Progenitor Cell Biology

Implications for Neural Regeneration

Mark F. Mehler, MD; John A. Kessler, MD

A few brief years ago, damage to the central nervous system was generally perceived to be irreparable, and loss of neurons was largely viewed as an irreversible process. However, major advances in the study of neural progenitor cells have altered these perceptions, and rational approaches to the repair of the damaged nervous system using transplanted progenitor cells now seem feasible. This review will discuss the basic biology of neural progenitor cells, the mechanisms regulating the generation of neurons and glia from these cells, and the techniques that are available for preparing such cells for transplantation into the nervous system. The potential uses for these cells in treating neurologic disease will then be reviewed, and the theoretical and technical problems that may be encountered will be discussed.

NEURAL PROGENITOR CELLS

The major cellular elements of the central nervous system (CNS) (neurons, astrocytes, and oligodendrocytes) arise from specialized germinative regions derived from the inner lining of the neural tube.1-3 These zones extend from periventricular regions of the telencephalon to the central canal of the spinal cord. They initially consist of a pseudostratified epithelium (ventricular zone [VZ]) during early embryonic life that contains progenitor cells with a range of neural lineage potentials.1,4 During later embryogenesis, the VZ gives rise to secondary paramedian subventricular zones that persist as restricted zones into the adult state (eg, subependymal region of the lateral ventricle).1,5 Neurons and radial glia are the initial cell types generated within the early embryonic VZ.6,7 By contrast, astrocytes and oligodendrocytes are generated during the late embryonic and the early postnatal periods within regional cortical subventricular zones.8 In addition, ongoing areas of neurogenesis during late postnatal and adult life occur in the olfactory bulb, in the dentate gyrus of the hippocampus, and in the Purkinje cell layer of the cerebellum.1,9

Early primordial multipotent progenitor cells (neural stem cells) can be identified by their ability to undergo continuous cellular proliferation, to regenerate exact copies of themselves (self-renew), to generate a large number of regional cellular progeny, and to elabrate new cells in response to injury or disease.1 Neural progenitor cells may generate different cellular species such as neurons, astrocytes, and oligodendroglia by several possible developmental mechanisms10 (Figure). First, cytokines and other environmental cues may instruct distinct early progenitor species to choose one specific neural lineage at the expense of other lineages (instructive process). Alternatively, additional classes of neural progenitors may generate different lineages by probabilistic mechanisms, and only selected cells may survive and proliferate depending on the types of environmental signals they encounter (selective process). Both of these mechanisms seem to be involved in progenitor cell development in the nervous system.1,2,10

NEUROGENESIS AND GlioGENESIS

Within the early embryonic cerebral cortical VZ, there is initially exponential expansion of neural progenitor populations, with the later elaboration of apical
and basal daughter cells that initiate the processes of cellular migration and neurogenesis.\textsuperscript{11-13} Proliferative neuroblasts are generated within the murine VZ by E13 (equivalent to about human embryonic stage 20 [51 days]), with subsequent migration to regions of the developing cortical plate and the evolution of columnar organization.\textsuperscript{14,15} During sequential phases of neurogenesis, there is progressive lengthening of the initial (G\textsubscript{1}) phase of the cell cycle, in part modulated by cytokines; these regulatory events are essential for neural fate determinations.\textsuperscript{16-19} The 6 laminae of the cerebral cortex are generated by successive waves of migrating neuroblasts in an “inside-out” manner.\textsuperscript{20,21} The laminar fate of neuroblasts is regulated by the final round of cell divisions within the VZ, and the commitment of neuroblasts to specific differentiated cell fates occurs at the time of delamination from the VZ.\textsuperscript{20,21}

