells expressed Ki67 by 27 days post-transplantation (**g**, lower inset is 27 days sham at T8; **h**, 27 days at the injury site at T8). Transplanted BMSC were often found surrounding the large spinal arteries in the subarachnoid space (**i**, 27 days at T12) and expression of nestin (**i**, 27 days at T12), GFAP (**j**, 27 days at T8), and NeuN (**k**, 27 days at T8) was not detected. BMSC expressed high levels of fibronectin at all times (**l**, 27 days at T12, inset shows high magnification).

and BMSC transplanted in rats with either a hemisection SCI[3] or after contusion SCI[2] were found only at the lesion site. In contrast, our data show that most cells remained in the intrathecal space and were distributed along the entire length of the spinal cord rostral to the lumbar injection site. Thus, in the present study, there was no exclusive 'homing' of NSPC and BMSC to the injury site. These discrepancies between previous studies and the present study may be explained by the differences in the type of SCI and variations in the transplant interval. We transplanted our cells at 1-week post-compression injury, whereas the other groups used a 10 and 14 days interval after either dorsal lateral funiculotomy or contusion injury.[2,3] We suggest that extensive incision and disruption of the dura was required to make the dorsal lateral funiculus lesions[3,4] and tearing of the dura in the contusion injury[2] allowed the transplanted cells to migrate freely into the injured spinal cord parenchyma. In contrast, the clip impact-compression model, a model more relevant to human SCI, does not disrupt the dura in most animals, although it does cause a minimal meningeal inflammatory reaction in the first 2 weeks after injury that gradually diminishes over time. This meningeal reaction or swelling of the spinal cord after injury with blockage of CSF flow might have attracted or trapped some of the intrathecally injected cells. In the present study, we observed only a few cells in the injured spinal parenchyma in

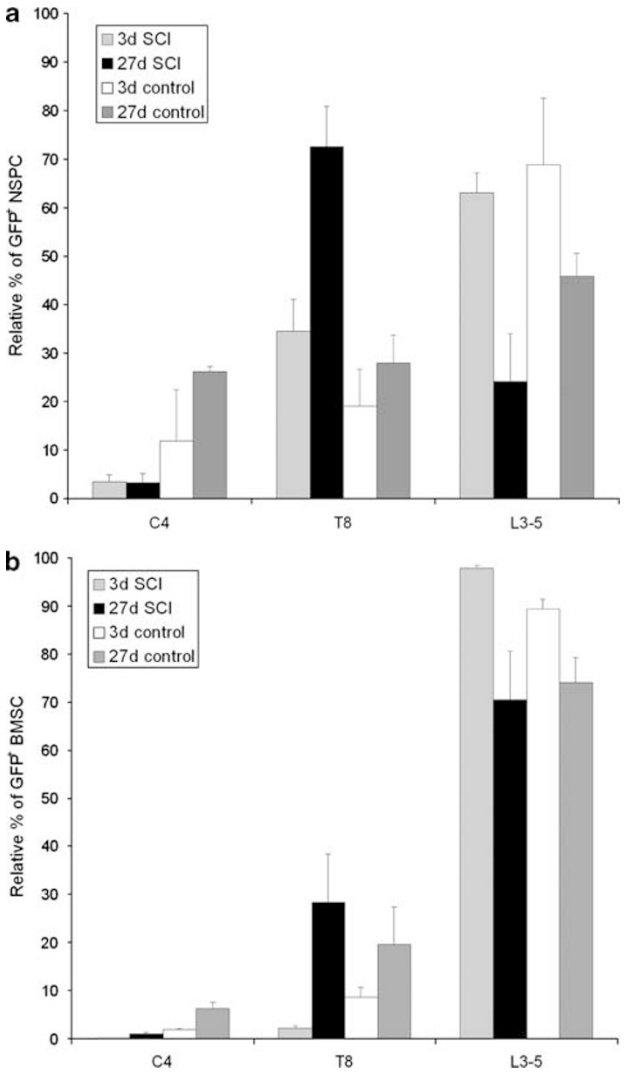

Figure 4 Relative distribution of NSPC (**a**) and BMSC (**b**) in the spinal cord. In SCI rats and uninjured transplanted controls, the total number of GFP^+ cells was counted in the three regions examined: C4 (cervical), T8 (lesion site in SCI animals) and L3–5 (injection site), and normalized to 100%. Data for the 27 days time point was combined with 17 days as described in the methods.

a minority of cases, which may have been due to minor disruption of the pia or meningeal inflammatory reaction. Also, in previous studies, a collagen matrix was implanted into the injury cavity following the hemisection,[4] which could have provided an adhesive substrate for the cells to attach, and also very likely caused a physical block of the subarachnoid space to interrupt the passage of the intrathecal cells.

