J Neurosurg (Spine 1) 3:330–337, 2004

Minimally invasive delivery of stem cells for spinal cord injury: advantages of the lumbar puncture technique

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Object. Stem cell therapy has been shown to have considerable therapeutic potential for spinal cord injuries (SCIs); however, most experiments in animals have been performed by injecting cells directly into the injured parenchyma. This invasive technique compromises the injured spinal cord, although it delivers cells into the hostile environment of the acutely injured cord. In this study, the authors tested the possibility of delivering stem cells to injured spinal cord by using three different minimally invasive techniques.

Methods. Bone marrow stromal cells (BMSCs) are clinically attractive because they have shown therapeutic potential in SCI and can be obtained in patients at the bedside, raising the possibility of autologous transplantation. In this study transgenically labeled cells were used for transplantation, facilitating posttransplantation tracking. Inbred Fisher-344 rats received partial cervical hemisection injury, and 2×10^6 BMSCs were intravenously, intraventricularly, or intrathecally transplanted 24 hours later via lumbar puncture (LP). The animals were killed 3, 10, or 14 days posttransplantation, and tissue samples were submitted to histochemical and immunofluorescence analyses. For additional comparison and validation, lineage restricted neural precursor (LRNP) cells obtained from E13.5 rat embryos were transplanted via LP, and these findings were also analyzed.

Conclusions. Both BMSCs and LRNP cells home toward injured spinal cord tissues. The use of LP and intraventricular routes allows more efficient delivery of cells to the injured cord compared with the intravenous route. Stem cells delivered via LP for treatment of SCI may potentially be applicable in humans after optimal protocols and safety profiles are established in further studies.

KEY WORDS • stem cell transplantation • spinal cord injury • minimally invasive surgery • rat

CELL transplantation has shown great promise in the treatment of many neurological disorders including SCI,[22,24] traumatic brain injury,[29] Parkinson disease,[3] and stroke.[30] During the past decade, several cell types have been investigated and many potential candidates have been identified. Embryonic stem cells,[22] olfactory ensheathing glial cells,[5] Schwann cells,[5] and BMSCs[7] are a few cell types that have shown therapeutic potential. Adult BMSCs, also known as mesenchymal stem cells, are found in the bone marrow and are thought to provide support to the hemopoietic stem cells. These cells have progenitor-like characteristics and they act as support cells by producing an array of trophic factors and cytokines.[7] Classically, BMSCs are described as having the potential to differentiate into several mesenchymal forms including muscle, cartilage, bone, and adipose tissue.[7] They have also been demonstrated to have homing properties evidenced by their movement toward injured tissues in the cardiovascular system.[2] Transplantation of BMSCs has been shown to have beneficial effects after traumatic SCI[8,13] and demyelinating-type injury.[1] Bone marrow stromal cells are particularly attractive clinically because they can be obtained in patients at bedside, expanded in culture, and transplanted into the patient, thus allowing for an autologous model of cell therapy.

In most animal studies, however, stem cells have been directly injected into the pathological CNS tissues to test the cell's therapeutic efficacy.[8,13] This paradigm is associated with many problems. It is known, for example, that injured spinal cord tissue releases many inflammatory and cytotoxic chemokines that make the local environment hostile to the transplanted cells.[27] Indeed, most investigators,[8] including us (unpublished data), have had difficulty in demonstrating long-term survival of stem cells transplanted directly into the injured spinal cord despite the presence of immunosuppression. In addition, even if proven to be efficacious, this model is very difficult to translate into clinical practice. Most neurosurgeons would be reluctant to perform a laminectomy and transplant cells directly into a patient's injured spinal cord for the fear of causing further

Abbreviations used in this paper: BBB = blood–brain barrier; BMSC = bone marrow stromal cell; CNS = central nervous system; CSF = cerebrospinal fluid; GRP = glial restricted progenitor; LRNP = lineage restricted neural precursor; NRP = neuronal restricted precursor; PBS = phosphate-buffered saline; SCI = spinal cord injury.

deterioration such as damaging spared cord tissue or producing anesthesia- or procedure-related complications in an already compromised individual. Furthermore, the technique of direct parenchymal cell transplantation does not allow delivery of multiple dosages of therapeutic cells.

In light of these drawbacks, we investigated alternative and less invasive routes for delivering stem cells in a rodent model of SCI. Additionally, we used a syngenic model (both host and transplanted cells belonged to the same inbred strain of Fisher-344 rats) without immunosuppression, therefore mimicking the autologous model of cell transplantation. We compared the efficiency of grafting cells into the injured spinal cord following different modes of minimally invasive delivery (intravenous, intraventricular, and intrathecal via an LP). We evaluated the temporal profile of graft-related efficiency with BMSCs. Finally, we tested the more general validity of this method by delivering another cell type, that is, the embryonic LRNP cells to the injured spinal cord by using the percutaneous LP technique.