The earliest wave of gliogenesis occurs at the time of embryonic neurogenesis with the generation of radial glia, specialized cells that act as a scaffold for neuroblast migration.\textsuperscript{7,22} By contrast, the peak periods of gliogenesis occur during late embryonic and early perinatal ages.\textsuperscript{8} Radial glia are initially present within the early embryonic (E10—equivalent to about human embryonic stage 15 [33 days]) cerebral cortical VZ.\textsuperscript{7} These specialized glial cells attain maximal representation within the forebrain during midembryonic life, and later become restricted as specialized regional glial populations during the first postnatal week.\textsuperscript{7} Within this developmental interval, there is a gradual transition of bipolar radial glia to unipolar and then multipolar species, with progressive expression of mature astroglial intermediate filament proteins.\textsuperscript{7} Although radial glia seem to be precursors of the astroglial lineage, most astrocytes are thought to be generated directly from multipotent cells.\textsuperscript{7,22}

Perinatal gliogenesis is a complex developmental process involving cells with a spectrum of increasingly restricted lineage fates (multipotent, bipotent, and unipotent) that differentiate into oligodendrocytes or astrocytes under the influence of microenvironmental signals present within white and gray matter tracts.\textsuperscript{8} Specific subsets of cytokines promote the generation of astrocytes or oligodendroglia from late embryonic subventricular zone–derived multipotent progenitors, and from perinatal cerebral bipotent oligodendroglial-type 2 astroglial (O-2A) progenitor cells during normal development and in specific pathological states.\textsuperscript{23,24}

### REGENERATIVE STRATEGIES

Over the past several years, there have been remarkable advances in neural regenerative strategies that have paralleled our increasing knowledge of the cellular and molecular mechanisms regulating neural development. Until recently, the experimental emphasis has been on the replacement of local neuromodulatory proteins (dopamine, neurotrophins) rendered deficient as a result of specific neurodegenerative disorders or accelerated aging. However, the reconstitution of more complex and widespread neural populations damaged by a variety of genetic or acquired neurologic disorders such as stroke or traumatic injury will require access to a broader array of neural lineage species and a greater understanding of the developmental signals that sanction integration into the host environments. Many studies have suggested that the normal adult brain may lack the appropriate environmental signals to allow neural progenitor species present in multiple mature CNS regions to realize their broad lineage potential.\textsuperscript{11-13} In addition, specific neuropathologic conditions may alter the normal balance of regional environmental signals, for example by releasing proinflammatory and other modulatory cytokines; the presence of these inappropriate cellular cues may predispose residual neural populations to undergo apopto-
sis. This suggests that it may be necessary to promote lineage commitment of progenitor cells in vitro prior to transplantation into a damaged brain.

Transplantation strategies have used regional embryonic tissue grafts or dissociated cellular preparations to promote the repair of lesional areas of the brain and spinal cord. For Parkinson disease, previous non-medical therapies had relied on stimulation of the appropriate CNS pathways to circumvent the cellular damage and, conversely, additive damage to the remaining disinhibitory circuits. More recently, the aim of different types of cell therapies has been the local replacement of dopamine or precursor molecules in target areas of dopaminergic terminals, the corpus striatum. The use of autologous renal chromaffin cell implantation has been associated with poor graft survival, and this treatment has shown a negligible effect when assessed during variable time periods. By contrast, allogenic fetal dopaminergic cell transplantation has shown more promising results, but there are a number of serious concerns and problems associated with this technique.

There are a limited number of cells that can be collected from fetal dissections, with variable postimplantation survival rates. In addition, the number of surviving dopamine cells measurably affects functional outcomes. Recent studies have suggested that the use of multiple fetal brains for each transplantation and the application of neurotrophic factors to support graft cell survival significantly increase the efficacy of the transplantation paradigms. Current experimental protocols have begun to focus on using tissue culture techniques to expand human fetal progenitor cells in vitro prior to transplantation. The aim of these investigations is to identify progenitor cells with broad lineage potential that can be directed toward the neuronal lineage and ultimately differentiate into dopaminergic precursor cells. Additional efforts are under way to genetically engineer target cells to express the synthetic machinery necessary to orchestrate the production and secretion of dopamine metabolites.

