The Changing Face of Neural Stem Cell Therapy in Neurologic Diseases

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New insights into the biology of neural stem cells (NSCs) have raised expectations for their use in the treatment of neurologic diseases. Originally, NSC transplantation was proposed as a means of replacing cells in central nervous system diseases that result in cell loss. However, recent data regarding their beneficial effects in various animal models of neurologic diseases indicate that transplanted NSCs may also attenuate deleterious inflammation, protect the central nervous system from degeneration, and enhance endogenous recovery processes. Herein, we review recent developments and future prospects of NSC therapy in neurologic diseases.

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A key issue in the treatment of neurologic diseases is that injurious processes are often irreversible because neurons in the brain and spinal cord are unable to spontaneously regenerate. This is especially frustrating because the adult central nervous system (CNS) harbors various cells with the potential to regenerate, but it fails to recruit them effectively. Therapeutic approaches that could address this include enhancing the number and function of endogenous regenerating cells or, alternatively, introducing regenerating cells into the diseased CNS by means of transplantation.

Neural stem cells (NSCs) and neural precursor cells (NPCs) can be isolated from the developing or adult CNS and can be safely expanded in chemically defined culture media for an extended period. The characteristics of restorative capacity and multipotentiality (Figure, A-D) suggest that NSCs may provide an unlimited source of neurons and glia for the treatment of neurologic disorders via cell replacement. Beneficial effects of NSC transplantation have been reported in several animal models of different neurologic diseases such as stroke, spinal cord injuries, Huntington disease, Parkinson disease (PD), multiple sclerosis (MS), and amyotrophic lateral sclerosis. However, the mechanisms by which transplanted cells are involved in clinical recovery are not fully understood. Until recently, the therapeutic potential of NSCs has focused primarily on their potential to replace damaged or missing cells. However, cell replacement-based strategies have encountered several as yet insurmountable obstacles because of the complexity of CNS structure and function. As will be reviewed herein, recent studies, focusing on reparative mechanisms, have shed light on the ways in which NSCs could promote neural recovery.

NSC TRANSPLANTATION FOR CELL REPLACEMENT

Given that neurologic dysfunction in CNS disorders is usually due to cell loss, the primary goal of NSC therapy is to replace missing cells and tissue. A fundamental precondition for a cell replacement approach is the generation of specialized cells specific for each neurologic disease. The first indication that brain cells could be replaced came from work in animal models of PD. Experimental data from rodents and nonhuman primates demonstrated that dopaminergic neurons derived from fetal ven-
central mesencephalon formed synaptic contacts, released dopamine, and ameliorated PD-like symptoms when grafted intrastriatally. Clinical trials of human fetal mesencephalic tissue transplantation in patients with PD were started in 1987 and showed improvement in some patients. These early studies raised many questions regarding the optimal site of grafting, dose of cell delivery, graft rejection, and adverse effects. Moreover, it became clear that fetal mesencephalic tissue, requiring up to 4 fetuses per patient, was not a practical source of transplantable dopamine neurons. Nevertheless, these studies provided proof of principle for cell replacement in PD and boosted the search for an ethically acceptable and inexhaustible source of dopamine neurons. There are no established methods by which specific neuronal types can be selectively enriched from adult NSCs. As an alternative, it has been suggested that stem cells from nonneural tissue could be used.6 Moreover, it remains controversial whether they are capable of acquiring the full range of neuronal functions. To date, the best candidate for mass generation of specialized neural cells is the embryonic stem cell (ESC). By recapitulation of developmental conditions in culture, it is possible to grow mouse and human ESCs and to generate cultures enriched with dopaminergic cells, motor neurons, oligodendrocytes, and retinal cells, among others.6 Moreover, mouse ESC–derived motor neurons can establish functional synapses with muscle fibers in vitro and extend axons to ventral roots after transplantation into motoneuron-injured adult rats.7 Although protocols for generation of dopaminergic neurons from human ESCs in vitro are available,8 their grafting in experimental animals has been hampered by poor survival of the transplanted cells, which is a major obstacle in the application of cell therapy for PD.

Huntington disease is another candidate for cell replacement therapy because it also results from a localized degenerative process involving a specific neuronal system. The ease of generation of γ-aminobutyric acid–ergic cells compared with dopaminergic cells means that clinical trials of human ESC–derived neuronal progenitors may progress faster in Huntington disease than in PD.

Cell replacement therapy for stroke or spinal cord injury is a bigger challenge because transplanted NSCs need to replace a range of neuronal types, remyelinate axons, and repair complex neural circuits. As a preliminary step toward this goal, a recent study9 showed that human fetal–derived NSCs transplanted into the brains of rodents after stroke survived, migrated, and differentiated into various types of neurons and glia.