Materials and Methods

Isolation and Labeling of BMSCs

The BMSCs used in these experiments were obtained in Fisher rats that were transgenically modified to express the human placental *alkaline phosphatase* gene. We have established in earlier experiments[12,23] that this gene product is a reliable marker of transplanted cells, it is expressed in the cell membrane and cytoplasm of all BMSCs, and the expression is not downregulated (unlike many other viral vectors that have been used in the past).[14] The cells were isolated from inbred, adult transgenic Fisher rat bone marrow obtained in the femur and tibia, as described previously.[13] Briefly, after resecting the bones, they were rinsed in saline, the epiphysial plates were excised, and bone marrow was flushed out using a syringe and needle with Hank buffered saline solution; the cells were pooled and counted. Following centrifugation (600 G for 10 minutes), the cells were plated at 120×10^6 cells/cm^2 in 10% fetal bovine serum (HyClone, Logan, UT), 45% Hams F-12 (Invitrogen, Carlsbad, CA), 45% α-MEM (Invitrogen), and supplemented with antibiotic agents (100 U/ml penicillin G and 100 μg/ml streptomycin sulfate [Invitrogen]). Flasks were incubated in a humidified atmosphere with 5% CO_2 at 37°C. After 8 days, nonadherent cells were removed, the remaining cells detached with 0.05% trypsin/0.53 mM ethylenediaminetetraacetic acid (Invitrogen), and the viable cells replated at 2000 cells/cm^2. Subsequently, cultures were passaged at 4-day intervals. The resulting stromal cell fraction represented a population of multipotential stemlike cells that have been well characterized in their ability to proliferate and differentiate into various phenotypes.[28] Material obtained in two animals was adequate to generate enough BMSCs for the entire experiment. All experiments were performed with cells passaged fewer than five times to maintain their phenotypic and growth potential.

Preparation of LNRP Cells

Embryos from transgenic Fischer-344 rats expressing the *alkaline phosphatase* marker gene were removed on embryonic Day 13.5. The embryos were transferred to dishes containing Hank buffered saline solution. The caudal regions of the embryonic spinal cords were dissected and incubated in 0.05% trypsin/ethylenediaminetetraacetic acid for 7 minutes, and surrounding connective tissue was then mechanically removed. The cords were placed in fresh medium, centrifuged at 1000 rpm for 5 minutes, and resuspended in fresh culture medium. The cords were gently triturated to a single-cell suspension, and cells were subsequently plated on poly-L-lysine and laminin-coated flasks. The media that was used to culture the LRNP cells consisted of Dulbecco modified Eagle medium/F-12 supplemented with bovine serum albumin, B27, 500 IU/ml penicillin, 500 μg/ml streptomycin, N2 supplement, basic fibroblast growth factor (10 ng/ml), and neurotrophic factor–3 (20 ng/ml). Mixed populations of NRP and GRP cells are together called LRNP cells; these were cultured for 48 hours at 37°C and 5% CO_2 prior to transplantation. The composition of the transplant population consisted of an approximate NRP/GRP ratio of 1:1. Two million LRNP cells were resuspended in 40 μl of complete medium prior to LP delivery.

Spinal Cord Injury Model

All surgeries were performed in adult Fisher-344 female rats according to protocols approved by an animal care and use committee at our institution. The rats were anesthetized using an intraperitoneal injection of xylazine-acepromazine-ketamine cocktail. A midline vertical incision was made over the cervical spine and the paraspinal muscles were retracted laterally. A C3–4 laminectomy was performed using the operating microscope. The dura mater was opened longitudinally, and the right lateral funiculus of the spinal cord was excised using microsurgical technique at C-3. We have previously established that this injury completely ablates the rubrospinal tracts without significantly compromising the respiratory or autonomic functions.[19] Hemostasis was completed and the surgical cavity filled with a hydrogel matrix (hydrogel was used to fill the injury cavity; this material is being developed for SCI repair but has no demonstrable effect on cell migration [unpublished data]). The dura was approximated with 9-0 nylon, and the wound was closed in layers.

Transplantation Techniques

All animals received 2×10^6 cells diluted in 40 μl of culture medium. Transplantation was performed 24 hours after SCI.

Lumbar Puncture. Lumbar puncture was performed after institution of inhalation (Isoflurane) anesthesia. Briefly, the rat was anesthetized and placed on an operating surface that flexes the animal's back. A small (1-cm) longitudinal incision was then made over the L3–5 spinous processes and the skin retracted. A neonatal LP needle (25-gauge) was advanced into the spinal canal at L3–4 or L4–5. Proper placement of the needle in the lumbar intradural space was indicated by three signs: 1) a feeling of "give" at the time of entry (soft sign); 2) a tail flick (more definitive sign); and 3) presence of CSF in the needle hub (most definitive sign). Once proper needle placement was confirmed, the CSF present in the needle hub was aspirated using a micropipette, and 2×10^6 BMSCs diluted in 40 μl culture medium were injected into the CSF during a 30-second period. Additionally 10 μl of saline was used to flush the needle, and the LP needle was withdrawn after replacing the stillette. The skin was stapled and the animal returned to its cage. There were 16 rats that received BMSCs injected via an LP; in 12 cervical injury was induced 24 hours earlier and four received transplanted cells without having undergone SCI. Six rats received LRNP cells 1 day after the SCI. Of the rats that received BMSCs, four (two injured and two control) were killed after 3 days, eight (six injured and two control) after 10 days, and four after 14 days. Of the rats that received LRNP cells, two rats each were killed after 10 days, 3 weeks, or 5 weeks of transplantation.

