Future of Cell and Gene Therapies for Parkinson's Disease

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The experimental field of restorative neurology continues to advance with implantation of cells or transfer of genes to treat patients with neurological disease. Both strategies have generated a consensus that demonstrates their capacity for structural and molecular brain modification in the adult brain. However, both approaches have yet to successfully address the complexities to make such novel therapeutic modalities work in the clinic. Prior experimental cell transplantation to patients with PD utilized dissected pieces of fetal midbrain tissue, containing mixtures of cells and neuronal types, as donor cells. Stem cell and progenitor cell biology provide new opportunities for selection and development of large batches of specific therapeutic cells. This may allow for cell composition analysis and dosing to optimize the benefit to an individual patient. The biotechnology used for cell and gene therapy for treatment of neurological disease may eventually be as advanced as today's pharmaceutical drug-related design processes. Current gene therapy phase 1 safety trials for PD include the delivery of a growth factor (neurturin via the glial cell line–derived neurotrophic factor receptor) and a transmitter enzyme (glutamic acid decarboxylase and aromatic acid decarboxylase). Many new insights from cell biological and molecular studies provide opportunities to selectively express or suppress factors relevant to neuroprotection and improved function of neurons involved in PD. Future gene and cell therapies are likely to coexist with classic pharmacological therapies because their use can be tailored to individual patients' underlying disease process and need for neuroprotective or restorative interventions.

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Clinical Application of the Emerging Cell-Based Treatment Approaches for Parkinson's Disease

New nonpharmacological treatment strategies involve cell and synaptic renewal or cell replacement in the living brain to restore the function of neuronal systems, including the dopaminergic (DA) system in Parkinson's disease (PD). Although recent laboratory work has focused on using stem cells as a starting point for exogenous or endogenous derivation of the optimal DA cells for repair, DA cell therapy using dissected fetal DA tissue containing neurons has already been explored in PD patients.[1–8] Open-label trials consistently demonstrated that functional motor deficits associated with PD can be reduced after application of this new technology, although double-blind trials failed to show evidence of significant benefit in comparison with placebo with the transplant variables that were selected.[1,6] Evidence shows that the underlying disease process does not destroy the transplanted fetal DA cells, although the patient's original DA system degeneration progresses.[4,5,7,9] In fact, most transplants containing DA midbrain neurons appear to be functional for at least a decade,[4,10,11] and there is evidence of graft and neuronal survival without pathology for at least 14 years after surgery.[11] Interestingly, in a few cases studied after 11 years, as some transplants grow older in ectopic putamen locations, a minor proportion of implanted DA neurons may show signs of protein aggregation and fibrillar changes indicative of Lewy bodies.[10,12] It is not clear whether the affected fraction of such implanted DA neurons are aging at a rate greater than normal adult non-PD brain DA neurons, perhaps because of ectopic, trophic, and inflammatory or immunological processes within the transplants, or whether PD pathological processes are directly or indirectly influencing the grafted neurons.

In clinical trials, despite technical shortcomings, human fetal dopamine (DA) ventral mesencephalic neu-

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rons are functional in PD patients.[1,3–5] Recently, it has been suggested that unregulated production of DA from grafted fetal neurons is the cause of unwanted "off"-medication dyskinesias seen in a subset of transplanted PD patients[1] despite the ability of grafts to reduce L-dopa–induced peak-dose dyskinesias. This pattern is quite possible, in a scenario of a "primed" dyskinetic circuitry produced by prior L-dopa treatment. The reduction of peak-dose dyskinesia may be because striatal DA terminal release from grafted fetal DA neurons is controlled by both cell intrinsic and extrinsic synaptic and autoreceptor mechanisms.[4,8,13] In animal models, fetal DA grafted neurons can reduce L-dopa–induced peak dyskinesias.[14] In grafted DA neurons, presynaptic DA autoreceptors regulate excess DA release,[15] and in vivo infusion of the full DA agonist apomorphine can block spontaneous DA release in the striatum.[13,16,17] An optimal DA cell regenerative system would reconstitute a normal network capable of restoring feedback-controlled release of DA in the nigrostriatal system.[8] The success of cell therapy for neurological diseases is, in our opinion, limited by access to highly specialized DA neurons found in the A9 and A10 regions of the substantia nigra (SN) in the ventral mesencephalon, as well as technical and surgical steps associated with the transplantation procedure.

Transfer of Appropriate Neurons and Glia for Cell Repair and Replacement in Neurological Diseases

PD has multiple causes (genetic and sporadic) and is characterized by the loss of selectively vulnerable groups of neurons. If possible, it is reasonable to initiate protective measures by drugs or gene therapy (see later) before onset and to prevent further degeneration, once the disease appears. The majority of patients are free of symptoms until most (70–80%) of striate DA connections are dysfunctional or degenerated, although the degree of actual neuronal cell loss is significantly less, thus providing a substrate for drugs and gene therapy to revitalize such dysfunctional or dormant cells. Still, unless disease modification by trophic factors is absolute, therapeutic cell transfer to replace lost neurons and glia is a rational approach. Such "live cell" replacement therapies are conceptually, and in practice, different from typical drug treatments now in the clinic.[18] Novel cell-based therapies therefore have generated many new questions in the neurological community and understandably been met with skepticism. However, novel cell repair and regeneration therapies are at this stage still largely exploratory, with limited data accumulated on techniques to optimize the therapeutic cell composition. Furthermore, the benefits of any novel therapeutic strategy will be optimized as knowledge about potentially responsive PD patient subgroups grows. In PD, individual patients have experienced significant and long-lasting benefits after DA fetal cell implantation in the basal ganglia. These clinical benefits are associated with evidence of physiological changes (fluorodopa positron emission tomography [PET] scans and functional magnetic resonance imaging), with long-lasting (beyond 14 years) and clinically meaningful (approximately 50–60% reduction in Unified Parkinson's Disease Rating Scale [UPDRS] scores off DA drug therapy) benefit.[4,5] Indeed, transfer of fetal DA neuron containing suspension (about 5% DA cell content) into putamen[3–5,7] has so far yielded positive results in some PD patients. In contrast, there may be a greater risk for adverse effects after implantation of solid tissue-piece fetal DA cells (also about 5–10% cell DA content).[1] It is necessary to consider a number of biological and technical challenges to comprehend the obstacles to developing a neurological cell therapy. Neuronal replacement therapies for PD have to date not successfully addressed the necessary details of making cell transfer therapies work in PD patients. Although fetal DA cell transfer has been validated via imaging and postmortem assessments, and appear to be effective in some PD patients, differences in potential critical parameters of the surgical procedure have not been addressed or explored. These techniques include the effective parameters for cell preparation, transplantation target, immunological treatments, or patient selection. In an ongoing collaboration with Dr E. Redmond using a nonhuman primate model of PD, we are evaluating a number of transplantation parameters that may be critical for future clinical efforts. As a prelude to ultimately testing the feasibility of stem cell transplants, we are evaluating fetal DA neurons implanted in primates with prior L-dopa–induced dyskinesias. Modern double-blind trial designs (which are, indeed, essential tools as *final* tests to establish the clinical efficacy of well-designed drugs) were perhaps applied too early in the transplantation field, before optimal neurobiological and technical parameters were fully understood. Nonetheless, much can still be learned from these "early" trials, particularly about adverse effects (off-medication dyskinesia in PD) and the need for appropriate patient selection based on preoperative evaluations of drug responses, as well as disease severity. As an example of differences between drug trials and cell-based studies, a double-blind cell transplantation trial used a 1-year end point as the conclusion of the blind study and final analysis of data.[1] The 1-year time point using live immature DA cells is insufficient for human fetal DA cells to fully grow and establish functional connections in animal models or patients.[2] Consequently, after the study was published, many patients continued to improve during year 2 and 3 after surgery, as presumably the fetal cells continued to mature, integrate, adapt, and improve the functional status of the PD patients.[4,7,8] These data suggest that longer

evaluation periods may be necessary to evaluate cell-based therapies.

