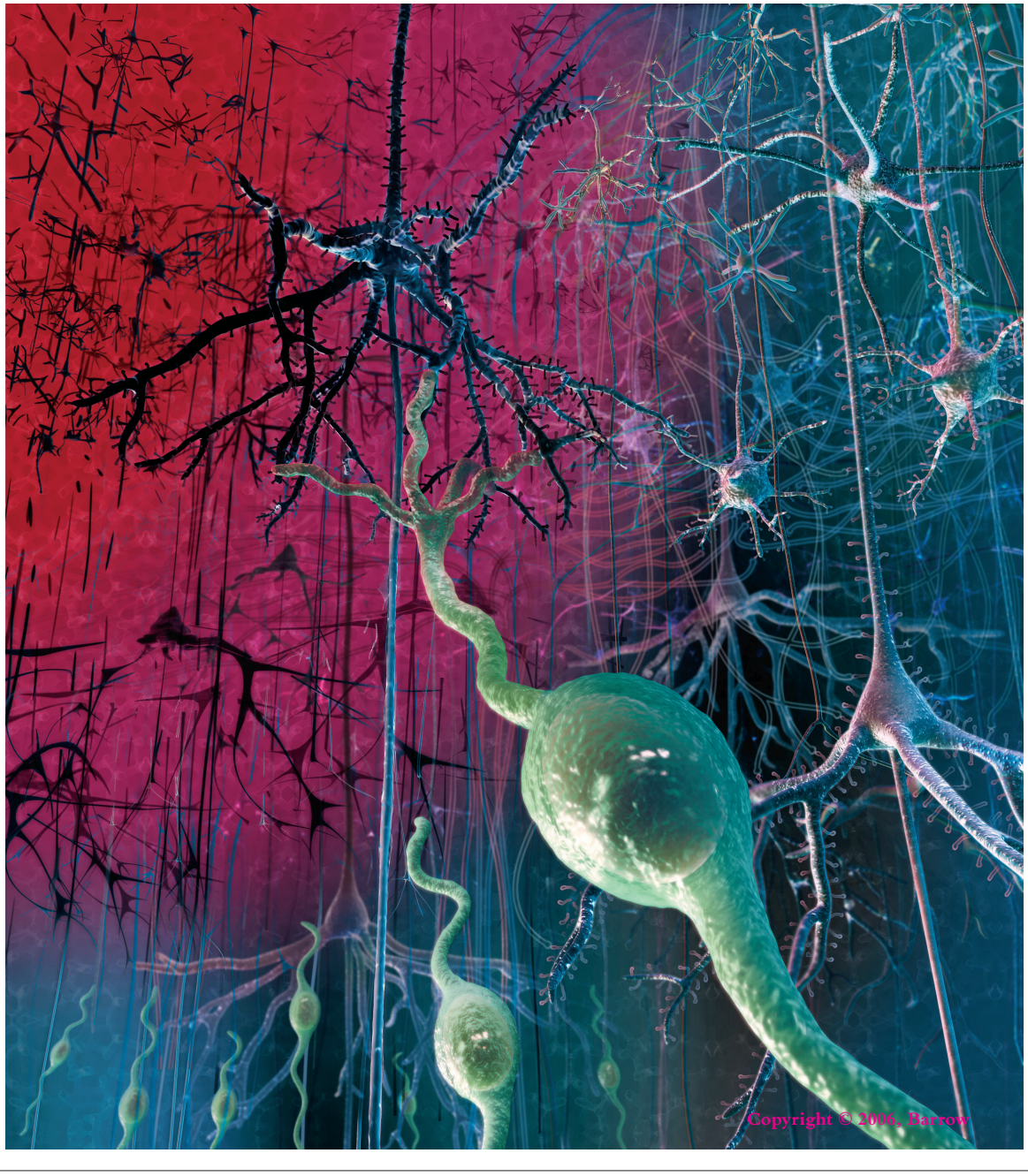
BARROW

QUARTERLY

VOLUME 23 ◊ NUMBER 1 ◊ 2007

BARROW NEUROLOGICAL INSTITUTE OF ST. JOSEPH'S HOSPITAL AND MEDICAL CENTER • PHOENIX, ARIZONA



NEURAL REGENERATION

C O M M E N T S

Despite great strides in the treatment of neurosurgical disorders over the past two decades, significant challenges remain. Unlike other organ systems, the capacity of the central nervous system to heal after injury is severely limited. Investigators confront major difficulties in learning to modify this healing response to allow cellular regeneration and restoration of function. The Neuroregeneration Laboratory at Barrow provides a critical forum in which clinicians and basic scientists can collaborate to develop new therapeutic modalities based on cellular regeneration.

This issue of the *Barrow Quarterly* summarizes research performed at Barrow and in collaboration with neurosurgical specialists at the Case Western Reserve University School of Medicine in Cleveland. Ischemic brain injury and stroke, traumatic brain injury, and spinal cord injury remain major national health issues and active arenas of research. Work in the Neuroregeneration Laboratory focuses on unlocking the potential of endogenous stem cells, which lie quiescent in the adult nervous system throughout life. By elucidating the complex relationships of these cells in their microscopic milieu, we hope to learn to modulate their behavior, allowing damaged areas of the nervous system to regenerate.

Neural regeneration could provide hope for thousands of patients with devastating neurological conditions. For example, Maughan et al. report a case that demonstrates the potential for recovery that the adult spinal cord possesses even in the presence of severe neurologic injury. Such cases inspire us to continue investigating the mechanisms underlying neural repair so that eventually good neurological outcomes may become the rule rather than the exception.

The work reported in this issue could not have been completed without collaboration with other scientists. As the scientific community in Phoenix expands, we anticipate further collaborations through partnerships with entities such as the newly formed medical school of Arizona State University and the Translational Genomics Project (T-Gen). Generous support from donors also fosters such work and helps unlock mysteries in neurosciences, which translates into the miracles of treatment for which Barrow is known. Please consider joining their ranks by using the enclosed self-addressed stamped envelope to share a tax-deductible donation that will help us to continue sharing such exciting findings with all those interested in the neurosciences.

Nicholas C. Bambakidis, MD
Guest Editor

The cover illustration is an artistic interpretation of neuronal stem cells migrating to an injury in the cerebral cortex and differentiating into functional neurons. This issue of the Barrow Quarterly is devoted to articles on neural regeneration. The illustration is by Michael Hickman and Mark Schornak.

Neural Regenerative Options in the Management of Ischemic Brain Injury

Shervin R. Dashti, MD, PhD†
Jason Wilson, MD†
Yin C. Hu, MD†
Warren R. Selman, MD†
Robert F. Spetzler, MD
Peter Nakaji, MD

Ischemic brain injury is a difficult clinical scenario for which adequate treatment paradigms are lacking. The formation of ischemic scar tissue in the cerebral cortex creates a barrier to regenerative efforts and restoration of function. Various agents that regulate the cell cycle hold promise for modulating the naturally progressive formation of this astrocytic scar tissue. Use of these agents will involve the development of strategies that influence the regenerative potential of neural progenitor cells.

Key Words: dentate gyrus, hippocampus, neural stem cells, neurogenesis

Abbreviations Used: BrdU, bromodeoxyuridine; CNS, central nervous system; DNA, deoxyribonucleic acid; G-CSF, granulocyte colony-stimulating factor; mRNA, messenger ribonucleic acid; NMDA, N-methyl-D-aspartate

Division of Neurological Surgery, Barrow Neurological Institute, St. Joseph's Hospital and Medical Center, Phoenix, Arizona

†*Department of Neurosurgery, University Hospitals of Cleveland, Case Western Reserve University School of Medicine, Cleveland, Ohio*

Until recently, it was widely believed that neuronal production in the mammalian brain occurred only during the prenatal period. Over the past 15 years, however, evidence has shown that neurogenesis continues in certain areas of the adult mammalian brain, namely, in the subventricular zone and hippocampal dentate gyrus (Fig. 1).[20] An obvious question is whether adult neurogenesis has the potential to replace dying neurons in the setting of brain injury. New evidence indicates that such neurogenesis is indeed possible. The logical clinical implication of this finding is the potential to augment the production of mature neurons in the injured brain. This ability would be of considerable value in victims of large strokes who seldom regain significant function in distributions where neural tissue has died.

In contrast to the recently discovered adult neurogenesis, hematopoietic stem cells have been studied extensively for a relatively long time. The hematopoietic system contains a pool of progenitor cells that differentiate into mature cells. Studying the molecular mechanisms governing the proliferation, differentiation, and apoptosis of the hematopoietic stem cell population provides valuable clues about the biology of neural progenitor cells.

This review examines neurogenesis in the normal brain and in response to ischemic insults. Factors that drive proliferation of stem cell populations into neural progenitor cells and those that drive differentiation of neural progenitor cells into neurons and, ultimately, integration into neural networks are discussed. Such therapeutic modalities can be broadly classified as using either an exogenous or an endogenous approach. These basic is-

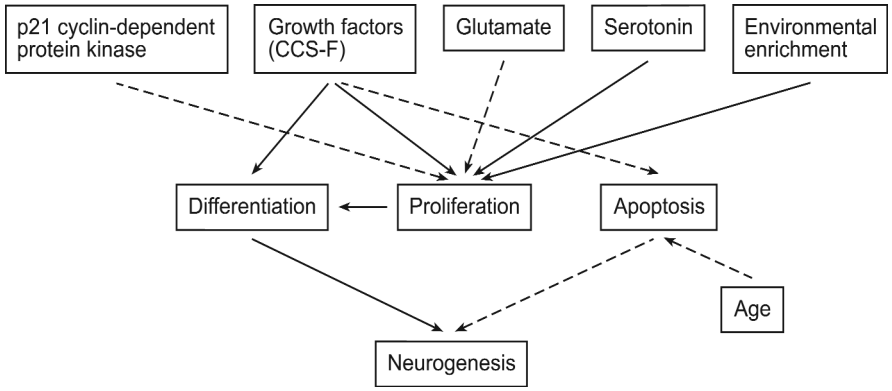


Figure1. Flow chart of the pathways involved in the differentiation of endogenous neural stem cells.

sues must be understood before strategies can be devised to improve outcomes based on repopulating neurons and achieving functional integration in the setting of acute ischemic injury.

Hippocampal Dentate Gyrus

Neural stem cells are located in the subgranular zone of the adult mammalian hippocampal dentate gyrus.[15,19] The primary neural progenitor cells, bipotent radial glia-like stem cells with astrocytic properties, differentiate into lineage-determined progenitor cells and then to mature neurons.[6,16] The mature neurons migrate a short distance and populate the granular cell layer of the hippocampal dentate gyrus. The S-phase marker bromodeoxyuridine (BrdU) has been used to track dividing cells in the adult rat dentate gyrus. Based on such studies, young adult rats generate 9,000 new cells each day or more than 250,000 per month. The new granule neurons generated each month constitute 6% of the total size of the granular cell population as well as 30 to 60% of afferent and efferent cells.[5]

Forebrain Subventricular Zone

Adult neurogenesis also occurs in the mammalian subventricular zone. Neurons are generated in the subependymal layer of the lateral ventricles and then migrate tangentially toward the olfactory bulb and striatum. In the olfactory bulb, these neurons climb radially into the granule and periglomerular cell layers. There, they assume the nuclear morphology of granule cells and express neuron-specific markers.[11] Most of these adult-generated granule neurons persist within the olfactory bulbs for at least 4 months.[10] Chains of neuroblasts also extend from the subventricular zone to the peri-infarct striatum. Many of these newly generated cells persist in the striatum and cortex adjacent to infarcts. However, 35 days after stroke, only the cells in the neostriatum express neuronal markers.[25] As with the hippocampal dentate gyrus, the persistent neurogenesis in the subventricular zone has been implicated in olfactory memory and odor discrimination.[1]

Response to Ischemic Insults

Stroke-Induced Neurogenesis

Recent evidence suggests that ischemia significantly induces neurogenesis in the brain of adult rodents.[20] Liu et al. first demonstrated a marked increase in cell birth in the dentate subgranular zone of gerbils in response to transient global ischemia.[21] Subsequent studies confirmed that focal[9,12,31,32] and global[30] cortical ischemia significantly increases the proliferation of neural stem cells in both the dentate gyrus and subventricular zone. This phenomenon peaks 7 to 10 days after ischemia is induced and returns to normal levels within a month.[20]