There is already substantial experience in using transplanted tissues to repair the diseased nervous system. In 1991, the Network of European CNS Transplantation and Restoration (NECTAR) was formed to focus on regenerative strategies for Parkinson disease. By 1994, the focus had broadened to include Huntington disease with the establishment of the European Network for Striatal Transplantation in Huntington's Disease (NEST-HD) in collaboration with the Huntington's Study Group. From these joint efforts came the Unified Huntington's Disease Rating Scale (UHDRS) and the Core Assessment Program for Intracerebral Transplantation in Huntington's Disease (CAPIT-HD). These initiatives established detailed cognitive, imaging, and neurophysiological criteria for evaluating the efficacy of regenerative strategies for specific neurodegenerative conditions.

The first study of human fetus-to-adult striatal transplantations has recently been performed in 3 nondemented patients with moderately advanced Huntington disease. These individuals each received bilateral implantations of donor tissue from multiple fetuses into the caudate and putamen. The patients experienced no adverse effects of the surgical procedure or the cyclosporin immunosuppression. Magnetic resonance imaging evaluation at 1 year documented graft survival and growth without displacement of surrounding tissues. Neuropsychological evaluations performed 4 to 6 months after surgery documented that all patients improved on some measures of cognitive function, although no uniform pattern was evident. Experimental studies are currently under way to genetically modify donor cells to secrete trophic factors to protect striatal neurons from neurodegeneration.

Another area in which human neural cell transplantation has already been used is in spinal cord injury. In posttraumatic syringomyelia, solid human embryonic spinal cord grafts have been used successfully to obliterate a large cystic cavity in a patient who was developing a notable myelopathy. Magnetic resonance imaging performed 7 months after surgery showed excellent graft visualization and localized obliteration of the cystic space. Prior to the institution of these human transplantation protocols, human fetal donor-to-adult animal recipient experimental studies had established that the lesion environment and the nature of the donor preparation significantly influence the pattern of neural differentiation and the degree of graft-host integration. For example, the elaboration of neurons over glia and significant neuronal differentiation is selectively promoted by the use of suspension as opposed to solid grafts, and by delayed graft implantation after injury. In addition, it has also been demonstrated that endogenous reparative responses to spinal cord contusion are also substantial. These inductive responses are associated with proliferation of progenitor cells from the ependymal zone surrounding the central canal of the spinal cord to form a scaffold within the lesioned area to promote the regeneration of axons. Further investigations have shown that homotypic fetal grafts are superior to heterotypic grafts, and that progressive cellular maturation, neuritic outgrowth, and graft-host integration occur in an orderly and progressive pattern over several months. Functional recovery seems to be directly related to graft survival, the temporal window between injury and transplantation, and the quality of debridement prior to transplantation.

**USE OF GENETICALLY MODIFIED CELLS**

The use of genetically modified cells has represented an early and ongoing strategy for neural regeneration. Initially, fibroblasts served as a relatively inert vehicle for retroviral transduction to allow the local supply of trophic factors. Fibroblasts have the advantage of being easily transfected, selected, and manipulated in vitro prior to transplantation. Retroviral transfection of neurotrophin genes has resulted in the correction of age-associated memory loss by enhanced nerve growth factor expression in the nucleus basalis magnocellularis in rodents, and in reductions in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced dopaminergic neuronal degeneration by augmentation of brain-derived neurotrophic factor expression. Further, cointransduction of basic fibroblast growth factor-expressing fibroblasts with fetal dopaminergic neuronal cells can potentiate cellular survival and has resulted in enhanced behavioral functions in a rodent model of Parkinson disease.
ever, new generations of retroviral vectors hold promise for allowing stable integration of foreign genes into the genome of postmitotic neurons, and the use of adenovirus and lentivirus vectors has overcome earlier problems associated with the efficient transfection of neural cells. These techniques now permit both direct and cell-mediated gene transfer into the mammalian CNS.