Cell therapy using fetal DA neurons is in a "prototype" technology phase because the quality of cell preparations using fetal DA cells is highly variable. For example, all the current PD transplantation studies have used rather crude cell preparations because the starting point for all work is dissected pieces from fetal tissue, which contain only about 10% newborn DA neurons. The remaining nonnigral cells are cells types not generally relevant to PD degeneration. Practically, it is not possible to use fetal tissue as a source for transplantation in more than rare experimental situations. For example, to replace a sufficient number of DA neurons, one needs six to eight fetal tissue pieces per patient, primarily because of low postoperative survival of grafted fetal DA neurons. In addition, current surgical techniques are inconsistent with respect to placement, volume, and type of cells grafted. It is encouraging, however, that recent research in stem cell biology may provide a solution to this problem of low cell access and yield.[19]

The most important factor in obtaining optimal functional effects (and minimal adverse effects) in PD by brain repair is probably the presence of new terminals and DA transmission that adequately adapt to the local milieu and provide physiologically appropriate DA release in the host caudate-putamen and SN.[4,5,7,15,18] Such grafts should have DA level feedback control provided by molecules such as dopamine transporter (DAT) and DA D2 receptors. Fetal DA neurons typically grow and establish functional connections with mature host striatal neurons. Synaptic contacts between transplanted fetal DA cells and host cells, as well as afferent contact by host neurons to transplanted cells, have been observed ultrastructurally.[20,21] The critical insight is that pharmacological delivery of DA into the striatum may not be as effective in ameliorating the motor symptoms of PD as cellular regeneration of the synaptic or terminal elements, which can regulate and synaptically control DA levels and cell contacts.[8] Data are available that illustrate this. When DA is directly administered into the ventricles of PD patients, serious motor abnormalities develop.[22] Second, the presence of high DA levels in vivo induce abnormal regulation of a large number of genes within the striatum.[23] Complications associated with unregulated DA levels are also obvious when observing effects of long-term L-dopa administration in patients. As PD progresses, and the midbrain DA neuron and its synapses continue to degenerate, nonphysiological levels of DA within the striatum and abnormal downstream activity in the basal ganglia produce severe motor abnormalities, such as dyskinesias. In neurophysiological recordings, it is clear that DA provides a modulatory role for the glutamate-mediated transmission, so that it appears to serve a gating function at that important striatal synapse.[24–30] Physiologically appropriate DA functions can be achieved by normal DA synapses or, alternatively, cells that express the complete set of feedback elements required to regulate release and uptake of DA.[15,16,31,32] DA-mediated involvement in the striatal neuronal network is such that unless there is tonic release of DA that is finely regulated at the synaptic cleft by afferents, glutamatergic synapses will be less effective in control of the striatal GABAergic output neurons.[24,25] Discontinuous stimulation of striatal DA receptors after loss of DA terminals or excessive L-dopa treatment is likely a major contributor of dyskinesia induction. Normally, basal changes in firing by mesencephalic DA neurons is limited, at least as has been tested in animal models.[26,27] More importantly, such frequency stimulation does not increase the extracellular concentration of putaminal DA because the synaptic network and terminals work to reduce fluctuations in DA concentration by reuptake mechanisms (DAT) and possibly other autoreceptor-mediated functions (D2).[24–30] Indeed, fetal DA transplants have been shown to reduce the incidence of L-dopa–induced peak dyskinesias in animal models.[14,15] Several clinical studies with positive outcomes in patients have also shown normalized metabolic and brain functional activity throughout the basal ganglia after DA neural transplantation.[4,5]

PET and carbon-11–labeled 2B-carbomethoxy-3B-(4-fluorophenyl)tropane (11C-CFT) can be used to visualize and quantify striatal presynaptic DATs in PD patients and degeneration models. In one such study, unilateral lesions of the SN DA system in rodents initially reduced the binding ratio to 15 to 35% of the intact side. After fetal DA neuronal transplantation, behavioral recovery occurred gradually and was first seen when the 11C-CFT binding ratio had increased above 35–50% of the intact side, demonstrating a threshold for functional recovery in the lesioned nigrostriatal system after neural transplantation that fits our understanding of the normal DA motor system requirements.[33] Also, critically, DAT control of reuptake, as well as autoregulation of DA release and metabolism by grafted DA neurons, has been shown by in vivo microdialysis in the striatum.[15,16] Infusion of a nonselective DA agonist (apomorphine) almost abolishes endogenous DA release in the grafted striatum,[16,34] showing a near-normal autoregulation of DA levels by the implanted DA neurons. The formation of effective DA terminals and synapses with adequate DA release and control has been determined in transplanted rodents after dyskinesia-inducing L-dopa injections.[14,35] L-Dopa–induced peak-dose dyskinesias in nonhuman primates are also reduced after fetal DA cell transplantation (H. Widner, personal communication), and in some PD patients after fetal nigral grafting.[9] These

data indicate that DA levels within the transplanted striatum can be regulated in a functional manner by correctly transplanted DA neurons if they act as the normal functional cellular regulators of DA neurotransmission in their normal target areas.[8,13,15,31]

Technical Developments for Regeneration of Neural Function and Pathways in Parkinson's Disease Patients

A number of critical variables determine the outcome of cell therapy for PD. Most of these variables have not been systematically evaluated in primates or patients. This is primarily a reflection of the technical and cellular challenges that new cell therapies present. Small exploratory, and lately placebo-controlled, trials have shown encouraging results in some PD patients (validated by analytic imaging and postmortem studies), but they have also highlighted problems in donor cell preparation resulting in highly variable individual responses. The preparations of donor cells and associated procedures are critically important in cell-based therapies, and there are major differences in cell preparations for transplantation in PD across clinical trials to date. The most successful method so far involves freshly dissected fetal tissue pieces (minute cubic millimeter pieces) that can be treated with proteolytic enzymes, then dissociated into a cell suspension.[3–5,7] Other procedures have included untreated tissue pieces, minced from the ventral mesencephalon of aborted fetuses.[9,36,37] A third type, the so-called noodle technique,[1] includes a long-term cell culture step. Such culture steps may alter the cells and select for cell types that are different than the populations obtained by fresh preparations. Furthermore, the "noodles" that were preselected for grafting contained the greatest levels of DA as measured by high-performance liquid chromatography, and may reflect abnormal DA biosynthesis and release. These three different techniques may produce different graft results.[3–5,7]