Differentiation of Stem Cells to Mature Neurons. Neural stem cells from adult rats co-cultured with primary neurons and astrocytes from neonatal hippocampus differentiate into electrically active neurons and integrate into neural networks with functional synaptic transmission (Fig. 2).[29] Preliminary evidence suggests that these cells play a role in hippocampal-dependent memory formation.[29] At 35 days after stroke, the chain of neuroblasts that migrates from the subventricular zone to the peri-infarct striatum also expresses neuronal markers.[25] As with the hippocampal dentate gyrus, the persistent neurogenesis in the subventricular zone has been implicated in olfactory memory and odor discrimination.[1]

Migration of Neural Precursors. Understanding the movement of neural precursors generated in the adult mammalian brain to their final destination has important implications for functional recovery after ischemic injury. Recall that normal neural stem cells in the hippocampal dentate gyrus differentiate into progenitor cells and then to mature neurons.[6,16] The latter migrate a short distance to populate the granular cell layer of the hippocampal dentate gyrus. Newly divided neuronal precursor cells also migrate from the subgranular zone into the granule cell layer and differentiate into neurons.[21] Migrating neuroblasts move as chains through a well-defined pathway, the rostral migratory stream. These chains contain only closely apposed neuroblasts. Another astrocytic cell type ensheathes the chains of migrating neuroblasts. Hence during chain migration, neural precursors move in association with each other; they are not guided by radial glial or axonal fibers.[22]

Parent et al. induced focal stroke in adult rats and assessed cellular proliferation and neurogenesis with BrdU labeling and immunostaining.[25] Brains examined 10 to 21 days after stroke showed chains of neuroblasts extending from the

subventricular zone to the peri-infarct striatum. These findings combined with those from several other studies indicate that focal and global ischemic injury directs the migration of the neuroblasts to the site of the infarct.[2,13]

Long-Term Survival of New Adult Neurons. An important question is how long the newly generated neurons in the ischemic adult brain persist. It is estimated that fewer than 1% of the ischemic and dying striatal neurons are replaced through adult neurogenesis.[2] This estimate suggests that most neurons generated in the adult brain in response to injury do not survive for a significant length of time. Understanding the mechanism of cell death in adult-born neurons is the first step in developing therapies intended to maximize functional recovery after stroke.

Neurogenesis in the Aging Brain

Most of the new neurons generated in response to stroke in adult rats do not survive, and stroke most often occurs in older populations. Therefore, an important question is whether aging influences the survival of adult-born neurons. Yagita et al. used a four-vessel transient ischemia model in rats to investigate the role of aging on proliferation of neural progenitor cells.[34] In both young and old rats, neurogenesis significantly increased in the subventricular zone in response to transient global ischemia. Advanced age, however, profoundly influenced the survival of the newly generated neural precursor cells. One month after the ischemia was induced, the number of surviving neural progenitor cells decreased seven-fold in the older rats. To develop strategies to maximize functional recovery after stroke in the aging population, we must first understand the molecular mechanism by which senescence decreases the survival of neurons. Is it through programmed cell death or tissue necrosis? What signaling pathways are involved? How can this cell death mechanism be down-regulated?

Neurotransmitters

Glutamate, the main excitatory neurotransmitter in the brain, appears to play a role in adult neurogenesis. Activation of NMDA-glutamate receptors rapidly decreases proliferation of neuronal stem cells in rats.[4] In contrast, administration of NMDA-receptor antagonists rapidly increases the number of cells during the S phase.[4] Lesioning of the entorhinal cortex, the main excitatory (glutamate-mediated) afferent input to the hippocampal granule neurons, also increases neurogenesis in the dentate gyrus. This finding suggests that glutamate negatively affects adult neurogenesis.

Serotonin is another neurotransmitter implicated in adult neurogenesis. Chronic antidepressant therapy with serotonin-reuptake inhibitors significantly increases proliferation of adult neuronal precursor cells in the dentate gyrus.[23] Conversely, inhibiting the serotoninergic pathway decreases neuronal proliferation in the dentate gyrus and subventricular zones.[3] However, the effect of serotonin on ischemia-induced proliferation of neural progenitor cells has not yet been established.

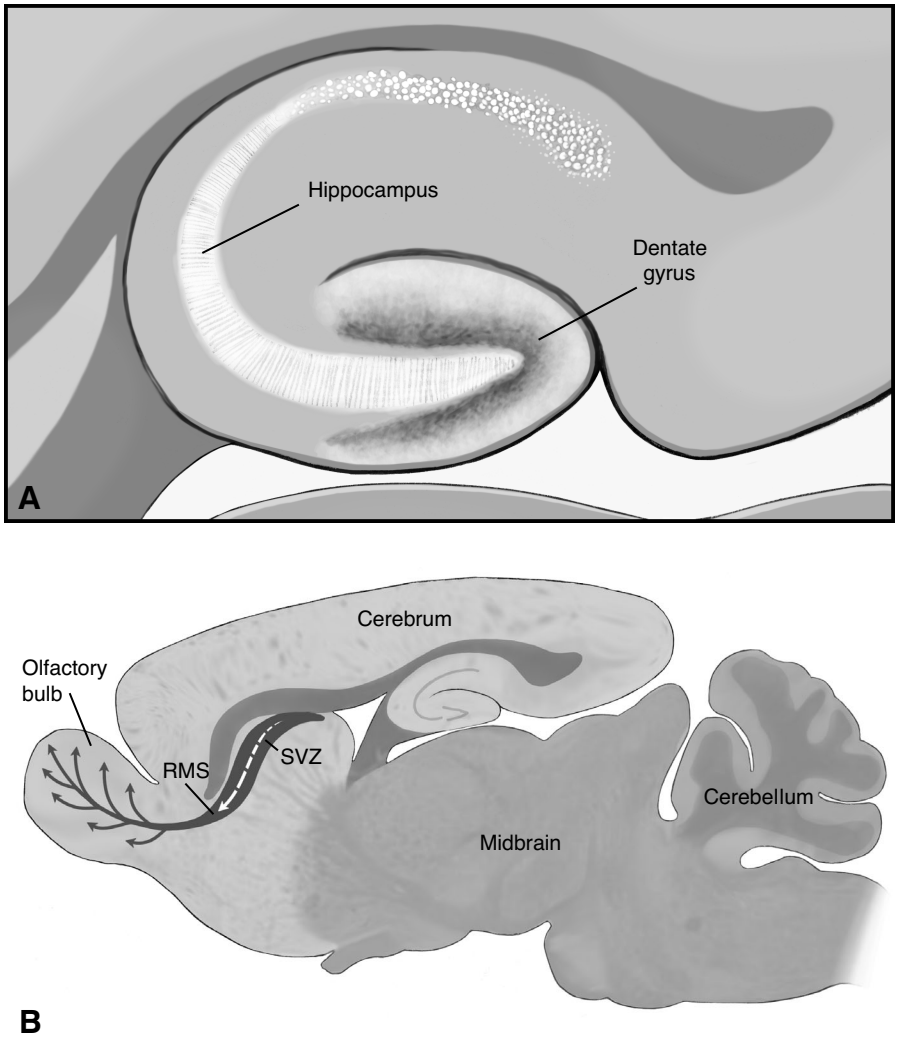


Figure 2. The two niches containing neural stem cells in the CNS of the adult mammal. (*A*) adult hippocampus. (*B*) Longitudinal section of the adult mouse brain in which the subventricular zone (SVZ) and the rostral migratory stream (RMS) are indicated. Neuronal precursors migrate tangetially along the rostral migratory stream from the SVZ to the olfactory bulb.

Environmental Enrichment

Environmental enrichment, consisting of social interactions, various physical activities, and exposure to novel objects, enhances functional recovery after stroke.[17,33] At the cellular level, environmental enrichment has increased dendritic branching and dendritic spines in pyramidal neurons of layers II–III in the contralateral cortex.[14] At the molecular level, environmental enrichment induces differential gene regulation in the injured brain.

Recent evidence suggests that postischemic environmental enrichment also enhances neurogenesis in the adult germinal zones. Komitova et al. induced stroke by ligating the middle cerebral artery of spontaneously hypertensive rats, which were then exposed to a standard or enriched environmental condition.[18] One and 7 days after stroke, rats exposed to the enriched environment performed significantly better than rats exposed to the standard environment. Five weeks after stroke, there was less cellular proliferation in the subventricular zone of the stroke-lesioned rats in the standard environment compared to intact animals.

Postischemic environmental enrichment counteracts the decreased level of cellular proliferation in the subventricular zone while increasing the pool of neural progenitor cells in the subventricular zone. The generation of neural precursor cells capable of recruitment to the stroke site also increases. The same investigators demonstrated similar findings in the hippocampi of rats with strokes in response to environmental enrichment.[24] One month after injury, these rats demonstrated improved spatial learning compared to control animals and a net increase in hippocampal neurogenesis.

Factors (G-CSF)

G-CSF, a 19.6 kDa growth factor, exerts neuroprotective effects after focal cerebral ischemia in the rat model.[27] It counteracts programmed cell death and enhances functional recovery. G-CSF is widely expressed in neurons in the CNS, and it is upregulated in response to cerebral ischemia, suggesting an autocrine neuroprotective signaling cascade.

In myeloid lineage cells, G-CSF induces enhanced survival and differentiation into mature neutrophilic granulocytes.[28] It appears that G-CSF exerts a similar effect on survival and differentiation of neural stem cells.[28] Adult neural stem cells isolated from the subventricular zone and hippocampus of rats express G-CSF in vitro.[28] G-CSF greatly induces promoter activity of the mature neuronal marker gene III-tubulin. In culture it also induces dose-dependent increases in levels of the mRNA of several differentiation markers. It does not, however, deplete the pool of undifferentiated cells.

Endogenous Suppression of Neural Regeneration (p21 Cyclin-Dependent Kinase Inhibitor)

The efficacy of neural stem cell expansion in response to injury depends on the proliferative capacity of the cells. Understanding the molecular kinetics of the cell cycle is the key to inducting the proliferation of stem cells for therapeutic purposes. In the hematopoietic stem cell model, there are two types of cellular proliferation: high-capacity proliferation of progenitor cells in response to cytokines and low-proliferation capacity quiescent stem cells. The latter are insensitive to cytokines but intermittently supply the proliferative pool. Likewise in the CNS, quiescent multipotent precursor cells or neural stem cells give rise to the proliferative adult neural progenitor cells. The inherently lower rate of turnover in the CNS may likewise be related to quiescence in neural progenitor cells.[26]

This possibility could explain the mild proliferation and migration of neural precursor cells and the subsequent minimal functional improvement in response to brain injury. Proteins such as cyclin-dependent kinase inhibitors, which down-regulate the cell cycle, mediate cellular quiescence. The cyclin-dependent kinase inhibitor p21cip1/waf1 specifically downregulates the cell cycle in hematopoietic stem cells (p21).[7,8] The p21 cyclin-dependent kinase inhibitor also prevents quiescent progenitor cells in the brain from entering the cell cycle.[26] Qiu and colleagues demonstrated that ischemic brain injury induces proliferation of quiescent neural progenitor cells in the hippocampus and in the subventricular zone of p21 knockout mice.[26] The increased proliferation of stem cells, however, did not occur in nonlesioned p21 knockout mice.

Conclusion

Future efforts aimed at investigating other regulators of the cell cycle, including the other members of the cyclin-dependent kinase-inhibitor family, will improve understanding of the relative quiescence of neural progenitor cells. Eventually, such studies may lead to improved functional recovery of the CNS in response to ischemic injury by spurring the development of strategies to enhance proliferative potential of neural progenitor cells.

