Perspective

# Neural Transplantation: A Call for Patience Rather Than Patients

JOHN R. SLADEK, JR., AND IRA SHOULSON

TRANSPLANTATION OF WHOLE AND MULTIPLE ORGANS IS now commonly used in the treatment of kidney, heart, and lung diseases, but application of this approach to neurological disease as yet is limited. Grafting of cells, particularly from (immature) embryonic tissue, holds promise as a therapeutic strategy for the many neurological disorders where treatments are either imperfect, palliative, or nonexistent. Attempts were made after the Chernobyl disaster to graft blood cell–producing tissue from human fetal liver into patients who suffered severe radiation damage of bone marrow (*1*). A procedure that results in reversal of diabetes mellitus in rodents (*2*) has been attempted in several human diabetics who now require less exogenous insulin after grafting of cells from human fetal pancreas. The possibilities for treating genetic and developmental defects are also indeed significant. With the aging of our population and the increasing prevalence of degenerative disorders, particularly of brain, the question of nerve cell replacement has arisen prominently. The medical, economic, and societal impact of truly devastating degenerative disorders, such as Alzheimer's and Parkinson's diseases, which affect millions of people in North America alone, have encouraged medical researchers to pursue transplantation procedures as a possible therapy. This idea is not new, however, and was attempted in the United States as early as 1944 when surgeons at Washington University implanted a piece of human spinal cord from Formalin-fixed cadaveric tissue into the damaged spinal cord of a young paraplegic in an unsuccessful attempt to reconstruct neural pathways (*3*). The ability of neural grafts to restore or improve neurological dysfunction was first shown in the 1970s in animals when L. Olson, Å. Seiger, and their colleagues at the Karolinska Institute and A. Björklund and U. Stenevi at the University of Lund were able to demonstrate the survival, growth, and functional integration of embryonic nerve cells grafted into either brain or anterior eye chamber (*4*). These and other studies provided important insights into not only the growth characteristics of immature neurons, but their ability to reverse motor, endocrine, cognitive, and other neurologic defects (*5*).

John Sladek is the Kilian and Caroline Schmitt Professor and Chairman of Neurobiology/Anatomy at the School of Medicine and Dentistry at the University of Rochester. Ira Shoulson is Professor of Neurology, Medicine, and Pharmacology and Director of the Unit of Movement and Inherited Neurologic Disorders at the same institution. Preparation of this perspective was supported by a PEW Neuroscience Award to the University of Rochester.

By 1982, neural transplantation research had progressed to a point where it could be considered for application, especially to diseases of central motor systems. Parkinson's disease (PD) appeared well suited for treatment with this approach because (i) it results largely from a profound loss of dopamine input from the substantia nigra to caudate nucleus and putamen, (ii) embryonic dopamine neurons showed excellent survival and neurite extension after grafting in rodents, (iii) the patient's own adrenal chromaffin cells could be used in place of fetal cells, avoiding the special problems associated with the use of embryonic tissue. Adrenal chromaffin cells, which are the cells of the adrenal medulla that synthesize and secrete epinephrine (adrenalin) and its precursors, norepinephrine and dopamine, were utilized in Scandinavia in the first human autograft attempts to ameliorate the symptoms of PD (*6*). This research was performed at a time when only sparse data supported the concept that grafting, as adapted from experiments in rodents, could be applied successfully in humans. In fact, a subsequent report on grafted adrenal chromaffin cells in four nonhuman primates suggested that there was minimal survival of cells (*7*). Published data did not exist at that time (and still do not) that indicate a high degree of confidence for this approach. Nevertheless, the autograft procedure was initiated in four patients with advanced PD for understandable reasons. Parkinson's disease is a debilitating, neurodegenerative disorder; although pharmacotherapy with dopaminergic agents ameliorates many symptoms and disabilities of early PD, benefits seldom endure and adverse effects frequently supervene. Many patients, particularly in advanced stages of illness, are quite willing and often desperate to participate in an experimental procedure, even if it is imperfect. Family members who share increasing care burdens may help fuel a growing sense of helplessness and despair. After unilateral transplantation of chromaffin cells into the caudate, slight improvement in limb rigidity was reported in one of two patients. This modest benefit was short-lived, and the patient needed to resume levodopa therapy. Subsequent chromaffin cell grafts into the putamen in two additional patients also resulted in a transient period of change that might have been due to a release of catecholamines from chromaffin cells, either spontaneously or as a consequence of cell death (*8*). These disappointing results prompted our Scandinavian colleagues to defer further implantation of autologous chromaffin cells in PD patients until a new strategy could be developed, such as infusion of nerve growth factor to enhance chromaffin cell survival and neurite outgrowth as reported in rodents (*9*).