When fetal ventral midbrain is dissected before transplantation, most published protocols do not make any distinction between the DA neurons residing in the ventral tegmental area (VTA) (A10) and those in the substantia nigra pars compacta (SNc) (A9).[1,38,39] These two midbrain subpopulations of DA neurons express different gene profiles and phenotypes,[40] including different levels of the DAT,[41] project to different areas,[42] and show different responses to growth factors.[40,43] In addition, PD patients show a relative sparing of DA neurons in the VTA compared with the SNc, indicating that the VTA DA neurons are significantly less vulnerable compared with their SNc counterparts.[44–47] When such VTA/A10 neurons are implanted into the putamen of PD patients, they may either fail to innervate the target region or form inappropriate connections because these are not their normal targets.[2,7,31,48] In addition, differences in DAT expression between subpopulations of DA neurons[41,49,50] may also result in abnormal DA release in the putamen[51] and uptake patterns that could cause suboptimal DA transmission. Another potential reason for uncontrolled motor responses after transplantation is that the location and size of tissue pieces implanted may, in some cases, create small lesions in the putamen with subsequent dysregulation of the GABAergic output neurons (as seen in Huntington's chorea). This theory is supported by the fact that small lesions in the striatum of primates (modeling Huntington's disease) make these animals severely dyskinetic in response to DA agonist treatment[52–54]; this may also occur in a situation of diffuse release from inappropriate DA neurons placed as tissue pieces in the lateral putamen.[1,6] Supporting such a view is the less frequent and less intense adverse effects from transplanted fetal DA cells to humans, when donor cells are prepared and placed as liquid cell suspension into PD putamen.[55] Such grafts typically reach less destructive size.[7]

Finally, of significance, the importance of appropriate cellular and biochemical characteristics of transplanted DA cells has also been shown by behavioral experiments. In a rodent model of parkinsonism, recovery from movement asymmetry is correlated with the rate of cellular maturation of the *donor* species.[13,31] Embryonic stem (ES) cells generating DA neurons also abide by such biological principles. Multiple anatomical analyses have demonstrated that specific axon guidance and cell differentiation factors remain in the adult and degenerating brain, providing growth and axonal guidance cues for fetal or ES cells.[56–58]

Deriving or Regenerating Optimal Stem or Progenitor Dopamine Neurons for Parkinson's Disease Patients

Recent discoveries elucidating the cell biology of DA neurons allow both sequential and parallel strategies for protection of remaining cells and treating with new cells to restore function in PD patients. The rapidly developing understanding of pathological mechanisms in PD and the life cycle of the DA neuron from stem cells, via progenitor cells, to adult and later aging DA neurons provides reasonable opportunities for new interventions to reverse the effects of this disease. Nonetheless, detailed knowledge is necessary about the following factors: (1) the appropriate neurons, (2) correct brain locations for repair, and (3) responsive PD patients for these regeneration therapies to become successful.

During midbrain fetal development, newborn DA cells migrate into their final positions and send projections to targets in the emerging striatum and cortex. The DA neuron is induced by cell signals emanating from factors released around the ventral midbrain from

neural ectoderm or ES cells.[59] Remarkably, of the three original germ layers, the neural ectoderm and the brain develop partly through a so-called default pathway.[60] Similarly, after ES or embryoid body cells have become neural precursors, under certain conditions, many will spontaneously acquire a neuronal midbrain-hindbrain identity, including DA cell specificity.[61–63] When there is an absence or blockade of mesodermal and endodermal signals, the ES cells will first become primitive and eventually differentiated neuronal cell types. This process was described initially in frogs and in knock-out gene cell systems, where bone morphogenetic protein and activin receptors (including SMAD pathways) were implicated.[60] One of the cell types that is derived from such spontaneous neural differentiation is the DA cell type.[61,62,64,65] The actual genetic subprograms controlling the midbrain DA cell-type development appear to include sequential and parallel action of several transcription factors. After proliferation in the ventricular zone, neuroblasts will migrate ventrally toward the central and lateral ventral mesencephalon. This fibroblast growth factor-8–rich zone, as well as sonic hedgehog protein,[59] induces a sequence and group of transcription factors in the progenitor cells, such as LMX1A[66,67] enzymes that establish most of the cell character of the midbrain neurons that share a DA phenotype. Engrail genes, Nurr1 and PitX3, are some of the critical transcription factors of the set that determines the final adult phenotype. Nurr1 certainly activates most of the transmitter-related genes and some of the trophic signaling pathways,[68] but PitX3 is also critical for the construction and survival of DA neurons that can reach motor control regions of the caudate-putamen.[66] In fact, the functional absence of PitX3 during development will cause the A9 DA neuron to become nonviable or nonfunctional in the midbrain, whereas the A10 VTA neuron can still survive, grow, and function in the limbic circuitry.

Can Stem Cell Biology Research Help Parkinson's Disease Patients?

In several experiments, the ES cell–derived DA neurons have been shown to reinnervate the brain and restore DA transmission. This was shown by DA release, behavioral correction of a motor syndrome, and functional integration (blood flow and restored activity in cerebral cortex). More recent works confirmed these findings and also enhanced the design of ES cells to more readily and reliably generate necessary fetal DA cells as replacement donor cells in PD.[19,62,69,70] Therefore, starting at the genetic level, a number of genes related to the development or control of DA precursor proliferation, identity, and specialization (eg, sonic hedgehog protein, LMX1b, Pitx3, Nurr-1, Engrail genes) must act in concert with other transcription factors to activate specific transmitter enzymes (eg, tyrosine hydroxylase [TH], DAT, and dopa-decarboxylase) in DA neurons to rationally derive DA neurons from ES cells.[71–73] An alternate route for cell repair is the possibility of manipulating inherent neurogenesis in the adult brain.[74] For example, in the adult brain, neural precursor cells are embedded in the subventricular zone and are capable of migration and differentiation into different neural cell types.[75] These neural progenitor cells can be expanded and studied, possibly even as a neuronal repair tool. The expansion of subventricular zone neuroprecursor cells is stimulated by delivery of basic fibroblast growth factor, brain-derived neurotrophic factor (BDNF),[76] noggin,[77] ciliary neurotrophic factor,[78] or epidermal growth factor[79] to the cerebrospinal fluid. Migration into the parenchyma can be stimulated by infusion of transforming growth factor-α into a target region.[80,81] Neuronal differentiation from precursors is enhanced by glial cell line–derived neurotrophic factor (GDNF), BDNF,[82] and natural bone morphogenetic protein receptor antagonists.[77] Expression of a DA phenotype can be driven by transcription factors such as Pitx3[83] and Nurr1, which drive genes of the full DA neuronal phenotype such as TH, DAT, and components of trophic factor receptors.[84–87] Furthermore, release of GDNF or BDNF by genetically modified cells in the caudate-putamen can increase survival of precursor cells and of DA neurons in the SN after lesions,[88,89] and the programmed cell death process can also be temporarily suspended by antiapoptotic factors (eg, XIAP).[90,91] Interestingly, for novel therapies, genetically modified cells can also serve as biological pumps to produce growth factors that sustain neurons on sites in the striatum or projecting DA neurons from the SN.[88,89,92–94] However, what has now become clear is that no procedure to date has induced reliable neurogenesis in the SN of the adult rat or monkey.

