Department of Veterans Affairs

Journal of Rehabilitation Research and Development
Vol. 39 No. 2, March/April 2002
Pages 287–298

Cell transplantation of peripheral-myelin-forming cells to repair the injured spinal cord

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Abstract—Much excitement has been generated by recent work showing that a variety of myelin-forming cell types can elicit remyelination and facilitate axonal regeneration in animal models of demyelination and axonal transection. These cells include peripheral-myelin-forming cells, such as Schwann cells and olfactory ensheathing cells. In addition, progenitor cells derived from the subventricular zone of the brain and from bone marrow (BM) can form myelin when transplanted into demyelinated lesions in rodents. Here, we discuss recent findings that examine the remyelination potential of transplantation of peripheral-myelin-forming cells and progenitor cells derived from brain and bone marrow. Better understanding of the repair potential of these cells in animal models may offer exciting opportunities to develop cells that may be used in future clinical studies.

Key words: *bone marrow, neural precursor cells, olfactory ensheathing cells, Schwann cells, spinal cord repair, transplantation.*

This material is based on supported in part by Rehabilitation Research and Development Service of the Department of Veterans Affairs, the National Institutes of Health, and the National Multiple Sclerosis Society and the Myelin Project (Washington, DC).
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INTRODUCTION

Multiple sclerosis (MS) and contusive spinal cord injury both present variable amounts of axonal demyelination and transection. Currently, therapeutic approaches for both neurological disorders have limited efficacy. Recent advances in cell biology have led to the preparation of a number of cell types in culture that, upon transplantation, can remyelinate demyelinated axons and can encourage axonal regeneration. While axonal regeneration is very limited in the normal injured mammalian central nervous system (CNS), substantial axonal regeneration can occur in peripheral nerves under certain conditions. Part of this regenerative capacity of peripheral nerve has been linked to several unique properties of Schwann cells, including their production of extracellular matrix and trophic factors and their lack of growth inhibitory proteins that are present on oligodendrocytes (1). While Schwann cells normally only form myelin on peripheral nerve, they can remyelinate demyelinated axons in the CNS (2,3). Another cell type, olfactory ensheathing cell (OEC), which is located in olfactory nerves and outer layers of the olfactory bulb and normally do not form myelin, can do so when transplanted into the CNS (4,5). An interesting observation of both Schwann cells and olfactory ensheathing cells is that they can enhance axonal regeneration in the spinal cord when transplanted into axonal transection lesion sites (6–8).

One practical difficulty with the potential use of either Schwann cells or olfactory ensheathing cells as an autologous cell therapy is the limitation of the number of cells that can be harvested. Recent work suggests that neural precursor cells derived from adult human subventricular zone and bone marrow (BM) cells both can form myelin when transplanted into the demyelinated spinal cord. A potential advantage of neural precursor cells is that they can be readily expanded in culture to increase cell number prior to transplantation. In the present review, we will discuss the potential of Schwann cells and olfactory ensheathing cells as cellular tools to remyelinate and to enhance axonal regeneration in the injured spinal cord, as well as the repair potential of cells derived from the adult human brain and BM, which can establish peripheral-like myelin in addition to central myelin when transplanted into the injured spinal cord.

Transplantation of Schwann Cells to Remyelinate Spinal Cord

Transplantation of Schwann cells into the demyelinated rodent spinal cord results in anatomically defined myelination (2,9,10). Moreover, when anatomical repair is achieved, near normal conduction properties of the remyelinated axons ensue (3,5,11). Endogenous remyelination of CNS demyelinated axons by oligodendrocytes (12) or Schwann cells (2) results in the reestablishment of relatively normal impulse conduction in animal models of demyelination (13–15). However, endogenous remyelination is very limited in human diseases such as MS (16–18). Given the success of cell transplantation to form functional myelin in animal models, myelin-forming cell transplantation has been suggested as a potential repair strategy for demyelinated CNS axons (3,5,19,20).

Transplantation of glial cells obtained from the adult human brain failed to achieve remyelination of demyelinated rat axons in the CNS (21), while transplantation of human Schwann cells (11) and olfactory ensheathing cells (22,23) elicited remyelination of demyelinated rat axons in the CNS. **Figure 1** shows low- and high-power micrographs of a rat demyelinated spinal cord 3 weeks after injection of reconstituted cryopreserved human Schwann cells. Note the relatively large number of myelinated axons with typical Schwann cell morphology, that is, large cytoplasmic and nuclear regions. Electron micrographs (not shown) reveal the presence of a basement membrane and extracellular collagen deposition (11). The conduction velocity of the axons remyelinated by the human Schwann cells was improved (see **Figure 2**), indicating that electrophysiological function of the remyelinated axons was restored.

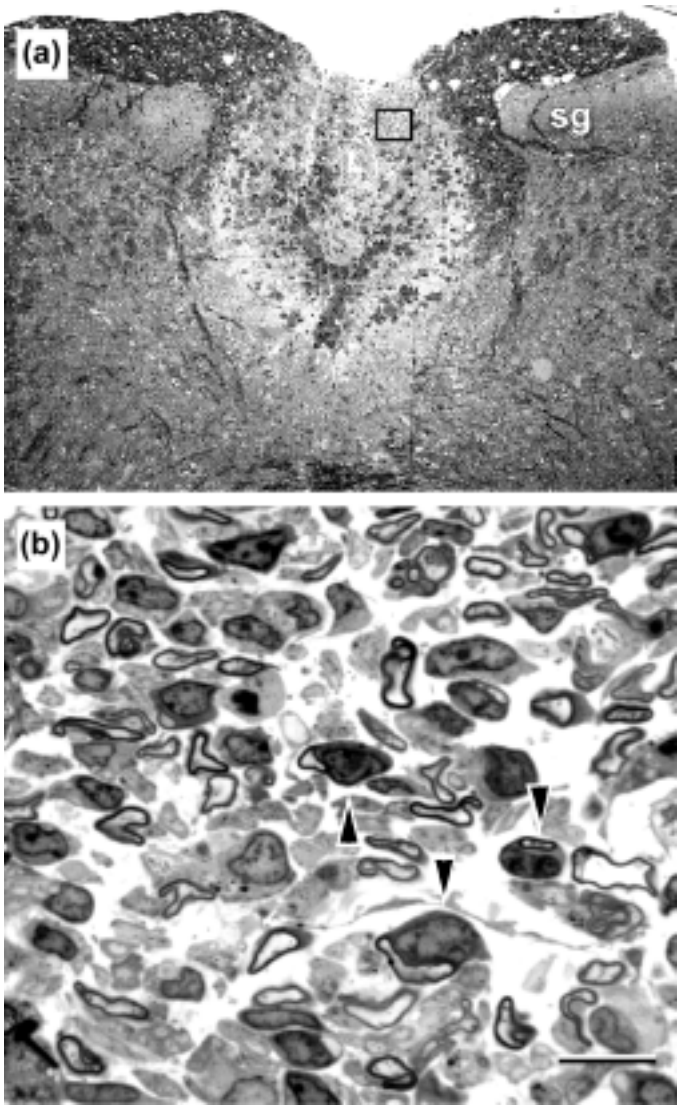

Figure 1.
Remyelinated axons following human Schwann cell transplantation show a peripheral pattern of myelination. Photomicrographs were obtained from spinal cords placed in fixative after in vitro electrophysiological recordings were obtained. (a) Lesion area of dorsal columns 3 weeks after induction of X-EB lesion. "Sg" refers to substantia gelatinosa in dorsal horn. (b) Higher power micrograph from boxed region of lesion showing remyelinated axons. Note myelinated axons surrounded by cells with large nuclear and cytoplasmic domains characteristic of peripheral myelin. Calibration in (b) corresponds to 100 µm in (a) and 10 µm in (b). Source: Modified from citation (11).