References

1. Alvarez-Buylla A, Garcia-Verdugo JM: Neurogenesis in adult subventricular zone. **J Neurosci 22:**629-634, 2002
2. 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
3. Brezun JM, Daszuta A: Depletion in serotonin decreases neurogenesis in the dentate gyrus and the subventricular zone of adult rats. **Neuroscience 89:**999-1002, 1999
4. Cameron HA, McEwen BS, Gould E: Regulation of adult neurogenesis by excitatory input and NMDA receptor activation in the dentate gyrus. **J Neurosci 15:**4687-4692, 1995
5. Cameron HA, McKay RD: Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. **J Comp Neurol 435:**406-417, 2001
6. Cameron HA, Woolley CS, McEwen BS, et al: Differentiation of newly born neurons and glia in the dentate gyrus of the adult rat. **Neuroscience 56:**337-344, 1993
7. Cheng T, Rodrigues N, Dombkowski D, et al: Stem cell repopulation efficiency but not pool size is governed by p27(kip1). **Nat Med 6:**1235-1240, 2000
8. Cheng T, Rodrigues N, Shen H, et al: Hematopoietic stem cell quiescence maintained by p21cip1/waf1. **Science 287:**1804-1808, 2000
9. Choi YS, Lee MY, Sung KW, et al: Regional differences in enhanced neurogenesis in the dentate gyrus of adult rats after transient forebrain ischemia. **Mol Cells 16:**232-238, 2003

10. Corotto FS, Henegar JA, Maruniak JA: Neurogenesis persists in the subependymal layer of the adult mouse brain. **Neurosci Lett 149:**111-114, 1993
11. Gheusi G, Cremer H, McLean H, et al: Importance of newly generated neurons in the adult olfactory bulb for odor discrimination. **Proc Natl Acad Sci USA 97:**1823-1828, 2000
12. Jin K, Minami M, Lan JQ, et al: Neurogenesis in dentate subgranular zone and rostral subventricular zone after focal cerebral ischemia in the rat. **Proc Natl Acad Sci USA 98:**4710-4715, 2001
13. Jin K, Sun Y, Xie L, et al: Neurogenesis and aging: FGF-2 and HB-EGF restore neurogenesis in hippocampus and subventricular zone of aged mice. **Aging Cell 2:**175-183, 2003
14. Johansson BB: Functional and cellular effects of environmental enrichment after experimental brain infarcts. **Restor Neurol Neurosci 22:**163-174, 2004
15. Kaplan MS, Hinds JW: Neurogenesis in the adult rat: Electron microscopic analysis of light radioautographs. **Science 197:**1092-1094, 1977
16. Kempermann G, Jessberger S, Steiner B, et al: Milestones of neuronal development in the adult hippocampus. **Trends Neurosci 27:**447-452, 2004
17. Keyvani K, Sachser N, Witte OW, et al: Gene expression profiling in the intact and injured brain following environmental enrichment. **J Neuropathol Exp Neurol 63:**598-609, 2004
18. Komitova M, Mattsson B, Johansson BB, et al: Enriched environment increases neural stem/progenitor cell proliferation and neurogenesis in the subventricular zone of stroke-lesioned adult rats. **Stroke 36:**1278-1282, 2005
19. Kuhn HG, Dickinson-Anson H, Gage FH: Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. **J Neurosci 16:**2027-2033, 1996
20. Lichtenwalner RJ, Parent JM: Adult neurogenesis and the ischemic forebrain. **J Cereb Blood Flow Metab 26:**1-20, 2006
21. Liu J, Solway K, Messing RO, et al: Increased neurogenesis in the dentate gyrus after transient global ischemia in gerbils. **J Neurosci 18:**7768-7778, 1998
22. Lois C, Garcia-Verdugo JM, Alvarez-Buylla A: Chain migration of neuronal precursors. **Science 271:**978-981, 1996
23. Malberg JE, Eisch AJ, Nestler EJ, et al: Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. **J Neurosci 20:**9104-9110, 2000
24. Nilsson M, Perfilieva E, Johansson U, et al: Enriched environment increases neurogenesis in the adult rat dentate gyrus and improves spatial memory. **J Neurobiol 39:**569-578, 1999
25. Parent JM, Vexler ZS, Gong C, et al: Rat forebrain neurogenesis and striatal neuron replacement after focal stroke. **Ann Neurol 52:**802-813, 2002
26. Qiu J, Takagi Y, Harada J, et al: Regenerative response in ischemic brain restricted by p21cip1/waf1. **J Exp Med 199:**937-945, 2004
27. Schabitz WR, Kollmar R, Schwaninger M, et al: Neuroprotective effect of granulocyte colony-stimulating factor after focal cerebral ischemia. **Stroke 34:**745-751, 2003
28. Schneider A, Kruger C, Steigleder T, et al: The hematopoietic factor G-CSF is a neuronal ligand that counteracts programmed cell death and drives neurogenesis. **J Clin Invest 115:**2083-2098, 2005
29. Shors TJ, Miesegaes G, Beylin A, et al: Neurogenesis in the adult is involved in the formation of trace memories. **Nature 410:**372-376, 2001
30. Takagi Y, Nozaki K, Takahashi J, et al: Proliferation of neuronal precursor cells in the dentate gyrus is accelerated after transient forebrain ischemia in mice. **Brain Res 831:**283-287, 1999
31. Takasawa K, Kitagawa K, Yagita Y, et al: Increased proliferation of neural progenitor cells but reduced survival of newborn cells in the contralateral hippocampus after focal cerebral ischemia in rats. **J Cereb Blood Flow Metab 22:**299-307, 2002
32. Tureyen K, Vemuganti R, Sailor KA, et al: Transient focal cerebral ischemia-induced neurogenesis in the dentate gyrus of the adult mouse. **J Neurosurg 101:**799-805, 2004
33. Will B, Galani R, Kelche C, et al: Recovery from brain injury in animals: Relative efficacy of environmental enrichment, physical exercise or formal training (1990-2002). **Prog Neurobiol 72:**167-182, 2004
34. Yagita Y, Kitagawa K, Ohtsuki T, et al: Neurogenesis by progenitor cells in the ischemic adult rat hippocampus. **Stroke 32:**1890-1896, 2001

Current Concepts in Neural Regeneration After Traumatic Brain Injury

Brian P. Gantwerker, MD†
Alan Hoffer, MD†
Mark C. Preul, MD
Nicholas Theodore, MD

TBI poses unique difficulties in terms of developing effective therapeutic modalities. The inflammatory cascade that results from TBI is distinct from that which follows other forms of injury to the CNS. This difference poses unique challenges to developing potential mechanisms of treatment based on the concepts of neural regeneration and repair.

Key Words: neural transplantation, neuroregeneration, trauma, traumatic brain injury

Abbreviations Used: BrdU, bromodeoxyuridine; CNS, central nervous system; DiO, dioctadecyloxacarbocyanine, DNA, deoxyribonucleic acid; GFAP, glial fibrillary acidic protein; MSC, marrow-derived stromal cells; NeuN, neuronalnuclei marker; TBI, traumatic brain injury

Division of Neurological Surgery, Barrow Neurological Institute, St. Joseph's Hospital and Medical Center, Phoenix, Arizona

†*Department of Neurosurgery, University Hospitals of Cleveland, Case Western Reserve University School of Medicine, Cleveland, Ohio*

Each year more than a million Americans are treated for TBI and released from emergency rooms. More than 5 million people live with permanent disabilities from TBI-related injuries, including behavioral disorders, cognitive difficulties, and motor impairment.[6] TBI is the leading cause of death in young people in America and costs billions of healthcare dollars each year. For many years, therapies aimed at regenerating lost brain tissue have been explored. The replacement of dead neurons by new cells derived from precursor cells, which has been the focus of recent efforts, remains a controversial ethical issue.

The primary damage caused by TBI is the result of the initial impact. Intracellular edema develops, and the electrochemical processes inherent to neural function are disrupted.[1,16,28] The secondary or delayed damage associated with TBI is less well understood but involves a complex cascade of events and pathophysiological interactions. Research once emphasized pharmacological therapies and early surgical intervention intended to limit this secondary constellation of events.

In the last decade, however, TBI has been recharacterized as a chronic, progressive disease involving the delayed death of neurons,[24] and the focus of research has shifted concomitantly. Recently, stimulation of latent neural progenitor cells and the introduction of exogenous precursor cells have been topics of investigation (Fig. 1).[21] Dead and damaged neural tissues have been replaced both *in vitro* and *in vivo* in rodents and nonhuman primates, but an effective and reproducible treatment for humans has yet to be developed. This article reviews current

concepts in the utilization of stem cell populations and their potential therapeutic role in the treatment of TBI.

Endogenous Stem Cells

Cellular replacement strategies have involved both endogenous and exogenous sources of progenitor cells. Endogenous neurogenesis has been a long-standing goal to minimize further trauma to the brain from transplantation and to avoid the subsequent need for immune suppressive therapies. Efforts to stimulate endogenous precursor cells to migrate to areas of injury have allowed the regions where these cells are found to be characterized. Adult mammals have neural stem cells that can be activated and that cause remodeling in the event of injury.[10] After TBI these proliferating stem cells continue this remodeling process.[5]

Remodeling occurs at two specific areas. The area of cortical injury nearest the site of impact involves glial remodeling. GFAP staining has shown that reactive astrocytes are upregulated and form a glial scar 3 weeks after injury. Second, staining with NeuN, a neuronal marker, has shown hypertrophy of neurons in the ipsilateral granular cell layer.[23]

Stem cells that supply the astrocytes originate in the subventricular zone. An intraventricular injection of the lipophilic tracer DiO reliably demonstrates labeling of this zone. These labeled cells migrate from the subventricular zone into the damaged area of cortex.[23] These cells co-localize with nestin, an intermediate filament specific to neuronal precursors. DiO labeling in the area of injured cortex persists 3 weeks, indicating that these cells survive. GFAP co-localizes with the DiO in this region. Morphologically, these cells appear to be the same reactive astrocytes that form the glial scar. Under normal circumstances, these cells supply replacement cells to the olfactory bulb, where they become glomerular neurons.[4] Therefore, these cells have the *potential* to differentiate along neuronal or glial cell lines. Recent studies have focused on delivering growth factors to this cell population to influence them to become new neurons in the presence of brain injury.

The adult nervous system hosts multiple regions of stem cells. Neural crest stem cells can be found in the peripheral nervous system. In the CNS, the retina, olfactory bulb, forebrain, hippocampus, and spinal cord contain stem cells (Fig. 2).[3,8,9,14] The largest repositories of neural stem cells are found in the subventricular zone and dentate gyrus of the hippocampus.[19]

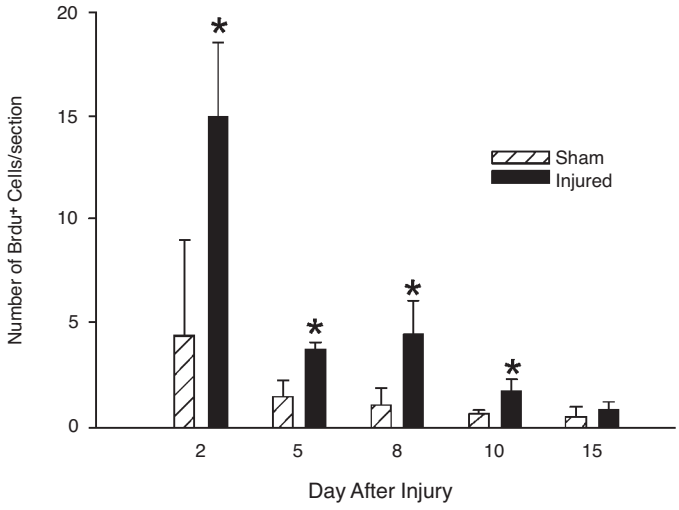


Figure 1. Graphic representation of BrdU positive cells per section in the SGZ (subgranular zone) and SEZ (subventricular zone) of animals injected with BrdU 18 and 20 hours after traumatic brain injury, then sacrificed at various times. The cells have a peak of proliferation at 2 and 8 days after injury. They then degrade or migrate away over 2 weeks or so. Most cells go on to form glial cells. These cells arise from the subgranular zone of the hippocampus and subependymal zones of the lateral ventricles. It is considered to be a normal response of the brain to injury. Modulation in the development of these cells away from an astrocytic (and therefore scar-forming) lineage is a potential key therapeutic target in ameliorating the damage following TBI. Data are presented as the number of cells per section ± standard deviation (SD). Injured animals in each group (3 to 6 animals) are compared with their respective shams using a *t* test. *Significant at $p < 0.05$. *From Rice AC, Khaldi A, Harvey HB, Salman NJ, White F, Fillmore H, Bullock MR: Proliferation and neuronal differentiation of mitotically active cells following traumatic brain injury. Exp Neurol 183(2):406-417, 2003. Used with permission from Elsevier Science.*

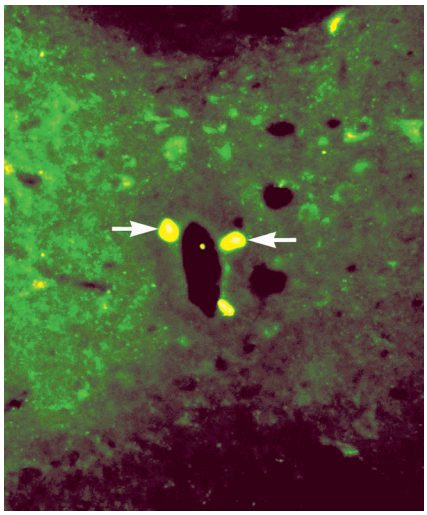


Figure 2. Photomicrograph of BrdU-stained cells dividing around the central canal in the spinal cord of an adult rodent exposed to recombinant Shh after sustaining a demyelinating lesion. These cells represent endogenous neural progenitors stimulated by the combination of demyelination and Shh. Fluorescent stain for BrdU (200x). *From Bambakidis NC, Theodore N, Nakaji P, Harvey A, Sonntag VK, Preul MC, Miller RH: Endogenous stem cell proliferation after central nervous system injury: Alternative therapeutic options. Neurosurg Focus 19(3):E1, 2005. Used with permission from Journal of Neurosurgery.*

Progenitor Cells versus Stem Cells

As an embryo develops, the pluripotent cells that compose the differentiating tissue gradually lose their ability to transdifferentiate. Embryonic stem cells are derived from the inner cell mass.[29] Adult stem cells are present in many different organ systems in the body. Tissue restriction limits the possible fates of these cells more than in their embryonic counterparts. This factor may explain the differences seen in experimental models that have tested the fates of cells in *in vitro* animal models. Some studies have shown that even these cells may cross fate lines and generate other tissues. For example, adult neural stem cells can become hematopoietic cells when transplanted into the marrow of irradiated mice. Whether this process represents actual transdifferentiation or cell-cell fusion is debated. The ideal population of adult stem cells would exhibit no *in vitro* or *in vivo* difference in their fate lines.