A Perspective on Remaining Challenges of Developing Novel Therapeutics Using Living Cells as Agents for Parkinson's Disease

Given the major efforts placed on understanding biological and therapeutic factors involved, there is renewed hope for novel cell-based procedures in PD. However, even for the most promising candidate neurological diseases for cell therapy (such as PD), where neuronal repair is conceptually and practically proved to work in some patients, we believe there is an underestimation of the biological, technical, and cellular sophistication required to make the treatment reliable and safe. Although neural repair for PD may be a more challenging therapeutic development than cell-based therapy for type 1 diabetes, that experience may provide insight and perspective. Several decades ago, there were reports of successful cell-based delivery (pancreatic islets) of insulin in diabetic rats (eg, Warnock and col-

leagues[95]). The new technology was quickly translated into clinical trials, where islet cells did not survive or function effectively. This methodology was retested in the late 1990s with more success. Several hundred patients with diabetes now have functioning islet-cell transplants, with limited need for insulin injections, and reduced risk for insulin drug delivery.[96] By analogy, generating, selecting, and transferring appropriate neurons and glia is challenging both from a technical and biological standpoint, and thus will require much work to optimize protocols and clinical procedures.

In a realistic future perspective, the most important challenges are to optimize functional effects and obviate grafted related side effects by providing physiologically appropriate DA release, whereas trophically maintaining connections in the nigrostriatal and related system. Notably, transplanted fetal DA neurons have been shown to grow synaptic connections with mature host striatal neurons.[20,21] Current evidence indicates that DA-mediated regulation of striatal neuronal and network interactions is of critical importance for normal motor control, and that if DA release is not regulated, glutamatergic synapses are less able to provide normal control of striatal GABAergic output neurons.[24,25,97] Physiological DA function is achieved by normal DA release and uptake. Implanted cells that express the complete set of feedback elements required to regulate the release and uptake of DA may function well.[15,16,31,32] Fetal DA transplants have been shown to reduce the incidence of L-dopa–induced dyskinesias in 6-hydroxydopamine (6-OHDA)–lesioned rodents.[14] Similarly, L-dopa–induced dyskinesias in nonhuman primates also are reduced after fetal DA cell transplantation. In several cases, after transplantation of fetal mesencephalic cell mixtures, PD patients have been able to eliminate their DA medication altogether.[3] However, such cases are rare, and although transplants can survive for at least 15 years, there is limited understanding of optimal cell transfer parameters and patient selection. For the patients who have received cell transplants in Sweden, Canada, and the United States, it is not clear why some transplants work whereas others do not.[1,3,6]

Current clinical studies have noted several mild-to-severe adverse effects caused by the implantation of DA cells. The primary problem has been the development of "off"-medication dyskinesias in some patients with otherwise good DA graft survival.[1,6] The mechanism(s) that underlie these "off"-medication dyskinesias are unknown. It has been shown that despite good graft viability, there is suboptimal reinnervation of the host target areas and suboptimal DA cell composition of the transplanted and surviving grafts. In addition, some transplanted neurons may, in effect, produce too much and/or continuous DA for the denervated striatum, thereby producing dyskinesia during "off" periods, analogous to the well-known DA-induced dyskinesia during patients' "on" periods. The normal midbrain adult DA neuron is a highly specialized functional unit that has evolved to release DA and be sustained in a physiological network with target neurons and structures. In that way, it is different from a transmitter pump or any other cell that could release and supply DA. More optimal cell therapy for PD may therefore include cells that function like A9 DA neurons, which are, indeed, specialized for function in putamen (limiting A10 cell types that work in mesolimbic and cortical settings). Finally, there are risks for other side effects and dyskinesia that depend on the final size and location of transplants, because posterior putamen lesions when exposed to DA will generate dyskinesias (perhaps by space-occupying lesions such as a grafts).[52] These latest exploratory clinical transplantation studies have found some evidence that the stage of the disease and responsiveness to L-dopa will influence the outcome of the transplantation procedures and the function of the new DA cells. It has become apparent that recent controlled clinical trials in PD demonstrate that the severity of PD influences transplant effects, or lack thereof. Less impaired patients (defined as <50 points on UPDRS during "off" medication) had significantly better or earlier functional responses to fetal grafts relative to placebo operated patients, whereas more severe patients (≤ 50 points on UPDRS during "off" medication) did not.[6] However, in a recent study including a severe PD case (UPDRS $>$ 80), there were also marked functional improvements that were associated with excellent graft innervation of the caudate-putamen.[7] In summary, in future cell therapy attempts for PD, it is probably necessary to identify responsive patients, and to obtain appropriate neurons for successful and optimal outcomes to occur. Such efforts also require information about optimal surgical and procedural applications, including cell implantation locations and cell dosage, cell preparations, and trophic factors, immunological and connectivity variables to allow functional reconstitution neurocircuitry. The current state-of-the-art cell therapy for PD will, therefore, require transfer of appropriate and selectively placed DA neurons, glia, or both in patients who are responsive to DA substitution therapy. Possibly, the emergence of selected neural or stem cell–generated DA neurons in concert with improved surgical and technical approaches may provide an improved cell therapy intervention.

Gene Therapy for Parkinson's Disease

The use of trophic factors or other therapeutic proteins has the ultimate goal of preventing the degeneration of existing host systems, strengthening their synaptic arrangement, and/or enhancing their DA phenotype. A number of approaches have been attempted to modify

host neural circuitry including the use of enzyme delivery to enhance DA production or the effectiveness of L-dopa, the delivery of inhibitory transmitter enzymes to reverse overactive brain sites within basal ganglia circuitry, and the delivery of trophic factors to the central nervous system. For trophic factors, it is clear that many trophic proteins potently protect neuronal cell types that are selectively vulnerable in PD from degenerating and augment the DA phenotype in the host nigrostriatal system. Trophic factors have an enormous history of biological investigation and a storied clinical history. However, identifying the appropriate trophic factor to sustain nigrostriatal systems is only part of the challenge. Identifying means to deliver trophic factors effectively, efficiently, and in a site-specific manner has been an equally compelling challenge. Infusion of trophic proteins has been tested clinically but, as discussed later, is fraught with technical problems. Gene therapy is a solid approach to deliver therapeutic molecules to the human brain. DNA is inserted into host cells using viral vectors encoding for specific proteins, and these cells then make the desired protein, theoretically, for the lifetime of that cell.