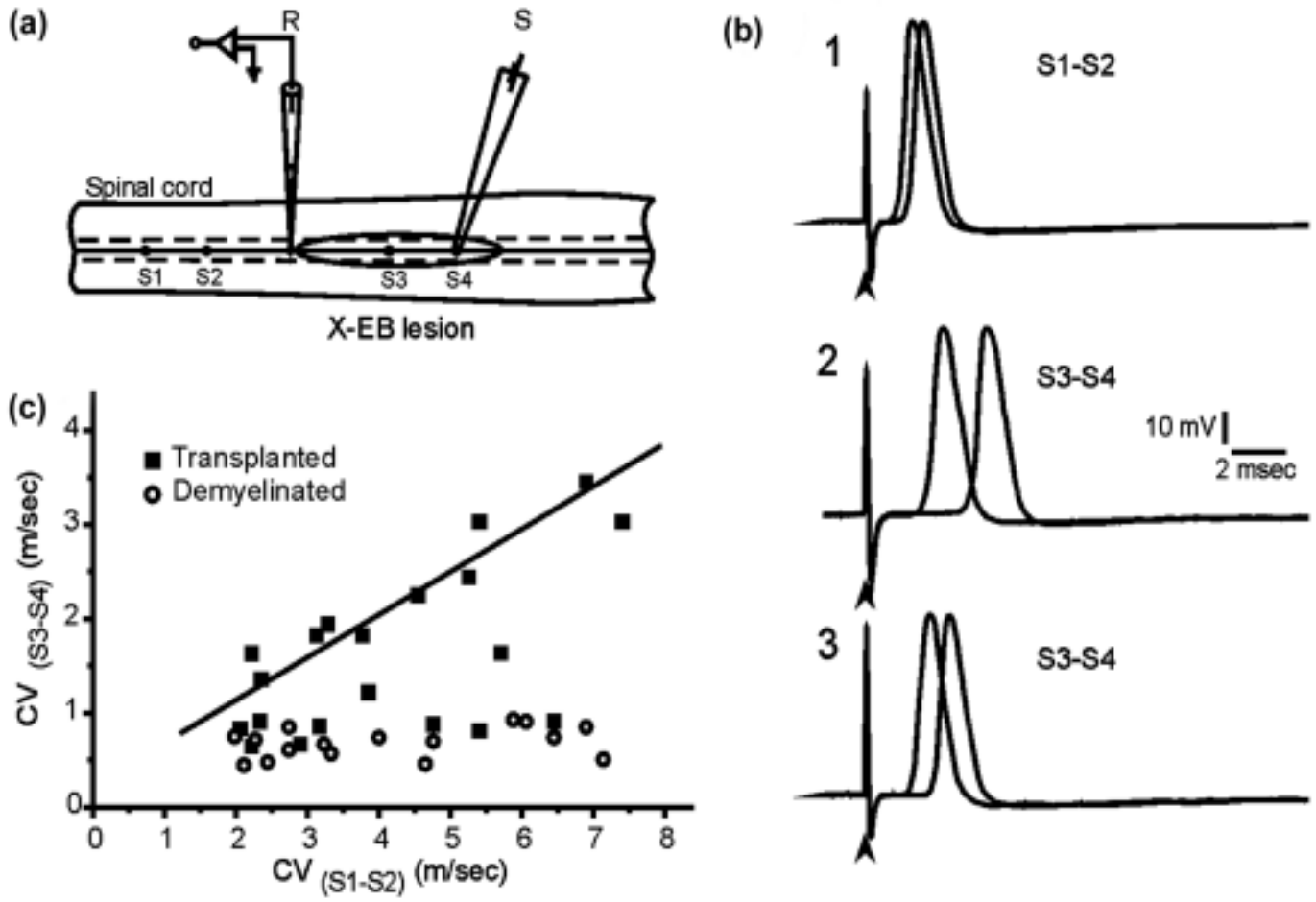

Figure 2.
Intra-axonal recordings from demyelinated and remyelinated dorsal column axons. (a) Schematic showing arrangement of intra-axonal recording and stimulation sites. Intra-axonal recordings were obtained from dorsal column axons outside of lesion where axons were normally myelinated. Stimulating electrodes were positioned outside (S1–S2) and within (S3–S4) X-EB lesion zone to assess single axon conduction velocity over both demyelinated or remyelinated axon segment and normally myelinated axon segment of same axon. (b) (1) Pairs of action potentials recorded from S1–S2 stimulation, (2) S3–S4 in the demyelinated dorsal columns, and (3) S3–S4 following cell transplantation. Recordings were obtained at comparable conduction distances. (c) Plot of the conduction velocity of axon segments within lesion (S3–S4) versus conduction velocity of axon segment outside of the lesion (S1–S2) for X-EB lesioned spinal cord without (open circles) and with (closed squares) transplantation. Source: Modified from citation (11).

Homologous or autologous tissue represents one possible source of Schwann cells for transplantation into patients with demyelinating disease. Presumably, Schwann cells are not antigenically predisposed to the immunological attack seen in MS as are oligodendrocytes. The demonstration of anatomical and electrophysiological repair of demyelinated axons by adult human Schwann cells and olfactory ensheathing cells is an important prerequisite for future consideration of these cells as candidates for autologous transplantation studies in humans.

Transplantation of Olfactory Ensheathing Cells to Remyelinate Spinal Cord

OECs are unique cells that belong to the glial lineage and cannot be classified within any of the known glial populations, even though OECs belong to the glial lineage (24). OECs are an unusual population of cells, in that they share characteristics with both astrocytes in the

CNS and Schwann cells in the peripheral nervous system (PNS) (24–27) and are the only glial cells known to cross the PNS-CNS transitional zone, accompanying the axons that they ensheath (28). In the normal nervous system, OECs surround or ensheath bundles of nonmyelinated axons in the olfactory nerve and do not form myelin. OECs share with Schwann cells the capability to support axonal ensheathment and regrowth (5,6,8,29–30), but they do not share the same developmental origin nor some immunocytochemical and morphological features of Schwann cells (24). The astrocyte-like characteristics of OECs include the expression of glial fibrillary acidic protein (GFAP), formation of the glial limitans at the PNS-CNS transitional zone, and end-feet at blood vessels (24,26). Although OECs and astrocytes share some phenotypic features, there are more differences than similarities between both cell types (24). They differ from each other in developmental origin, mode of association with axons, ultrastructure, and antigenic characteristics (24).