Stated simply, stem cells can differentiate into many different types of cells (Fig. 3). They have an unlimited capacity for self-renewal and are found in many different kinds of tissue. Progenitor cells also have multiple potential destinies. However, they have far fewer potential fates than stem cells. Although much greater than that of differentiated cells, the capacity of progenitor cells for renewal is more limited than that of stem cells. Stem cells are the best candidates for treating TBI because they are robust and more flexible than progenitor lines. Furthermore, neural progenitor cells must be isolated from embryos, a process with controversial ethical and sociological implications.

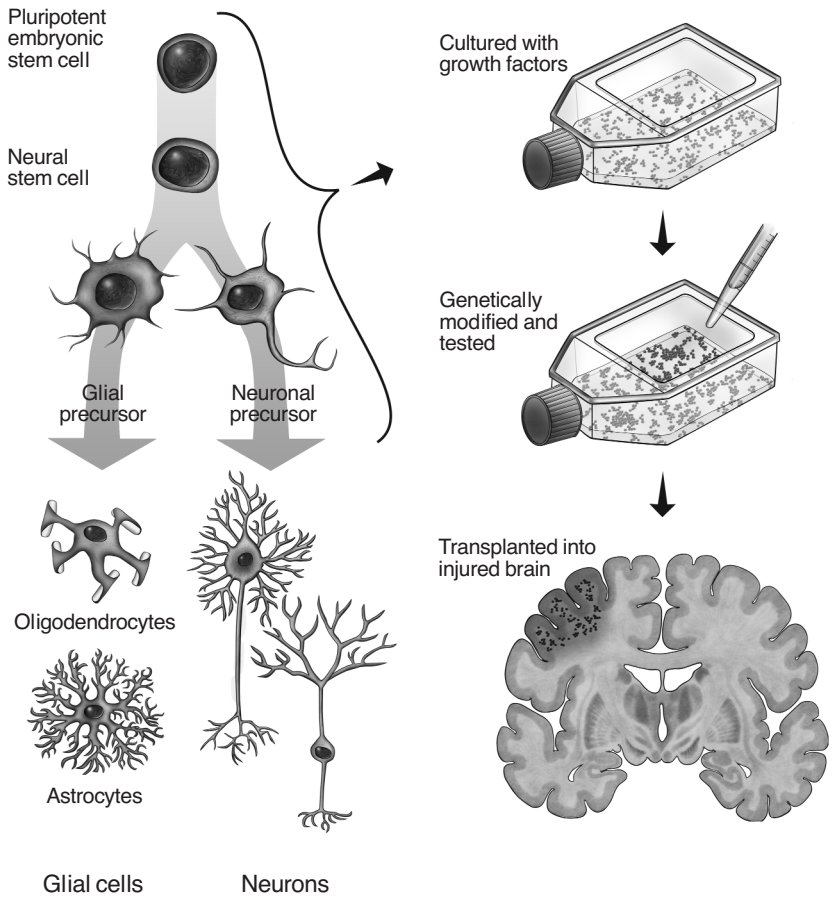


Figure 3. Diagram demonstrating the pluripotent nature of embryonic stem cells as well as their potential therapeutic roles in traumatic brain injury. Pluripotent stem cells develop under the influence of various growth factors into multipotent progenitors which can then form either neuronal, oligodendroglial, or astrocytic cells. This process can be modulated *in vitro* and subsequently can result in cells which can be transplanted at the site of injury with a resultant potential for therapy.

Exogenous Delivery of Cells

Regeneration in the CNS requires one or more of the following processes to occur: neurotrophic factor elaboration, cell replacement, axonal guidance, removal of growth inhibition (characteristic of damage in the CNS), modulation of the immune response, a substrate for bridging, and cell signaling manipulation.[10] Injections into the bloodstream and intraparenchymal delivery have been used to transplant cells in an attempt to rebuild a damaged nervous system. To be effective, the transplanted cells must be self-renewing and pluripotent. They must exhibit tropism for the damaged area and form functional connections. The survival and integration of these cells depend on both the location of the transplant and the local environment.[27]

Fetal Tissue Transplantation

The direct implantation of fetal cells into damaged cortex was first evaluated more than 20 years ago. Recent studies based on the fluid percussion model of TBI found that the optimal window for transplantation is within 2 weeks of injury.[26] Transplanted fetal tissue is able to integrate into damaged tissue and thereby restore function. This capability may hold particular promise after an injured brain is exposed to retraining activities. In this case transplanted cells may help re-establish injured neuronal connections.

Keyvani et al. performed DNA microarray analysis of intact and injured brains.[13] They examined the nonnecrotic ipsilateral cortex and homotropic contralateral cortex in brain-

injured rats exposed to environmental enrichment after injury. In the brain-injured rats, 59 genes were differentially regulated compared to 17 genes regulated in analogous areas in the brains of intact rats. This finding suggests an increased propensity for enrichment-induced plasticity after injury. Such research is promising, but significant ethical and immunological dilemmas remain.

Infusional Delivery

In a hemorrhagic brain injury model, injection of blood from human umbilical cords has been found to reduce the effects of neurological damage.[18] Rats injected with human umbilical cord blood 24 hours after intracerebral hemorrhage were evaluated immediately before receiving the injection, 1 day after injury, and then 7 and 14 days after injury. On Days 7 and 14, the neurological severity test scores of these experimental rats were better than those of saline-treated rats. Umbilical cord blood has been used successfully to repopulate depleted immune systems. Therefore, it is thought that hematopoietic stem cells are able to transdifferentiate and to perform a similar function in the nervous system.[18]

Mahmood et al.[17] used MSCs as a source of neural growth factors to facilitate intrinsic stem cells to grow and differentiate. MSCs can differentiate into endodermal, mesodermal, and ectodermal derivatives.[12] MSCs were delivered by an intravenous injection of marrow cells. Long bones from normal rats were obtained and cleaned, and the cells were cultured and labeled with BrdU. One day after the rats received a pneumatic injury intended to simulate a concussive impact, the cell mixture was injected. Rats were assessed via standard neurological function tests at various endpoints. Histological analysis was performed when the rats were sacrificed 15 days after injury. Levels of three different growth factors were measured on Days 2, 5, and 8: nerve growth factor, brain-derived nerve growth factor, and basic fibroblast growth factor.

Compared to untreated control animals, the animal receiving intravenous MSC had significantly better scores on the Rotarod motor test and neurological severity test. Furthermore, MSCs migrated into the injured hemisphere where they formed oligodendrocytes and some neurons. The levels of nerve growth factor peaked on Days 5 and 8. Brain-derived nerve growth factor peaked on Day 8 after injury. Levels of basic fibroblast growth factor were not statistically different at any of the time points. In this model, the cells provided useful growth factors and eventually became functional cells in the damaged hemisphere. The increased level of these factors alone may partially explain the functional recovery seen in experimental models of TBI.[15]

Intraparenchymal Injection

Direct injection of stem cells has been the focus of recent research. In 2002 Riess et al.[22] concluded that the injection of neural stem cells attenuates motor dysfunction but not cognitive dysfunction after TBI. In 2004 Shear at al.[25] directly injected neural progenitor cells into the ipsilateral caudate of injured cortex of rats 1 week after injury. Motor abilities improved as early as 1 week after transplantation and were still present at 1 year. The transplanted cells persisted as long as 14 months and remained in areas of the hippocampus and adjacent cortex. Interestingly, the transplanted cells labeled with NG2, a marker of oligodendrocytic precursors. Neural stem cells and progenitor cells still need to be harvested from embryonic, adult, or dead donors. However, nonneural sources of progenitor cells have also been injected.

MSCs have been used successfully to attenuate the effects of neural injury via intraparenchymal injury. Chen et al.[7] found that MSCs actually elaborate neurotrophic factors that were measured in the study by Mahmood et al.[17] MSC-derived neurotrophins had a protective effect on the native neurons. Again, nerve growth factor was the most significantly elevated factor and was responsible for the maintenance of cholinergic neurons. Thus, not only do the transplanted cells integrate into the CNS, they also synthesize diffusible substances that protect uninjured tissue. Cholinergic neurons are partially responsible for memory formation and may play a large role in recall.

MSCs may fuse with neurons rather than undergo transdifferentiation.[2] Tissue replacement may play a role in this particular model although the maintenance of native cells via the elaboration of neurotrophins is becoming a more central focus in research.

Other nonmurine sources of stem cells have also been studied. Embryonic stem cells from cynomolgus monkeys have also been transplanted successfully into mice after brain injury.[11] Embryonic stem cells were preinduced by *in vitro* treatment with retinoic acid and differentiated into neurons. The cells were transplanted 1 week after injury. Mice were evaluated by the Rotarod test and the beam walking test. The scores of the mice receiving the transplants were significantly better than those of the mice without transplants. This study was the first to use nonhuman primates as a source of stem cells. Although the mechanism of injury was cryogenic, the idea of using a genetically similar but nonhuman donor might solve certain ethical problems. Nonetheless, other issues, such as the danger of transplant rejection, persist.

Mechanisms of Action

In the context of cellular transdifferentiation, stem cells act as a source of new neurons. Native stem cells in the subventricular zone contribute to brain remodeling, resulting in the familiar glial scar. Neurotrophins such as nerve growth factor are protective of cholinergic neurons and other neuronal populations. Phillips et al.[20] used immortalized embryonic rat hippocampus cells (HiB5) and transfected HiB5 cells that produced mouse nerve growth factor. The performance of both transplant groups on motor and cognitive tests improved significantly compared to control animals. However, the nerve growth factor-secreting transplants were associated with the least cell death in the hip-

pocampal CA3 region. Nerve growth factor may contribute to neuronal plasticity and regeneration and also may act as a suppressor of free radicals.

Conclusion

Although TBI remains a challenging clinical problem, therapeutic interventions based on the concepts of neural regeneration and repair hold promise. Further work is needed to translate such concepts from the bench to the bedside to ameliorate the effects of and to promote recovery from TBI.