In the present study, we transplanted nestin expressing, proliferating neurospheres that were spinal cord-derived NSPC, and after LP transplantation, the majority of the transplanted NSPC expressed oligodendrocyte markers. This is consistent with our earlier studies in which NSPC transplanted directly into the cord after impact-compression injury[13] or into focal demyelination lesions induced by ethidium bromide[12] differentiated primarily into oligodendrocytes. LP transplanted NSPC did not express neuronal markers. Also, there was no expression of any neuronal antigens by either cultured or transplanted BMSC, supporting the findings of most other studies.[14,16,21] In contrast, some reports suggest that BMSC express neuronal markers either *in vitro* or after transplantation into the injured spinal cord.[22,23] Indeed, our LP transplanted BMSC did not express any neural antigens and showed high levels of fibronectin expression as in culture. We also found that BMSC continued to proliferate 3 days post-transplantation unlike NSPCs that were primarily post-mitotic at that time. LP transplanted BMSC and NSPC were often located surrounding the spinal arteries in the spinal subarachnoid space, suggesting a positive influence of vascular cells for attraction and retention of these transplanted cells in this niche environment. The results of this study indicate that additional experimental work is necessary to assess the utility of LP delivery of stem cells for SCI. We are planning to assess the effects of LP transplantation of NSPC on functional recovery after SCI.

There are differences between the anatomical relationships of the spinal cord and vertebral column in the rat versus humans. In the rat, the spinal cord terminates at L3–L4 vertebral level, whereas in the human it terminates at L1–L2 vertebral level.[24] It is unknown whether there are functional differences between the intrathecal space and flow of cerebrospinal fluid between rats and humans.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported by the Canadian Institutes of Health Research (CIHR NET), the Multiple Sclerosis Society of Canada, and by the Canadian Paraplegic Association (Ontario Branch). We thank Kurt van Bendegem for preparing the digitized schematic diagram in Figure 1. We also thank Linda Lee and Rita van Bendegem for tissue processing and Kenny Zhan for help with data analysis.

References

1 Tator CH. Review of treatment trials in human spinal cord injury: issues, difficulties, and recommendations. *Neurosurgery* 2006; **59**: 957–982; discussion 982–987.
2 Bakshi A, Barshinger AL, Swanger SA, Madhavani V, Shumsky JS, Neuhuber B *et al*. Lumbar puncture delivery of bone marrow stromal cells in spinal cord contusion: a novel method for minimally invasive cell transplantation. *J Neurotrauma* 2006; **23**: 55–65.
3 Bakshi A, Hunter C, Swanger S, Lepore A, Fischer I. Minimally invasive delivery of stem cells for spinal cord injury: advantages of the lumbar puncture technique. *J Neurosurg Spine* 2004; **1**: 330–337.
4 Lepore AC, Bakshi A, Swanger SA, Rao MS, Fischer I. Neural precursor cells can be delivered into the injured cervical spinal cord by intrathecal injection at the lumbar cord. *Brain Res* 2005; **1045**: 206–216.
5 Jung DI, Ha J, Kang BT, Kim JW, Quan FS, Lee JH *et al*. A comparison of autologous and allogenic bone marrow-derived