In contrast to this lack of success with autologous chromaffin cell grafts, by 1985 there were reports that experimental PD in monkeys, induced by the neurotoxin *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) could be treated by grafting embryonic dopamine neurons from the substantia nigra. MPTP-treated African Green monkeys showed major improvement in tremor, motor freezing, and other parkinsonian features in two animals that received grafts of fetal substantia nigra bilaterally into the caudate nuclei (*10*). In these experiments the monkeys were examined for only 10 weeks after surgery. A follow-up study showed reversal of symptoms in the most severely debilitated animals, graft survival, neuritic extension into host striatum by catecholaminergic (grafted) neurons, and correspondence between the presence of grafts and elevated dopamine levels in adjacent striatal regions at 7.5 months after grafting (*11*). Thus, data from nonhuman primate models reveal dramatic improvement in neurotoxic-induced features of PD after transplantation of embryonic tissue.

Because of the disappointing results of the chromaffin cell grafts in Scandinavia and the apparent success of the use of fetal neural cells in experimental PD, conventional wisdom might have suggested that further experimental surgery in humans should be preceded by

confirmatory studies of autologous adrenal autografts in parkinsonian monkeys and extension of studies with fetal cells to reverse experimental PD. To the contrary, human experimentation moved ahead rapidly in Mexico City (*12*), China, Cuba, and the United States. Most researchers adopted a procedure that involved cavitation of a portion of the head of the caudate nucleus unilaterally prior to surgical attachment of several large pieces of adrenal tissue. To date, nearly 100 adrenal medulla-to-caudate autografts have been performed in PD patients by at least 15 groups of investigators in the United States. Preliminary results collectively indicate only modest to moderate benefits in only a proportion of patients (*13*). There is also growing disillusionment regarding the report of dramatic improvement in two Mexican patients (*14*).

These disappointing developments and the public attention accorded to neural grafting prompt a critical reevaluation of this experimental procedure in humans as well as consideration of some basic medical values and scientific justifications. Is the mere replication of findings (even those published in a prestigious medical journal) based on only two patients (*12*) sufficient justification for wide expansion of adrenal medullary autograft studies in PD patients? Is it justified for investigators to operate on two or three patients to gain a first-hand experience of this unproven research procedure? Is patient and family desperation sufficient reason for launching into uncontrolled investigations? Is it justifiable to expand experimental use of a procedure attended by postoperative mortality rates that may exceed the expected annual mortality from natural causes? We think not.

Perhaps scientific history repeats itself. In 1940, Russell H. Meyers presented exciting new information on relief of PD tremor in three patients who had undergone partial or complete unilateral removal of part of the caudate nucleus (*15*). This operation provided immediate amelioration of debilitating tremors, even while the patient was still under local anesthesia. Considerable discussion followed this presentation at the New York Neurological Society Meeting, with each discussant praising what was viewed "as a brilliant attack on an old and extremely troublesome problem." One comment by Dr. Joseph E. King was particularly noteworthy: "It is to be hoped that none of my colleagues will attempt this operation—I am sure that I shall not. . . . It is a splendid operation, and I think that we should wait, watch, and learn until they are satisfied with their own results; otherwise, the operation may fall into disrepute as a result of being improperly done or carried out for the wrong condition." Two years later, Dr. Meyers presented a more complete report of his findings in the form of case studies on eight patients (*16*). Each procedure involved variations on extirpation or sectioning of portions of the brain; varying improvement was noted. Of particular note were his follow-up observations of the 39-year-old patient who had earlier been viewed as a success. With time, that patient underwent a second, and then a third operation, because tremors grew progressively worse on the operated side, while the improvement on the contralateral side was maintained. After the second operation, which removed the left caudate nucleus, tremors on the left side increased to the point where a third operation was attempted. By 1942, Dr. Meyers listed this case as a "rank failure."

Our points are that considerable time is needed for clinical evaluation and that early judgments can be flawed. A combination of effects that have little to do with dopamine release by adrenal cell grafts may have accounted for the initial striking "success" reported by the investigators from Mexico City. First, some improvement in PD signs and symptoms might be realized by even a small amount of injury or stimulation to the caudate, perhaps by means of the cavitation procedure for adrenal attachment. Second, the use and adjustment of medications for PD before and after surgery may greatly influence the clinical outcome and confound interpretation of experimental interventions. Third, surgical intervention or insertion of adrenal medullary tissue might stimulate regeneration in the remaining host dopamine systems, as has been reported in rodents and monkeys (*17*); and fourth, robust placebo effects continue to astonish investigators who carry out controlled clinical trials. The spectacular nature of this procedure and the heavy emotional investment by patient, family, and clinician could predispose to a major placebo effect. All of these factors combined might produce some level of improvement, particularly in younger patients. That the dramatic results reported initially have not been replicated in the United States would support this suspicion.