OEC tranplantation has several potential advantages to elicit remyelination within CNS compared to other glial types (4,5). First, OECs are easily cultured from the adult olfactory system in contrast to other glial cells from the mature CNS (29). Second, OECs can migrate through the adult CNS, cross the PNS-CNS transitional zone, and properly integrate within the CNS upon transplantation (29). A major obstacle to migration of myelin-forming glia in the CNS after damage is the so-called glial scar, which is predominantly formed by reactive astrocytes and microglia (31,32). OECs allow the regenerating axons to grow through known inhibitory substrates such as gliotic tissue (29), though neither CNS nor PNS axons are able to grow through this barrier (33). Thus, they might be able to navigate glial scars and remyelinate axons in the damaged CNS (29). Third, OECs are a source of neurotrophic factors, such as nerve growth factor, platelet-derived growth factor and neuropeptide Y (34–36), suggesting that trophic factor, production by OECs might enhance the survival of damaged axons. Recent work indicates that transplantation of both rodent and human (22,23) OECs can remyelinate demyelinated rat spinal cord axons (5,22,23).

The graphs in **Figure 3** show dorsal column area, the demyelinating lesion area, and the number of myelinated axons in a control lesion (**Figure 3a**) and a lesion following transplantation of OECs at a single point in the longitudinal center of the lesion. Virtually no axons are myelinated in the control lesion, but myelinated axons can be found

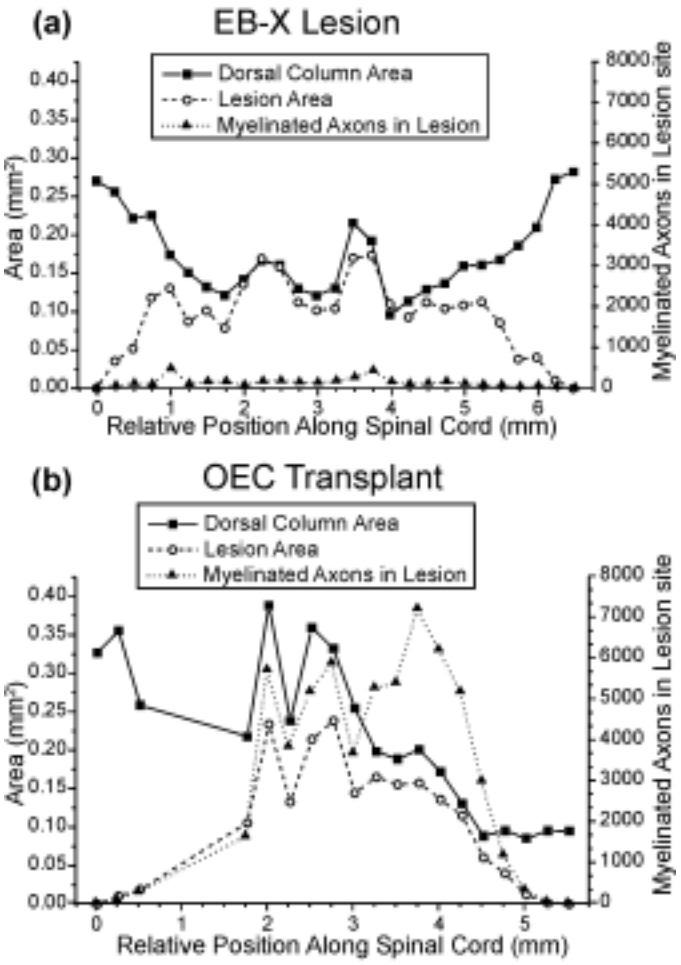

Figure 3.
Spatial dimensions of normal myelinated, demyelinated, and remyelinated (OEC transplantation) areas and number of axons in dorsal funiculus. *x*-axis corresponds to longitudinal length of spinal cord segment under study and *y*-axis to the area of target zone (left) and the number of myelinated axons (right). (a) Data indicate that lesion constitutes a significant proportion of dorsal funiculus and very few fibers are myelinated in control lesion. (b) Similar dimensions for lesion site as in (a), but a substantial number of fibers are myelinated throughout lesion following transplantation of OECs. Source: Modified from citation (5).

throughout the entire longitudinal dimension following OEC transplantation (**Figure 3b**). Similar results are obtained with Schwann cell transplantation where the donor cells were transfected with LacZ and identified in vivo (3). These data indicate that both OECs and Schwann cells are able to elicit relatively extensive peripheral-like myelination upon transplantation into a spinal cord demyelinating lesion. In the X-irradiation/ethidium bromide lesion model,

no endogenous repair of the demyelinated lesion is observed before 6 to 8 weeks. In addition to identification of a reporter gene in some of the transplant studies, the observation of extensive remyelination at 3 weeks postlesion strongly suggests that the donor cells were responsible for the repair.

Neural Precursor Cells Derived from Adult Brain as Source of Peripheral-Myelin-Forming Cells

Multipotent neural precursor or stem cells are present in the mammalian CNS during development and in the adult brain (37–42). Neurospheres, clusters multipotent/progenitor cells, can be developed from neurogenic regions of the adult human brain (43,44). Neural precursor cells can be isolated and expanded in culture in the presence of mitogens, such as epidermal growth factor (EGF) or basic fibroblast growth factor (bFGF) (45–49). After withdrawal of the mitogens and with appropriate growth factors or substrates, these cells can differentiate into neurons or glia (43,50). When transplanted into the embryonic or neonatal CNS, both neurons (51,52) and oligodendrocytes (47,53) have been generated. These cells appear to differentiate and integrate into the host CNS because they form functional synapses (neurons) and myelinate (oligodendrocytes) axons. However, when injected into the normal adult CNS, stem cells differentiate primarily into astrocytes (54). These results indicate that environmental signals may direct the specification of cell lineage.

Multipotential neural progenitor cells derived from the fetal human brain propagate and differentiate in culture and in vivo (55-57). Progenitor cells from adult animals have been cultured from the subependymal zone (SEZ) (38,40,42,58), the subventricular zone (SVZ) (39), the hippocampus (37,41), and the spinal cord (48,59). A recent study suggested that ependymal cells may be a source of progenitor cells (58), but a GFAP-positive cell distinct from, but adjacent to, ependymal cells has been recently implicated as the primary neural progenitor cell type of the subventricular region (60). Although the precise cell type within the subventricular zone giving rise to neural precursor cells is still under investigation, it is clear that with appropriate culture and mitogen conditions, neural precursor cells can be developed and clonally expanded in culture.