References

1. Adams JH, Graham DI, Jennett B: The neuropathology of the vegetative state after an acute brain insult. **Brain 123 (Pt 7):**1327-1338, 2000
2. Alvarez-Dolado M, Pardal R, Garcia-Verdugo JM, et al: Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. **Nature 425:**968-973, 2003
3. Bambakidis NC, Theodore N, Nakaji P, Harvey A, Sonntag VK, Preul MC, Miller RH: Endogenous stem cell proliferation after central nervous system injury: Alternative therapeutic options. **Neurosurg Focus. 19(3):**E1, 2005.
4. Brock SC, Bonsall J, Luskin MB: The neuronal progenitor cells of the forebrain subventricular zone: Intrinsic properties in vitro and following transplantation. **Methods 16:**268-281, 1998
5. Cao Q, Benton RL, Whittemore SR: Stem cell repair of central nervous system injury. **J Neurosci Res 68:**501-510, 2002
6. CDC National Center for Injury Prevention and Control: Traumatic brain injury in the United States: A report to Congress (Abstract). **www.cdc.gov/ncipc/tbi/tbi_congress/tbi_congress.htm**, 1996
7. Chen Q, Long Y, Yuan X, et al: Protective effects of bone marrow stromal cell transplantation in injured rodent brain: synthesis of neurotrophic factors. **J Neurosci Res 80:**611-619, 2005
8. Clarke D, Frisen J: Differentiation potential of adult stem cells. **Curr Opin Genet Dev 11:**575-580, 2001
9. Gritti A, Bonfanti L, Doetsch F, et al: Multipotent neural stem cells reside into the rostral extension and olfactory bulb of adult rodents. **J Neurosci 22:**437-445, 2002
10. Horner PJ, Gage FH: Regenerating the damaged central nervous system. **Nature 407:**963-970, 2000
11. Ikeda R, Kurokawa MS, Chiba S, et al: Transplantation of neural cells derived from retinoic acid-treated cynomolgus monkey embryonic stem cells successfully improved motor function of hemiplegic mice with experimental brain injury. **Neurobiol Dis 20:**38-48, 2005
12. Jiang Y, Jahagirdar BN, Reinhardt RL, et al: Pluripotency of mesenchymal stem cells derived from adult marrow. **Nature 418:**41-49, 2002
13. Keyvani K, Sachser N, Witte OW, et al: Gene expression profiling in the intact and injured brain following environmental enrichment. **J Neuropathol Exp Neurol 63:**598-609, 2004
14. Kruger GM, Mosher JT, Bixby S, et al: Neural crest stem cells persist in the adult gut but undergo changes in self-renewal, neuronal subtype potential, and factor responsiveness. **Neuron 35:**657-669, 2002
15. Longhi L, Zanier ER, Royo N, et al: Stem cell transplantation as a therapeutic strategy for traumatic brain injury. **Transpl Immunol 15:**143-148, 2005
16. Maas AI: Neuroprotective agents in traumatic brain injury. **Expert Opin Investig Drugs 10:**753-767, 2001
17. Mahmood A, Lu D, Chopp M: Intravenous administration of marrow stromal cells (MSCs) increases the expression of growth factors in rat brain after traumatic brain injury. **J Neurotrauma 21:**33-39, 2004
18. Nan Z, Grande A, Sanberg CD, et al: Infusion of human umbilical cord blood ameliorates neurologic deficits in rats with hemorrhagic brain injury. **Ann N Y Acad Sci 1049:**84-96, 2005
19. Palmer TD, Takahashi J, Gage FH: The adult rat hippocampus contains primordial neural stem cells. **Mol Cell Neurosci 8:**389-404, 1997
20. 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
21. Rice AC, Khaldi A, Harvey HB, Salman NJ, White F, Fillmore H, Bullock MR: Proliferation and neuronal differentiation of mitotically active cells following traumatic brain injury. **Exp Neurol 183(2):**406-417, 2003.
22. 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:**1043-1052, 2002
23. Salman H, Ghosh P, Kernie SG: Subventricular zone neural stem cells remodel the brain following traumatic injury in adult mice. **J Neurotrauma 21:**283-292, 2004
24. Schouten JW, Fulp CT, Royo NC, et al: A review and rationale for the use of cellular transplantation as a therapeutic strategy for traumatic brain injury. **J Neurotrauma 21:**1501-1538, 2004
25. 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
26. Soares H, McIntosh TK: Fetal cortical transplants in adult rats subjected to experimental brain injury. **J Neural Transplant Plast 2:**207-220, 1991
27. 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
28. Teasdale GM, Graham DI: Craniocerebral trauma: protection and retrieval of the neuronal population after injury. **Neurosurgery 43:**723-737, 1998
29. Thomson JA, Itskovitz-Eldor J, Shapiro SS, et al: Embryonic stem cell lines derived from human blastocysts. **Science 282:**1145-1147, 1998

Multimodality Treatment of Spinal Cord Injury: Endogenous Stem Cells and Other Magic Bullets

Eric M. Horn, MD, PhD
Mark C. Preul, MD
Volker K. H. Sonntag, MD
Nicholas C. Bambakidis, MD

Acute traumatic SCI initiates a complex cascade of inflammation and ischemia that leads to scar formation. After injury this scar formation provides a strong inhibition to regeneration. Because the overall injury occurs on multiple levels, both spatially and temporally, a multimodality approach to treatment is needed. Only by combining neuroprotective and neuroregenerative treatments can significant advances be made to overcome SCI. Furthermore, new techniques of manipulating endogenous stem cells show great promise in promoting neuroregeneration.

Key Words: neuroprotection, neuroregeneration, spinal cord injury, trauma

Abbreviations Used: 4-AP, 4-aminopyridine; bFGF, fibroblast growth factor beta; EGF, epidermal growth factor; FGF2, fibroblast growth factor 2; NMDA, N-methyl-D-aspartate; SCI, spinal cord injury; Shh, sonic hedgehog

Division of Neurological Surgery, Barrow Neurological Institute, St. Joseph's Hospital and Medical Center, Phoenix, Arizona

Each year in the United States, SCI affects 10,000 to 14,000 persons. The mean age at injury is 30 years. Consequently, at any given time, 150,000 to 300,000 people are living with significant disabilities related to SCI. Estimates of the lifetime costs to care for an individual with a SCI range from $325,000 to $1.35 million, and the annual cost to society reaches $8 billion. As long-term care technologies improve, these costs are expected to continue to increase. There have been significant advances in accessibility for persons with disabilities. Nonetheless, the goal of medical science is to overcome the physiological barriers imposed by the injury itself to allow these individuals to regain their preinjury level of neurological function.

The severity of these injuries ranges from complete paralysis to mild myelopathy, depending on the mechanism. Injuries from acute trauma such as automobile accidents tend to garner the most attention, but insidious injuries from degenerative spinal disease are far more prevalent. When treatment of these various types of injuries is considered, it is important to consider the mechanism of injury.

In severe traumatic injuries associated with significant physical force at the time of injury, the initial trauma causes most of the destruction, which is related to shearing and to laceration and disruption of neurons, axons, and supporting tissue (e.g., vascular, connective). After the initial injury, significant scar tissue forms and acts as a barrier to the repair of injured tissue. For such injuries, the ideal treatment should include realignment of the spinal column to minimize further physical trauma to the spinal cord, prevention of sub-

sequent ischemia from the secondary injury cascade, and promotion of neural regeneration.

The same principles apply to lower-impact SCIs (e.g., from degeneration, spinal tumors), but there are significant and important differences in treatment. The first step in treating this type of SCI is to decompress the offending pathology. Because the long-standing compression has led to chronic ischemia, the next step is to prevent further ischemia by promoting adequate tissue perfusion of the spinal cord. Finally, promoting either regeneration or remyelination of the damaged neural elements is needed for further recovery of function.

Until 25 years ago, the prevailing wisdom was that SCIs were irreversible. Consequently, the focus was on helping patients with disabilities to become integrated into society. However, in 1980 one of the first demonstrations of the regenerative ability of injured spinal cord tissue was published.[36] Thereafter, such research expanded exponentially. Although various treatment schemes have been successful in rodent models of SCI, no treatment has yet been effective in humans.

A potential reason for this lack of success has been the focus on finding the 'magic bullet' treatment that will allow an injured spinal cord to recover. The mechanism of SCI is as complex as it is varied, especially the temporal sequence of events after injury. Most likely, a multimodality approach to SCI is needed to make meaningful gains in the clinical treatment of humans. Most research on treatment of SCI falls into two broad categories, which serve as natural starting points for attempting multimodality treatment regimens. The first treatment strategy is to attenuate the secondary injury cascade (neuroprotection); the second strategy is to promote remyelination and regeneration of axons (neuroregeneration).

The secondary injury cascade, which begins soon after the primary injury has occurred, can be influenced by many factors such as hypoxia, hypotension, and the extent of the primary injury (Fig. 1). The initial insult disrupts the microvasculature, which leads to tissue hypoperfusion.[11] The hypoperfusion can be severely accentuated by systemic variables such as pulmonary and cardiovascular dysfunction related to the inability of the spinal cord tissue to autoregulate perfusion after traumatic injury.[40] The resulting profound tissue ischemia persists hours to days after injury. In addition to the initial injury, the ischemia initiates a cascade of cellular destruction due to the breakdown of cellular membranes and to the release of multiple factors such as calcium and glutamate.[1] These factors further potentiate the breakdown of cellular membranes by activating proteases and phospholipases in a positive-feedback loop.

The role ischemia plays in the secondary injury cascade is well studied in animal models.[11] To date, the most effective way to limit the extent of spinal cord ischemia after injury is to limit systemic hypoxia and hypotension. In various experimental animal models of SCI, neuroprotective agents that limit excitotoxicity and membrane breakdown caused by ischemia have been studied extensively. Significant neurological improvement has followed treatment with sodium-channel modulators, glutamate-receptor blockers, glucocorticoids, and gangliosides.[5,13,20,27] Only a handful of treatments, however, has been tested in human trials of acute SCI. Moreover, the primary issue with spinal cord ischemia is disruption of the vasculature itself. This disruption creates a physical barrier to tissue perfusion. In turn, the barrier limits the ability to deliver pharmacological agents to the site of injury. This limitation is one possible reason why many of these agents are unsuccessful in treating SCI.

Neuroprotection

A controversial treatment for SCI is the use of high-dose methylprednisolone. Glucocorticoids such as methylprednisolone stabilize cellular membranes, reduce vasogenic edema, enhance spinal cord blood flow, alter the concentration of electrolytes at the site of injury, inhibit en-

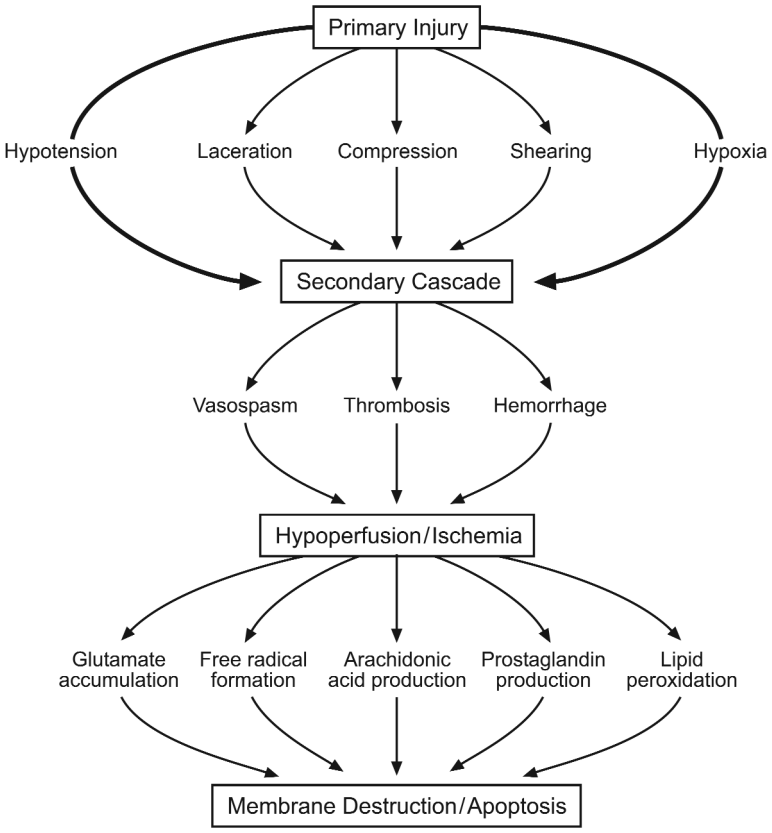


Figure 1. Outline of the primary and secondary injury cascades after acute traumatic SCI.

dorphin release, scavenge damaging free radicals, and limit the inflammatory response after injury.[21] Based on these basic properties of methylprednisolone and on the promising results from animal trials, the first randomized trial in humans was reported in 1984.[6] One year after injury, however, this study showed no differences in the neurologic outcomes of patients receiving low or high doses of methylprednisolone. Subsequent animal studies, however, indicated that the dose used in the trial was too low to produce significant differences in long-term functional outcomes.[9]