In attempts to mimic L-dopa production in cells, viral vectors were used in the late 1980s to transduce fibroblasts with TH.[98] This resulted in a modest reversal of rotational deficits in 6-OHDA–lesioned rats but had only limited and transient transgene expression. Fibroblasts were tested initially in many neural systems with the hope that, ultimately, patients' own fibroblasts could be used to deliver therapeutic proteins without the concern of developing an immune response. In one example of such a study, BDNF-releasing fibroblasts were able to prevent (3-methyl-phosphinico-propionic acid) MPP+-induced nigrostriatal degeneration in the rat.[89] The first in vivo gene therapy studies using viral vectors as delivery systems used an adenovirus (Ad) vector. However, initial studies attempting to directly deliver TH to the striatum also failed to produce a complete reversal of motor dysfunction using this model, probably because of the use of a single gene to produce DA and the type of vector used.[99] Subsequent studies utilizing bicistronic viral vectors to transfer guanosine triphosphate cyclohydrolase and aromatic amino acid decarboxylase together with TH proved to be more effective in reversing motor dysfunction in both rodents and primates.[71,100–102] This combination provided benefits in DA-lesioned rats that were observable for 12 months, but striatal DA levels were only modestly increased, demonstrating the need for long-term expression of molecules after transfection.[98]

The use of early generations of viral vectors failed to provide long-term gene expression because they induced a strong inflammatory response.[103,104] Ad- and herpes simplex virus (HSV)–based vectors have been modified to make them safer and more efficient for transgene expression to avoid this problem.[105,106] In initial studies, transgene expression rarely lasted for more than 1 month because of immune responses within host cells. The development of gutless Ad has minimized their immunogenicity, but because Ad is prevalent among humans, the risks for an immune reaction might still be high. Other viral vectors, such as adeno-associated virus (AAV) and lentivirus, have proved to be more suitable for human use. Initially, constructs of AAV had a low level of transgene expression, and lentivirus had the stigma of being associated with human immunodeficiency virus. Lentivirus was initially considered by some to be the vector of choice for clinical trials because of its robust expression profile and lack of immunotoxicity. However, there has been a remarkable improvement in the safety, transfection rates, and duration of transgene expression of AAV vectors. Novel AAV serotypes have been shown to have robust long-term gene expression (more than 2 years) in rats and monkeys, and appear equal to lentivirus for those characteristics deemed important for gene delivery: long-term expression, neuronal preference, and absence of immunogenicity and inflammation. A significant difference between AAV and lentivirus is that the AAV itself is retrogradely transported whereas lentivirus is not, although the functional relevance of this difference remains unclear. Currently, only AAV vectors have been approved for clinical trials in PD.

Gene Therapy for the Delivery of Trophic Factors

Because PD is a long-term degenerative disorder and gene delivery requires a surgical intervention, a single surgical procedure that provides long-term or permanent benefits is desirable. Gene delivery of therapeutic DA enzymes has not produced results in animal models of PD sufficient to suggest that they might reverse the symptoms of the disease, and this strategy would not be expected to influence disease progression.

Currently, trophic factors are the only transferable genes that are thought to have the capability to provide neuroprotective or restorative effects. The transfer of neurotrophic factor DNA can result in the expression of large numbers of active molecules, depending on the viral vector used. Initially, the effects of trophic molecules on DA nigrostriatal neurons were examined by direct bolus administrations to animal models of PD. In these studies, many DA trophic molecules, such as epidermal growth factor, basic fibroblast growth factor, BDNF, sonic hedgehog, neurturin (NTN), and GDNF prevent DA neuronal degeneration (see Collier and Sortwell[107] for review). Of these, GDNF has proved to be the most potent and the most consistent in producing antiparkinsonian benefits in animal models, and was the first trophic factor to be tested in patients with PD.

The successful reversal of nigrostriatal degeneration in animal model experiments led to the initiation of open-label and double-blind studies examining the safety and efficacy of intraventricular injections of GDNF in PD.[108,109] In these studies, GDNF failed to improve the symptoms of PD, and serious adverse effects were noted. These results should not have been unexpected because even chronic intraventricular infusions of GDNF results in only trivial distribution of the GDNF protein beyond the ventricular ependyma. Thus, this approach method failed to deliver the trophic factor to the nigral neurons.[108] The challenge of finding the correct trophic factor was not combined with a realistic effort to find an appropriate delivery system; thus, this approach failed. Indeed, these initial studies demonstrated that despite the potency of GDNF to enhance the DA nigrostriatal system in the laboratory, poor delivery methods result in poor outcomes. As a general rule, intraventricular delivery for any trophic factor should be avoided because of numerous adverse effects and poor penetration of the trophic factor into brain parenchyma (eg, Eriksdotter and coauthors[110]).

After the failure of intracerebroventricular administration of GDNF to affect PD, two open-label trials infused GDNF chronically through a catheter into the postcommissural putamen of PD patients.[111,112] As opposed to the intraventricular delivery route, infusion of GDNF into the postcommissural putamen has a stronger theoretical basis. DA loss in PD is greatest in the postcommissural putamen relative to the anterior putamen or caudate nucleus. In addition, the postcommissural putamen is anatomically connected with motor cortex, whereas the anterior putamen and caudate nucleus have reciprocal connections with nonmotor association cortices. Both studies reported sustained functional benefit, and one trial also reported increases in fluorodopa uptake on PET scanning that progressed through 1 year. The only patient who came to autopsy, however, showed only marginal changes in TH immunoreactivity (IR), and fluorodopa PET changes were primarily at the catheter tip.[113]

Recently, a multicenter, double-blind, placebo-controlled trial was completed that investigated the safety and efficacy of bilateral intraputamenal GDNF in patients with PD.[114] The trial failed to meet its primary end point (improvement on the UPDRS in the "off" state) at an interim analysis. In addition, neutralizing antibodies against GDNF were detected in approximately 10% of patients, and some monkeys infused with high doses GDNF displayed profound cerebellar degeneration. As a consequence of these findings, the study was prematurely terminated. Based on the results of this study, one cannot conclude that GDNF will be effective in PD, despite the promising laboratory profile and open-label studies using this trophic factor. It is important to bear in mind that the model systems in which GDNF and other putative neuroprotective agents are tested may not reflect the etiopathogenesis of PD, and results in the laboratory after infusion of GDNF may not be transferable to the clinic. A possible explanation for the difference between the open-label and double-blind studies could include a placebo effect and experimenter bias, which are commonly observed in clinical trials. Alternatively, there were potentially critical methodological differences in the open-label and double-blind trials.[115] These included dosage used (greater dosages ultimately used in the open-label trials), catheter design (a thicker catheter [Bristol] and multiport catheter [Kentucky] in the open-label studies), and rate of trophic factor delivery (convection-enhanced delivery in the open-label trials and nonconvection-enhanced delivery in the double-blind trial). Limited benefit may also have been related to the use of a catheter with point delivery of GDNF and inadequate distribution of the GDNF protein throughout the target area. Indeed, it is likely that when the infusion parameters are all considered in total, it is unlikely that appreciable levels of GDNF were delivered throughout the putamen in the double-blind trial. In addition, antibodies might have developed because of leakage of protein into the abdomen during refilling of the pump or the use of glycosylated rather than native GDNF. The cerebellar damage seen in monkeys and antibodies in PD patients may also have been related to the use of a thin catheter with nonconvection delivery resulting in GDNF backing up outside of the catheter and then flowing over the brain convexities to the cerebellum. The functional effect of the presence of antibodies to GDNF is unclear, and to date, no related side effects have been reported in patients who received intraputamenal GDNF. Similarly, no patient who received intraputamenal GDNF has been reported to display signs of cerebellar degeneration. Gene therapy offers a major advantage here because it permits diffuse delivery of the protein throughout the target region, is less likely to leak into the cerebrospinal fluid, does not have the hardware problems associated with catheters and pumps, and does not require frequent pump refills over long periods. Again, gene therapy approaches more likely meet the challenge of delivering therapeutic proteins to the human brain than infusions of native protein.

