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Brain repair by cell replacement and regeneration

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Not so long ago the adult brain was thought to be a slowly decaying organ, a sophisticated but flawed machine condemned to inevitable decline. Several studies now suggest that stem cells can be isolated and used to restore function in the adult brain. There is also recent evidence that neurons can be generated from endogenous cells after injury to the brain. We discuss here new data from Zhao *et al.* (1) in this issue of PNAS suggesting that dopamine neurons are continuously formed in the adult substantia nigra. These cells and regenerative responses might provide a path to functional recovery in neurodegenerative disease and brain injury.

In neurodegenerative diseases, a loss of specific cells causes patients to present with psychiatric or neurological symptoms. The prospect of replacing the missing or damaged cells is attractive. The loss of a specific type of dopamine neuron in the substantia nigra is a major feature of the pathology in Parkinson's disease. Embryonic tissue from this region, rich in dopamine neuroblasts, has been grafted to the striatum in Parkinson's patients. These clinical trials provide proof-of-principle for the cell replacement strategy in the human brain (2), but the technique is not ready for general use (3). What are the problems here? Is there really a prospect for regenerating the damaged brain? Here we discuss two fundamental issues for a regenerative approach to neurology: (*i*) Can we identify and acquire the cells of interest? (*ii*) Does the damaged brain allow regenerative responses?

There are major logistical difficulties with the routine clinical use of human cells or tissue. These problems are amplified when the tissue comes from a small region of the developing brain, such as the substantia nigra. The development of techniques to expand the precursors provides a possible solution. Self-renewing stem cells have been identified in both the fetal and adult nervous systems. These stem cells can be grown in the lab for long periods and differentiate into neurons and glia. Two recent studies analyze the responses of stem cells from the adult mouse brain when they are grafted into a model of immune mediated demyelination,

experimental allergic encephalomyelitis (EAE) (4, 5). Demyelinating diseases, like multiple sclerosis, attack many sites in the brain, and it has not been clear how cells could easily be delivered to these diffuse locations. An important feature of both studies is that the grafted cells become widely dispersed. The presence of new cells at many sites is likely necessary for the behavioral recovery reported in one of the papers (5). EAE causes local inflammation that may be required to open the blood–brain barrier and stimulate cytokines that attract stem cells to the injury. Previous work with adult and fetal CNS stem cells suggests that they have an unusual ability to move through brain tissue. New methods of labeling cells are available to follow the cells precisely as they track down the lesion and differentiate to form new myelin. What are these adult cells? More than 10 years of study suggests that multipotent cells can be isolated from the nervous system. The stem cell is not the immediate precursor to oligodendrocytes, as a more committed oligo-precursor is also well defined. Perhaps the stem cell takes the initial steps of finding the lesion and then generates an oligo-precursor that goes through a local expansion and differentiation. Whatever the mechanism, it is important to know the details of this regenerative process and whether regeneration occurs in chronic models of demyelination that are more similar to multiple sclerosis.

Dopamine neurons are continuously formed in the adult substantia nigra.

Could these multipotent CNS stem cells be used to repair damage to any cell in the nervous system? There are two possible reasons for a negative answer to this question. First, CNS stem cells can differentiate into oligodendrocytes, but they may not efficiently generate all neural cell types of clinical interest. Second, successful engraftment also depends on the host tissue incorporating the donor cells. The adult brain may allow glial but not neuronal replacement. We discuss these two points in relation to Parkinson's disease.

To study and treat Parkinson's disease we must focus on a specific type of neuron, a neuron with special features that is in the substantia nigra. The substantia nigra is in the ventral midbrain and contains neurons that use dopamine as a neurotransmitter and send their axons to the striatum. In Parkinson's disease, there is a gradual loss of these neurons leading to severe depletion of striatal dopamine levels and motor abnormalities. The cell replacement strategy is based on a bulk of studies in animal models showing that restoration of dopamine levels in the striatum by embryonic neural grafts can lead to substantial and long-lasting functional recovery (2). The clinical studies have demonstrated that the grafted dopamine neurons can survive and reinnervate the striatum for at least 10 years despite an ongoing disease process, which destroys the patient's own dopamine neurons (6, 7). The grafts can also normalize striatal dopamine release (7) and restore frontal cortical activation associated with movements (8). However, although some patients have exhibited major clinical improvement (7), there is a variability in the functional outcome, and other patients have shown no or only modest improvement (3). Troublesome involuntary movements, so-called dyskinesias, have occurred in 7–15% of grafted patients (3, 9), but there is no evidence that these dyskinesias are caused by dopaminergic overgrowth or are a general feature of dopamine neuron replacement *per se* (9). The most important goals for cell therapy research in Parkinson's disease are to develop strategies to generate large numbers of viable dopamine neurons and to avoid graft-induced dyskinesias.