While oligodendrocytes normally myelinate CNS axons, Schwann cells can remyelinate CNS axons after injury (14) and following transplantation into the demyelinated CNS (3,9). Schwann cells can be derived from single cell clones of neural crest cells (61). Mujtaba et al. have distinguished a common neural progenitor for the PNS and the CNS (59). They found that cultured neuroepithelial cells derived from embryonic rat spinal cords can differentiate into CNS precursors, giving rise to CNS neurons and glia, and into PNS precursors, which can differentiate into neural crest cells, giving rise to peripheral neurons, Schwann cells, and smooth muscle. Recently, Keirstead et al. demonstrated that immunoselected precursor cells from the neonatal rat forebrain expressing polysialyated (PSA) form of the neural cell adhesion molecule (NCAM), which mostly generates oligodendrocytes and astrocytes in vitro, can also produce peripheral myelin in vivo (62). This suggests that both peripheral and central myelin can be produced from neural precursor cells derived from the CNS.

To test the capability of neural precursor cells derived from the adult brain to differentiate into myelin-forming cells and repair the adult demyelinated CNS, we transplanted clonal neural progenitor cells derived from the adult human brain into an experimentally established glial-free zone in the dorsal columns of the rat spinal cord (44). Although these precursor cells differentiated upon mitogen withdrawal in culture into neurons and astrocytes—and to a lesser extent oligodendrocytes—when transplanted into a demyelinated glial-free zone of the adult rat spinal cord, they extensively remyelinated the axons and restored conduction velocity toward normal values (**Figure 4**). The majority of the myelinated axons displayed a peripheral pattern of myelination characterized by large nuclear and cytoplasmic regions of the myelin-forming cells surrounding the axons and a basement membrane. These data provide evidence that clonal neural precursor cells derived from the adult brain can elicit Schwann cells that form functional myelin when transplanted into an axon-enriched, glial-free environment of adult central white matter. Taken together these data suggest that a common neural progenitor cell for both the CNS and the PNS described for embryonic neuroepithelial cells (59) also may be present in the adult human brain. While our lesion model does not normally lead to the beginning of remyelination for 6 to 8 weeks—and we observed extensive remyelination at 3 weeks postlesion and transplantation—we cannot rule out that the cell transplantation may facilitate endogenous repair. We view this unlikely because of the nature of the lesion

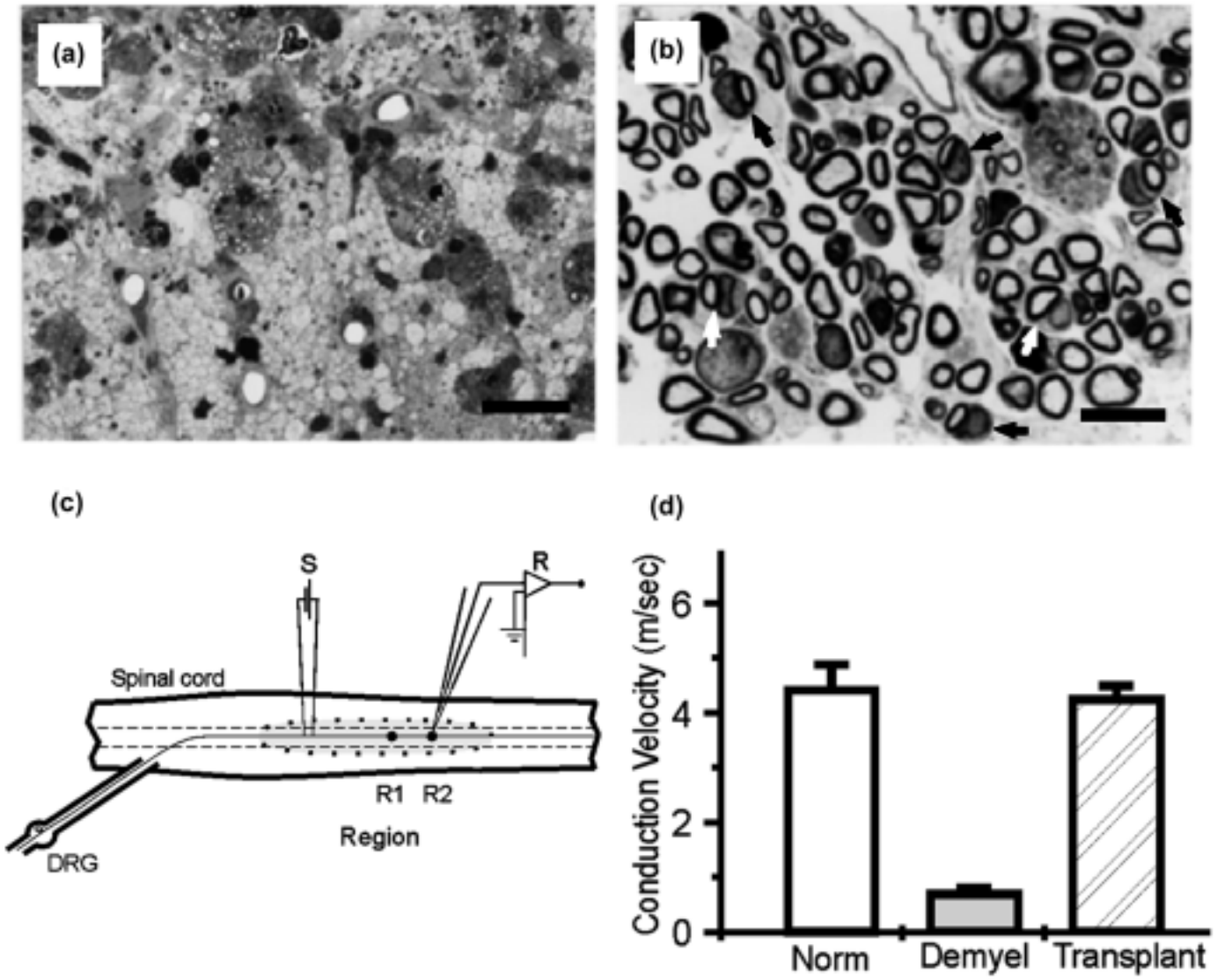

Figure 4.
Remyelination and improved conduction velocity of rat spinal cord following transplantation of adult human neural precursor cells. (a) Demyelinated and (b) remyelinated axons of the dorsal column. The anatomical pattern of myelination elicited by transplantation of human neural precursor cells was similar to that produced by Schwann cells. (c) Schematic showing dorsal surface of spinal cord with positions of stimulating (S) and recording (R) electrodes. Shaded region indicates area of demyelination or remyelination. (d) Conduction velocity for control, EB-X demyelinated and transplant-induced remyelinated axons (n = 5: each group) recorded at 26 °C. Error bars indicate SEM. Source: Modified from citation (44).

induction, but more efficient cell marking techniques will help resolve this issue in the future.

