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Brief Communication

Effects of Direct Transplantation of Multipotent Mesenchymal Stromal/Stem Cells Into the Demyelinated Spinal Cord

D. P. J. Hunt,* K. A. Irvine,† D. J. Webber,* D. A. S. Compston,* W. F. Blakemore,† and S. Chandran*

*Cambridge Centre for Brain Repair, Department of Clinical Neuroscience, Forvie Site, Addenbrookes Hospital, University of Cambridge, Cambridge, UK
†Department of Veterinary Medicine, University of Cambridge, Cambridge, UK

The adult bone marrow contains a population of multipotent mesenchymal stromal cells (MSCs), defined by plastic adherence, expression of stromal cell surface markers, and differentiation into mesenchymal lineages. There has been much interest in the possible therapeutic use of MSCs in the treatment of demyelinating diseases of the central nervous system. One therapeutic possibility is that these cells may be able to remyelinate when directly injected into the demyelinated spinal cord. Here we examine the effects of direct transplantation of green fluorescent protein (GFP)-labeled MSCs into a model of focal spinal cord demyelination induced by ethidium bromide. We demonstrate that direct intralesional injection of undifferentiated MSCs does not lead to remyelination. Furthermore, we report that transplanted MSCs migrate into areas of normal tissue, deposit collagen, and are associated with axonal damage. These findings support the need for further experimental evaluation of the safety and efficacy of direct parenchymal injection of MSCs into demyelinated lesions and highlight an important issue regarding potential clinical consequences of culture heterogeneity of MSCs between centers.

Key words: Bone marrow; Mesenchymal stromal cell; Remyelination; Demyelination; Multiple sclerosis

INTRODUCTION

The bone marrow contains a well-characterized mesenchymal stromal cell (MSC) population (27). These cells, also called mesenchymal stem cells or marrow stromal cells, are expanded in vitro as plastic adherent monolayers in serum-containing media and can be differentiated into mesenchymal cell types such as osteocytes, adipocytes, and chondrocytes (10,13,27). They maintain a stable phenotype over 40–50 population doublings. MSCs express stromal cell surface markers including CD90, CD44, and CD105 and do not express the hematopoetic markers CD45 or CD34 (10).

MSCs are attractive candidates for cell-based therapies because of their comparative ease of isolation, expansion, and potential autologous application. MSCs display a number of potential mechanisms of therapeutic action (10,13,20,21,24,25,27). These include a broad differentiation repertoire, trophic support, and immunomodulatory properties (17,19,21,31). A number of studies have reported in vitro and in vivo differentiation of MSCs into neuronal cells (17,34) and Schwann cells (19) as well as cardiomyocytes and hepatocytes (17,31). However, interpretation of these findings may be confounded by alternative explanations such as cellular fusion (33).

Against this background, a series of reports has suggested that bone marrow-derived cells are able to remyelinate the demyelinated spinal cord (1,2,15,30), a finding of potential relevance to multiple sclerosis (6,8). These studies have shown that acutely isolated bone marrow (1,15,30) and cultured marrow stromal cells (2) can remyelinate axons when injected intravenously or directly into a focal demyelinating lesion induced by ethidium bromide in X-irradiated tissue (X-EB lesion). In this model, demyelination is induced by focal injection of ethidium bromide and endogenous remyelination is suppressed by X-irradiation-mediated depletion of oligodendrocyte precursor cells. Consequently, any remy-

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Address correspondence to Dr. David Hunt, Cambridge Centre for Brain Repair, Forvie Site, Robinson Way, University of Cambridge, Cambridge CB2 2PY, UK. Tel: 01223 331160; Fax: 01223 331174; E-mail: dpjh2@cam.ac.uk

elination observed following transplantation can be attributed to the transplanted cells (4). These results raise the possibility of the use of MSCs as remyelinating cells.

A fundamental question in the development of novel cell therapies is the route of delivery of cells. Mesenchymal stromal cells may be administered systemically by intravenous infusion, intrathecally, or focally into a demyelinated lesion. However, there is limited data upon which to base assessment of the safety and efficacy of these different approaches. Further difficulty arises because different populations of MSCs have been used in both experimental and clinical settings. Experimental studies of the behavior of bone marrow-derived stromal cells in a demyelinated environment have used bone marrow stromal cells isolated and cultured in media specialized for the growth of neural progenitor cells with ex vivo culture of cells with EGF and FGF-2 (2). In contrast, most clinical studies to date have used MSCs isolated and expanded in basal media (typically low-glucose DMEM) and 10% FCS (9,10) without the addition of growth factors. It is possible that different culture conditions lead to heterogeneity of both culture-initiating cells and subsequent in vitro and in vivo behavior of MSCs. The problem of MSC heterogeneity has recently been addressed by a working party of the International Society for Cellular Therapy (ISCT), which has proposed minimal criteria for the definition of multipotent mesenchymal stromal cells (10). These include: (i) plastic adherence in standard culture conditions, (ii) expression of stromal cell surface markers and absence of defined cell surface markers such as hematopoetic markers, and (iii) functional differentiation of cells into mesenchymal lineages.

There are no reports of the effects of direct transplantation of bone marrow-derived MSCs, cultured and characterized according to the principles of the ISCT criteria, into an area of focal demyelination. We therefore sought to investigate the remyelinating potential of such MSCs following focal transplantation into the adult X-EB model of demyelination.

