Endogenous stem cell proliferation after central nervous system injury: alternative therapeutic options

NICHOLAS C. BAMBAKIDIS, M.D., NICHOLAS THEODORE, M.D., PETER NAKAJI, M.D.,
ADRIAN HARVEY, B.S., VOLKER K. H. SONNTAG, M.D., MARK C. PREUL, M.D.,
AND ROBERT H. MILLER, PH.D.

Division of Neurological Surgery, Barrow Neurological Institute, St. Joseph's Hospital and Medical Center, Phoenix, Arizona; and Department of Neurosciences, Case Western Reserve University, Cleveland, Ohio

The continuous regeneration of glial cells arising from endogenous stem cell populations in the central nervous system (CNS) occurs throughout life in mammals. In the ongoing research to apply stem cell therapy to neurological diseases, the capacity to harness the multipotential ability of endogenous stem cell populations has become apparent. Such cell populations proliferate in response to a variety of injury states in the CNS, but in the absence of a supportive microenvironment they contribute little to any significant behavioral recovery. In the authors' laboratory and elsewhere, recent research on the regenerative potential of these stem cells in disease states such as spinal cord injury has demonstrated that the cells' proliferative potential may be greatly upregulated in response to appropriate growth signals and exogenously applied trophic factors. Further understanding of the potential of such multipotent cells and the mechanisms responsible for creating a favorable microenvironment for them may lead to additional therapeutic alternatives in the setting of neurological diseases. These therapies would require no exogenous stem cell sources and thus would avoid the ethical and moral considerations regarding their use. In this review the authors provide a brief overview of the enhancement of endogenous stem cell proliferation following neurological insult.

KEY WORDS • endogenous stem cell • spinal cord injury • sonic hedgehog glycoprotein

OVERVIEW

Stem cell therapy in the setting of CNS injury has become increasingly promising. Theoretically, the pluripotent nature of these precursor cell populations allows them to develop along any neural lineage. Consequently, their progeny potentially can contribute to repair mechanisms, which may take the form of neuronal regeneration, remyelination, prevention of demyelination, or amelioration of scar formation. Embryonic stem cells also maintain a highly proliferative and migratory nature, both of which contribute to the role that they may play in the restoration of function.^{2,7} These cells have been driven to terminal differentiation along all glial lineages in animal models, and motor neurons have been derived in vitro from pluripotent neural progenitors.^{20,49}

For most of the twentieth century, it was believed that stem cells existed only transiently during mammalian development. Subsequent investigation has demonstrated neural progenitor cell rests in the CNS of adult mammals. Although usually quiescent and localized, these cells

remain available for replication and maturation throughout life, not just during embryonal development.^{10,14,22}

Endogenous Stem Cells in the Adult CNS

The presence of endogenous stem cell populations in the adult mammalian CNS has led to the discovery that these cells primarily reside in discrete anatomical zones (Fig. 1). In the brain, these cells are concentrated in the hippocampus and the subventricular zone along the lateral wall of the lateral ventricle.^{10,22} Some investigators also have identified neuronal cell-specific progenitors within the dentate gyrus.⁴¹ Endogenous stem cell populations have been identified around the central canal of the spinal cord; in the ependymal layer of the fourth ventricle;^{14,43,45} and in the hypothalamus,²⁷ optic nerve,³³ and substantia nigra.²¹ In many of these locations, the exposure of these cell populations to injury in the surrounding tissue results in a proliferative response wherein most of the progenitor cells mature along an astroglial lineage.³⁵

The signals that influence the development of neural progenitor cells along a particular lineage have been partially elucidated. Factors such as EGF, insulin-like growth factor-I, and FGF-2 have direct effects on the development of adult neural stem cells.^{2,35} For example, administration of FGF-2 and EGF results in a 50-fold increase in the degree

Abbreviations used in this paper: BMP = bone morphogenetic protein; CNS = central nervous system; EGF = epidermal growth factor; FGF-2 = fibroblast growth factor-2; Shh = sonic hedgehog; TBI = traumatic brain injury.

of neurogenesis resulting from ischemic injury.³¹ The tissue levels of these signals are elevated substantially after spinal cord injury, and the interaction of these cytokines is critical in influencing stem cells to develop down a particular lin-

age.⁹ The modification of such microenvironmental cues is critical so that the previously observed tendency of endogenous neural progenitor cells to differentiate mostly into astrocytes after injury can be altered.