It is likely that attention now will be focused on the use of human embryonic cells as the source of grafted tissue. Animal experiments suggest that fetal tissue, particularly from brain, would be more effective than adult adrenal cells as donor tissue for PD. Immature nerve cells better endure the grafting procedure because of their robust abilities to grow and integrate with host brain, abilities that in part reflect the timing of the genetic program for cellular growth and maturation. A debate has now been sparked by an upcoming review by the National Institutes of Health, called by the Assistant Secretary for Health and Human Services, to examine in detail the potential use of human fetal tissue from induced abortions for transplantation purposes. Ethical, legal, and scientific questions will be addressed by an external advisory committee to provide a more informed background for evaluation of a request by NIH to perform human fetal neural tissue transplants from induced abortions into PD patients. This committee, together with the apparent failure to replicate the reported success from Mexico City should serve to raise a public and scientific awareness of the questions attendant to the use of embryonic cell grafting in PD patients. Our present scientific knowledge suggests that the best source of graft tissue for the amelioration of PD is the embryonic cell that most closely resembles that which is deficient in the disease, that is, the dopamine neuron of the substantia nigra. Both human and nonhuman cells might be effective; however, considerable study of this question is needed. We should not repeat the experience of the adrenal autograft experiments wherein far more humans than nonhuman primates were operated upon as a result of a single unconfirmed report of two patients.

It also seems timely to reassess our values regarding the scope of animal research. Has the animal rights lobby intimidated investigators to the point where they are being unduly pressured to carry out animal research in humans? After all, there is a very reasonable animal model for the study of parkinsonism (*18*). Although the application of basic research findings must be translated inevitably by clinical research, we should first be persuaded that the immediate scientific and practical questions regarding grafting are unanswerable in animal experiments. However, an insufficient number of animal studies have been performed at present to accurately predict, for example, the crucial amount and age of embryonic brain tissue necessary to produce lasting relief from parkinsonian symptoms, while at the same time not producing overgrowth of this rapidly developing tissue. We do not know yet whether grafts will need to be placed in both hemispheres and whether both targets of the nigrostriatal system, that is, caudate nucleus and putamen need to receive cells. Because survival of human embryonic neurons may be as low as 10 to 20 percent and because the human striatum is an extremely large subcortical mass, cells from several donors might need to be pooled to provide a sufficient replenishment of lost dopamine neurons to eliminate symptoms and to prevent reoccurrence of the symptoms should a continued decline in the host system occur during the progression of the disease. Should cells be pooled from more than one fetal donor, and if so, is cryopreservation of cells sufficiently advanced to accomplish this? The long-term efficacy

of transplantation also is unknown, and we should not forget that embryonic brain tissue during normal maturation continues to grow in size with growth of the human skull well into the second decade of life. Does this mean that embryonic nerve cells grafted into the fluid-filled sacs of the ventricular system will grow for several years to a point where hydrocephalus might result from the production of tumorous masses; we simply do not know. Graft-host immunology of neural transplants is largely unstudied in primates, and the possibility that newly growing tissue could influence neural systems beyond the implanted brain centers is one that cannot be overlooked; in fact, many of the adrenal autograft recipients are exhibiting behavioral changes (*13*). These basic science questions are linked to important issues regarding the standards of clinical investigation. If this type of research is to go forward, serious consideration should be given to controlled clinical trials aimed at clarifying efficacy, adversity, and mechanism of any substantive findings. To this end, consideration should be given to the basic tenets of a controlled clinical trial including clear definitions of hypothesis, end point, subject selection criteria, sample size requirements, proper controls, blindness, duration of follow-up, uniformity of surgical techniques, verification of pathology, viability of graft, costs, benefits and risks, and ethical considerations.

The societal issues are equally important. Conclusions reached at a conference held at Case Western Reserve University in December 1986 on the ethics, law, and science of neural transplantation reveal that human embryonic cell grafting could be performed legally in the majority of states in the United States by applying present guidelines on the use of cadaver tissue for organ donor programs (*19*). However, other sources of donor tissue exist that would circumvent the need to use embryonic human cells. For example, Gash and colleagues demonstrated survival after transplantation of neuroblastoma cells that had been rendered amitotic (*20*); these cells synthesize various transmitters and might be genetically altered to produce a specific transmitter. Autologous grafting might be accomplished if, for example, the dopamineric neurons of the carotid body prove effective in reversing experimental parkinsonism in animal models. Cross-species transplantation of embryonic dopamine neurons from the substantia nigra could be utilized if the immune response is suppressed. Perhaps most importantly, the power of genetic engineering is being harnessed in attempts to transfect oncogenes to produce desired cell lines. Such cells could arise from the patient by modification of an active tissue component such as fibroblasts, or they could be produced from an immortalized cell line, perhaps of human embryologic origin. These scientific possibilities provide hope that, with time, a donor cell line will be produced for PD that will be effective, readily available, and generally acceptable to society.

The issues encompassed by fetal grafting research and its application to humans deserve our dispassionate and timely attention. As a society we have not yet had sufficient time to fully explore and understand the many issues attendant to embryonic cell grafting for neurodegenerative and other disorders. Unfortunately, real therapeutic benefits of grafting neural tissue, whether autologous or embryonic, may be overlooked in the growing disillusionment with the current experience of adrenal implantation in PD patients. These concerns may further confound what appears to be an extraordinary approach to experimental therapeutics. We hope not, particularly because the scientific rationale continues to build for neural grafting as a therapy for neurological disease. Now, however, we could benefit from more **patience** rather than more **patients**.

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