An attractive alternative to the use of genetically engineered fibroblasts has been the advent of cell-mediated transplantation paradigms using immortalized neural progenitor cells. Experimental protocols for immortalization initially used different isoforms of oncogenes, and more recently “conditional” immortalization has been employed using temperature-sensitive alleles of the SV40 large tumor antigen. Immortalized cells have the advantage of representing a genetically homogeneous neural progenitor population that can be expanded to yield a virtually limitless supply of donor cells. After transplantation, immortalized progenitor cells can migrate over extended distances, differentiate in a site-specific manner into neuronal and glial species, fully integrate into the regional microenvironment, and establish neural connections and exhibit stable phenotypic characteristics for prolonged periods in vivo. Immortalized cells are not transformed and cease proliferation on transplantation. Conditionally immortalized cells have the added advantage that they can proliferate in vitro when cultured at a temperature permissive for SV40 tumor antigen expression (33°C), but exit the cell cycle and differentiate when subsequently switched to a temperature not permissive for SV40 tumor antigen expression (39°C), which corresponds to mammalian core body temperature. This added cellular feature allows manipulation of the developmental state of the donor cells in vitro prior to transplantation. The recent introduction of the CRE-lox-P system now even permits removal of the immortalizing agent prior to transplantation. Using cell-mediated gene transfer strategies, neurotrophin-expressing immortalized cells have been used to rescue axotomized cholinergic neurons after fimbria-fornix lesions, to reverse age-associated behavioral impairments after transplantation into the nucleus basalis magnocellularis of unlesioned rats, and to improve neuronal survival and cellular maturation by activation of an autocrine loop. Transplantation of wild-type or genetically engineered immortalized progenitor cells has also been used experimentally to improve biochemical and morphologic parameters in mice with specific lysosomal storage diseases (Tay-Sachs disease and mucopolysaccharidosis type VII). Immortalized cells represent a source of glia as well as of neurons. Oligodendrocyte progenitor species can be immortalized at multiple stages of the oligodendrocyte developmental cycle, and such progenitor cells have been used to reverse the pathologic sequelae of experimental demyelinating and hypomyelinating diseases. Immortalized oligodendroglial–type 2 astroglial progenitor cells can undergo cellular maturation and myelinate host axons in neonatal shiverer mice that do not produce myelin. In addition, within the spinal cords of myelin-deficient rats, these immortalized progenitor cells can proliferate, migrate, and myelinate axons for several weeks following transplantation.

More recent regenerative strategies have focused on the use of primary, unmodified neural progenitor cells. Many of these paradigms were developed because of the myriad problems associated with human fetal transplantation. As discussed above, embryonic stem cells are pluripotent progenitors that can generate mature neuronal and glial phenotypes after manipulation in cell culture. In addition, multipotent and bipotent progenitor cell species can be propagated for prolonged periods in tissue culture without losing their potential for undergoing neural maturation. These primary neural progenitor cell species can also be genetically modified prior to transplantation to facilitate regional targeting or to enhance expression of a specific mature neural phenotype. Increasing experimental evidence also suggests that the adult CNS retains a complement of multipotent progenitor cells capable of exponential growth, cellular migration to specific target regions, and the generation of mature neuronal and glial progenies, neurotrophin-expressing immortalized cells have been used to rescue axotomized cholinergic neurons after fimbria-fornix lesions, to reverse age-associated behavioral impairments after transplantation into the nucleus basalis magnocellularis of unlesioned rats, and to improve neuronal survival and cellular maturation by activation of an autocrine loop. Transplantation of wild-type or genetically engineered immortalized progenitor cells has also been used experimentally to improve biochemical and morphologic parameters in mice with specific lysosomal storage diseases (Tay-Sachs disease and mucopolysaccharidosis type VII). Immortalized cells represent a source of glia as well as of neurons. Oligodendrocyte progenitor species can be immortalized at multiple stages of the oligodendrocyte developmental cycle, and such progenitor cells have been used to reverse the pathologic sequelae of experimental demyelinating and hypomyelinating diseases. Immortalized oligodendroglial–type 2 astroglial progenitor cells can undergo cellular maturation and myelinate host axons in neonatal shiverer mice that do not produce myelin. In addition, within the spinal cords of myelin-deficient rats, these immortalized progenitor cells can proliferate, migrate, and myelinate axons for several weeks following transplantation.

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