Sonic Hedgehog Induces Endogenous Stem Cell Proliferation

During development, the relative concentration of a variety of small molecules has considerable influence over the particular fate of these multipotent progenitors. The BMPs play critical roles in the proliferation and maturation of progenitor cells in adults.^{23,35} The administration of BMP⁷ induces neuronal repair and provides a neuroprotective effect in ischemic rat brains.⁸ The administration of Shh has a crucial role in the modulation of stem cell proliferation in the hippocampus.¹⁸ A member of the hedgehog family of secreted glycoproteins, which affect the development of many cell groups and organs, Shh is expressed throughout the CNS and profoundly affects the development of the embryonic notochord.^{6,17,29,40} This glycoprotein mediates notochord-influenced anterior spinal cord motor neuron induction and induces the migration and proliferation of oligodendrocyte precursors.^{15,17,31,36} Constitutive expression of Shh in the developing rat brain results in bursts of stem cell proliferation in the dorsal columns of the spinal cord.³⁹ The administration of clonal DNA encoding Shh expression in the adult rat brain produces a threefold increase in the proliferation of endogenous stem cells that later mature into neurons.¹⁸ The removal of Shh signaling during development dramatically reduces the number of neural progenitors in the postnatal subventricular zone and hippocampus, whereas stimulation of the hedgehog pathway in the mature rodent brain by using oral Shh agonists increases the number of proliferating cells in these brain regions.²⁵

Neurological Disorders and Response of Endogenous Stem Cells to Injury

As noted, endogenous stem cell populations respond to injury primarily through proliferation and maturation into astrocytes.³⁵ Much smaller numbers differentiate into oligodendrocytes and neurons. Microenvironmental cues play an important role in the development and maturation of these cells in the context of CNS injury. For example, although embryonically derived oligodendrocyte progenitors readily myelinate in the injured spinal cord after cellular transplantation, the capacity of similar endogenously derived precursors to become myelinated seems limited. This limitation is found despite a marked increase in the number of oligodendrocyte progenitors observed after spinal cord injury.²⁸ Yamamoto, et al.,⁴⁷ hypothesized that the lack of neuronal differentiation after CNS injury is related to up-regulation of the Notch signal pathway. The increased levels of various cytokines within the microenvironment surrounding the area of injury also may contribute a lack of trophic support for maturation into nonastrocytic lineages.^{9,16,32,46}

Despite these obstacles, the induction and manipulation of endogenous stem cell populations have shown promise in a variety of laboratory investigations pertinent to neurological disorders and are targets of intensive investigation. Researchers continue to explore the potential for neural regeneration through endogenous stem cell manipulation in

Fig. 1. Schematic illustration of endogenous stem cell rests in adult mammals and of the signals inducing proliferation and maturation along definitive glial lines. Endogenous progenitors have been identified in the hippocampal dentate gyrus, the subventricular zone, and the central canal of the spinal cord. Proliferation of primitive-appearing cells with bipolar processes can be stimulated by factors such as FGF-2 and EGF-1. These progenitors then differentiate into a particular neural form after exposure to factors such as Shh or retinoic acid. Exposure of stem cells to BMPs promotes astrocytic differentiation, whereas delivery of the BMP antagonist Noggin supports neuronal differentiation. An undifferentiated progenitor cell may therefore give rise to either astroglial, oligodendroglial, or neuronal offspring. IGF-1 = insulin-like growth factor-I.

disorders of the basal ganglia such as Huntington and Parkinson diseases.^{13,30} Evidence of neurogenesis, stem cell proliferation, migration, and stem cell-mediated improvement of recovery has also been found in animal models of ischemic brain injury.^{1,4,11,26,31} Research has focused on the ability of stem cells to alter mechanisms of neural repair in models of traumatic injury in the CNS.