Most neurologic diseases such as MS are not limited to pathologic conditions at one site or system. This raises a major concern regarding appropriate cell delivery. It is well established that transplanted myelin-forming cells efficiently remyelinate focal lesions of demyelination.10 However, MS is a chronic multifocal disease that requires study in a more clinically relevant model such as experimental autoimmune encephalomyelitis (EAE). Neural precursor cells cultured in the form of neurospheres survived for prolonged periods in the ventricular space of naive animals and responded to induction of EAE by migration into the inflamed white matter tracts and integration into the tissue (Figure, E and F).11 Therefore, transplanting the cells intrathecally and intraventricularly is a promising approach for cell delivery because this brings the cells into proximity with the periventricular and spinal tracts, which are most commonly involved in MS. They can then migrate to the active sites of disease under the direction of inflammatory cues. Alternatively, a small fraction of intravenously supplied NPCs may be able to cross the blood-brain barrier into sites of active disease.12 Transplanted human oligodendrocyte progenitors accomplished widespread myelination in animal models of dysmyelinating disease such as the shiverer mouse.13 However, the developing CNS provides a much more permissive environment for cell migration and remyelination, and to date there is no evidence that NSCs can remyelinate demyelinated axons in EAE. Achieving this is
a major challenge and a prerequisite for cell replacement therapy in MS.

Degenerative diseases of the adult CNS such as Alzheimer disease and amyotrophic lateral sclerosis have a limited inflammatory component and a nonpermissive environment for regenerative processes, rendering significant NSC migration unlikely. Therefore, the prospect of therapeutic cell replacement in this scenario with appropriate cell migration and integration into existing neural circuitries is remote.

In summary, regarding diseases that seem amenable to cell replacement therapy, the delineation of the optimal developmental stage of transplanted cells remains a key issue. Candidate cells need to be committed to their target specialization but must retain the plasticity of their precursors that is necessary for effective integration in the CNS.

**IMMUNOSUPPRESSIVE EFFECTS OF TRANSPPLANTED NSCs**

Although NSCs may exert their therapeutic effects by directly replacing missing cells, transplantation rarely results in significant numbers of transplant-derived terminally differentiated neurons. This raises the suspicion that, in addition to their potential for cell replacement, the beneficial effect of NSCs in disease models may be attributable to alternative biologic properties.

The first indication of an anti-inflammatory effect of NPCs came from transplantation experiments in rats with EAE. It was shown that intraventricular transplantation of NPCs reduced brain inflammation and clinical disease severity. Because clinical signs in this model result from disseminated brain inflammation without demyelination, it was suggested that the benefit of NPC transplantation was mediated by an anti-inflammatory effect. Additional investigations confirmed the immunomodulatory properties of NPCs in EAE in mice. The use of the mouse model of EAE demonstrated that amelioration of brain inflammation in transplanted animals was associated with reduced demyelination and axonal loss. The exact mechanisms by which transplanted NPCs attenuate brain inflammation are unclear. One school of thought suggests an immunomodulatory effect by which NPCs promote apoptosis of type 1 T-helper cells, shifting the inflammatory process in the brain toward a more favorable climate of dominant type 2 T-helper cells. To our knowledge, there are no data yet on whether NSCs specifically affect the type 17 population of T-helper cells, which were recently found to have an important role in EAE pathogenesis. Alternatively, a nonspecific bystander immunosuppressive effect of NPCs on T-cell activation and proliferation has been suggested. Intravenously administered NPCs were transiently found in peripheral lymphoid organs, where they interacted with T cells to reduce their encephalitogenicity. This suggests that the beneficial effects of intravenous NPC injection in EAE were mediated by a peripheral immunosuppressive mechanism, resulting in reduced immune cell infiltration into the CNS and milder CNS damage (Figure, G and H). Coculture experiments that mimic the direct interactions of lymph node cells and NPCs in vivo showed a striking inhibition of EAE-derived, as well as naive, T-cell activation and proliferation by NPCs following various stimulations. The suppressive effect of NPCs on T cells was accompanied by a significant suppression of proinflammatory cytokines. This nonspecific anti-inflammatory mechanism may be of major importance in the application of transplantation therapy in immune-mediated diseases because it can protect the host CNS and graft from additional immune attacks.

A recent study has shown a similar beneficial immunosuppressive effect of bone marrow stromal cells in attenuating EAE, adding another potential source of cells for therapy in MS. Bone marrow stromal cells can be derived from the patient, expanded in vitro, and reintroduced intrathecally as an autologous graft. Initial clinical experience from our institution suggests that this approach is feasible and without overt complications.