MATERIALS AND METHODS

Isolation of Rat Bone Marrow Mesenchymal Stromal Cells

Whole bone marrow was removed from the long bones of an adult Sprague-Dawley rat, which expressed green fluorescent protein (GFP) under the control of chicken actin promoter (26) (kind gift, M. Okabe, University of Osaka), using a heparinized 23-gauge needle (2,16). Bone marrow aspirate was diluted in 5 ml of DMEM and was loaded onto a 10-ml Ficoll solution using a Pasteur pipette. The cells were centrifuged at $560 \times g$ for 25 min and collected from the mononuclear layer. The resulting cells were resuspended in low-glucose DMEM and 10% FCS and were plated in T25 culture dishes. The medium was changed at 48 h and floating cells removed. The stromal layer grew out from adherent cells and subsequently grew as a monolayer. The cells were passaged using 0.1% trypsin/EDTA at 80% confluence and replated at a density of 1:3. The cells were characterized at passage 5. Passage 5 cells for transplantation were removed from the flasks with trypsin, washed twice in PBS, and resuspended in DMEM at a concentration of 5000 cells/μl. Cell viability was assessed using trypan blue and hemocytometer after trypsinisation with >99% viability.

Characterization of Rat Bone Marrow MSCs

GFP fluorescence was analyzed using a FACSCalibur machine (Becton-Dickinson). The phenotype of the stromal cells was confirmed by immunocytochemical analysis. Cells were plated on PDL/laminin-coated coverslips. They were washed in PBS and fixed with 4% paraformaldehyde for 15 min at room temperature. Cells were washed, blocked, and permeablized with 5% normal goat serum (NGS), 0.1% Triton/PBS for 30 min. Primary antibodies were incubated overnight at 4°C with 2% NGS. Primary antibodies used were fibronectin (1:800 rabbit polyclonal, Sigma), CD90/Thy1 (mouse monoclonal clone MRC OX-7 Abcam Ltd, Cambridge), CD34 (Serotec mouse monoclonal), CD45 (1:400, Leucocyte common antigen; Serotec, Oxford, UK) Negative controls with omission of primary antibody were performed for all immunocytochemical experiments.

Functional differentiation into adipogenic and osteogeneic lineages was assessed according to established protocols (16). Briefly, cells were plated at a density of 3.7×10^4 cells/cm^2 on coverslips coated with poly-D-lysine and fibronectin (1 μg/ml). Cells were differentiated for 14 days in the following media: adipogenic media containing αMEM, 10% FCS, 1% penicillin-streptomycin with adipogenic supplement (R&D Systems) containing hydrocortisone isobutylmethylxanthine and indomethacin. Cells were also differentiated for 14–21 days in osteogenic media containing αMEM, 10% FCS, 1%penicillin-streptomycin with osteogenic supplement (R&D Systems) containing dexamethasone, ascorbate-phosphate, and β-glycerolphosphate. The presence of adipocytes was confirmed by phase contrast microscopy with the appearance of lipid droplets. Coverslips were fixed at day 14 for 20 min with fresh 4% paraformaldehyde. Cells were incubated overnight with the following antibodies: 10 μg/ml goat anti-mouse fatty-acid binding protein (FABP-4) and10 μg/ml mouse anti-osteocalcin. Calcium deposition in osteocyte cultures was visualized with Alizarin red staining.

Animal Preparation and Transplantation

All experiments were conducted under a Home Office Project License after review by Cambridge Universities Ethical Review Panel. Demyelinating lesions were induced in rat spinal cord ($n = 8$) by injection of ethidium bromide (EB). Briefly, a laminectomy was performed at the level of T13 vertebra and the spinal cord exposed. Using a micromanipulator, a Hamilton syringe tipped with a fine glass micropipette was introduced into the dorsal funiculus to a depth of 0.75 mm and 1 μl of 0.1% EB injected. To abolish the inherent capacity of the spinal cord to generate remyelinating cells, the demyelinating lesion was induced in tissue that was exposed to 40 Gy of X-irradiation 1 day after EB injection, a procedure that causes rapid and complete loss of oligodendrocyte progenitor cells. For X-irradiation rats were anesthetized with intramuscular injection of Hypnorm (Jenssen Pharmaceuticals, NJ), consisting of a combination of fentanyl citrate (0.0785 mg/kg) and fluanisone (2.5 mg/kg). When the animals were anesthetized they were secured in lateral recumbency by adhesive tape and a 4-cm (craniocaudal) × 2-cm (dorsoventral) window in lead sheeting was centered on T13 and positioned in such a way to prevent X-irradiation of the abdominal cavity. Following radiography to confirm positioning of the lead sheet animals were then subjected to 40 Gy of X-irradiation (225 kV) using an X-ray machine (Pantak, UK). Two days later, 1 μl of cell suspension containing 5×10^3 cells was injected into the EB lesion site ($n = 5$). Control rats were injected with DMEM alone ($n = 3$).

Processing of Tissue for Immunohistochemical and Ultrastructural Analysis

Rats were perfused with 4% paraformaldehyde in phosphate buffer under deep anesthesia 3 weeks following transplantation. The length of the spinal cord containing the lesion was removed and cut into 1-mm transverse slices; alternate blocks were postfixed in 4% glutaldehyde and embedded in TAAB resin while the others were cryoprotected in 30% sucrose solution and rapidly frozen using dry ice. Sections (20 μm) were cut from the frozen blocks and mounted onto polylysine-coated glass slides (VWR International Ltd, Lutterworth, UK) for immunohistochemical analysis. The resin blocks were sectioned at 1 μm with sections stained with toluidine blue. Selected areas were examined by electron microscopy.