To address the issue of underdosing with methylprednisolone in the first trial, a second trial was undertaken with a high dose of methylprednisolone to assess neurological improvement after acute SCI.[7] This trial demonstrated a small but significant improvement in motor scores 1 year after injury compared to a placebo group. However, several aspects of the study have been criticized strongly. The primary complaints were the lack of a standardized assessment of functional outcome (as opposed to basic motor scores) and the use of post hoc analysis to determine statistical significance.[23] A third trial then found that methylprednisolone had a greater benefit if administered within 3 hours rather than within 8 hours of injury.[8] Because of the significant problems associated with these studies, methylprednisolone for the treatment of acute SCI is only considered an option.[18,19] Methylprednisolone has also been associated with medical complications, primarily an increased incidence of infections, gastrointestinal problems, and pulmonary issues.[28] Evidence concerning its long-term effects is mixed.[16]

Other agents tested in human clinical trials include tirilazad, naloxone, and GM-1 ganglioside. The opiate antagonist naloxone was included in the second methylprednisolone trial, but its use was associated with no significant clinical benefit.[7] In the third trial, the 21-aminosteroid tirilazad was compared to methylprednisolone. No benefit was found, but the trial lacked a true placebo group.[8] Two randomized clinical trials have analyzed the effectiveness of the ganglioside GM-1 on neurological improvement after SCI. The first, smaller study showed a marked improvement in functional neurological outcomes in the GM-1 group compared to the control patients.[15] The larger study failed to detect this improvement. Consequently, ganglioside GM-1 is only considered an option for acute SCI.[14]

Other promising neuroprotective agents are thyrotropin-releasing hormone, the NMDA-receptor antagonist gacyclidine, and the calcium-channel antagonist nimodipine.[26,33,34] These agents were tested in clinical trials to determine their effect on outcomes when used to treat SCI in humans. Unfortunately, none showed any benefit compared to the placebo and all have been abandoned. One agent that has shown promise in human trials is the potassium-channel antagonist, 4-AP. Although 4-AP failed to benefit patients with chronic SCI, this agent may have the potential to stabilize damaged axonal membranes during the acute period of injury.[17]

Several treatments are being developed to provide neuroprotection after SCI. Although these agents have only been tested in animal models of SCI, they represent the next wave for clinical trials in humans. The sodium-channel antagonist riluzole has significantly improved the outcome of SCI in rats. The Food and Drug Administration recently approved its use as a treatment for amyotrophic lateral sclerosis.[37] Attenuation of the inflammatory response after acute SCI has also shown great promise in animal models. When used to treat animals with SCIs, COX-2 inhibitors, ibuprofen, tetracycline, and erythropoietin have all improved functional recovery.[10,22,35,38]

Neuroregeneration

Once the secondary injury has evolved, the process of neuroregeneration begins. Unfortunately, the central nervous system is not a permissive site for neuroregeneration because inhibitors of axonal growth are derived from the formation of scar tissue. The goal for regeneration is to attenuate or overcome this inhibition to allow repair and regeneration at the site of injury. Several strategies have been used to help the spinal cord to allow regeneration.

One such strategy is to inject activated macrophages into the site of injury to reduce the concentration of inhibitory factors after injury. The macrophages, activated with autologous peripheral nerve tissue, help clean up the cellular debris and damaged myelin that contribute to the strong inhibition to regeneration after injury. Using this therapy in patients with a complete SCI in a Phase I safety trial, three of eight patients improved without significant side effects.[25] This treatment is now being evaluated in Phase II clinical trials at multiple sites worldwide.

Another regenerative treatment being used in clinical trials modifies the cellular cascade that leads to the inhibition of regenerating axons. The activity of the second-messenger pathway that uses the Rho protein in injured axons increases after injury and is partially responsible for the inability of these axons to grow through the glial scar. A Rho antagonist (C3 transferase) that has been developed has a robust ability to allow axonal regeneration and functional recovery in animal models of SCI.[39] This agent (Cethrin, BioAxone, Therapeutic, Inc.; Montreal, Quebec, Canada) is undergoing Phase I/IIa safety and efficacy trials in patients with complete SCIs. As long as 2 weeks after injury, the drug is applied at surgery. It then diffuses across the dura to deliver a high concentration locally at the site of injury.

An exciting treatment potential for promoting neuroregeneration after SCI is stem cell transplantation or stimulation. Although transplantation of stem cells into the injured spinal cord has shown great promise in animal models, work in humans has been limited.[12] One reason for this slow progress is the ethical dilemma inherent when working with embryonic stem cells. Consequently, other solutions, including stem cells derived from bone marrow and stimulation of endogenous stem cells, have been investigated.

Activation and promotion of endogenous stem cells are particularly attractive

because 500,000 and 2 million new cells are produced at the site of injury during the first month after injury.[31] After a contusion injury in animal models, endogenous neural progenitor cells are up-regulated.[24,29,30] Most of these cells originate near the ependyma of the central canal. The greatest level of induction occurs 3 to 7 days after injury. However, most of these cells develop into non-neuronal cells and actually contribute to the inhibition of neuroregeneration. Therefore, this line of research focuses on how to promote endogenous stem cells to develop into cell types that help injured axons to survive and regain function.

The process of differentiation of endogenous stem cells in the adult spinal cord after injury is yet to be determined. Several agents, however, have been used to control the differentiation of these stem cells. Based on existing knowledge, the goal of this treatment is to steer the endogenous stem cells away from the astrocytic pathway and toward a neuronal or oligodendrocytic pathway. By studying the genetic profile of early spinal cord development, proteins such as sonic hedgehog (Shh) and fibroblast growth factor beta (bFGF) have shown promise in controlling this differentiation. The Shh protein, which is involved in early neuronal differentiation, dramatically increases the number of neuronal progenitor cells in the spinal cord after a demyelination injury.[3,4] In rats with a contusion injury to the spinal cord, the neuronal progenitor cells increase after Shh is administered.[3] The combination of Shh with oligodendrocyte precursors also reduces the amount of cellular damage and improves functional recovery in rodents after SCI

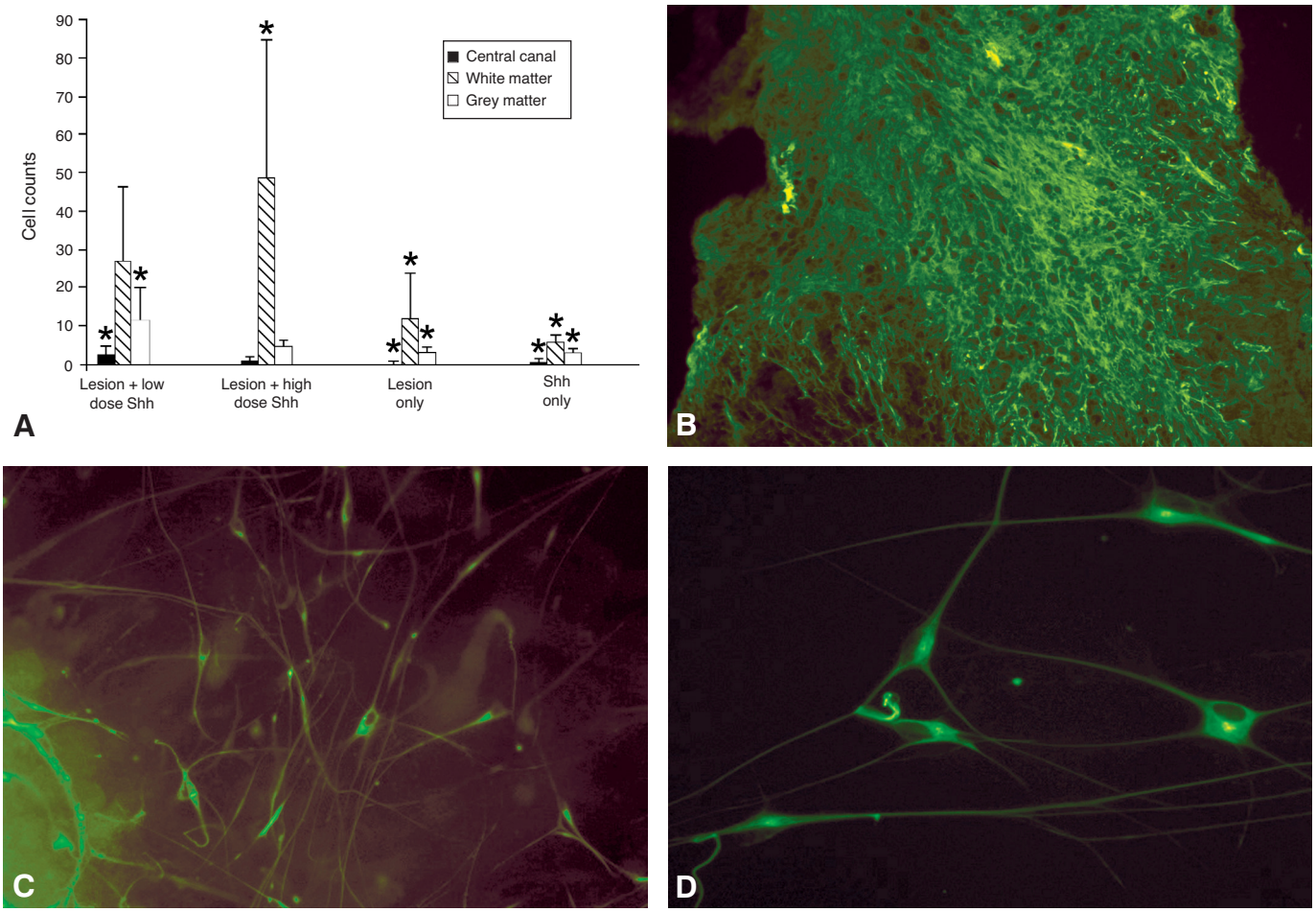


Figure 2. (*A*) Bar graph demonstrates elevation in the cell counts of actively dividing cells in the spinal cord sections of adult rats. Rats with a spinal cord lesion were treated with a low dose (3.0 μL) or high dose (6.0μL) of Shh. Rats without a lesion were given a high dose of Shh. The number of proliferating cells significantly increased after rats with a lesion were exposed to Shh. Data were analyzed with repeated-measures analysis of variance, followed by the Student-Newman-Keuls post hoc t-test (*$p < 0.01$). (*B*) Photomicrograph shows diffuse positivity for nestin in dorsal regions of hyperproliferation. Nestin is an intermediate filament protein found in central nervous system precursors (original magnification, 100x; nestin). (*C*) Low-power (original magnification, 50x, nestin) and (*D*) high-power (original magnification, 200x, nestin) photomicrographs of nestin-positive neural precursors from dorsal explant cultures from the spinal cords of rats that received a contusive spinal cord lesion and were treated with Shh. These primitive-appearing cells characteristically demonstrate bipolar morphologies and are highly motile and proliferative. [From Bambakidis NC et al: Endogenous stem cell proliferation after central nervous system injury: alternative therapeutic options. Neurosurg Focus 19 (3):E1, 2005]. *Used with permission from Neurosurgical Focus.*

(Fig. 2).[2] Likewise in rodents, the expression of bFGF in spinal cord cells increases after traumatic injury. In cell cultures, the bFGF derived from these cells caused them to differentiate into neuronal phenotypes.[41] After a contusion is induced in genetically engineered mice, other growth factors such as EGF, FGF2, neurogenin2 and Mash1 promote neuronal differentiation of endogenous stem cells at the site of injury.[32]

Conclusions

The complexity of the cellular destruction after SCI belies a multimodality approach to treatment. Temporally, the three hallmarks of this treatment are segregated into (1) the acute period during which the best clinical treatment is needed, (2) the subacute period during which neuroprotective treatment is needed, and the (3) delayed period during which neuroregenerative treatment is needed (Fig. 3). The burgeoning field of neuroregeneration, especially the manipulation of endogenous stem cells, may promote significant advances in the treatment of this devastating clinical condition.