Gene Delivery of Glial Cell Line–Derived Neurotrophic Factor Protects against 6-Hydroxydopamine

The preclinical data supporting the gene delivery of therapeutic trophic factors are strong. Numerous studies have shown that gene delivery of GDNF before neurotoxin administration can provide neuroprotection and neuroaugmentation for degenerating neurons of

the SN. Initial studies, pioneered by Martha Bohn and colleagues, used an Ad to deliver GDNF before 6-OHDA lesions in rats.[104] They found that the supranigral administration of Ad-GDNF before a striatal 6-OHDA lesion protected 75% of retrogradely labeled nigral DA neurons. It should be noted, however, that striatal DA levels were unchanged. In contrast, injection of Ad-GDNF into the rat striatum resulted in enhanced striatal TH IR and functional improvement on amphetamine-induced rotation.[103] Significantly greater numbers of DA neurons were observed in the SNc than in control animals, although again complete protection was not achieved. These two studies illustrate that the site-specific administration of GDNF is critical to the degree of protection of the nigrostriatal pathway that can be obtained after 6-OHDA lesions. In support of this concept, Kirik and coworkers[116] compared the degree of neuroprotection seen with GDNF delivery by intrastriatal and intranigral routes in the 6-OHDA rat. GDNF was able to preserve motor function only when it was delivered to the striatum where it was able to preserve striatal TH innervation. Nigral delivery prevented nigral cell death but did not influence striatal reinnervation or functional recovery. Bohn and colleagues repeated their initial study using Ad-GDNF, but now injected the vector intrastriatally after the creation of a partial 6-OHDA lesion.[117] In this model, behavioral asymmetry was reduced and nigral TH-IR cell numbers were protected, but close examination of the striatum demonstrated no improvement in the density of TH-IR fibers. In a subsequent study, Ad-GDNF delivery to the nigra or striatum before a striatal 6-OHDA lesion was examined in aged rats, and again functional benefit was observed only in animals receiving striatal Ad-GDNF.[118] This concept is supported in monkey models where functional recovery is also not seen without preservation of striatal DA.[119] Thus, it can be concluded that if a gene delivery for GDNF is to be pursued clinically for PD, intrastriatal delivery, and recovery of striatal DA, is required for functional benefit.

Ad was the first vector utilized for gene transfer studies in PD models. However, this vector induces severe inflammatory responses in the area of injection. Thus, in the early studies by Choi-Lundberg and coworkers[104] and Bilang-Bleuel and researchers,[103] both groups reported adverse cellular reactions to the administration of "first-generation" Ad, regardless of whether trophic factor or control DNA sequence was transfected by the vector. As an alternative to Ad, HSV has been tested in an effort to transfer GDNF DNA, in an attempt to protect cells from neurotoxin-induced degeneration.[120] Striatal administration into 6-OHDA rat striatum of HSV vectors encoding GDNF reduced amphetamine-induced rotational behavior. Fluorogold and TH-positive nigral cell counts were doubled in HSV-GDNF–treated animals, and coadministration of HSV-GDNF with HSV delivery of the antiapoptotic gene Bcl-2 resulted in even greater neuroprotection. However, this study failed to examine for any cytotoxic effects after recombinant HSV administration and looked for only transgene expression at 1 week after transfection. Thus, conclusions about the suitability of HSV for further gene therapy experiments from this study cannot be made. However, marked inflammatory responses with HSV have been noted in other experiments,[121,122] leading to the conclusion that this is not likely to be a viable vector for use in patients.

Soon after the initial proof-of-concept experiments with Ad-GDNF, the use of AAV vectors increased markedly, as they offered the ability to circumvent the Ad-mediated immunogenic response. Three particular studies highlighted how GDNF, when expressed after transfection by an AAV, can prevent DA cell death in the SNc and reduce behavioral deficits in 6-OHDA–lesioned rats.[123–125] Once again, the site of GDNF delivery and the augmentation of striatal DA were found to be critical for functional benefits.[123] Stable expression of GDNF was achieved for more than 6 months in 6-OHDA–lesioned rats when AAV-GDNF was injected into the SN, but no protection of the striatal DA-IR terminals was observed, and behavioral recovery did not occur. In contrast, when AAV-GDNF was administered to the striatum of rats before a 6-OHDA lesion, the DA nigrostriatal pathway was completely protected; this was accompanied by a reduction in amphetamine-induced asymmetry.

Lentivirus is another vector that induces little inflammation, preferentially infects neurons, and has been studied in the rat 6-OHDA model of nigrostriatal degeneration. Lentiviruses of human or equine origin have both been tested, and both have been demonstrated to be effective in providing neuroprotection in animal models of PD. When GDNF is delivered 4 weeks before a 6-OHDA lesion into the median forebrain bundle in rats by an equine infectious anemia virus, a relative of human lentivirus, preservation of nondrug-induced complex behaviors has been observed. This includes such behavioral assays as operant, corridor, staircase, stepping, and cylinder task.[126] GDNF gene transfer by a lentivirus, 3 weeks before a striatal injection of 6-OHDA, also preserved nigral neurons in comparison with rats receiving a control vector.[127] Intense sprouting of fibers was observed in the medial parts of the striatum, globus pallidus, and entopeduncular nucleus corresponding to the presence of GDNF IR. This indicated that the GDNF protein was transported and had remote effects because lentivirus itself is incapable of either retrograde or anterograde transport. Georgievska and colleagues[128] repeated this experiment using a single titer of lenti-GDNF administered into the striatum, and similarly showed

preservation of TH-positive cells in the nigra and correction of amphetamine-induced behavioral abnormalities. In addition, using fluorogold retrograde labeling from the striatum or AAV-GFP anterograde labeling from the nigra, this group demonstrated that lenti-GDNF administration protected axonal projections of the SNc into the striatum. However, the authors note reduced TH fiber IR in the striatum. They speculate that this was due to downregulation of TH after high levels of GDNF expression. They also observed that nondrug-induced motor asymmetry was unchanged by lenti-GDNF administration, and attributed this to aberrant sprouting of TH-positive fibers from the striatum to the globus pallidus, entopeduncular nucleus, and SN. They then examined whether this downregulation of TH expression was replicated in naive rats, and observed that treatment with lenti-GDNF into the striatum or nigra resulted in 60 to 70% decreases in TH expression and TH messenger RNA levels. L-Dopa production remained at normal levels, indicating that GDNF might have increased the phosphorylation of TH, thus increasing its activity.[129,130] Downregulation of TH after gene delivery of GDNF or its functional analogue NTN in rats has been seen by others but appears to be species specific because gene delivery of GDNF does not cause a similar downregulation in primates. In contrast, as detailed later, gene delivery of GDNF consistently upregulates TH in a number of nonhuman primate studies.[119,131]

Although gene delivery of GDNF protects against 6-OHDA, 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP), and age-related changes in the nigrostriatal system, lenti-GDNF delivery to the supranigral region did not protect SNc neurons in rats from toxicity induced by lentiviral delivery of A30P α-synuclein mutation.[132] Although excellent expression of GDNF was observed in this study, gene delivery of this trophic factor did not prevent the loss of nigrostriatal neurons. It should be noted that levels of α-synuclein achieved after lentiviral administration are supraphysiological, and GDNF trophism may not be able to overcome such high levels of this abnormal protein. However, the failure for GDNF to provide neuroprotection in this model needs to be considered seriously with respect to its potential value in PD because mutant α-synuclein is associated with a familial form of PD, and α-synuclein aggregation in the SN identifies the disease.