Traumatic Brain Injury. Significant work has focused on the ability of neural progenitor cells to promote functional recovery after TBI. The ability of pluripotent cells to mature into multiple cell types may be of particular benefit in the setting of trauma in which a combination of demyelination, disruption of neuronal networks, and astrocytic scar formation all contribute to injury in various degrees. The transplantation of exogenous neural progenitor cells in animal models of TBI has shown promise in promoting behavioral and motor recovery.^{24,42} These cells respond to signals in injured brain tissue and differentiate into neurons and glia. Other studies have shown rapid (though incomplete) motor recovery during the initial few weeks following transplantation of immortalized or stromal cell transplants after TBI.^{34,38,44}

Neural progenitor cells derived from E14.5 mouse brains and transplanted 1 week after TBI survived for 1 year and stained positively for chondroitin sulfate proteoglycan, also called NG2 (oligodendrocyte progenitor marker) but not for neuronal, astrocytic, or microglial markers.⁴² These cells migrate to the site of injury and enhance motor and cognitive recovery. These findings imply that neuronal recovery is not a prerequisite for functional improvement.

The data are less clear for endogenous stem cells. Rice, et al.,³⁷ demonstrated proliferation and neuronal differentiation in rodent brains as long as 10 days postinjury, although no evidence of behavioral improvement was available. It is likely that the numbers of such cells remain too low to contribute to a significant healing response and that they are inhibited by the trophic factors released after severe trauma. The injection of exogenous progenitor cells attenuates neuronal degeneration and the proliferation of endogenous progenitor cells.¹²

Spinal Cord Injury. Analogous to research on exogenous stem cell transplantation in TBI, functional improvement has followed such transplantation in animal models of spinal cord injury.^{32,35} Similar data regarding the ability of stromal cells to support axonal growth and ameliorate demyelination have been presented.³ Direct cellular transplantation of neural stem cells that have been committed to an oligodendrocytic lineage but retain the migratory and proliferative capacity of progenitor cells results in clinically significant functional recovery and axonal conduction after injury.^{5,19} Investigations into the manipulation and fate of endogenous stem cell populations within the spinal cord have demonstrated that these cells proliferate in response to injury, but have provided no evidence of significant neurogenesis.^{16,22,48} This proliferative potential is increased by the addition of stimulating factors such as Shh.⁶

In models of demyelinating injury in the dorsal column of adult rats, the numbers of progenitor cells have increased in the gray and white matter of the spinal cord around the level of injury as well as around the central canal (Figs. 2 and 3). These areas of proliferation extend into the dorsal columns and stain positively for nestin (Fig. 4). In models

Fig. 2. Photomicrograph of bromodeoxyuridine-stained dividing cells (arrows) located around the central canal in adult rodent spinal cords after a demyelinating lesion that was exposed to recombinant Shh. These cells represent endogenous neural progenitors stimulated by the combination of demyelination and Shh. Fluorescent stain for bromodeoxyuridine, original magnification $\times 200$.

of spinal cord contusion in adult rats, the addition of Shh to the site of injury has been associated with a strong trend toward improved functional recovery and axonal conduction compared with findings in control animals.⁵ Likewise, the numbers of neural progenitors observed in the spinal cords of treated animals are greatly increased, both in the dorsal columns and in dissociated cell cultures incubated for 7 days (Fig. 5). Although the ultimate fate and plasticity of these progenitors have yet to be determined, the functional improvement and partial recovery observed seem to indicate that a future therapeutic role is possible.