Intravenous Delivery. After induction of anesthesia, a 0.5-in incision was made over the groin, and the femoral vein was exposed. This vein was cannulated under magnification, and 2×10^6 BMSCs, diluted in 40 μl culture medium, were injected during a 5- to 7-minute period. Of seven rats that received BMSCs intravenously, four underwent injury 24 hours prior to transplantation and three that received BMSCs but no spinal injury were used as controls.

Intraventricular Delivery. Rats were anesthetized and positioned in a stereotactic apparatus. A vertical skin incision was made and a 1-mm-diameter hole was made in the skull at a site 3.5 mm caudal to the lambda in the midline. A stereotactic needle was advanced 6 mm into the dura of the fourth ventricle and 2×10^6 BMSCs, diluted in 40 μl culture medium, were injected during a 30-second period according to previously described methods.[32] Four rats received

BMSCs intraventricularly, and results were analyzed 3 (two rats) or 10 (two rats) days after injury.

Histopathological Examination

Rats were killed by an overdose of Nembutal and were perfused with 4% paraformaldehyde. Brain and spinal cord tissue were dissected and cryoprotected in 30% sucrose. Tissue specimens were then frozen in TissueTek, and axial sections were cut through the injured segment in a cryotome. Transplanted cells were identified using alkaline phosphatase histochemical (because the gene expresses human alkaline phosphatase enzyme) and immunofluorescence techniques, following previously described protocols.[12,23]

Histochemical Evaluation

Sections were washed three times in PBS, heat treated at 60˚C in PBS for 1 hour to inactivate endogenous alkaline phosphatase, briefly washed in alkaline phosphatase buffer (100 mM Tris, 100 mM NaCl, 50 mM MgCl2, pH 9.5), and incubated for 2 hours at room temperature in the dark with alkaline phosphatase buffer containing 1 mg/ml NBT, 0.1 mg/ml BCIP, and 5 mM levamisole. Slides were placed on cover slips in aqueous mounting media and observed using a light microscope. The alkaline phosphatase–positive cells stained bluish black.

Immunofluorescence Testing

Selected slides were ringed with rubber cement and hydrated with PBS. Nonspecific immunoaffinity was blocked using 10% goat serum for 1 hour and primary antibody diluted in PBS–0.2% Triton was then applied (polyclonal rabbit anti–human alkaline phosphatase; 1:200) for overnight incubation at room temperature. The following morning, primary antibody was rinsed three times with PBS and secondary antibody (rhodamine-conjugated goat anti–rabbit) was applied at a dilution of 1:200 for 2 hours in the dark. The secondary antibody was then rinsed and slides were placed on cover slips with Vectashield-Hardset containing DAPI for nuclear staining. Images were acquired using a microscope with fluorescent and DIC optics, fitted with a high-resolution Photometrics Sensys cooled charged coupled device digital camera connected to a computer for acquisition and processing of 12-bit images.

Results

Homing of Stem Cells Toward the Injured Spinal Cord

We observed that BMSCs selectively homed toward the injured tissues of the spinal cord after delivery via all three routes. No cells were demonstrated around the cervical segments of the uninjured animals that received BMSC transplants (data not shown). In contrast, all specimens obtained in spinal cord–injured rats had evidence of BMSC penetration into the injured tissues (Fig. 1), although some variability was noted in the number of cells reaching the spinal cord in different animals. Most importantly, all BMSCs were located in or around the injured parenchyma and no cells were observed on the contralateral side of the injured cervical segment, indicating that the homing phenomenon operated with some degree of specificity (Fig. 1). Immunofluorescence confirmed that the alkaline phosphatase–positive stain was composed of viable cells with distinctive cytoplasmic and nuclear morphological features (Fig. 2). Axonal growth was noted around the transplanted cells.

Intravenous Route: Least Efficient

Very few to no cells were present within the injured spinal segments in rats that received BMSCs intravenously (Fig. 3). In contrast, considerably more cells were detected in the injured tissues after both intrathecal and intraventricular delivery (Fig. 3). These data confirm the hypothesis that transplanting cells into the CSF leads to more successful grafting when injection is via an intrathecal or -ventricular rather than intravenous route. It should be noted that the same cell dosage (2 million cells) was used for all routes and varying cell dosages might lead to improved grafting efficacy.