Neuroprotection by Gene Delivery of Glial Cell Line–Derived Neurotrophic Factor in Primate Models of Parkinson's Disease

To date, the MPTP model in nonhuman primates remains the best animal model for testing new therapies for PD. Being relatively close phylogenetically to humans, monkeys provide natural advantages for the evaluation of novel therapeutic strategies. These include an advanced behavioral repertoire to allow for motor testing in a manner more analogous to humans. The structure of the primate brain also closely resembles the human brain, with a clear separation of the caudate nucleus and putamen, and delineation of the globus pallidus into internal and external segments. In addition, MPTP causes a parkinsonian syndrome with selective loss of SNc DA neurons in both monkeys and humans in a pattern that resembles PD. Accordingly, we have tested the safety and efficacy of gene delivery in MPTP-treated monkeys before the initiation of clinical trials in PD patients.

It should be noted that the MPTP model also has significant limitations. Principal among them is the fact that MPTP affects only the nigrostriatal system and is an acute toxin that may not specifically reflect the cause or pathogenesis of PD. Still, the cardinal features of PD are mediated by the nigrostriatal system, and for these the MPTP monkey provides an extremely useful model. About neuroprotection/restoration studies, there is also the problem that each monkey is differentially sensitive to MPTP, and special consideration must be given to experimental design issues to establish the veracity of any functional effect and the responsible mechanisms. For these reasons, some groups use 6-OHDA rather than MPTP in nonhuman primate studies because this toxin provides a more consistent lesion across animals.[133] Indeed, when delivered via stereotaxic administrations into the SN or medial forebrain bundle of smaller primates, such as marmosets, 6-OHDA virtually guarantees the destruction of the nigrostriatal pathway. AAV-GDNF was injected into the striatum and SN of marmosets, before an injection of 6-OHDA into the median forebrain bundle.[133] This treatment provided modest protection of DA cells in the SN, although motor function assessed by a modified clinical rating scale was reversed to prelesion levels after 5 weeks. Also (+)-amphetamine–induced rotational behavior and head positional bias was attenuated at all time points after lesion, indicating that AAV-transfected GDNF protected motor function in the primates.

Neurorestoration of Nigrostriatal Function in Aged Nonhuman Primates by Gene Delivery of Glial Cell Line–Derived Neurotrophic Factor

Aging is the most well-established risk factor for the development of PD. It is well established that as a function of age, there is a phenotypic loss of DA in the striatum and the SN, although there is no structural loss of nigral neurons.[134,135] This age-related loss of nigrostriatal DA is associated with the age-related accumulation of α-synuclein.[136] Age-related pathology in the substantia used to be thought to occur in a pattern that differed from that seen in PD. However, a recent reevaluation of the pattern of age-related degenerative

processes seen in the SN of young, middle-aged, and aged monkeys proved this was not true because diminished TH expression and markers of proteasome dysfunction are seen predominantly in the ventral tier of the nigra, followed by the dorsal tier and VTA, exactly the same pattern as is seen in PD.[137] These data, coupled by the age-related motor dysfunction seen in aged monkeys and aged humans, make testing gene therapy approaches of trophic factors in aged monkeys a crucial preclinical step before initiating phase 1 clinical trials. We injected lenti-GDNF into the caudate and putamen of aged monkeys.[119] Three months later, these monkeys displayed robust increases in fluorodopa uptake on PET scan ipsilateral to the injections. After death, postmortem evaluations demonstrated robust transgene expression, highly significant increases in striatal DA, and hypertrophy and increased TH optical density within nigral neurons. These data clearly indicate that the aged primate nervous system is receptive and responsive to gene delivery of GDNF.

Neurorestoration by Glial Cell Line–Derived Neurotrophic Factor Gene Delivery after MPTP Treatment in Primates

MPTP-lesioned animals display clear motor disabilities and quantifiable neurochemical deficits, and offer an excellent model in which to assess the functional restoration of DA nigrostriatal neurotransmission by gene therapy. In collaboration with Patrick Aebischer's group, who generated a lentivirus expressing GDNF, Kordower's laboratory[119] injected lenti-GDNF or lenti-β-gal into the caudate, putamen, and SN of young adult rhesus monkeys 1 week after a unilateral intracarotid MPTP lesion. Importantly, the administration of lenti-GDNF reduced motor disability over the next 3 months. Dexterity, as measured by an objective hand reach task, was improved by the administration of lenti-GDNF compared with control animals. A 300% increase in striatal fluorodopa uptake in the putamen was seen in PET scans of lenti-GDNF–treated animals taken at 3 months after vector injection compared with control animals. Robust GDNF IR was consistently observed at injection sites coupled with increased TH IR and DA/homovanillic acid content. IR for TH in the striatum was significantly greater in lenti-GDNF–treated monkeys compared with control monkeys, mimicked the profile of GDNF staining in the caudate and putamen, and correlated with improvement in dexterity. IR for GDNF was also observed in the globus pallidus and SN pars reticulata, indicating anterograde transport. An increase in the number of TH-positive cells and cell intensity was observed in the SN after lenti-GDNF administration in comparison with controls and to the intact hemisphere relative to controls. The lentiviral construct also caused no immunogenicity, as determined by CD45, CD3, and CD8 immunohistochemistry.

Examination of the effect of lenti-GDNF in the striatum of aged monkeys and unilaterally MPTP-lesioned monkeys show additional findings of interest. MPTP treatment alone increased the numbers of intrinsic DA neurons in the striatum, and the administration of lenti-GDNF further increased the intrinsic striatal TH-IR cell counts by sevenfold.[138] There was also a large increase in TH and DAT IR in aged monkeys that correlated with GDNF expression, suggesting an autotrophic effect. These results suggest that lenti-GDNF is effective regardless of the level of downregulation of the DA phenotype. We also treated two young unlesioned rhesus monkeys with lenti-GDNF injected into the right caudate and putamen and left SNc, and looked at 8 months for gene expression. There were many GDNF-IR cells in the nigra, and tissue levels of 2.5 to 3.5ng/mg GDNF were found in the caudate and putamen, proving long-term transgene expression.