Immunohistochemistry

All sections were blocked with 10% NGS (Sigma-Aldrich. Poole, UK) in Triton (0.3% in PBS) for 1 h and then incubated overnight at 4°C with primary antibody diluted in 0.3% Triton in PBS containing 2.5% NGS. The primary antibodies used were polyclonal rabbit anti-GFAP (1:200, glial fibrillary acid protein; DAKO, Ely, UK), polyclonal rabbit anti-NG2 (1:200, Chemicon, Hampshire, UK), monoclonal mouse SMI 31 antibody (1:3000, Sternberger, CA, USA), monoclonal mouse SMI 32 antibody (1:1000, Sternberger, CA, USA), monoclonal human anti-fibronectin (1:800, Sigma, Gillingham, Dorset, UK), monoclonal mouse anti-CNPase (1:100, Sigma), monoclonal p75 (1:100, Chemicon), polyclonal rabbit anti-S100 (1:200, DAKO), and monoclonal rat anti-CD45 (1:400, Leucocyte common antigen; Serotec). All sections were rinsed and then incubated for 2 h with the relevant biotinylated (1:200, Vector Laboratories, Peterborough, UK; Southern Biotech, AL, USA) or Alexa Fluor 555 secondary antibodies (Invitrogen, Ely, UK). Sections were then coverslipped using vectashield with DAPI (Vector Laboratories).

RESULTS

Characterization of GFP-Expressing Rat Bone Marrow MSCs

The mononuclear layer of bone marrow was isolated by Ficoll density gradient and plated on tissue culture plastic in media containing low-glucose DMEM and 10% FCS. Adherent cells were maintained in culture and passaged when 80% confluent. The cells displayed the typical morphology of rat MSCs (16,27) at confluence (Fig. 1a) and maintained a stable phenotype over 40 population doublings with a constant doubling time of 4–5 days. Prior to transplantation FACS analysis confirmed that 93% of cells expressed GFP (Fig. 1c). Cells expressed the cell surface markers CD44 and CD90 as well as fibronectin (all >95%). Cells did not express CD34 and CD45 (11) (Fig. 1c). Functional differentiation of MSCs along osteogenic and adipogenic lineages was demonstrated using standard differentiation protocols (16,27). Adipogenic differentiation was shown by immunostaining for fatty acid binding protein (FABP) and the identification of lipid droplets characteristic of adipocytes (Fig. 1e). The presence of osteocytes was determined by immunostaining for osteocalcin and the identification of calcium deposition by Alizarin red staining (Fig. 1f). The cells therefore displayed a phenotype consistent with multipotent MSCs.

Transplanted Mesenchymal Stromal Cells Do Not Remyelinate

Focal demyelination was induced by injection of EB into the dorsal funiculus of the adult spinal cord. Intralesional injection of MSCs (5000 cells/μl DMEM,