Neurons are generated in a very precise sequence during brain development. It became clear that in Parkinson's models, grafting occurred most efficiently if the implanted issue was from the specific stage of development when dopamine neurons are normally generated. Functional midbrain dopamine neurons cannot be efficiently obtained from the adult stem cells used in the EAE model. A proliferating precursor for dopamine neurons can be isolated if the substantia nigra is dissected at this time, but this cell cannot be generated in large numbers (10). However, unlimited numbers of midbrain dopamine neurons can be obtained from pluripotent embryonic stem cells from the mouse. These cells form functional dopamine neurons in the brain

when they are taken to the appropriate developmental stage in tissue culture (11) Similar methods have been used to generate other cell types of clinical interest, including motor neurons and pancreatic endocrine cells (12–15). These results suggest that access to suitable numbers and types of cells will be possible by improving the technologies associated with manipulating embryonic and somatic stem cells.

The transplantation approach would be weak if the adult tissue has no regenerative capacity. No matter how many cells can be generated in the lab, they would be useless if the adult brain did not accept them. Increasing anatomical evidence suggests that endogenous stem cells can also regenerate neurons in several regions of the mammalian CNS in response to injury. Targeted apoptotic degeneration of cortical neurons in mice promotes the formation of new cortical neurons that extend axons to the thalamus (16). Stroke increases neurogenesis in the rat subventricular zone, and the new neurons migrate into the damaged striatum, where they express markers of medium-sized spiny striatal neurons (17, 18). In mice, infusion of epidermal growth factor (EGF) after stroke promotes a neurogenic response and replacement of parvalbumin-expressing striatal interneurons (19). Finally, intraventricular infusion of EGF and fibro-blast growth factor 2 after global fore-brain ischemia in rats gave rise to regeneration of hippocampal CA1 pyramidal neurons from neural stem cells located around the posterior periventricle adjacent to the hippocampus (20). These new neurons seemed to form afferent and efferent connections and reverse some functional deficits. All of these studies suggest that the injured adult brain can support the production and incorporation of new neurons. Even if the numbers are small, these results show the adult brain can regenerate neurons after injury.

We must analyze the mechanisms controlling these remarkable regeneration processes.

In this issue of PNAS, Zhao *et al.* (1) provide the first experimental evidence that self-repair mechanisms may operate also in the adult substantia nigra. The results suggest that dopamine neurons are constantly turned over, they die and are replaced at a very low rate (20 new cells per day). Perhaps the most intriguing result is that a significant portion of the new neurons seemed to reestablish the connection to the striatum. Also, that rate of replacement doubles when the mature dopamine neurons were deleted by the toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. These new data are exciting because if neurogensis occurs also in the human substantia nigra it would have important implications both for the cell replacement strategy and for the pathogenesis of Parkinson's disease. Progression of this disorder would then be determined not only by the rate of degeneration of substantia nigra neurons but also on the efficacy in the formation of new dopamine neurons. Cell therapeutic interventions might involve both transplantation and stimulation of the endogenous neurogenic response. However, it is important to emphasize that other investigators have failed to detect nigral neurogenesis. Lie *et al.* (21) found that the adult rat substantia nigra contains a population of progenitors, but they only gave rise to glial cells *in situ*. The reason for this discrepancy is currently unclear. To firmly establish the occurrence of nigral neurogenesis, genetic or retroviral labeling of stem cell progeny could be used to show the origin, migration, and differentiation of the presumed new dopamine neurons. We also need to know whether the new neurons function and contribute to behavioral recovery.

The data reviewed here suggest that (*i*) the controlled and scaleable production of specific cell types from precursors is technically feasible and (*ii*) the adult tissue retains at least some of the growth potential that is so active at early stages of development. Clinical trials in Parkinson's disease suggest that the transition to clinical efficacy may not be simple. However, we argue here that to succeed we must systematically analyze the mechanisms controlling these remarkable regeneration processes. In the short term, we need to establish the technology to generate and regenerate the cells of interest. Right now, most of the work has been done in animal model systems, but we obviously need to know whether human cells respond to similar mechanisms.