Other Trophic Factors as an Alternative to Glial Cell Line–Derived Neurotrophic Factor

NTN was discovered in 1996 in Jeffrey Milbrandt's laboratory and identified as the second trophic factor in the GDNF family of ligands. Its amino acid sequence shares a 42% homology to GDNF, and they share similar signaling pathways.[139] Both GDNF and NTN signal through the RET receptor, but GDNF preferentially binds to growth factor receptor α1 (GFRα1), whereas NTN prefers GFRα2.[140–142] Because there are no GFRα2 receptors in the striatum, this would suggest that the delivery of NTN would not be effective. However, at the levels achieved after gene delivery, NTN binds to GFRα1 and provides potent neuroprotection for nigrostriatal neurons. NTN delivery using a lentivirus to four sites in the rat striatum, 2 weeks before a 6-OHDA lesion, resulted in the protection of more than 90% of the nigral TH-IR cells.[143] However, DA striatal denervation was not prevented, and amphetamine-induced rotational behavior was not ameliorated. The Kordower laboratory, in collaboration with Ceregene, has performed a series of experiments in rats and monkeys that indicate that NTN can provide structural and functional protection of nigrostriatal neurons. When injected into 6-OHDA–lesioned rats, AAV2-NTN (also called CERE-120) prevents motor deficits, as well as the loss of nigral neurons, with a potency equal to that seen with AAV2-GDNF.[144] We have found that when injected into the striatum of young adult monkeys, AAV2-NTN robustly augments striatal TH expression in a dose-dependent manner[145] (Dass and colleagues, Soc Neurosci, abstract 2004). Transgene gene expression is exceptional, and numerous nigral neurons demonstrate

retrogradely transmitted NTN IR. The injections of AAV2-NTN induced a profound upregulation of phospho-erk, indicating the initiation of critical signaling in the mitogen-activated protein kinase trophic factor cascade. Similar results were found in aged monkeys.[146] Increased fluorodopa expression was seen 4 and 8 months after AAV2-NTN treatment. At death, these imaging parameters were confirmed by an increase in optical density of striatal TH IR with a pattern similar to that found in young adult monkeys. In addition, gene delivery of this trophic factor activated phospho-erk. Finally, AAV2-NTN delivery to the striatum and SN prevented the emergence of motor symptoms that are destined to occur after MPTP administration for up to 10 months.[147] In this model, gene delivery of NTN prevented the loss of TH-IR nigral neurons, attenuated the loss of striatal TH-IR innervation, and induced the expression of phospho-erk in preserved neurons. These data, taken together with a series of comprehensive evaluations of the safety of AAV2-NTN (CERE-120), indicated that gene delivery of this compound is safe and effective for preclinical studies, and led to the initiation of phase 1 and 2 clinical trial in patients with PD. Indeed, a clinical trial of AAV-NTN injected bilaterally into the striatum of 12 patients with advanced PD demonstrated significant benefits in UPDRS motor score during "off" stage with reduced dyskinesias.[145] No serious adverse events were encountered, and specifically none of the patients experienced off-medication dyskinesia.

Gene Therapy and the Future

The development of gene therapy as a potential strategy to treat any human disease requires that two major questions about the treatment must be answered: Is the method of gene transfer and the gene itself safe? Is the transgene product efficacious at treating the disease after gene transfer?

Safety remains a primary concern regarding gene therapy trials. Two gene therapy trials have ended in failure because of adverse events. In 2002, a gene therapy trial using a retrovirus for patients with severe combined immune deficiency was halted after 1 of 11 patients experienced development of leukemia.[148] DNA analysis demonstrated that the retrovirus had integrated into the host genome at 40 different sites including LMO-2, which is related to oncogenesis and contributed to the abnormal growth of a T cell. Insertional mutagenesis is therefore a major concern with the use of integrating viral vectors, especially those such as AAV/5 or lentivirus, which are capable of infecting glial cells. However, it has recently been shown in mice that AAV serotype 2 vectors preferentially integrate into active rather than quiescent genes.[149] In addition, this vector tends to transfer DNA to an episome outside of the host genome, which should preclude adverse events.

In another trial, a patient had a severe immune reaction to an Ad vector and died 5 days after treatment; the patient had suffered from ornithine transcarbamylase deficiency and was infused into the right hepatic artery with an Ad encoding the human ornithine transcarbamylase gene.[150] The fatal outcome might have occurred because the patient had been previously exposed to Ad, which might have contributed toward an increased autoimmune response to the vector. Other patients in this trial, who received lower titers of Ad, also rapidly developed fevers, supporting the theory that previous exposure Ad followed by a second administration of the virus could cause an immune response. Therefore, rigorous screening of patients must be conducted before commencing gene therapy.

The best site for administration of viral vectors also must be established, to ascertain the method of getting the greatest efficacy when treating PD. For therapies that aim to enhance DA nigrostriatal function, it has been thought that the postcommissural putamen is the most critical site (see Olanow and colleagues[151]). SNc neurons, which degenerate in PD, project to this region. The loss of DA in PD is greatest in the postcommissural putamen, and this region of the striatum is reciprocally connected with motor cortex. The caudate nucleus and anterior putamen are similar embryologically, have dense interconnections with association cortex, and are linked to orbitofrontal regions rather than motor areas. Thus, previous clinical trials aiming to increase striatal DA by using human or porcine fetal transplantation strategies have grafted cells exclusively to the postcommissural putamen. However, these trials have failed to elicit functional recovery in patients. Several factors could account for these results, including the number of surviving cells and immune rejection. It is also possible that other transplant sites such as the anterior putamen/caudate or the SN itself may provide enhanced results.

The ability of the viral vector or gene product to be transported away from the site of administration must also be considered. AAV has a capability for retrograde and anterograde transport that could have beneficial or adverse effects. For example, although it may be favorable to transport a trophic factor or DA-synthesizing enzyme to the SNc, it is unclear whether transport to the pallidum, thalamus, or neocortex would be beneficial, harmful, or inconsequential. Potential problems with diffusion should also be considered. Transgene placed into the nigral region could extend to VTA neurons and induce hallucinations. Similarly, trophic factors delivered into the striatum by gene therapy could extend to the ventricle and cause side effects. Thus, the optimal target site and the presence of transgene remote from the site of administration need to be

fully investigated. One strategy to localize the expression of gene products is to transplant cells that have been infected with a viral vector, but this strategy has had mixed results in 6-OHDA–lesioned rats.

The ideal candidate for gene therapy delivery in PD is another issue that requires attention. We believe that transfection of trophic factors has great promise in that it could prevent the progressive degeneration of the DA nigrostriatal system, whereas enhancing the function of surviving neurons. The failed trial with direct GDNF infusion by catheter should not discourage investigations into the potential value of gene therapy to provide more diffuse delivery throughout the target region of this trophic molecule. Still, the use of viral vectors to deliver GDNF safely, locally, and over a long period must be carefully examined.

In the past 2 years, three clinical trials using viral vectors to deliver therapeutic genes for PD have begun: AAV-GAD delivered to the subthalamic nucleus (Neurologix), AAV-aromatic amino acid decarboxylase delivered to the striatum (Avigen), and AAV-NTN delivered to the striatum (Ceregene). It is interesting to note that each of these trials has utilized AAV to transfer DNA, reflecting the lesser immunogenic response and greater safety profile of this vector. Some level of efficacy has been reported for each approach in non–peer-review form. However, there have been no reports of safety concerns. The optimal vector, therapeutic protein, target site, and gene delivery treatment protocol remain to be fully defined, as does, of course, the safety and efficacy associated with each specific treatment paradigm.

In closing, the future of gene and cell therapy for PD, and the potential for developing novel and effective therapies for this condition, is promising. The high rate of discovery and identification of genes associated with PD, as well as molecular pathways that when modified protect DA neurons, will allow many "gain or loss" of molecular function experiments in vitro and in animal models. Such studies will establish which molecules can best limit DA neuron degeneration or enhance the function of remaining circuitry, or both. In conjunction with the current pharmacologically based clinical treatment paradigms, cell and gene therapy may emerge as tools that address individual patient's needs for neuroprotection, neuroenhancement, or neurorestoration.

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