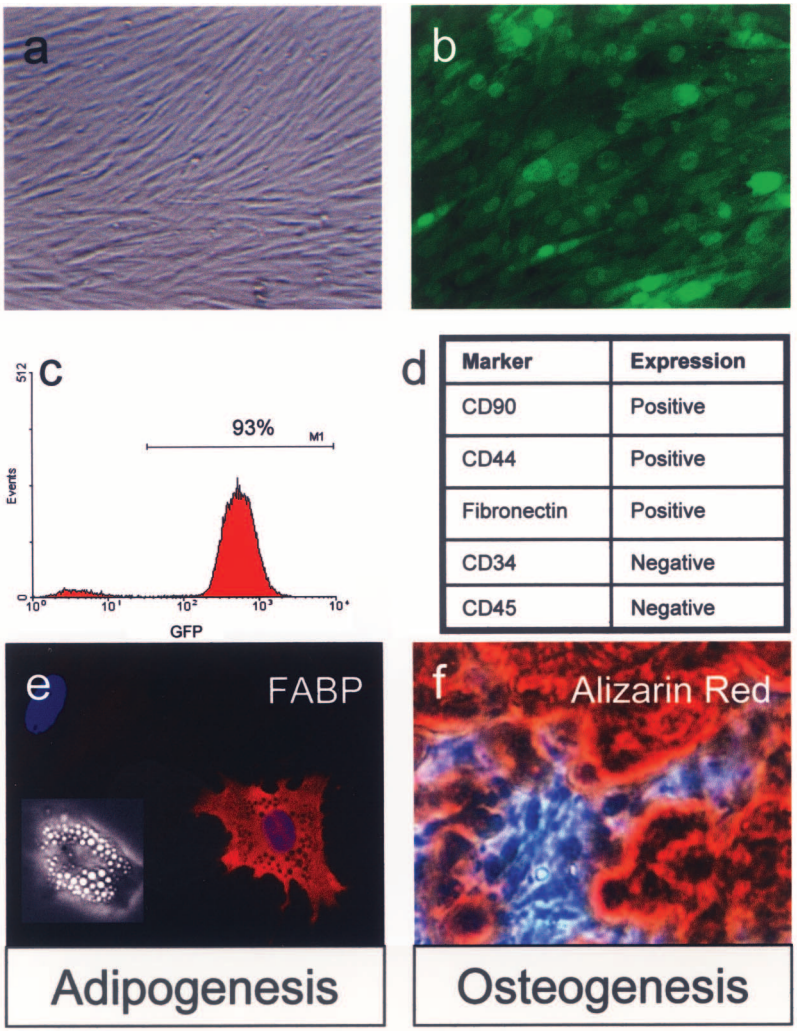

Figure 1. In vitro characterization of GFP-labeled rat mesenchymal stromal cells (MSCs). (a) Phase contrast microscopy of MSCs at confluence demonstrating morphology of plastic-adherent monolayers. (b, c) MSCs express GFP as determined by both immunocytochemistry and FACS analysis. FACS analysis confirms 93% GFP expression in MSCs. (d) Cells express stromal cell surface markers and fibronectin (>95%) and do not express CD34 or CD45. (e, f) MSCs differentiate into adipocytes (high power, fatty acid binding protein, FABP, red) and osteocytes (Alizarin red staining, medium power, calcium deposition orange).

$n = 5$) or control injections (DMEM alone, $n = 3$) were performed 2 days after X-irradiation. Animals were sacrificed 3 weeks after transplantation with adjacent blocks processed for preparation of frozen and plastic-embedded sections.

Analysis of toluidine blue semithin sections of control animals (DMEM microinjection) revealed demyelinated axons clumped together and separated by macrophages containing myelin debris. As expected, no remyelination of the dorsal funiculus was seen in the control group (Fig. 2a).

In MSC-transplanted animals, immunohistochemical analysis revealed GFP+ cells within the demyelinated dorsal funiculus lesion in all five rats. Migration of GFP+ cells beyond the demyelinated area into adjacent normal white matter was observed in three of the five animals. Cells migrated distances of up to 150 μm in all directions from the site of injection (Fig. 3f). Analysis of the adjacent toluidine blue semithin sections revealed no evidence of remyelination in regions of transplanted MSCs (Fig. 2b). Furthermore, compared to control treated lesions (Fig. 2a) there was considerable disruption of cytoarchitecture where transplanted MSCs were present. Electron microscopy of these regions revealed the presence of large cells with a fibroblastic appearance, only present in transplanted lesions. These cells