More Transplanted Cells Present at Later Time Points

The number of cells within the injured spinal cord tissues increased with passage of time. Few cells were noted 3 days after transplantation (Fig. 1B); however, many more cells were seen 10 (Fig. 1C) and 14 days (Fig. 1D) after transplantation. This result was consistently found in all three methods of cell delivery and when using either cell type (Fig. 4). Because this study provides descriptive preliminary data, however, a more thorough experiment involving a larger number of animals as well as quantitative estimation of cell numbers is being performed to determine the temporal profile of migrating transplanted cells.

Delivery of Different Cell Types

To confirm and validate the efficacy of the LP delivery method, we transplanted LRNP cells, which are a different cell type embryologically and phenotypically compared with BMSCs. We found that, after LP transplantation, LRNP cells also migrated toward the injured tissues of the spinal cord with considerable specificity (Fig. 4). Because the number of cells increased with passage of time, more cells were observed at later time points during the study period. The migration of LRNP cells appeared to be a bit slower than BMSCs (Fig. 4) because not many of the former were noted at the injury site 10 days following transplantation (in contrast to BMSCs), but these numbers appeared to increase at 3 and 5 weeks.

Discussion

Cell Transplantation Techniques

A major unresolved problem in the context of SCI is the delivery of cells to an already compromised spinal cord without causing further damage. Most investigators have undertaken direct injection into the injured spinal cord parenchyma.[8,13] Although this is acceptable in animal experiments, its extrapolation to humans may be difficult because a major neurosurgical operation will be required. This difficulty in translation will limit clinical trials, at least initially, to patients with complete SCIs in whom further deterioration cannot occur but in whom significant benefit from transplantation therapies is also least likely.[6] Another problem associated with direct parenchymal injections is the likelihood of damaging spared spinal tissues with the injecting needle. It is a well-known principle of neurosurgery that injured tissues do not tolerate operative manipulation as well as normal tissues because of the presence of edema, altered blood flow, and injury-related cytokines. Finally, direct injection of cells into the parenchyma does not allow suitable delivery of multiple therapeutic doses because of its invasive nature and because injecting cells into multifocal diseases presents many logistical and technical challenges.