Bone Marrow as a Cell Source to Induce CNS Remyelination

Bone marrow stroll cells have recently been reported to differentiate into neurons and astrocytes in cell culture and into astrocytes when transplanted into normal and ischemic brain (63–65). Systemic (intravenous) application of a BM fraction into terminally X-irradiated rats results in neuronal phenotypes in brain, which were derived from the injected BM (66,67). These investigators have suggested that BM or isolated BM stromal cells, which can be harvested from a patient, may be useful for potential cell therapy approaches in neurological disease. Our preliminary work indicates that transplantation of an

acutely isolated BM cell fraction (devoid of erythrocytes, platelets, and debris) into the demyelinated rat spinal cord results in relatively extensive remyelination (68). These results indicate that an acutely prepared BM cell fraction can develop a myelinating phenotype in vivo and anatomically repair demyelinated axons when injected into the spinal cord. This suggests the potential of developing an easily accessible and renewable source of autologous donor cells for cell transplantation studies in demyelinating disease.

Many of the myelinated axons induced by transplantation of BM cells are characteristic of peripheral myelin. While bone marrow-derived-peripheral-like myelinating cells have many similarities to Schwann cell myelination, a number of noted differences exist. In particular, the BM-induced remyelinating cells show larger and multilobular nuclei and larger cytoplasmic regions. In addition, while no Schwann cells were observed to engage more than one axon, some of the BM cells (2.7 percent) myelinated two to three adjacent axons. Interestingly, neural precursor cells and olfactory ensheathing cells transplanted into our lesion model show peripheral-like myelin and some of the features such as multilobular nuclei and multiple axonal myelination described here for BM cells (22,44). Given that both OECs and Schwann cells can remyelinate and encourage axonal regeneration of transected long tracts in the spinal cord, the peripheral-like differentiation of many of the BM cells may be advantageous for the development of a renewable and autologous source of cells for the treatment of spinal cord injury and demyelinating disorders.

Cell Transplantation to Encourage Axonal Regeneration of Spinal Cord Tracts

Long tract axons in the mammalian spinal cord do not normally regenerate for an appreciable distance within the denervated host tract after they are transected. However, a number of experimental approaches have been reported to improve elongative regeneration of axons in the transected mammalian spinal cord. These include blockade of inhibitory proteins on glial cells and introduction of neurotrophic factor-enhanced peripheral nerve bridges (69,70). Recent attention has focused on transplants of cultured OECs into ablated corticospinal tract axons (6) and into nerve bridges in the spinal cord (7) to enhance regeneration. OECs have several unique properties that provide a rationale for their potential to enhance CNS axonal regeneration. They are specialized cells that support axons that leave the olfactory epithelium and project through the peripheral nervous system into the olfactory bulb of the CNS; they are pluripotential cells that can show Schwann cell or astrocyte-like cell properties (71,72). Interest has focused on these cells because olfactory epithelial neurons are continuously replaced and regenerate axons in the adult (72,73). It has been reasoned that the unique properties of OECs may allow them to guide and enhance regenerating CNS axons through a normally growth inhibitory environment (6,24).

OEC transplantation can enhance regeneration of transected spinal cord axons, improve forepaw reaching behavior, and remyelinate demyelinated axons in the spinal cord (4–6). Following transplantation of either OECs or Schwann cells into transected dorsal funiculus of the adult rat spinal cord, we found that the regenerating ascending sensory axons displayed stable conduction properties with regard to conduction velocity (**Figure 5**) and frequency-response properties (8,30). However, the conduction velocity of the regenerated axons was significantly greater than normal axons; morphological assessment of the regenerated axons indicates that they are myelinated with a peripheral pattern of myelin (**Figure 6**) and of a larger caliber than control axons (8). These results indicate that the regenerated spinal cord axons reconstitute electrophysiological function, an important requirement for an intervention therapy to enhance axonal regeneration after spinal cord injury, but there is a preferential regeneration of large myelinated and rapidly conducting axons. Therefore, while the number of regenerated axons induced by cell transplantation of OECs or Schwann cells is limited, a rapidly and securely conducting new information line is established which may contribute to the observed behavioral recovery of function.

CONCLUSION

Animal models of demyelination and axonal transection of the spinal cord indicate feasibility of cell transplantation as an approach to elicit at least some degree of functional recovery. One of the primary challenges to such therapies will be selecting the appropriate cell type and delivery method for the appropriate neurological condition. While oligodendrocytes normally form myelin in the CNS, the prospect of using Schwann cells to remyelinate CNS lesions in MS patients is intriguing because they can

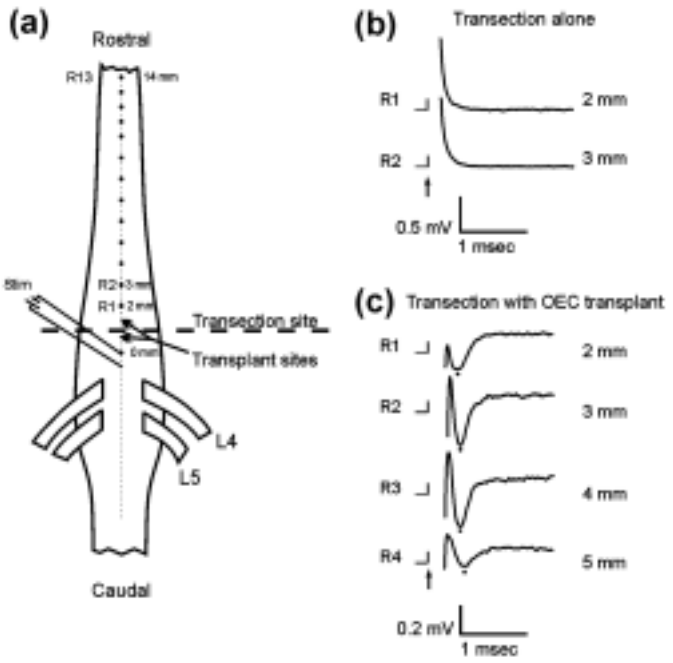

Figure 5.
Olfactory ensheathing cell transplantation facilitates axonal regeneration in transected dorsal funiculus. (a) Schematic showing transection site, position of cell injections, and of stimulating and recording electrodes. Stimulating electrodes were positioned 1-mm caudal to transection site (0-mm), and recordings were obtained at 1-mm intervals beyond lesion site; schematic shows recordings up to 4 mm, but recordings were obtained up to 15 mm. (b) CAP recordings for control lesion. At 2-mm rostral to stimulating site (i.e., 1-mm beyond transection site) in a transected spinal cord without cell transplantation, no clear response could be obtained. At 3 mm (2-mm beyond the transection site), no CAP could be detected; note flat baseline following stimulus artifact. (c) CAP recording for OEC transplanted lesion. In a transected spinal cord that had been transplanted with OECs, propagating CAPs could be detected for several milimeters beyond transection site; note distinct negatives recorded at various distances beyond lesion site. Peak negativities are indicated by solid squares. Source: Modified from citation (30).