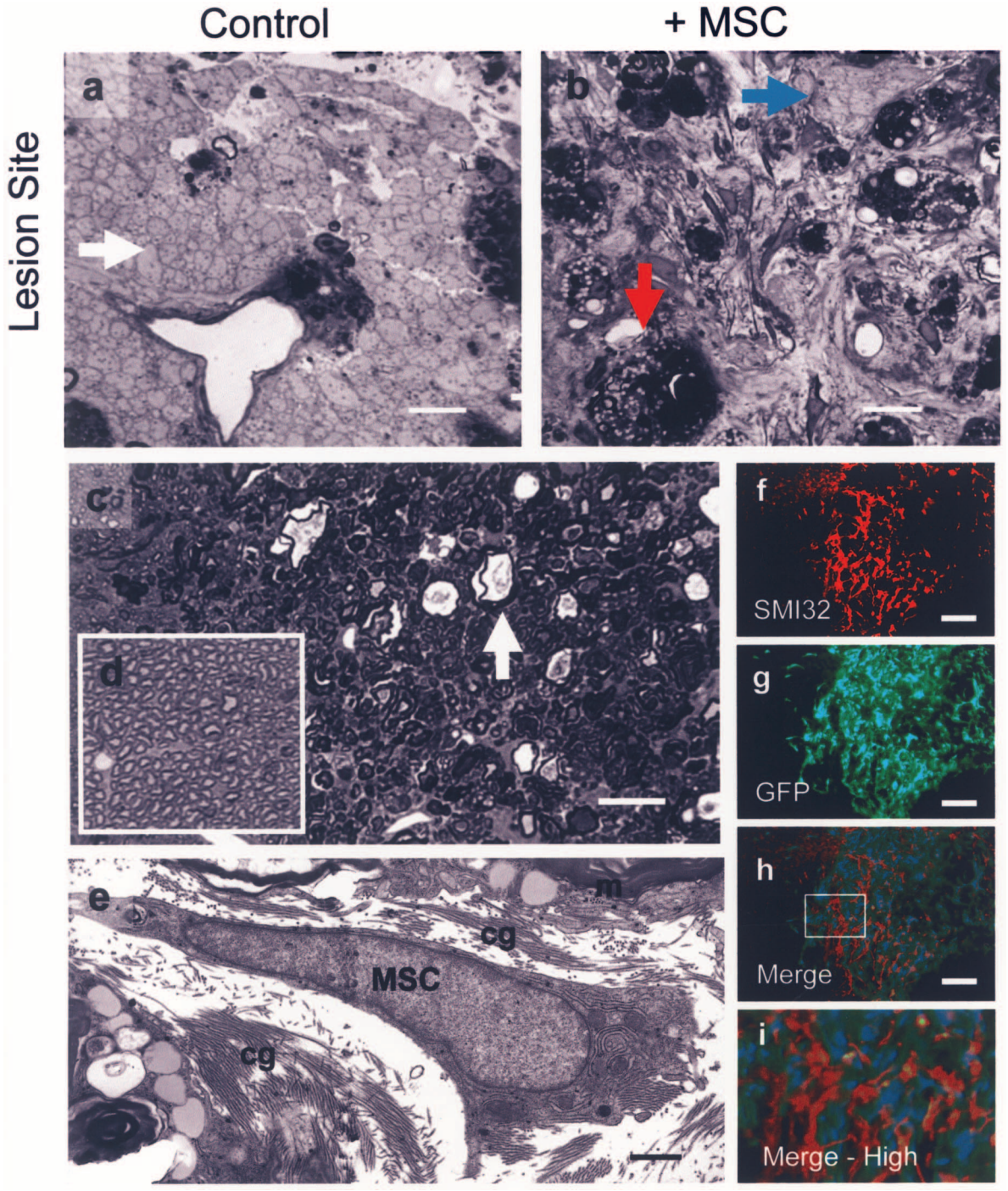

Figure 2. MSCs transplanted into the X-EB demyelinated lesion do not remyelinate, deposit collagen, and are associated with axonal damage.(a) High-power micrograph of toluidine blue resin section demonstrating demyelinated axons in control lesioned animals (white arrow) (scale bar: 10 μm). (b) No remyelination is seen in lesions where MSCs were injected. In MSC-transplanted animals, there are smaller clumps of demyelinated axons (green arrow), macrophages (red arrow), and widespread disruption of cytoarchitecture. (c) Morphological changes in the dorsal funiculi characteristic of Wallerian degeneration in resin section taken cranial to the transplant site (white arrow) (scale bar: 20 μm) (d, inset) Normal dorsal funiculus in control spinal cord showing myelinated axons. (e) Electron micrograph of transplanted MSC in demyelinated lesion (MSC) reveals no interaction of transplanted cells with axons. There is evidence of extracellular matrix and collagen deposition surrounding the transplanted MSC (cg). (f–i) In areas of high transplanted MSC density, neighboring axons expressed SMI32, a marker of axonal damage, and exhibited a dystrophic morphology compared to nontransplanted animals (scale bar: 50 μm). (i) Higher power magnification confirms GFP and SMI32 expression do not colocalize.

showed no association with demyelinated axons and there was no evidence of remyelination. Transplanted MSCs were surrounded by extensive collagen deposition (Fig. 2e).

Transplanted Mesenchymal Stromal Cells Are Associated With Axonal Damage

In regions of high GFP immunoreactivity, intense SMI32 (dephosphorylated neurofilament) staining was seen, indicating the presence of axonal damage (Fig. 2f–i). This pattern of strong immunoreactivity was not seen in control lesions. GFP expression did not colocalize with SMI32 expression (Fig. 2i). In these regions axons expressing SMI32 appeared displaced by GFP-expressing transplanted MSCs and exhibited a dystrophic morphology (Fig. 2f). This was not observed in control lesions. The presence of morphological changes in the dorsal funiculi characteristic of Wallerian degeneration in resin sections taken rostral to the transplant site were consistent with axon transection in the transplanted lesions (Fig. 2c).

Transplanted Cells Express Mesenchymal Markers and Do Not Express Neuronal or Glial Markers

We examined whether the transplanted MSCs expressed markers of mesenchymal, neuronal, or glial cell lineages by immunohistochemistry. GFP+ cells strongly expressed fibronectin, a marker of mesenchymal stromal cells (Fig. 3a–c, colocalization Fig. 3d). GFP+ cells did not express markers for astrocytes (GFAP, Fig. 3e–h), neurons (SMI31), Schwann cells (p75, S100), oligodendrocyte precursor cells (NG2), or oligodendrocytes (CNPase, MBP).

DISCUSSION

We report that direct transplantation of multipotent bone marrow MSCs into the adult demyelinated spinal cord did not lead to remyelination. Transplanted cells expressed mesenchymal markers and did not express any markers of neuronal or glial differentiation. In addition, transplantation of MSCs into the demyelinated spinal cord led to a number of potentially adverse effects. Transplanted MSCs migrated beyond the lesion site into areas of normal appearing white matter and ultrastructural analysis suggested transplanted MSCs deposited collagen and disrupted cellular architecture. In addition, grafted MSCs were associated with extensive axon degeneration.

Our results contrast with previous published reports that demonstrated robust Schwann cell remyelination following bone marrow stromal cell injection into the demyelinated X-EB lesion (2). This study reported no deleterious effects of such transplants. A potential explanation for this important difference is the distinct culture methodologies used to generate MSCs. Although there are some minor interspecies differences in the properties and characterization of rat and human MSCs (16), we adopted and followed the key principles and recommendations of the ISCT when characterizing MSCs. This included demonstration of functional differentiation of MSCs into cells of a mesenchymal lineage, which has not been demonstrated by previous studies (2). In addi-