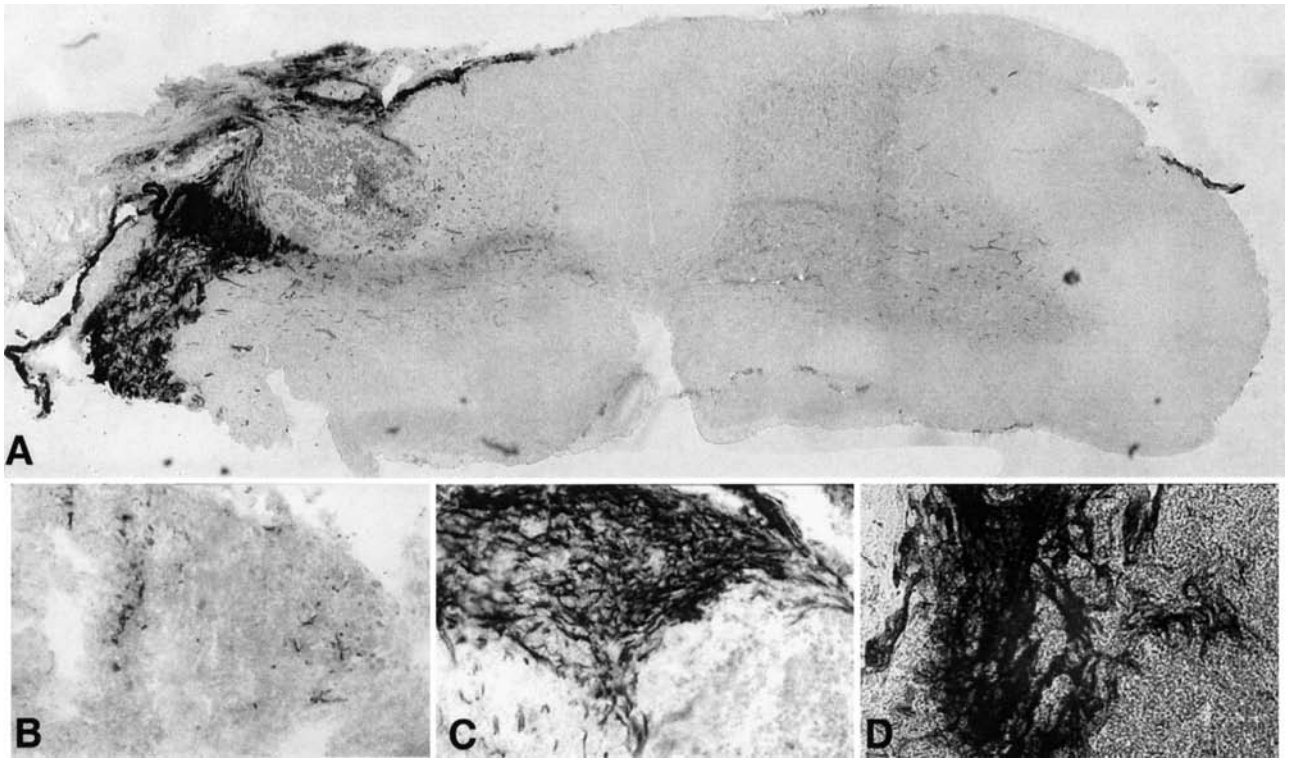

FIG. 1. Photomicrographs. A: A composite of a transverse section of an injured spinal cord obtained in an animal that received BMSCs via LP. Transplanted cells are noted within the parenchyma of the injured spinal cord and the root entry zone. Transplanted cells appear dark black. Transplanted BMSCs are observed only at the injury site, indicating injury-specific homing behavior. B–D: Higher-powered views of the injured spinal cord parenchyma. The temporal profile of BMSCs homing to the injured spinal cord is shown with few cells noted 3 days after transplant (B); many more cells are seen 10 (C) and 14 (D) days after transplantation. Alkaline phosphatase histochemistry technique, original magnification × 50 (A); × 100 (B–D).

To overcome these limitations, some groups have evaluated intravascular delivery of stem cells.[20,26] Intraarterial delivery of stem cells for the treatment of spinal cord disorders is limited by the multisegmental arterial supply of the spinal cord, which requires highly selective and technically challenging cannulation of the spinal arteries. One advantage of intravenous stem cell delivery is that it is comparatively the least invasive approach and has been investigated in several studies.[11,26] The authors of most studies have demonstrated some cellular homing into the pathological CNS tissues after intravenous injection, but as our results indicate that intravenous delivery is not efficient compared with other methods of intra-CSF delivery. Additional problems associated with intravenous stem cell delivery include reliance on injury-mediated opening of the BBB to allow cells access to the CNS parenchyma (or the need for additional drugs such as lipopolysaccharide to open the BBB[26]), transplanted cells being subjected to major first-pass effects and becoming trapped in extra-CNS tissues such as lung and liver, and prolonged exposure of the transplanted cells to the reticuloendothelial and immune systems while circulating in the blood.

The direct delivery of stem cells into the CSF has also been explored,[31] and intraventricular injection has been the favored delivery method.[33] This technique, however, is too invasive for clinical applicability, which makes its translation challenging. One group of investigators has demonstrated that neurosphere-derived stem cells delivered into the ventricular CSF can reach the injured tissue in a spinal contusion model.[32] We reasoned, however, that BMSCs are more appropriate because of their evidenced therapeutic effect, their availability, and the possibility of an autologous model in humans. Injection of cells into the lumbar CSF via an indwelling cannula has been shown to be effective for delivering embryonic germ cell derivatives.[16] Another group has used the LP technique in rats to deliver neuroblastoma cells to alleviate neuropathic pain.[9] In addition, Lazorthes, et al.,[18] have performed LP delivery of allogenic adrenal medullary tissue to alleviate pain in patients with terminal cancer. Their study was focused on delivery of analgesic chemicals into the CSF without any need or demonstration of the cell-migration phenomenon. Theoretically and clinically, LP delivery of stem cells is extremely attractive. Lumbar puncture is a minimally invasive procedure that can be performed at the bedside after injection of a local anesthetic. In humans LP is performed at the L3–4 level, far away from the cervical or thoracic spinal cord, which are the regions most commonly effected by SCI. This makes LP delivery of stem cells relatively safe and unlikely to worsen compromised patients as a direct result of the intervention. Additional advantages of LP delivery are related to several factors: 1) the cells are delivered across the BBB into the CSF, making it far more efficient than intravenous delivery; 2) CSF