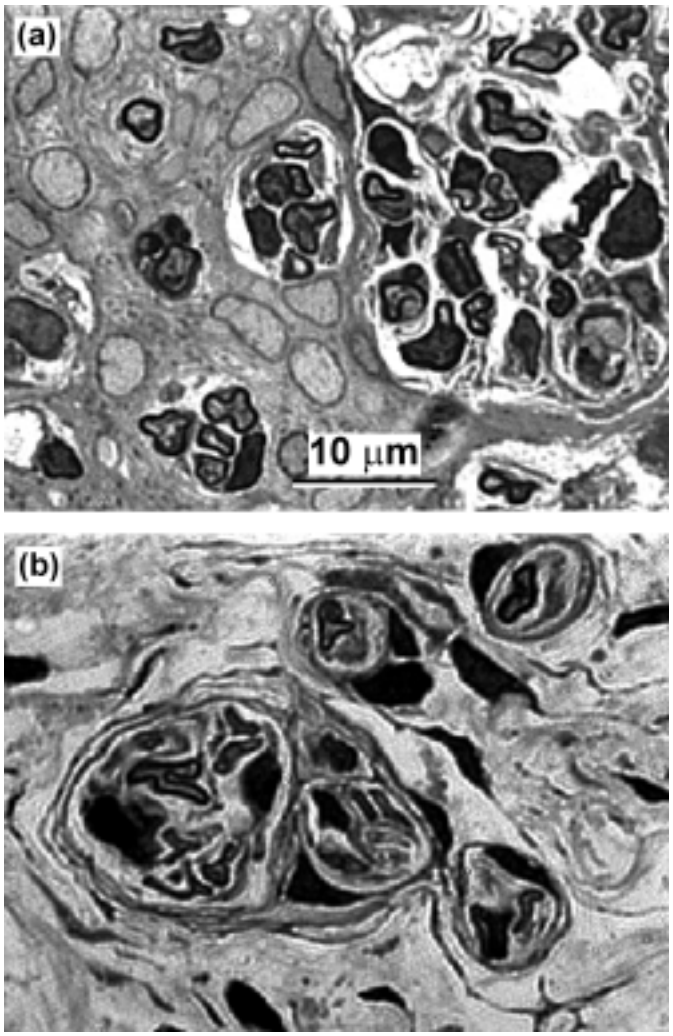

Figure 6.
Regenerated axons following dorsal funiculus transection and OEC transplantation are myelinated. Photomicrographs obtained at (a) 0.75 mm, and (b) 0.25 mm rostral to the lesion of rat dorsal column following transplantation of OECs into the completely transected spinal cord 5 weeks earlier. Note the small bundles of axons with Schwann cell-like patterns of myelination. Source: Modified from citation (8).

be harvesting autologously, and they may not have antigens present on oligodendrocytes that may be immunogenic. Yet the use of Schwann cells has limitations. One is that Schwann cell remyelination in the CNS does not precisely recapitulate the pattern of remyelination by oligodendroctyes. The density of axonal spacing is less with Schwann cell myelination, and one must ask what potential negative effects this could have on the system, such as a reduction in axon number. Indeed, it has been speculated that a selective force for the evolution of oligodendrocytes was to provide for maximum conduction velocity conferred by myelin deposition, with the most economic use of space. The advantage this organization achieves with oligodendrocyte myelination is a relatively compact CNS, but a disadvantage is that pathology to a single oligodendrocyte will effect a number of myelin segments. In spite of this limitation, it is reasoned that restoration of myelin and conduction in even a limited subset of axons could potentially result in significant functional recovery.

Another important concern with regard to cell transplantation therapies is the harvesting of sufficient numbers of cells for transplantation. While a number of cell types can be expanded in culture with trophic factors and mitogens, it is not clear if such expansion will alter the physiology of the cells in a way that impairs their capability to form functional myelin. Further studies to examine the myelinogenic potential of expanded cells will be required. Another concern with expanded cells is the potential risk of tumor formation. It will be absolutely essential to determine if experimental in vivo transplantation of expanded cells not only retain their capability to carry out neural repair but also do not form tumors. It is encouraging that expanded neural precursor cells derived from human subventricular zone and BM, as just reported, were able to form myelin in a rat model of demyelination and that no obvious tumor formation was observed.

Another concern is that cell-induced axonal regeneration and remyelination may not recapitulate uninjured structures but may establish new neuronal circuits and conduction channels. Such newly organized neural structures could be maladaptive and elicit neurological problems, such as inappropriate movement, pain, or paresthesae. An example of maladaptive plasticity has been well-characterized following peripheral nerve injury. A section of a peripheral nerve can result in dying back of C-fibers and A-delta fibers from the substantia gelatinosa with reoccupation of these synapses with A-beta fibers (74–77). Normally A-beta fibers are associated with nonnoxious mechanical inputs to the spinal cord and terminal in lamina III of the dorsal horn. It has been hypothesized that this aberrant regeneration of these fibers and new synapse formation may contribute to tactile allodynia, a condition that normally nonnoxious stimuli result in pain (74–77). Care must be taken to assure that new pathways and myelination elicited by interventional approaches such as cell transplantation do not result in maladaptive changes.

Rehabilitation medicine may take on an important role both in the evaluation of cell therapy studies in man and in the therapeutic design of the studies. Clearly, appropriate design of functional experiments to assess efficacy will be paramount to evaluating these approaches in clinical studies. Additionally, because patterns of remyelination and potentially partial restoration of axon white matter tracts and synapses may not recapitulate preinjury structures, rehabilitation strategies may be needed to better understand and maximize the extent of achievable functional recovery.

REFERENCES

1. Ide, C. Peripheral nerve regeneration. Neurosci Res 1996; 25:101–21.
2. Blakemore WF. Remyelination of CNS axons by Schwann cells transplanted from the sciatic nerve. Nature 1977;266: 68–69.
3. Honmou O, Felts PA, Waxman SG, Kocsis JD. Restoration of normal conduction properties in demyelinated spinal cord axons in the adult rat by transplantation of exogenous Schwann cells. J Neurosci 1996;16:3199–208.
4. Franklin RJ, Gilson JM, Franceschini IA, Barnett SC. Schwann cell-like myelination following transplantation of an olfactory bulb-ensheathing cell line into areas of demyelination in the adult CNS. Glia 1996;17:217–24.
5. Imaizumi T, Lankford KL, Waxman SG, Greer CA, Kocsis JD. Transplanted olfactory ensheathing cells remyelinate and enhance axonal conduction in the demyelinated dorsal columns of the rat spinal cord. J Neurosci 1998;18:6176–85.
6. Li Y, Field PM, Raisman G. Repair of adult rat corticospinal tract by transplants of olfactory ensheathing cells. Science 1997;277:2000–2.
7. Ramon-Cueto A, Plant GW, Avila J, Bunge MB. Long-distance axonal regeneration in the transected adult rat spinal cord is promoted by olfactory ensheathing glia transplants. J Neurosci 1998;18:3803–15.
8. Imaizumi T, Lankford KL, Kocsis JD. Transplantation of olfactory ensheathing cells or Schwann cells restored rapid and secure conduction across the transected spinal cord. Brain Res 2000;854:70–78.
9. Blakemore WF, Crang AJ. The use of cultured autologous Schwann cells to remyelinate areas of persistent demyelination in the central nervous system. J Neurol Sci 1985;70: 207–23.
10. Duncan ID, Hammang JP, Jackson KF, Wood PM, Bunge RP, Langford L. Transplantation of oligodendrocytes and Schwann cells into the spinal cord of the myelin-deficient rat. J Neurocytol 1988;17:351–60.
11. Kohama I, Lankford KL, Preiningerova, J, White FA, Vollmer TL, Kocsis JD. Transplantation of cryopreserved adult human Schwann cells enhances axonal conduction in demyelinated spinal cord. J Neurosci 2001;21:944–50.
12. Glendhill RF, Harrison BM, McDonald WI. Pattern of remyelination in the CNS. Nature 1973;244:443–44.
13. Blight AR, Young W. Central axons in injured cat spinal cord recover electrophysiological function following remyelination by Schwann cells. J Neurol Sci 1989;91:15–34.