mesenchymal stem cell transplantation in canine spinal cord injury. *J Neurol Sci* 2009; **285**: 67–77.
6 Shi E, Jiang X, Wang L, Akuzawa S, Nakajima Y, Kazui T. Intrathecal injection of hepatocyte growth factor gene-modified marrow stromal cells attenuates neurologic injury induced by transient spinal cord ischemia in rabbits. *Anesthesiology* 2010; **113**: 1109–1117.
7 Takahashi Y, Tsuji O, Kumagai G, Hara CM, Okano HJ, Miyawaki A *et al*. Comparative study of methods for administering neural stem/progenitor cells to treat spinal cord injury in mice. *Cell Transplant* 2010.
8 Pluchino S, Quattrini A, Brambilla E, Gritti A, Salani G, Dina G *et al*. Injection of adult neurospheres induces recovery in a chronic model of multiple sclerosis. *Nature* 2003; **422**: 688–694.
9 Weiss S, Dunne C, Hewson J, Wohl C, Wheatley M, Peterson AC *et al*. Multipotent CNS stem cells are present in the adult mammalian spinal cord and ventricular neuroaxis. *J Neurosci* 1996; **16**: 7599–7609.
10 Kulbatski I, Mothe AJ, Keating A, Hakamata Y, Kobayashi E, Tator CH. Oligodendrocytes and radial glia derived from adult rat spinal cord progenitors: morphological and immunocytochemical characterization. *J Histochem Cytochem* 2007; **55**: 209–222.
11 Martens DJ, Seaberg RM, van der Kooy D. *In vivo* infusions of exogenous growth factors into the fourth ventricle of the adult mouse brain increase the proliferation of neural progenitors around the fourth ventricle and the central canal of the spinal cord. *Eur J Neurosci* 2002; **16**: 1045–1057.
12 Mothe AJ, Tator CH. Transplanted neural stem/progenitor cells generate myelinating oligodendrocytes and Schwann cells in spinal cord demyelination and dysmyelination. *Exp Neurol* 2008; **213**: 176–190.
13 Parr AM, Kulbatski I, Zahir T, Wang X, Yue C, Keating A *et al*. Transplanted adult spinal cord-derived neural stem/progenitor cells promote early functional recovery after rat spinal cord injury. *Neuroscience* 2008; **155**: 760–770.
14 Ohta M, Suzuki Y, Noda T, Ejiri Y, Dezawa M, Kataoka K *et al*. Bone marrow stromal cells infused into the cerebrospinal fluid promote functional recovery of the injured rat spinal cord with reduced cavity formation. *Exp Neurol* 2004; **187**: 266–278.
15 Clark BR, Jamieson C, Keating A. Human long-term bone marrow culture. In: Walker JM, Pollard JW (eds). *Basic Cell Culture Protocols*, 2nd edn. The Humana Press Inc: Totowa, NJ, pp 75:249–256, 1997.
16 Parr AM, Kulbatski I, Wang XH, Keating A, Tator CH. Fate of transplanted adult neural stem/progenitor cells and bone marrow-derived mesenchymal stromal cells in the injured adult rat spinal cord and impact on functional recovery. *Surg Neurol* 2008; **70**: 600–607; discussion 607.
17 Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D *et al*. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006; **8**: 315–317.
18 Rivlin AS, Tator CH. Effect of duration of acute spinal cord compression in a new acute cord injury model in the rat. *Surg Neurol* 1978; **10**: 38–43.
19 De la Calle JL, Paino CL. A procedure for direct lumbar puncture in rats. *Brain Res Bull* 2002; **59**: 245–250.
20 Mothe AJ, Kulbatski I, Parr A, Mohareb M, Tator CH. Adult spinal cord stem/progenitor cells transplanted as neurospheres preferentially differentiate into oligodendrocytes in the adult rat spinal cord. *Cell Transplant* 2008; **17**: 735–751.
21 Ankeny DP, McTigue DM, Jakeman LB. Bone marrow transplants provide tissue protection and directional guidance for axons after contusive spinal cord injury in rats. *Exp Neurol* 2004; **190**: 17–31.
22 Chopp M, Zhang XH, Li Y, Wang L, Chen J, Lu D *et al*. Spinal cord injury in rat: treatment with bone marrow stromal cell transplantation. *Neuroreport* 2000; **11**: 3001–3005.
23 Woodbury D, Schwarz EJ, Prockop DJ, Black IB. Adult rat and human bone marrow stromal cells differentiate into neurons. *J Neurosci Res* 2000; **61**: 364–370.
24 Gelderd JB, Chopin SF. The vertebral level of origin of spinal nerves in the rat. *Anat Rec* 1977; **188**: 45–47.