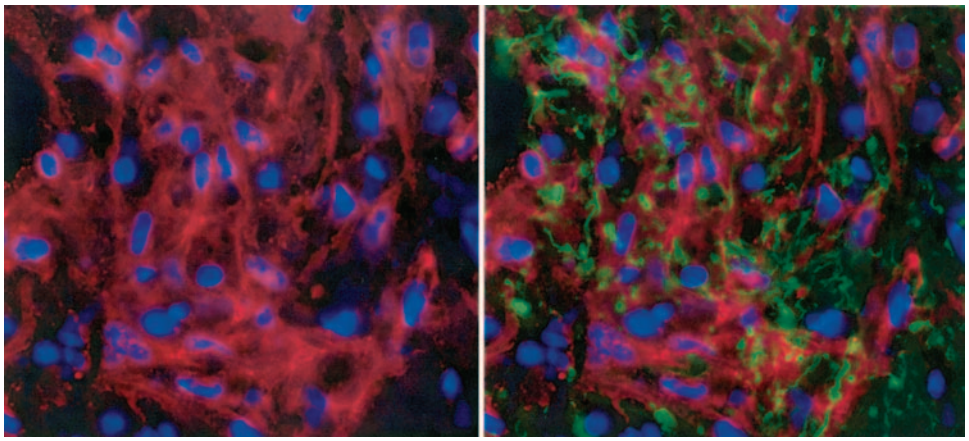

FIG. 2. Immunofluorescence double staining was performed to confirm the nature of transplanted cells 10 days after transplantation. High-powered images were obtained of a transverse section of an injured spinal cord (lateral funiculus) in a rat that received BMSC transplanted via the CSF. *Left:* Nuclei (blue) and transgenic alkaline phosphate enzyme expression on the transplanted cells (red). Polyclonal anti–human alkaline phosphatase antibody and rhodamine-labeled secondary antibody, original magnification × 200. *Right:* Axons (green) growing around the transplanted cells. Polyclonal anti–human alkaline phosphatase antibody, RT-97, and fluorescein isothiocyanate–labeled secondary antibody, original magnification × 200.

circulates within the CNS, allowing transplanted cells to home into the injured tissues, and cells are preserved in a relatively immune-privileged environment; and 3) because the transplanted cells are delivered away from the hostile environment of the injured tissue, they are given a greater opportunity to survive and migrate to the injury site.

Injury Model

Many different animal models of SCI have been devised to test different aspects of the injury and its repair. Admittedly, the spinal contusion model most closely approximates the sequence of events that occurs in patients. In this study, however, we chose to use the partial hemisection model for a few reasons. As has been noted in our earlier studies,[19] partial hemisection as our group has performed is a very limited injury that minimizes the animal's morbidity and allows careful analysis of axon regeneration and transplant survival. Eventually, the findings of this study will be validated in a contusion model of SCI.

Cell Type and Mechanisms of Therapeutic Efficacy

Cell transplantation paradigms have shown great promise in improving outcomes after SCIs; different cell types have been tested in various injury models.[24] Adult BMSCs (also known as mesenchymal stem cells) are thought to provide support to the hemopoietic stem cells. These cells have progenitor-like characteristics and act as support cells by producing an array of trophic factors and cytokines[7] (unpublished data). Bone marrow stromal cells have the potential to differentiate into many mesenchymal tissues (muscle, fat, and blood vessels). There are reports in the recent literature regarding the ability of these cells to "transdifferentiate" into neural tissue.[21] These reports are controversial and may be characteristic of a very small population of multipotential cells[15] or a rare event that does not necessarily produce functional differentiation and is unlikely to be a major mechanism of therapeutic efficacy of BMSCs.[7,28] A more likely factor contributing to their efficacy is their production of growth factors, which can support regenerating axons. Bone marrow stromal cells may also provide a cellular matrix that provides a permissive environment at the injury site. Regardless of the nature of its repair mechanisms, we and others have demonstrated improvement in outcomes after spinal contusion[8,13] and demyelinating injury[1] following BMSC transplantation. Therefore, in this study we evaluated the possibility of delivering BMSCs by using minimally invasive methods. For additional comparison and technique validation, we also demonstrated the possibility of delivery through LP of LRNP cells leading to their grafting in the injured spinal cord. Our group has demonstrated that grafted NRP cells differentiate into mature neurons, survive for at least 1 month, appear to integrate within the host spinal cord, and extend processes in both gray and white matter.[12] In addition, it has been postulated that GRP cell transplantation might assist in recovery from SCI by improving remyelination and supplying astrocytes that support regenerating neurons.[12] Although we have provided preliminary proof of a principle of minimally invasive LRNP cell delivery, an optimized protocol for minimally invasive delivery of a mixture of NRP and GRP cells will be a significant advance in this field.

Transplanted Cell Survival

An important finding in this study was that a consistently greater number of transplanted cells were found within the injured spinal cord tissues at later time points compared with earlier ones after injection. This finding is in direct contrast to that reported after direct parenchymal injection studies in which most investigators reported a decline in the number of cells present at injury sites with passage of time.[8] There are two possible explanations for this. First, it may be reasoned that circulating transplanted cells gradually home to the injured tissues and lead to an increase in numbers, or, second, the few cells that do manage to home and divide rapidly after reaching their destination. It is not possible to differentiate between these two