**NEUROPROTECTIVE EFFECT OF TRANSPPLANTED NSCs**

The immunosuppressive effect of transplanted NPCs protects the brain from deleterious consequences of autoimmune processes in EAE. A more general neuroprotective effect was observed in other nonautoimmune experimental disease models. Neural stem cells rescued dopaminergic neurons of the mesostriatal system in a PD model in rodents. These findings led to the concept that NSCs are endowed with inherent mechanisms for rescuing dysfunctional neurons. This effect was found to be important in other neurologic diseases. Neural stem cells seeded on a synthetic biodegradable scaffold and grafted into the hemicorrection adult rat spinal cord induced significant improvement in animal movement by reduction of necrosis in the surrounding parenchyma and by prevention of inflammation, glial scar formation, and extensive secondary cell loss. Recent findings in amyotrophic lateral sclerosis models have shifted the therapeutic target of NSC transplantation from neuron replacement to motor neuron salvage. A neuroprotective effect of NSC transplantation was observed in other models of neurodegeneration. In mutant mice in which Purkinje neurons die in the fourth to fifth week of life, transplanted NSCs rescued host Purkinje cell function and restored motor coordination. Subretinal injection of human NPCs provided almost full protection of visual function in a rat model of retinal degeneration. Therefore, the key principle for fully using the neuroprotective properties of NSCs in the clinical setting is early intervention, whether mediated by degenerative, immunologic, or traumatic pathogenesis. This might prevent or slow down progression of disease and reduce the need for later cell replacement.

**NEUROTROPHIC EFFECTS OF TRANSPPLANTED NPCs**

As explained, insufficient repair capacity of the adult CNS results from an inability of local progenitors to respond appropriately to disease states by replacing damaged cells, coupled with a lack of regenerative capacity of injured axons. Neural stem cell therapy is emerging as a mode of treatment that can enhance the ability of the host brain to repair itself in both aspects.
After sectioning of the adult spinal cord, NSC transplantation induced a permissive environment for axonal regeneration. Similarly, in a model of retinal degeneration, NPC transplantation promoted neurite growth in the optic nerve. In both cases, this effect was mediated by induction of matrix metalloproteinases that degrade the impeding extracellular matrix and cell surface molecules, enabling axons to extend through the glial scar. Transplantation of olfactory-ensheathing cells into the sectioned spinal cord also promoted axonal regeneration in long fiber tracts, with a return of lost function. This was explained by the creation of proper realignment, enabling axonal growth through a permissive tract. In addition, the cells increased axonal sprouting, remyelination, and vascularization of the injured spinal cord. Substantial endogenous reconstitution of brain structural connectivity was observed when NSCs were transferred by biodegradable scaffolds into regions of extensive brain degeneration caused by hypoxia.

Recent work indicates that transplanted NPCs can enhance endogenous neurogenesis in pathologic conditions. Mice exposed prenatally to opioids display impaired learning associated with reduced neurogenesis; transplantation of NPCs improved learning functions and host brain-derived neurogenesis in the hippocampus. A similar neurotrophic effect was reported in physiologic aging. Although neurogenesis in the dentate gyrus declines severely by middle age, transplantation of NSCs stimulated the endogenous NSCs in the subgranular zone to produce new dentate granule cells. Candidate molecules that may affect endogenous brain repair are neurotrophins. The multiple roles of neurotrophins as mediators in cell cycle regulation, cell survival, and differentiation during development and adulthood make them potential candidates for the regulation of endogenous NSC proliferation and differentiation following brain injury. For example, neurotrophins secreted by NPCs help promote corticospinal axon growth after transplantation onto an organotypic coculture system containing dissected brain cortex and spinal cord.

### SUMMARY

Cell replacement therapy in neurologic diseases may be applicable in some situations such as PD, Huntington disease, and demyelinating diseases. However, this is still far from practical in situations in which cells must recapitulate complex neural circuitry with multiple cellular specializations and long-distance connections within the hostile environment of the adult CNS. Recent investigations highlight novel neuroprotective and neurotrophic mechanisms by which NSCs are beneficial to the host brain and can be manipulated for therapeutic indications (Table). Neural stem cells inhibit inflammation to protect the brain from its deleterious consequences, protect host neurons from degenerative processes, and enhance the latent capacities of endogenous CNS progenitors for repair and of severed axons for regeneration. The underlying molecular mechanisms are not fully understood but relate in part to the production of myriad growth factors and to the modulation of the host environment to be permissive for regeneration. With its neuroprotective and trophic properties, NSC transplantation can be viewed as a potential fountain of youth. It remains to be seen whether this will be translated into practical therapy for the aging or diseased CNS.

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### REFERENCES