CONCLUSIONS

The study of endogenous stem cell populations in the adult mammalian CNS continues to demonstrate potential therapeutic benefits for the treatment of neurological diseases. Further study to delineate the trophic factors contributing to a supportive microenvironment is required to

Fig. 3. Bar graph showing the increase in the numbers of actively dividing cells in spinal cord sections of adult rodents. Rats with a lesion were treated with a low dose (3 μ l) or a high dose (6 μ l) of Shh, and rodents without a lesion were given a high dose of Shh. Consistently significant increases in the number of proliferating cells followed exposure to Shh in rats with a lesion. Data were analyzed using repeated-measures analysis of variance, followed by the Student-Newman-Keuls post hoc t-test. Asterisks designate probability values of less than 0.01. LPC = lysophosphatidyl choline. (Reprinted from Bambakidis NC, Wang R-Z, Franic L, Miller RH: Sonic hedgehog-induced neural precursor proliferation after adult rodent spinal cord injury. *J Neurosurg* 99 (Suppl 1):70–75, 2003.)

allow cellular manipulation. Such studies would be designed to maximize the cells' contribution to a longlasting healing response. The development of systemically administered growth factors such as intravenously delivered Shh agonists and other agents will allow study of the effects of

Fig. 4. Photomicrograph showing diffuse positivity for nestin in dorsal regions of hyperproliferation after administration of exogenous recombinant Shh in a rat with a demyelinating spinal cord injury. Nestin is an intermediate filament protein found in CNS precursors. Nestin staining, original magnification $\times 100$.

chronic growth factor activity within the CNS after injury without requiring direct injection of the material into the spinal cord. By manipulating microenvironments, it may be discovered that innate reparative mechanisms are adequate to restore functional abilities. Endogenous therapy potentially obviates the need to harvest exogenous stem cells. Currently, this area of study is limited by our knowledge of the delicate balance of cellular homeostasis. The more knowledge we acquire about the innate potential for regeneration, the less we may have to rely on exogenous cell lines. Further research is required to determine whether such cellular manipulation is feasible as a treatment strate-

Fig. 5. Photomicrographs showing dorsal explant cultures from the spinal cords of rats after a contusive spinal cord lesion that was treated with Shh. These are low-power and high-power images of nestin-positive neural precursors. These primitive-appearing cells characteristically demonstrate a bipolar morphology and are highly motile and proliferative. Nestin staining, original magnifications $\times 50$ (A) and $\times 200$ (B).

gy and whether it can sustain functional improvements after a severe injury to the CNS.