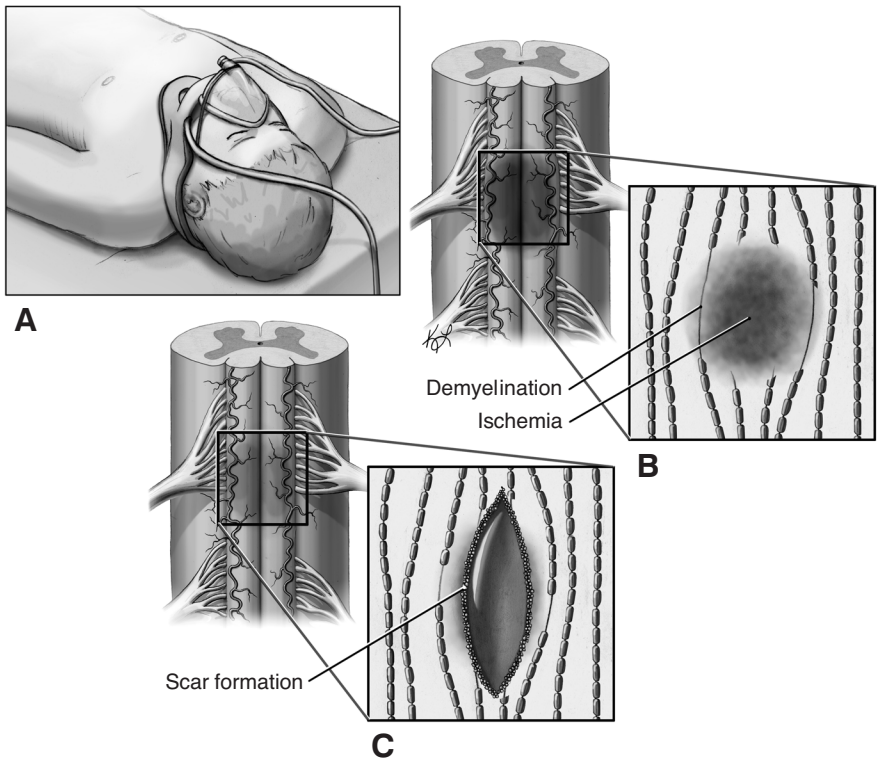


Figure 3. Triphasic treatment paradigm for SCI. (*A*) Illustration shows optimum clinical management during early minutes after spinal cord injury. Spinal protection during prehospital treatment, surgical stabilization, and spinal cord perfusion and oxygenation are key. (*B*) The second step is neuroprotection by the use of methylprednisolone, minocycline, erythropoeitin, riluzole, and 4-AP. (*C*) During the weeks after injury, the last phase includes neuroregeneration using endogenous stem cell promotion, Cethrin, and ProCord (Proneuron Biotechnologies, Inc.; Los Angeles, California) to prevent scar formation.

References

1. Amar AP, Levy ML: Pathogenesis and pharmacological strategies for mitigating secondary damage in acute spinal cord injury. **Neurosurgery 44:**1027-1039, 1999
2. 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 4:**16-26, 2004
3. Bambakidis NC, Theodore N, Nakaji P, et al: Endogenous stem cell proliferation after central nervous system injury: Alternative therapeutic options. **Neurosurg Focus 19:**E1, 2005
4. Bambakidis NC, Wang RZ, Franic L, et al: Sonic hedgehog-induced neural precursor proliferation after adult rodent spinal cord injury. **J Neurosurg 99:**70-75, 2003
5. Behrmann DL, Bresnahan JC, Beattie MS: Modeling of acute spinal cord injury in the rat: Neuroprotection and enhanced recovery with methylprednisolone, U-74006F and YM-14673. **Exp Neurol 126:**61-75, 1994
6. Bracken MB, Collins WF, Freeman DF, et al: Efficacy of methylprednisolone in acute spinal cord injury. **JAMA 251:**45-52, 1984
7. Bracken MB, Shepard MJ, Collins WF, et al: A randomized, controlled trial of methylprednisolone or naloxone in the treatment of acute spinal-cord injury. Results of the Second National Acute Spinal Cord Injury Study. **N Engl J Med 322:**1405-1411, 1990
8. Bracken MB, Shepard MJ, Holford TR, et al: Administration of methylprednisolone for 24 or 48 hours or tirilazad mesylate for 48 hours in the treatment of acute spinal cord injury. Results of the Third National Acute Spinal Cord Injury Randomized Controlled Trial. National Acute Spinal Cord Injury Study. **JAMA 277:**1597-1604, 1997
9. Braughler JM, Hall ED, Means ED, et al: Evaluation of an intensive methylprednisolone sodium succinate dosing regimen in experimental spinal cord injury. **J Neurosurg 67:**102-105, 1987
10. Celik M, Gokmen N, Erbayraktar S, et al: Erythropoietin prevents motor neuron apoptosis and neurologic disability in experimental spinal cord ischemic injury. **Proc Natl Acad Sci U S A 99:**2258-2263, 2002
11. Fehlings MG, Tator CH, Linden RD: The relationships among the severity of spinal cord injury, motor and somatosensory evoked potentials and spinal cord blood flow. **Electroencephalogr Clin Neurophysiol 74:**241-259, 1989
12. Garbossa D, Fontanella M, Fronda C, et al: New strategies for repairing the injured spinal cord: The role of stem cells. **Neurol Res 28:**500-504, 2006
13. Gaviria M, Privat A, D'Arbigny P, et al: Neuroprotective effects of a novel NMDA antagonist, Gacyclidine, after experimental contusive spinal cord injury in adult rats. **Brain Res 874:**200-209, 2000
14. Geisler FH, Coleman WP, Grieco G, et al: The Sygen multicenter acute spinal cord injury study. **Spine 26:**S87-S98, 2001
15. Geisler FH, Dorsey FC, Coleman WP: Recovery of motor function after spinal-cord injury—a randomized, placebo-controlled trial with GM-1 ganglioside. **N Engl J Med 324:**1829-1838, 1991
16. Gerndt SJ, Rodriguez JL, Pawlik JW, et al: Consequences of high-dose steroid therapy for acute spinal cord injury. **J Trauma 42:**279-284, 1997

17. Grijalva I, Guizar-Sahagun G, Castaneda-Hernandez G, et al: Efficacy and safety of 4-aminopyridine in patients with long-term spinal cord injury: A randomized, double-blind, placebo-controlled trial. **Pharmacotherapy 23:**823-834, 2003
18. Hadley MN: Initial closed reduction of cervical spine fracture-dislocation injuries. **Neurosurgery 50:**S44-S50, 2002
19. Hadley MN: Pharmacological therapy after acute cervical spinal cord injury. **Neurosurgery 50:**S63-S72, 2002
20. Hains BC, Saab CY, Lo AC, et al: Sodium channel blockade with phenytoin protects spinal cord axons, enhances axonal conduction, and improves functional motor recovery after contusion SCI. **Exp Neurol 188:**365-377, 2004
21. Hall ED, Braughler JM: Glucocorticoid mechanisms in acute spinal cord injury: A review and therapeutic rationale. **Surg Neurol 18:**320-327, 1982
22. Hall ED, Wolf DL: A pharmacological analysis of the pathophysiological mechanisms of posttraumatic spinal cord ischemia. **J Neurosurg 64:**951-961, 1986
23. Hurlbert RJ: Methylprednisolone for acute spinal cord injury: An inappropriate standard of care. **J Neurosurg 93:**1-7, 2000
24. Ke Y, Chi L, Xu R, et al: Early response of endogenous adult neural progenitor cells to acute spinal cord injury in mice. **Stem Cells 24:**1011-1019, 2006
25. Knoller N, Auerbach G, Fulga V, et al: Clinical experience using incubated autologous macrophages as a treatment for complete spinal cord injury: Phase I study results. **J Neurosurg Spine 3:**173-181, 2005
26. Lepeintre JF, D'Arbigny P, Mathe JF, et al: Neuroprotective effect of gacyclidine. A multicenter double-blind pilot trial in patients with acute traumatic brain injury. **Neurochirurgie 50:**83-95, 2004
27. Mao J, Hayes RL, Price DD, et al: Post-injury treatment with GM1 ganglioside reduces nociceptive behaviors and spinal cord metabolic activity in rats with experimental peripheral mononeuropathy. **Brain Res 584:**18-27, 1992
28. Matsumoto T, Tamaki T, Kawakami M, et al: Early complications of high-dose methylprednisolone sodium succinate treatment in the follow-up of acute cervical spinal cord injury. **Spine 26:**426-430, 2001
29. McTigue DM, Wei P, Stokes BT: Proliferation of NG2-positive cells and altered oligodendrocyte numbers in the contused rat spinal cord. **J Neurosci 21:**3392-3400, 2001
30. Mothe AJ, Tator CH: Proliferation, migration, and differentiation of endogenous ependymal region stem/progenitor cells following minimal spinal cord injury in the adult rat. **Neuroscience 131:**177-187, 2005
31. Myckatyn TM, Mackinnon SE, McDonald JW: Stem cell transplantation and other novel techniques for promoting recovery from spinal cord injury. **Transpl Immunol 12:**343-358, 2004
32. Ohori Y, Yamamoto S, Nagao M, et al: Growth factor treatment and genetic manipulation stimulate neurogenesis and oligodendrogenesis by endogenous neural progenitors in the injured adult spinal cord. **J Neurosci 26:**11948-11960, 2006
33. Petitjean ME, Pointillart V, Dixmerias F, et al: [Medical treatment of spinal cord injury in the acute stage]. **Ann Fr Anesth Reanim 17:**114-122, 1998
34. Pitts LH, Ross A, Chase GA, et al: Treatment with thyrotropin-releasing hormone (TRH) in patients with traumatic spinal cord injuries. **J Neurotrauma 12:**235-243, 1995
35. Resnick DK, Graham SH, Dixon CE, et al: Role of cyclooxygenase 2 in acute spinal cord injury. **J Neurotrauma 15:**1005-1013, 1998
36. Richardson PM, McGuinness UM, Aguayo AJ: Axons from CNS neurons regenerate into PNS grafts. **Nature 284:**264-265, 1980
37. Schwartz G, Fehlings MG: Evaluation of the neuroprotective effects of sodium channel blockers after spinal cord injury: Improved behavioral and neuroanatomical recovery with riluzole. **J Neurosurg 94:**245-256, 2001
38. Stirling DP, Khodarahmi K, Liu J, et al: Minocycline treatment reduces delayed oligodendrocyte death, attenuates axonal dieback, and improves functional outcome after spinal cord injury. **J Neurosci 24:**2182-2190, 2004
39. Sung JK, Miao L, Calvert JW, et al: A possible role of RhoA/Rho-kinase in experimental spinal cord injury in rat. **Brain Res 959:**29-38, 2003
40. Tator CH, Fehlings MG: Review of the secondary injury theory of acute spinal cord trauma with emphasis on vascular mechanisms. **J Neurosurg 75:**15-26, 1991
41. Xu Y, Kitada M, Yamaguchi M, et al: Increase in bFGF-responsive neural progenitor population following contusion injury of the adult rodent spinal cord. **Neurosci Lett 397:**174-179, 2006

Management of a Cervical Spine Infection: Case Report

Peter H. Maughan, MD
Steven W. Chang, MD
Nicholas C. Bambakidis, MD

Increasingly, neurosurgeons are treating patients with spinal infections. Management of these patients requires antibiotic therapy and often surgical intervention. We report a patient with a severe infection who was initially treated with an external orthosis and antibiotics due to his multiple medical comorbidities. After a surprising neurologic improvement, he underwent surgical fixation and made an excellent recovery.

Key Words: infection, instrumentation, osteomyelitis, spinal fusion, spine

Abbreviations Used: AIDS, autoimmune deficiency syndrome; CT, computed tomography; MR, magnetic resonance

Division of Neurological Surgery, Barrow Neurological Institute, St. Joseph's Hospital and Medical Center, Phoenix, Arizona

Spinal infections can have devastating consequences, including paralysis and death. Administration of antibiotics is a mainstay of treatment. When neural elements are compressed or deformed, surgery may be required. We report a patient who developed a neurologic deficit related to a cervical spine infection and his subsequent treatment.

Illustrative Case

A 47-year-old man with a complicated medical history sought treatment at an outside hospital complaining of progressive paraparesis. Three months earlier his right arm had been amputated to treat necrotizing fasciitis, and he had been discharged to a skilled nursing facility on oral antibiotics. His medical history was significant for hepatitis C, liver cirrhosis, hypertension, bipolar disorder, and chronic pain syndrome. His paraparesis rapidly progressed to complete paralysis of the lower extremities with involvement of his left, and only remaining, upper extremity. The patient was placed in a halo brace at the outside hospital, and he was transferred to our institution for further treatment.