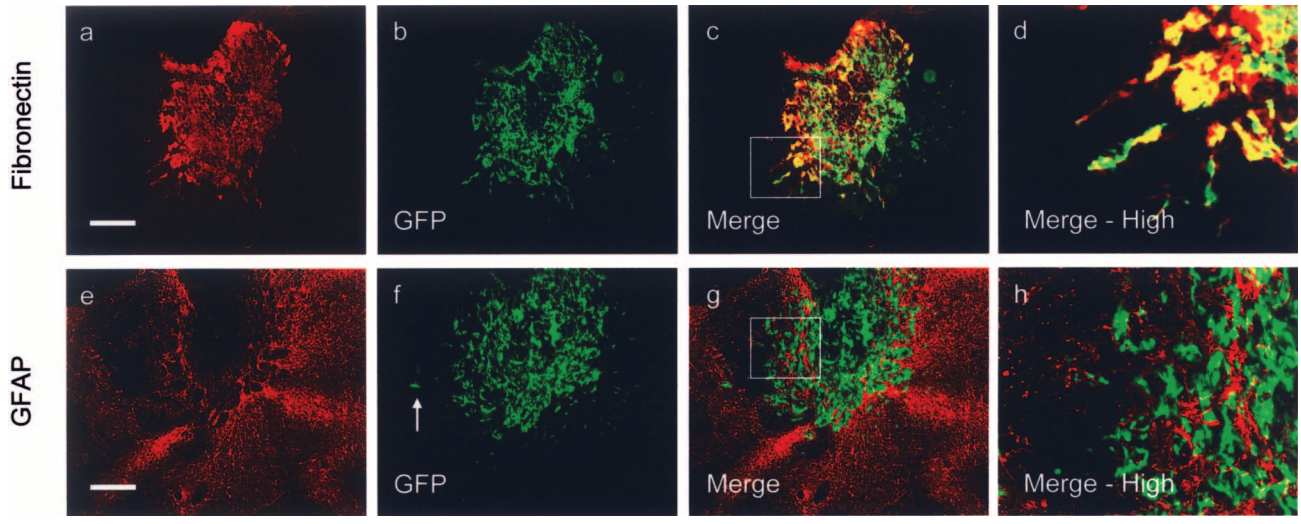

Figure 3. Transplanted MSCs express mesenchymal markers, migrate from the lesion site, and do not express glial markers. (a–d) Colocalization of fibronectin with transplanted GFP+ MSCs 3 weeks following transplantation. Low-power fluorescence photomicrograph, scale bar: 50 μm. (e–h) Transplanted cells do not colocalize with GFAP. GFP+ MSCs were identified migrating beyond the lesion site and site of transplantation (f, white arrow).

tion, Akiyama and coworkers cultured bone marrow stromal cells in a media specialized for the growth of neural precursor cells, which contained serum, EGF, FGF, and proprietary neural survival factors (2). In contrast, MSCs in the present study were grown according to established protocols using low-glucose DMEM and 10% fetal calf serum. Similar protocols are used in virtually all clinical studies using human MSCs (10,13,21). It is possible that prior in vitro treatment with media specialized for the growth of neuronal cells may alter initial adherence, selection, and growth of colony-forming cells from the bone marrow, allowing the generation of cells with a myelinating phenotype. Further study of the consequences of such differences in culture methodology will be of considerable interest.

The behavior of transplanted MSCs is similar to that observed when Schwann cell cultures contaminated with fibroblasts were injected into the X-EB lesion (7). In these studies, when significant numbers of contaminating fibroblasts were present in transplanted human Schwann cells cultures, a failure of remyelination was observed and was associated with deposition of large amounts of collagen by fibroblasts and extensive axonal degeneration. Undifferentiated MSCs have a fibroblastic morphology and are derived from colony-forming unit fibroblasts (CFU-F) from the bone marrow. MSCs may therefore behave in a similar manner to fibroblasts in the X-EB model of demyelination.

Although this is a small study, our results reported here are representative of a variety of different cells with mesenchymal characteristics that we have transplanted into the X-EB lesion. These include (i) human bone marrow-derived MSCs, (ii) cells with MSC features derived from human dermis (18), and (iii) rat bone marrow stromal cells cultured in neural progenitor medium. In all cases we have observed a failure of remyelination, extracellular matrix deposition, and axonal damage (Hunt, Joannides, Chandran, and Blakemore, unpublished results). In vitro neural and neural crest differentiation has been reported with a number of these cell types (14,17,34). It is possible that, although MSCs may possess in vitro neural differentiation capacity, their in vivo behavior when directly injected into an acutely demyelinating CNS environment may be unpredictable, possibly detrimental, and more similar to the behavior of fibroblasts than transplanted neural precursors (7).

MSCs are a promising tool for the treatment of neurological disorders and have shown significant preclinical therapeutic efficacy for diseases such as stroke and multiple sclerosis, particularly when injected intravenously (32,35). The basis of the therapeutic effect of MSCs is unlikely to be due to "transdifferentiation" to a neural phenotype but due to immunomodulatory, neuroprotective, and trophic effects. Of particular interest is the observation that MSCs migrate and selectively home to sites of inflammation and tissue damage, both in animal models and patients with severe tissue damage (29).

There is considerable interest in developing MSC-based treatments for patients with multiple sclerosis, and experimental trials are currently recruiting patients for this purpose (12,32). The rationale for the use of MSCs in the treatment of multiple sclerosis is varied and includes remyelinating, neuroprotective, and immunomodulatory mechanisms of action (5,32,35,36). A key question is whether MSCs should be delivered focally (by intralesional injection) or systemically (by intravenous infusion). The safety of intraspinal injection of MSCs is unknown and to our knowledge there are no clinical case reports of direct intraspinal injection of MSCs into a demyelinated lesion. There has been a small uncontrolled trial of intraspinal injection of autologous MSCs in patients with motor neuron disease (23). One recent report of intrathecal delivery of GFP rodent MSCs in a model of multiple sclerosis reports an abnormal cellular reaction surrounding transplanted cells, despite efficacy of the cellular therapy in improving the functional outcome of the animals with EAE (28). Other preclinical reports of direct injection of MSCs into the nondemyelinated spinal cord do not report adverse events (3,12,22,24).