14. Felts PA, Smith KJ. Conduction properties of central nerve fibers remyelinated by Schwann cells. Brain Res 1992;574: 178–92.
15. Smith KJ, McDonald WI, Blakemore WF. Restoration of secure conduction by central demyelination. Trans AM Neurol Assoc 1979;104:25–29.
16. Ghatak NR, Hirano A, Doron Y, Zimmerman HM. Remyelination in multiple sclerosis with peripheral type myelin. Arch Neurol 1973;29:262–67.
17. Itoyama Y, Ohnishi A, Tateishi J, Kuroiwa Y, Webster HD. Spinal cord multiple sclerosis lesions in Japanese patients: Schwann cell remyelination occurs in areas that lack glial fibrillary acidic protein (GFAP). Acta Neuropathol (Berl) 1985;65:217–23.
18. Prineas JW, Connell F. Remyelination in multiple sclerosis. Ann Neurol 1979;5:22–31.
19. Groves AK, Barnett SC, Franklin RJ, Crang AJ, Mayer M, Blakemore WF, Noble M. Repair of demyelinated lesions by transplantation of purified 0-2A progenitor cells. Nature 1993;362:453–55.
20. Kocsis JD. Restoration of function by glial cell transplantation into demyelinated spinal cord. J Neurotrauma 1999; 6:695–702.
21. Targett MP, Sussman J, Scolding N, O'Leary MT, Compston DA, Blakemore WF. Failure to achieve remyelination of demyelinated rat axons following transplantation of glial cells obtained from the adult human brain. Neuropathol Appl Neurobiol 1996;22:199–206.
22. Kato T, Honmou O, Uede T, Hashi K. Transplantation of human olfactory ensheathing cells elicits remyelination of demyelinated rat spinal cord. Glia 2000;30:209–18.
23. Barnett SC, Alexander CL, Iwashita Y, Gilson J, Crowther J, Clark L. Identification of a human olfactory ensheathing cell that can effect transplant-mediated remyelination of demyelinated CNS axons. Brain 2000;123:1581–88.
24. Ramon-Cueto A, Valverde F. Olfactory bulb ensheathing glia: a unique cell type with axonal growth-promoting properties. Glia 1995;14:163–73.
25. Barnett SC, Hutchins AM, Noble M. Purification of olfactory nerve ensheathing cells from the olfactory bulb. Dev Biol 1993:155:337–50.
26. 26. Doucette R. PNS-CNS transitional zone of the first cranial nerve. J Comp Neurol 1991;312:451–66.
27. Doucette R. Olfactory ensheathing cells: potential for glial cell transplantation into areas of CNS injury. Histol Histopathol 1995;10:503–7.
28. Fraher JP. The CNS-PNS transitional zone of the rat: Morphometric studies at cranial and spinal levels. Prog Neurobiol 1992;38:261–316.
29. Ramon-Cueto A, Nieto-Sampedro M. Regeneration into the spinal cord by transected dorsal root axons is promoted by ensheathing glia cells. Exp Neurol 1994;127:232–44.
30. Imaizumi T, Lankford KL, Burton WV, Foder WL, Kocsis JD. Xenotransplantation of transgenic pig olfactory ensheathing cells promotes axonal regeneration in rat spinal cord. Nature Biotechnol 2000;18:949–53.
31. Fernaud-Espinosa I, Nieto-Sampedro M, Bovolenta P. Differential activation of microglia and astrocytes in aniso- and isomorphic gliotic tissue. Glia 1993;8:277–91.
32. Hatten ME, Liem RK, Shelanski ML, Mason CA. Astroglia in CNS injury. Glia 1991;4:233–43.
33. Rudge JS, Silver J. Inhibition of neurite outgrowth on astroglial scars in vitro. J Neurosci 1990;10:3594–603.
34. Knott JN, Westrum LE, Raines EW, Sasahara M, Ross R. Olfactory ensheathing glia and platelet-derived growth factor B-chain reactivity in the transplanted rat olfactory bulb. Int J Dev Neurosci 1994;12:315–23.
35. Ubink R, Halasz N, Zhang X, Dagerlind A, Hokfelt T. Neuropeptide tyrosine is expressed in ensheathing cells around the olfactory nerves in the rat olfactory bulb. Neuroscience 1994;60:709–26.
36. Williams R, Rush RA. Electron microscopic immunocytochemical localization of nerve growth factor in developing mouse olfactory neurons. Brain Res 1988;463:21–27.
37. Gage FH, Coates PW, Palmer TD, Kuhn HG, Fisher LJ, Suhonen JO, Peterson DA, Suhr ST, Ray J. Survival and differentiation of adult neuronal progenitor cells transplanted to the adult brain. Proc Natl Acad Sci USA 1995; 92:11879–83.
38. Johe KK, Hazel TG, Muller T, Dugich-Djordjevic MM, McKay RD. Single factors direct the differentiation of stem cells from the fetal and adult central nervous system. Genes Dev 1996;10:3129–40.
39. Lois C, Alvarez-Buylla. Proliferating subventricular zone cells in the adult mammalian forebrain can differentiate into neurons and glia. Proc Natl Acad Sci USA 1993;90: 2074–77.
40. Morshead CM, Reynolds BA, Craig CG, McBurney MW, Staines WA, Morassutti D. Neural stem cells in the adult mammalian forebrain: A relatively quiescent subpopulation of subependymal cells. Neuron 1994;13:1071–82.
41. Palmer TD, Takahashi J, Gage FH. The adult rat hippocampus contains primordial neural stem cells. Mol Cell Neurosci 1997;8:389–404.
42. Reynolds BA, Weiss S. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science 1992;255:1707–10.
43. Kukekov VG, Laywell ED, Suslov O, Davies K, Scheffler B, Thomas LB. Multipotent stem/progenitor cells with similar properties arise from two neurogenic regions of adult human brain. Exp Neurol 1999;156:333–44.
44. Akiyama Y, Honmou O, Kato T, Uede T, Hashi K, Kocsis JD. Transplantation of clonal neural precursor cells derived