On arrival, the patient was awake and alert. Bilaterally, his lower extremity motor function was graded 0/5. His left upper extremity function was graded as follows: deltoid 2/5, biceps 4/5, triceps 2/5, and grip 1/5. He had no sensation in his lower extremities. CT of the cervical spine showed cervical kyphosis and osteolytic changes in the vertebral bodies of C3 to C6 (Fig. 1). MR imaging showed osteomyelitis, diskitis, and an epidural abscess posterior to the C5 and C6 vertebral bodies (Fig. 2). Because the pa-

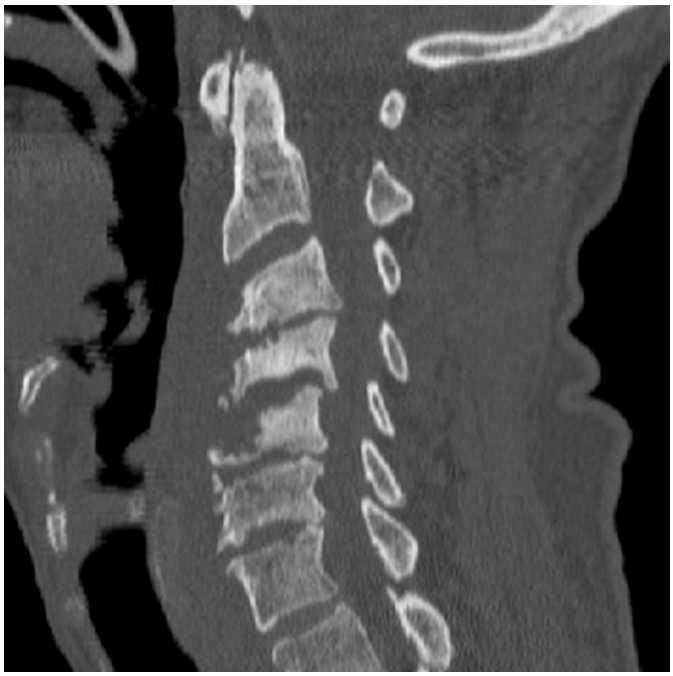


Figure 1. Sagittal CT reconstruction of the cervical spine shows extensive infection involving the anterior vertebral bodies and the resulting kyphotic deformity.

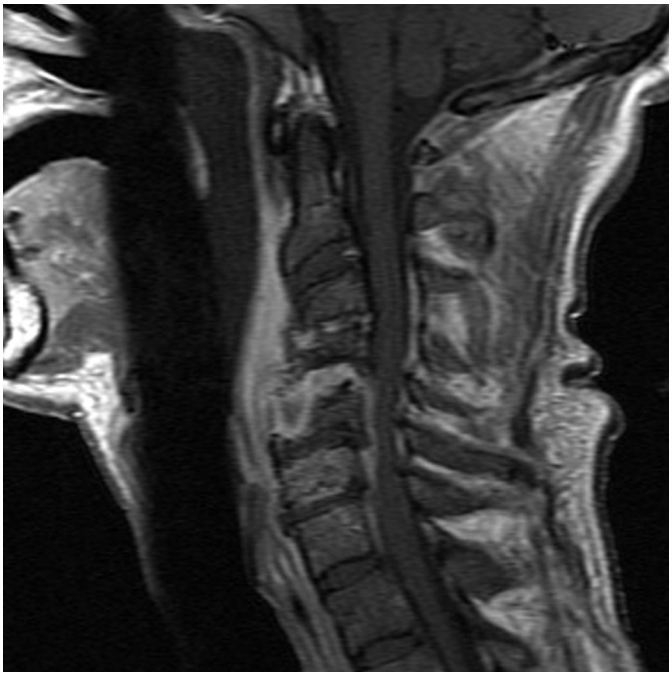


Figure 2. Sagittal T1-weighted MR image with gadolinium shows infection involving the vertebral bodies, disc spaces, prevertebral tissues, and epidural space.

tient had no motor function for 3 days before his arrival and because he had multiple medical comorbidities, we elected to treat him conservatively. The patient's blood cultures grew oxacillin-resistant *Staphylococcus aureus*. He was treated with linezolid and rifampin, as recommended by our infectious disease service. He was maintained in halo fixation. He experienced respiratory failure due to pneumonia and required a tracheostomy for long-term ventilation. He was discharged to a skilled nursing facility.

Over the next 3 months, the patient's physical examination improved. Sensation returned to his lower extremities. His motor function also improved. With the exception of the deltoid muscle, which was graded 4/5, strength in his upper left extremity returned to normal. His lower extremity function improved to 3/5 on the right and to 1 to 2/5 on the left. By this time the patient had stabilized medically, his infection was thought to be treated. Reimaging of the patient's cervical spine showed no changes, and he continued to wear the halo brace.

Because the bony destruction caused by the infection was extensive, we recommended cervical fusion. The patient underwent a C2 to T1 posterior fusion. We performed a C2 to C7 laminectomy and placed C2 pars interarticularis, C3 to C6 lateral mass, and T1 pedicle screws. Bone morphogenic protein and locally harvested autograft were used for a lateral fusion (Fig. 3A and B). After surgery the halo brace was removed, and the patient was placed in a Miami-J collar (Jerome Medical, Moorestown, NJ). He was discharged to a rehabilitation facility 1 week later.

At his 3-month follow-up examination, the patient's motor function had improved and was graded 5/5 in his left upper extremity and 4/5 in both lower extremities. He had a spastic gait and hypertonia but was ambulatory with a walker. A follow-up CT scan showed bony fusion, and the collar was removed.

Discussion

Spinal infections are often classified by anatomic location as diskitis, osteomyelitis, or epidural abscess. As in our patient, an infection commonly involves more than one location. Although spinal infections are relatively rare, their incidence, which is about 1/100,000, is increasing.[5] This increase reflects the growing number of patients affected by AIDS, those using intravenous drugs, and those with medical conditions like diabetes and renal disease. Mortality rates as high as 20% have been reported.[4]

Diagnosis

Prompt diagnosis of a spinal infection is important to minimize neurologic complications. Patients typically become symptomatic with back pain and are often febrile. An elevated erythrocyte sedimentation rate and C-reactive protein level can also help make the diagnosis. Diagnosis may be easier in patients with a predisposing condition like AIDS or other immunosuppressive states.

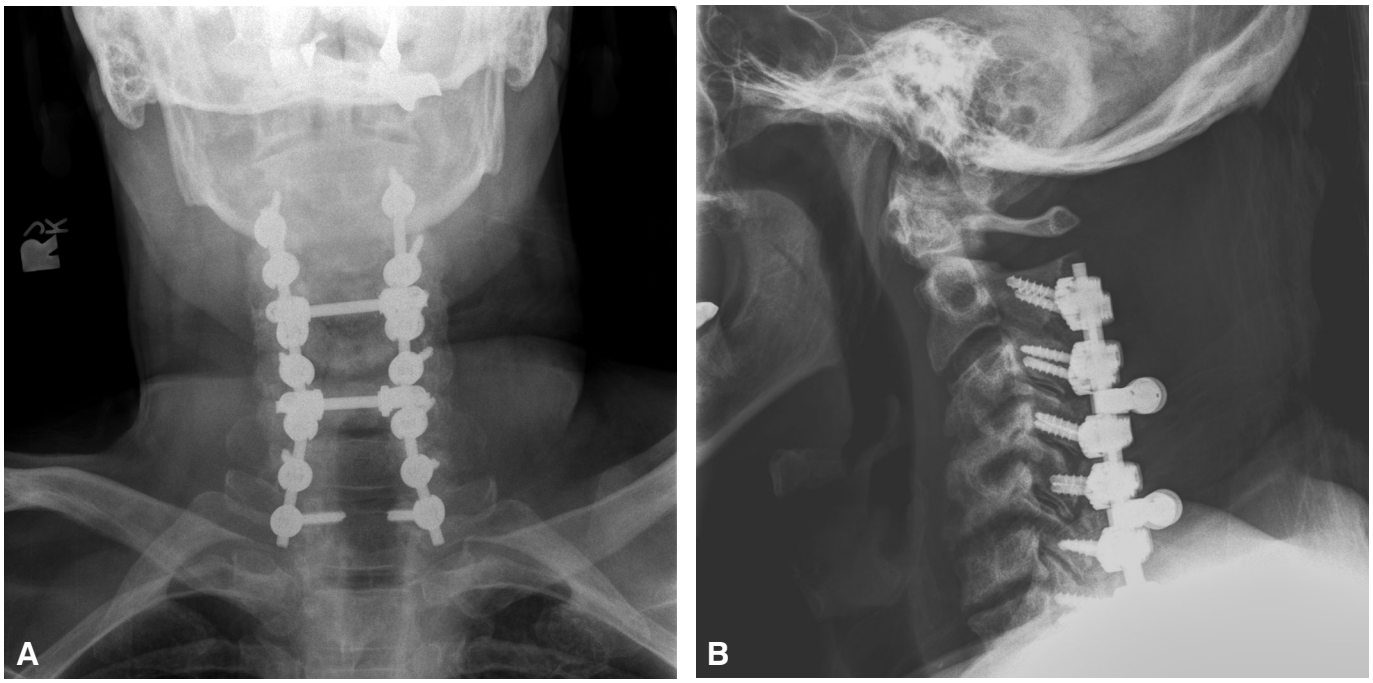


Figure 3. Postoperative (*A*) anteroposterior and (*B*) lateral radiographs show posterior fixation from C2 to T1.

However, the index of suspicion must be high when patients present with this constellation of signs and symptoms.

MR imaging has proven to be the most sensitive modality for the diagnosis of spinal infections.[3] On T1-weighted MR images, infection tends to appear as low to intermediate signal intensity while on T2-weighted MR images it appears as high to intermediate signal intensity. Gadolinium enhancement of infection is usually diffuse. CT can help determine bone involvement and deformity. If MR imaging is unavailable, the extent of soft tissue involvement may be visualized with contrast CT.

Microbiology

Gram-positive organisms are the most common cause of spinal infections, and *Staphylococcus aureus* is the most common organism involved.[1] Gram-negative organisms are less common but are likely to be associated with genitourinary or gastrointestinal sources of infection. Every attempt should be made to isolate a causative organism before antibiotic treatment is initiated. Direct culture, CT-guided biopsy, or blood cultures can be used to obtain the diagnosis. In septic or deteriorating patients, awaiting the results of cultures should not delay treatment. In such cases, broad-spectrum antibiotics should be started immediately.

Treatment

Once a spinal infection has been diagnosed, appropriate antibiotic therapy must be instituted. Some patients require surgical intervention for decompression, debridement, or fixation. Most patients with acute or progressive neurologic deficits should undergo emergent decompression and debridement of their infection. When spinal instability is obvious, internal fixation may be performed during the initial surgery.[2] Some patients require delayed fixation, especially those who develop progressive deformity despite appropriate medical treatment. Because our patient had suffered a fixed neurological deficit for 3 days and because he had multiple medical comorbidities, we elected to treat him with an external orthosis and antibiotics. Despite his poor medical and neurologic condition at presentation, he made an excellent recovery.

Conclusions

The severity and presentation of spinal infections vary. Appropriate treatment requires an individualized approach that includes antibiotic therapy. Surgical intervention may be required for immediate decompression or may be delayed for spinal stabilization.

References

1. Acosta FL, Jr., Chin CT, Quinones-Hinojosa A, et al: Diagnosis and management of adult pyogenic osteomyelitis of the cervical spine. **Neurosurg Focus 17:**E2, 2004
2. Dietze DD, Jr., Fessler RG, Jacob RP: Primary reconstruction for spinal infections. **J Neurosurg 86:**981-989, 1997
3. Parkinson JF, Sekhon LH: Spinal epidural abscess: Appearance on magnetic resonance imaging as a guide to surgical management. Report of five cases. **Neurosurg Focus 17:**E12, 2004
4. Quinones-Hinojosa A, Jun P, Jacobs R, et al: General principles in the medical and surgical management of spinal infections: A multidisciplinary approach. **Neurosurg Focus 17:**E1, 2004
5. Tandon N, Vollmer DG: Infections of the spine and spinal cord, in Winn HR (ed): *Youmans Neurological Surgery*. Philadelphia: WB Saunders, 2004, pp 4363-4394