References

- Abrahams JM, Gokhan S, Flamm ES, et al: De novo neurogenesis and acute stroke: are exogenous stem cells really necessary? **Neurosurgery** **54**:150–156, 2004
- Agrawal S, Schaffer DV: In situ stem cell therapy: novel targets, familiar challenges. **Trends Biotechnol** **23**:78–83, 2005
- Ankeny DP, McTigue DM, Jakeman LB: Bone marrow transplants provide tissue protection and directional guidance for axons after contusive spinal cord injury in rats. **Exp Neurol** **190**:17–31, 2004
- Arvidsson A, Collin T, Kirik D, et al: Neuronal replacement from endogenous precursors in the adult brain after stroke. **Nat Med** **8**:963–970, 2002
- Bambakidis NC, Miller RH: Transplantation of oligodendrocyte precursors and sonic hedgehog results in improved function and white matter sparing in the spinal cords of adult rats after contusion. **Spine J** **14**:16–26, 2004
- Bambakidis NC, Wang RZ, Franic L, et al: Sonic hedgehog-induced neural precursor proliferation after adult rodent spinal cord injury. **J Neurosurg** **99** (Suppl 1):70–75, 2003
- Bottai D, Fiocco R, Gelain F, et al: Neural stem cells in the adult nervous system. **J Hematother Stem Cell Res** **12**:655–670, 2003
- Chang CF, Lin SZ, Chiang YH, et al: Intravenous administration of bone morphogenetic protein-7 after ischemia improves motor function in stroke rats. **Stroke** **34**:558–564, 2003
- Frisen J, Johansson CB, Torok C, et al: Rapid, widespread, and longlasting induction of nestin contributes to the generation of glial scar tissue after CNS injury. **J Cell Biol** **131**:453–464, 1995
- Gage FH: Mammalian neural stem cells. **Science** **287**:1433–1438, 2000
- Haas S, Weidner N, Winkler J: Adult stem cell therapy in stroke. **Curr Opin Neurol** **18**:59–64, 2005
- Hagan M, Wennersten A, Meijer X, et al: Neuroprotection by human neural progenitor cells after experimental contusion in rats. **Neurosci Lett** **351**:149–152, 2003
- Hermann A, Gerlach M, Schwarz J, et al: Neurorestoration in Parkinson's disease by cell replacement and endogenous regeneration. **Expert Opin Biol Ther** **4**:131–143, 2004
- Horner PJ, Power AE, Kempermann G, et al: Proliferation and differentiation of progenitor cells throughout the intact adult rat spinal cord. **J Neurosci** **20**:2218–2228, 2000
- Jessell TM: Neuronal specification in the spinal cord: inductive signals and transcriptional codes. **Nat Rev Genet** **1**:20–29, 2000
- Johansson CB, Momma S, Clarke DL, et al: Identification of a neural stem cell in the adult mammalian central nervous system. **Cell** **96**:25–34, 1999
- Krauss S, Concordet JP, Ingham PW: A functionally conserved homolog of the *Drosophila* segment polarity gene *hh* is expressed in tissues with polarizing activity in zebrafish embryos. **Cell** **75**:1431–1444, 1993
- Lai K, Kaspar BK, Gage FH, et al: Sonic hedgehog regulates adult neural progenitor proliferation in vitro and in vivo. **Nat Neurosci** **6**:21–27, 2003
- Lee KH, Yoon do H, Park YG, et al: Effects of glial transplantation on functional recovery following acute spinal cord injury. **J Neurotrauma** **22**:575–589, 2005
- Li XJ, Du ZW, Zarnowska ED, et al: Specification of motoneurons from human embryonic stem cells. **Nat Biotechnol** **23**:215–221, 2005
- Lie DC, Dziewczapolski G, Willhoite AR, et al: The adult substantia nigra contains progenitor cells with neurogenic potential. **J Neurosci** **22**:6639–6649, 2002
- Lie DC, Song H, Colamarino SA, et al: Neurogenesis in the adult brain: new strategies for central nervous system diseases. **Annu Rev Pharmacol Toxicol** **44**:399–421, 2004
- Lim DA, Tramontin AD, Trevejo JM, et al: Noggin antagonizes BMP signaling to create a niche for adult neurogenesis. **Neuron** **28**:713–726, 2000
- Lu D, Li Y, Mahmood A, et al: Neural and marrow-derived stromal cell sphere transplantation in a rat model of traumatic brain injury. **J Neurosurg** **97**:935–940, 2002
- Machold R, Hayashi S, Rutlin M, et al: Sonic hedgehog is required for progenitor cell maintenance in telencephalic stem cell niches. **Neuron** **39**:937–950, 2003
- Magavi SS, Leavitt BR, Macklis JD: Induction of neurogenesis in the neocortex of adult mice. **Nature** **405**:951–955, 2000
- Markakis EA, Palmer TD, Randolph-Moore L, et al: Novel neuronal phenotypes from neural progenitor cells. **J Neurosci** **24**:2886–2897, 2004
- McDonald JW, Becker D, Holekamp TF, et al: Repair of the injured spinal cord and the potential of embryonic stem cell transplantation. **J Neurotrauma** **21**:383–393, 2004
- Miao N, Wang M, Ott JA, et al: Sonic hedgehog promotes the survival of specific CNS neuron populations and protects these cells from toxic insult in vitro. **J Neurosci** **17**:5891–5899, 1997
- Mohapel P, Brundin P: Harnessing endogenous stem cells to treat neurodegenerative disorders of the basal ganglia. **Parkinsonism. Relat Disord** **10**:259–264, 2004
- Nakatomi H, Kuriu T, Okabe S, et al: Regeneration of hippocampal pyramidal neurons after ischemic brain injury by recruitment of endogenous neural progenitors. **Cell** **110**:429–441, 2002
- Okano H, Ogawa Y, Nakamura M, et al: Transplantation of neural stem cells into the spinal cord after injury. **Semin Cell Dev Biol** **14**:191–198, 2003
- Palmer TD, Markakis EA, Willhoite AR, et al: Fibroblast growth factor-2 activates a latent neurogenic program in neural stem cells from diverse regions of the adult CNS. **J Neurosci** **19**:8487–8497, 1999
- Philips MF, Mattiasson G, Wieloch T, et al: Neuroprotective and behavioral efficacy of nerve growth factor-transfected hippocampal progenitor cell transplants after experimental traumatic brain injury. **J Neurosurg** **94**:765–774, 2001
- Picard-Riera N, Nait-Oumesmar B, Baron-Van Evercooren A: Endogenous adult neural stem cells: limits and potential to repair the injured central nervous system. **J Neurosci Res** **76**:223–231, 2004
- Porter JA, Young KE, Beachy PA: Cholesterol modification of hedgehog signaling proteins in animal development. **Science** **274**:255–259, 1996
- Rice AC, Khaldi A, Harvey HB, et al: Proliferation and neuronal differentiation of mitotically active cells following traumatic brain injury. **Exp Neurol** **183**:406–417, 2003
- Riess P, Zhang C, Saatman KE, et al: Transplanted neural stem cells survive, differentiate, and improve neurological motor function after experimental traumatic brain injury. **Neurosurgery** **51**:1043–1054, 2002
- Rowitch DH, St-Jacques B, Lee SM, et al: *Sonic hedgehog* regulates proliferation and inhibits differentiation of CNS precursor cells. **J Neurosci** **19**:8954–8965, 1999
- Ruiz I, Altaba A, Palma V, Dahmane N: Hedgehog-Gli signalling and the growth of the brain. **Nat Rev Neurosci** **3**:24–33, 2002
- Seaberg RM, van der Kooy D: Adult rodent neurogenic regions: the ventricular subependyma contains neural stem cells, but the dentate gyrus contains restricted progenitors. **J Neurosci** **22**:1784–1793, 2002
- Shear DA, Tate MC, Archer DR, et al: Neural progenitor cell transplants promote long-term functional recovery after traumatic brain injury. **Brain Res** **1026**:11–22, 2004
- Shihabuddin LS, Horner PJ, Ray J, et al: Adult spinal cord stem cells generate neurons after transplantation in the adult dentate gyrus. **J Neurosci** **20**:8727–8735, 2000