from adult human brain establishes functional peripheral myelin in the rat spinal cord. Exp Neurol 2001;167:27–39.
45. Cattaneo E, McKay R. Proliferation and differentiation of neuronal stem cells regulated by nerve growth factor. Nature 1990;347:762–65.
46. Gensburger C, Labourdette G, Sensenbrenner M. Brain basic fibroblast growth factor stimulates the proliferation of rat neuronal precursor cells in vitro. FEBS Lett 1987; 217:1–5.
47. Hammang JP, Archer DR, Duncan ID. Myelination following transplantation of EGF-responsive neural stem cells into a myelin-deficient environment. Exp Neurol 1997; 147:84–95.
48. Kalyani A, Hobson K, Rao MS. Neuroepithelial stem cells from the embryonic spinal cord: Isolation, characterization, and clonal analysis. Dev Biol 1997;186:202–23.
49. Svendsen CN, Clarke DJ, Rosser AE, Dunnett SB. Survival and differentiation of rat and human epidermal growth factor-responsive precursor cells following grafting into the lesioned adult central nervous system. Exp Neural 1996;137:376–88.
50. Qian X, Davis AA, Goderie SK, Temple S. FGF2 concentration regulates the generation of neurons and glia from multipotent cortical stem cells. Neuron 1997;18:81–93.
51. Brustle O, Maskos U, Mc Kay RD. Host-guided migration allows targeted introduction of neurons into the embryonic brain. Neuron 1995;15:1275–85.
52. Snyder EY, Yoon CH, Flax JD, Macklis JD. Multipotent neural progenitors can differentiate toward replacement of neurons undergoing targeted apoptotic degeneration in adult mouse neocortex. Proc Natl Acad Sci USA 1997;94: 11663–68.
53. Milward EA, Lundberg CG, Ge B, Lipsitz D, Zhao M, Hajihosseini M. Isolation and transplantation of multipotential populations of epidermal growth factor-responsive neural progenitor cells from the canine brain. J Neurosci Res 1997;50:862–71.
54. Lundberg C, Bjorklund A. Host regulation of glial markers in intrastriatal grafts of conditionally immortalized neural stem cell lines. Neuroreport 1996;7:847–52.
55. Chalmers-Redman RM, Priestley T, Kemp JA, Fine A. In vitro propagation and inducible differentiation of multipotential progenitor cells from human fetal brain. Neuroscience 1997;76:1121–28.
56. Moyer MP, Johnson RA, Zompa EA, Cain L, Morshed T, Hulsebosch CE. Culture, expansion and transplantation of human fetal neural progenitors cells. Transplant Proc 1997; 29:2040–1.
57. Svendsen CN, Caldwell MA, Shen J, Ter Borg MG, Rosser AE, Tyers P. Long-term survival of human central nervous system progenitor cells transplanted into a rat model of Parkinson's disease. Exp Neurol 1997;148:135–46.
58. Johansson CB, Momma S, Clarke DL, Risling M, Lendahl U, Frisen J. Identification of a neural stem cell in the adult mammalian central nervous system. Cell 1999;96:25–34.
59. Mujtaba T, Mayer-Proschel M, Rao SM. A common neural progenitor for the CNS and PNS. Dev Biol 1998;200:1–15.
60. Doetsch F, Caille I, Lim DA, Garcia-Verdugo JM, Alvarez-Buylla A. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. Cell 1999;97: 703–16.
61. Le Douarin N, Dulac C, Dupin E, Cameron-Curry P. Glial cell lineages in the neural crest. Glia 1991;4:175–84.
62. Keirstead HS, Ben-Hur T, Rogister B, O'Leary MT, Dubois-Dalcq M, Blakemore WF. Polysialylated neural cell adhesion molecule-positive CNS precursors generate both oligodendrocytes and Schwann cells to remyelinate the CNS after transplantation. J Neurosci 1999;19:7529–36.
63. Woodbury D, Schwarz EJ, Prockop DJ, Black IB. Adult rat and human bone marrow stromal cells differentiate into neurons. J Neurosci Res 2000;61:364–70.
64. Azizi SA, Stokes D, Augelli BJ, DiGirolamo C, Prockop DJ. Engraftment and migration of human bone marrow stromal cells implanted in the brains of albino rats similarities to astrocyte grafts. Proc Natl Acad Sci USA 1998;95: 3908–13.
65. Eglitis MA, Dawson D, Park KW, Mouradian MM. Targeting of marrow-derived astrocytes to the ischemic brain. Neuroreport 1999;10:1289–92.
66. Brazelton TR, Rossi RM, Keshet GI, Blau HM. From marrow to brain; expression of neuronal phenotypes in adult mice. Science 2000;290:1775–79.
67. Mezey E, Chandross KJ, Harta G, Maki RA, McKercher SR. Turning blood into brain: cells bearing neuronal antigens generated in vivo from bone marrow. Science 2000; 290:1779–82.
68. Sasaki M, Honmou O, Akiyama Y, Uede T, Hashi K, Kocsis JD. Transplantation of an acutely isolated bone marrow fraction repairs demyelinated adult rat spinal cord axons. Glia 2001;35:26–34.
69. Schwab ME, Kapfhammer JP, Bandtlow CE. Inhibitors of neurite growth. Annu Rev Neurosci 1993;16:565–95.
70. Cheng H. Coa Y, Olson L. Spinal cord repair in adult paraplegic rats: partial restoration of hid limb function. Science 1996;273:510–13.
71. Devon R, Doucette R. Olfactory ensheathing cells myelinate dorsal root ganglion neurites. Brain Res 1992;589: 175–79.
72. Moulton DG. Dynamic of cell population in the olfactory epithelium. Ann N Y Acad Sci 1974:237.
73. Graziadei PPC, Montigraziadei GA. Neurogenesis and neuron regeneration in the olfactory system of mammals: I. Morphological aspects of differentiation and structural organization of the olfactory system. J Neurocytol 1979;8: 197–213.

74. Woolf CJ, Shortland P, Coggeshall RE. Peripheral nerve injury triggers central sprouting of myelinated afferents. Nature 1992;355:75–78.
75. Shortland P, Woolf CJ. Chronic peripheral nerve section results in a rearrangement of the central axonal arborizations of axotomized A beta primary afferent neurons in therat spinal cord. J Comp Neurol 1993;330:65–82.
76. Koerber HR, Mirnics K, Brown PB, Mendel LM. Central sprouting and functional plasticity of regenerated primary afferents. J Neurosci 1994;14:3655–71.
77. Kohama I, Ishikawa K, Kocsis JD. Synaptic reorganization in the substantia gelatinosa following peripheral nerve neuroma formation: aberrant innervation of lamina II neurons by Aß afferents. J Neurosci 2000;20:1538–49.