This study cautions against direct focal injection of MSCs into demyelinated lesions as a strategy for remyelination. Further studies are required to examine the factors affecting the in vivo behavior of MSCs in experimental models of demyelination. Such studies should include careful and systematic analysis of the effects of minor alterations in cell culture technique and reagents on both the in vitro and in vivo behavior of these cells.

REFERENCES

1. Akiyama, Y.; Radtke, C.; Honmou, O.; Kocsis, J. D. Remyelination of the spinal cord following intravenous delivery of bone marrow cells. Glia 39(3):229–236; 2002.
2. Akiyama, Y.; Radtke, C.; Kocsis, J. D. Remyelination of the rat spinal cord by transplantation of identified bone marrow stromal cells. J. Neurosci. 22(15):6623–6630; 2002.
3. Ankeny, D. P.; McTigue, D. M.; Jakeman, L. B. Bone marrow transplants provide tissue protection and directional guidance for axons after contusive spinal cord injury in rats. Exp. Neurol. 190(1):17–31; 2004.
4. Baron-Van Evercooren, A.; Blakemore, W. Remyelination through engraftment. In: Lazzarini, R. A., ed. Myelin biology and disorders. New York: Elsevier Science; 2004: 143–172.
5. Beggs, K. J.; Lyubimov, A.; Borneman, J. N.; Bartholomew, A.; Moseley, A.; Dodds, R.; Archambault, M. P.; Smith, A. K.; McIntosh, K. R. Immunologic consequences of multiple, high-dose administration of allogeneic mesenchymal stem cells to baboons. Cell Transplant. 15(8–9): 711–721; 2006.
6. Blakemore, W. F. Remyelination of CNS axons by