44. Tate MC, Shear DA, Hoffman SW, et al: Fibronectin promotes survival and migration of primary neural stem cells transplanted into the traumatically injured mouse brain. **Cell Transplant** **11**:283–295, 2002
45. Weiss S, Dunne C, Hewson J, et al: Multipotent CNS stem cells are present in the adult mammalian spinal cord and ventricular neuroaxis. **J Neurosci** **16**:7599–7609, 1996
46. Widenfalk J, Lundstromer K, Jubran M, et al: Neurotrophic factors and receptors in the immature and adult spinal cord after mechanical injury or kainic acid. **J Neurosci** **21**:3457–3475, 2001
47. Yamamoto S, Nagao M, Sugimori M, et al: Transcription factor expression and Notch-dependent regulation of neural progenitors in the adult rat spinal cord. **J Neurosci** **21**:9814–9823, 2001
48. Yamamoto S, Yamamoto N, Kitamura T, et al: Proliferation of parenchymal neural progenitors in response to injury in the adult rat spinal cord. **Exp Neurol** **172**:115–127, 2001
49. Zhang SC, Wernig M, Duncan ID, et al: In vitro differentiation of transplantable neural precursors from human embryonic stem cells. **Nat Biotechnol** **19**:1129–1133, 2001

Manuscript received July 15, 2005.

Accepted in final form August 16, 2005.

Address reprint requests to: Nicholas C. Bambakidis, M.D., Neuroscience Publications, Barrow Neurological Institute, 350 West Thomas Road, Phoenix, Arizona 85013. email: neuropub@chw.edu.