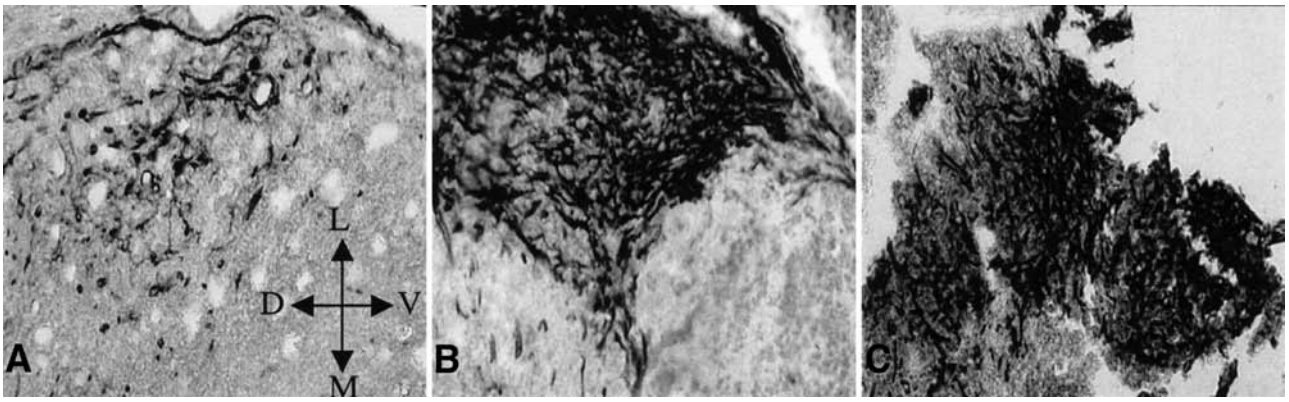

FIG. 3. Photomicrographs showing transverse sections through injured spinal cords of rats that received 2×10^6 BMSCs via intravenous (A), LP (B), or intraventricular (C) routes. All sections are from animals killed at the 10-day time point, and orientation of all the sections is depicted by the graphic in A. Few cells are noted within the injury site after intravenous delivery (A), whereas more cells reached the injured cord tissues after LP (B) or intraventricular (C) delivery indicating greater efficiency of intra-CSF delivery. D = dorsal; L = lateral; M = medial; V = ventral. Alkaline phosphatase histochemistry, original magnification $\times$ 100.

possibilities within the experimental framework presented here and more studies involving markers of proliferation are needed to resolve this issue. This finding, however, can have major therapeutic implications because it allows minimally invasive cell therapy to be used for delivering therapeutic molecules to the injured tissues in a specific manner.

Autologous Model of Transplantation

Marrow stromal cells are particularly attractive clinically because autologous transplantation can be performed in patients with SCI, especially if combined with the minimally invasive delivery techniques described in this paper. Considerable clinical experience has been collected in the administration of autologous BMSCs in patients suffering from hematological disorders and other malignancies.[10] These studies have established the feasibility and safety of harvesting BMSCs in patients, expanding their numbers in vitro, and retransplanting them for therapeutic benefit.[17] It should be noted that the experiments reported in this paper represent a syngenic model of transplantation because both the transplated cells and host animals belonged to the same inbred Fisher-344 strain of rats. Syngenic transplantation approximates the autologous model by eliminating most of the immune-mediated reactions that are responsible for graft rejection after transplantation. By using the autologous model, we can eliminate or minimize the problems associated with embryonic stem cells that include the ethical and technical considerations of obtaining embryonic tissues, the need for long-term immunosuppression of the recipients, and the possibility of transmitting infectious agents with the transplanted cells. One of the problems of developing autologous therapies is the absence of a large-scale model to deliver them effectively to the patients. We believe, however, that with the increasing number of autologous therapies in diverse fields such as hematological malignancies,[10] cancer vaccines,[25] and cell therapies for bone and cartilage regeneration,[4] among many others, there will soon be a critical mass that will allow the establishment of dedicated facilities, similar to in-hospital blood banks, to provide autologous therapies. The theoretical, scientific, and clinical advantages of autologous therapies are too great to be left unexplored for the lack of a business model.

Further Studies

This preliminary study provides proof-of-concept data, but detailed experiments are required. The number of rats was small, and variability was noted in the number of cells found in the injured tissues, precluding accurate quantitative analysis. This variability may be reflective of the small size of the rat, making the LP technique quite challenging, particularly compared with its application in humans. Although the LP technique has been shown to be more efficient than intravenous delivery in this study, the homing phenomenon mechanism needs to be determined. It appears that the cells delivered via the CSF only enter the cord in regions of breach to the glia limitans. It is possible that the injured tissues are releasing certain chemokines that attract the cells circulating into the CSF to migrate toward the injury. This question will need to be resolved in carefully designed experiments. Our findings stimulate additional questions. What happens to the cells after they are injected into the CSF? For how long do they circulate? Is there a risk of causing hydrocephalus by blocking the CSF absorption paths? What is the optimal cell dosage? What is the optimal timing of their injection? Will multiple small doses of cells be safer and more efficacious? All these issues need further experimentation and optimization of protocols. Nevertheless, we believe that the results of this study are important in the efforts to find acceptable delivery techniques and optimal protocols. This technique has possible applications in diverse diseases such as traumatic brain injury, stroke, multiple sclerosis, and amyotrophic lateral sclerosis in addition to SCI. It is particularly suitable for multifocal diseases in which local parenchymal delivery will produce inordinate logistical and safety problems. We propose that this type of minimally invasive delivery of cells in the treatment of neurological disorders will not only make it easier to achieve therapeutic efficacy in animal models but also eventually in humans.