Schwann cells transplanted from the sciatic nerve. Nature 266(5597):68–69; 1977.
7. Brierley, C. M.; Crang, A. J.; Iwashita, Y.; Gilson, J. M.; Scolding, N. J.; Compston, D. A.; Blakemore, W. F. Remyelination of demyelinated CNS axons by transplanted human schwann cells: The deleterious effect of contaminating fibroblasts. Cell Transplant. 10(3):305–315; 2001.
8. Chandran, S.; Hunt, D.; Joannides, A.; Zhao, C.; Compston, A.; Franklin, R. J. Myelin repair: The role of stem and precursor cells in multiple sclerosis. Philos. Trans. R. Soc. Lond. B Biol. Sci. 363(1489):171–183; 2008.
9. Connick, P.; Hunt, D. P. J.; Compston, D. A.; Chandran, S. Multipotent mesenchymal stromal cells in experimental neurology; safety considerations. J. Neurol. 254(Suppl. 3): 93; 2007.
10. Dominici, M.; Le Blanc, K.; Mueller, I.; Slaper-Cortenbach, I.; Marini, F.; Krause, D.; Deans, R.; Keating, A.; Prockop, D.; Horwitz, E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8(4):315–317; 2006.
11. Giordano, A.; Galderisi, U.; Marino, I. R. From the laboratory bench to the patient's bedside: An update on clinical trials with mesenchymal stem cells. J. Cell Physiol. 211(1):27–35; 2007.
12. Hofstetter, C. P.; Schwarz, E. J.; Hess, D.; Widenfalk, J.; El Manira, A.; Prockop, D. J.; Olson, L. Marrow stromal cells form guiding strands in the injured spinal cord and promote recovery. Proc. Natl. Acad. Sci. USA 99(4): 2199–2204; 2002.
13. Horwitz, E. M.; Le Blanc, K.; Dominici, M.; Mueller, I.; Slaper-Cortenbach, I.; Marini, F. C.; Deans, R. J.; Krause, D. S.; Keating, A. Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. Cytotherapy 7(5):393–395; 2005.
14. Hunt, D. P.; Morris, P. N.; Sterling, J.; Anderson, J. A.; Joannides, A.; Jahoda, C.; Compston, A.; Chandran, S. A highly enriched niche of precursor cells with neuronal and glial potential within the hair follicle dermal papilla of adult skin. Stem Cells 26(1):163–172; 2008.
15. Inoue, M.; Honmou, O.; Oka, S.; Houkin, K.; Hashi, K.; Kocsis, J. D. Comparative analysis of remyelinating potential of focal and intravenous administration of autologous bone marrow cells into the rat demyelinated spinal cord. Glia 44(2):111–118; 2003.
16. Javazon, E. H.; Colter, D. C.; Schwarz, E. J.; Prockop, D. J. Rat marrow stromal cells are more sensitive to plating density and expand more rapidly from single-cell-derived colonies than human marrow stromal cells. Stem Cells 19(3):219–225; 2001.
17. Jiang, Y.; Jahagirdar, B. N.; Reinhardt, R. L.; Schwartz, R. E.; Keene, C. D.; Ortiz-Gonzalez, X. R.; Reyes, M.; Lenvik, T.; Lund, T.; Blackstad, M.; Du, J.; Aldrich, S.; Lisberg, A.; Low, W. C.; Largaespada, D. A.; Verfaillie, C. M. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 418(6893):41–49; 2002.
18. Joannides, A.; Gaughwin, P.; Schwiening, C.; Majed, H.; Sterling, J.; Compston, A.; Chandran, S. Efficient generation of neural precursors from adult human skin: Astrocytes promote neurogenesis from skin-derived stem cells. Lancet 364(9429):172–178; 2004.
19. Keilhoff, G.; Goihl, A.; Langnase, K.; Fansa, H.; Wolf, G. Transdifferentiation of mesenchymal stem cells into Schwann cell-like myelinating cells. Eur. J. Cell Biol. 85(1):11–24; 2006.
20. Kim, B. G.; Hwang, D. H.; Lee, S. I.; Kim, E. J.; Kim, S. U. Stem cell-based cell therapy for spinal cord injury. Cell Transplant. 16(4):355–364; 2007.
21. Le Blanc, K.; Ringden, O. Immunobiology of human mesenchymal stem cells and future use in hematopoietic stem cell transplantation. Biol. Blood Marrow Transplant. 11(5):321–334; 2005.
22. Lee, J.; Kuroda, S.; Shichinohe, H.; Ikeda, J.; Seki, T.; Hida, K.; Tada, M.; Sawada, K.; Iwasaki, Y. Migration and differentiation of nuclear fluorescence-labeled bone marrow stromal cells after transplantation into cerebral infarct and spinal cord injury in mice. Neuropathology 23(3):169–180; 2003.
23. Mazzini, L.; Fagioli, F.; Boccaletti, R.; Mareschi, K.; Oliveri, G.; Olivieri, C.; Pastore, I.; Marasso, R.; Madon, E. Stem cell therapy in amyotrophic lateral sclerosis: A methodological approach in humans. Amyotroph. Lateral Scler. Other Motor Neuron Disord. 4(3):158–161; 2003.
24. Nandoe Tewarie, R. D.; Hurtado, A.; Levi, A. D.; Grotenhuis, J. A.; Oudega, M. Bone marrow stromal cells for repair of the spinal cord: Towards clinical application. Cell Transplant. 15(7):563–577; 2006.
25. Oh, T.; Peister, A.; Ohashi, K.; Park, F. Transplantation of murine bone marrow stromal cells under the kidney capsule to secrete coagulation factor VIII. Cell Transplant. 15(7):637–645; 2006.
26. Okabe, M.; Ikawa, M.; Kominami, K.; Nakanishi, T.; Nishimune, Y. 'Green mice' as a source of ubiquitous green cells. FEBS Lett. 407(3):313–319; 1997.
27. Pittenger, M. F.; Mackay, A. M.; Beck, S. C.; Jaiswal, R. K.; Douglas, R.; Mosca, J. D.; Moorman, M. A.; Simonetti, D. W.; Craig, S.; Marshak, D. R. Multilineage potential of adult human mesenchymal stem cells. Science 284(5411):143–147; 1999.
28. Polyzoidou, E.; Karrussis, D. Host brain reaction following transplantation of bone marrow stromal cells in experimental allergic encephalomyelitis. J. Neurol. 254(Suppl. 3):36; 2007.
29. Ringden, O.; Uzunel, M.; Sundberg, B.; Lonnies, L.; Nava, S.; Gustafsson, J.; Henningsohn, L.; Le Blanc, K. Tissue repair using allogeneic mesenchymal stem cells for hemorrhagic cystitis, pneumomediastinum and perforated colon. Leukemia 21(11):2271–2276; 2007.
30. Sasaki, M.; Honmou, O.; Akiyama, Y.; Uede, T.; Hashi, K.; Kocsis, J. D. Transplantation of an acutely isolated bone marrow fraction repairs demyelinated adult rat spinal cord axons. Glia 35(1):26–34; 2001.
31. Schwartz, R. E.; Reyes, M.; Koodie, L.; Jiang, Y.; Blackstad, M.; Lund, T.; Lenvik, T.; Johnson, S.; Hu, W. S.; Verfaillie, C. M. Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells. J. Clin. Invest. 109(10):1291–1302; 2002.
32. Uccelli, A.; Zappia, E.; Benvenuto, F.; Frassoni, F.; Mancardi, G. Stem cells in inflammatory demyelinating disorders: A dual role for immunosuppression and neuroprotection. Expert Opin. Biol. Ther. 6(1):17–22; 2006.
33. Wang, X.; Willenbring, H.; Akkari, Y.; Torimaru, Y.; Foster, M.; Al-Dhalimy, M.; Lagasse, E.; Finegold, M.; Olson, S.; Grompe, M. Cell fusion is the principal source of bone-marrow-derived hepatocytes. Nature 422(6934): 897–901; 2003.
34. Woodbury, D.; Schwarz, E. J.; Prockop, D. J.; Black, I. B. Adult rat and human bone marrow stromal cells differentiate into neurons. J. Neurosci. Res. 61(4):364–370; 2000.

35. Zappia, E.; Casazza, S.; Pedemonte, E.; Benvenuto, F.; Bonanni, I.; Gerdoni, E.; Giunti, D.; Ceravolo, A.; Cazzanti, F.; Frassoni, F.; Mancardi, G.; Uccelli, A. Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. Blood 106(5): 1755–1761; 2005.

36. Zhang, J.; Li, Y.; Chen, J.; Cui, Y.; Lu, M.; Elias, S. B.; Mitchell, J. B.; Hammill, L.; Vanguri, P.; Chopp, M. Human bone marrow stromal cell treatment improves neurological functional recovery in EAE mice. Exp. Neurol. 195(1):16–26; 2005.