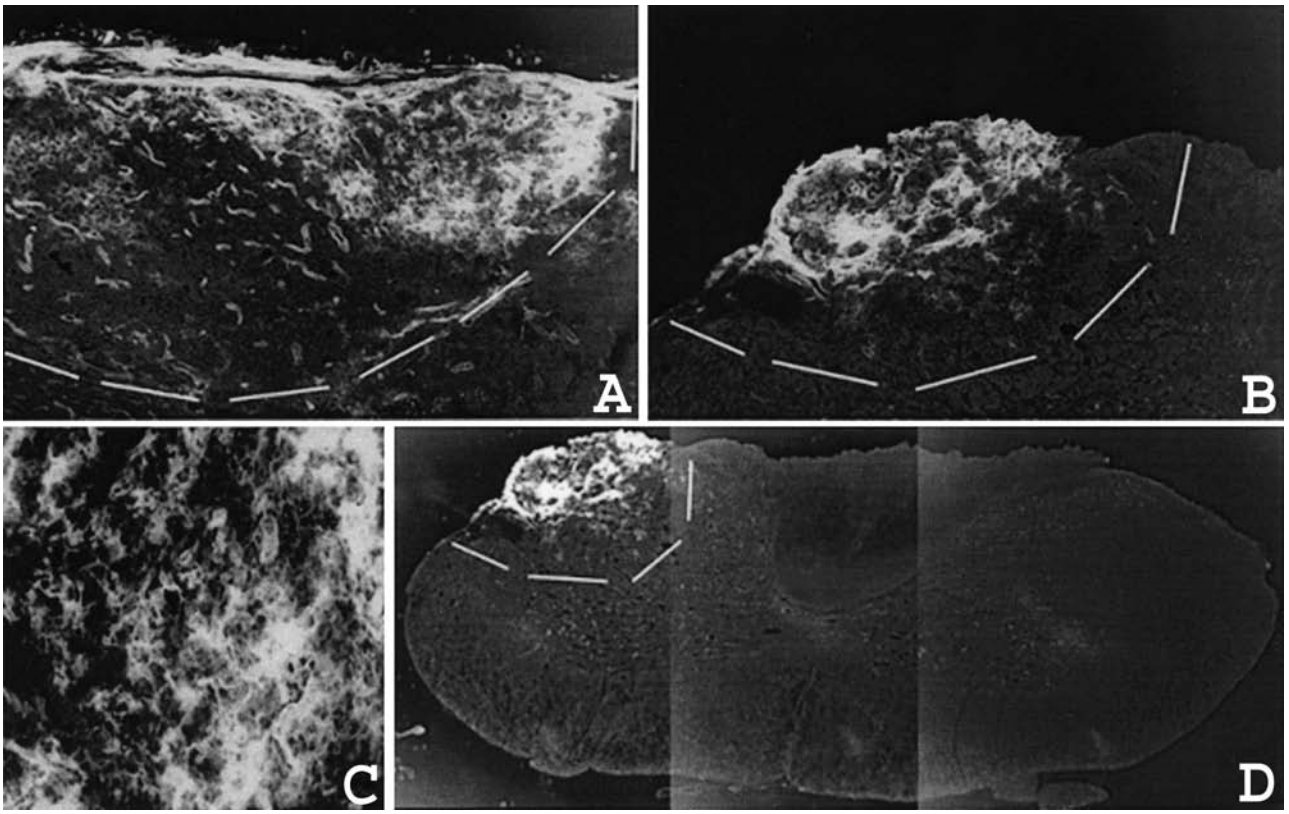

FIG. 4. Migration and survival of LRNP cells in the injured spinal cord following LP delivery. The LRNP cells were transplanted via LP 24 hours after generation of a partial C3–4 hemisection. A and B: The LRNP cells (seen as white in this section) are present throughout the injury site at both 3 (A; sagittal) and 5 (B; transverse) weeks following transplantation. C: High-power image showing some cells with processes indicating differentiation into downstream phenotypes. D: A composite transverse section of spinal cord obtained at the injury level. Grafted cells can be seen at the injury site but not in the surrounding uninjured tissue. *Dashed lines* in each panel represent the interface between host spinal cord and hydrogel matrix within the injury site. Alkaline phosphatase histochemistry, original magnifications × 100 (A); × 50 (B); × 200 (C); × 8 (D).

Conclusions

We have demonstrated the possibility of delivering two different types of stem cells to injured spinal cord tissues; the delivery mechanism involved a minimally invasive percutaneous LP. In comparison with intrathecal or -ventricular routes, intravenous delivery of stem cells is far less efficient. Cell grafting efficiency seems to increase with the passage of time after transplantation. Further experiments are needed to determine the optimal delivery protocols and to demonstrate histological and behavioral improvement in injured animals as a result of minimally invasive stem cell therapy.

Disclosure

The two senior authors (A.B. and I.F.) are coinventors of the stem cell delivery technology partly described in this paper and are seeking a patent.

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Manuscript received November 14, 2003.
Accepted in final form May 25, 2004.
Dr. Fischer received support from the National Institutes of Health (Grant Nos. NS24707, NS37515, and NS43882).

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