

Clinical applications of cell therapy

José López-Barneo

INSTITUTO DE BIOMEDICINA DE SEVILLA-IBIS

Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla. Sevilla

Cell therapy: a challenge of translational medicine

Replacement of damaged organs, tissues, and cells is one of the fundamental objectives of modern medicine, and the last decades have witnessed spectacular advances in organ/tissue transplantation and related disciplines (organ preservation, immunosuppression, patient clinical care, etc). Within this field, cell therapy still remains in its initial stages of translation to medicine, although it is the area that should profit more of the numerous developments in cell biology occurred in recent years. It is over forty years that cell therapy is applied routinely in hematology to replace bone marrow cells damaged by accidental irradiation, mutations, or cancer. There have been also clinical advances in cell therapy related with other areas of medicine such as skin or bone/cartilage replacement and regeneration. However, it is in the neurological diseases where the promises of cell therapy have probably created the highest expectations, as the ability of regeneration of the mammalian central nervous system is null, or very low. In addition, neurological disorders, and particularly neurodegenerative diseases, have high individual, social, and economic costs, thus representing one of the major challenges of developed societies.

Cell therapy in diseases of the central nervous system; introductory remarks

Many diseases of the central nervous system (CNS) are a consequence of the acute or chronic loss of neurons and/or glial cells owing, among other causes, to degenerative, toxic, traumatic, ischemic or inflammatory aggressions. Therefore, the replacement of damaged cells by new ones has been considered as a potential therapeutic strategy in these CNS disorders. Neurologic cell-based therapy implies not only the transplantation of exogenous tissues to repair or restore function (cell replacement), but also the use of cells for the delivery of trophic factors with a protective action on the neurons affected by the ongoing pathological processes (neuroprotection) (1-3). Routes of cell or tissue administration include direct intracerebral grafting by open surgery or through stereotaxic needles, as well as intrathecal or intraventricular delivery. Systemic intravascular (arterial or venous) injections are used in some cases and migration of the putative therapeutic cells to the site of lesion has been reported. Another plausible form of cell therapy is the activation of pre-existing neuronal precursors located in

neurogenic centers (i.e. the subventricular zone of the lateral ventricles or the dentate gyrus of the hippocampus) that could migrate and differentiate to replace destroyed cells in some parts of the brain. This possibility is speculative at present and based solely on preliminary experimental observations (4, 5).

In theory, the ultimate goal of neurologic cell therapy is the histological and functional integration of grafts within the neighboring brain parenchyma. In this regard, synaptic connections between grafted neurons and the host tissue have been described in experimental studies. However, it has become evident that physiological restoration of the intricate synaptic circuits of the brain cannot be achieved through the cell replacement protocols currently used to treat CNS disorders. In contrast, the beneficial effects of transplants are, in most cases, a consequence of gross anatomical or neurochemical modifications in the recipient organ. For instance, the amelioration of parkinsonism after intrastriatal grafting of dopaminergic tissue is due to the tonic release of dopamine and/or trophic factors from grafted cells which, respectively, activate dopamine receptors in neighboring neurons and induce sprouting of the dopaminergic nigrostriatal fibers in the host brain. Therefore, the best results of cell therapy are obtained in CNS disorders, such as Parkinson's disease (PD), with relatively focalized lesions and affecting diffuse synaptic circuits in which pre-post synaptic specification is not an absolute requisite for the restoration of function. At present, it is difficult to envisage how therapies based on cell replacement or the activation of preexisting neurons could restore the delicate cortical and hippocampal synaptic circuits underlying memory storage and retrieval extensively damaged in Alzheimer's disease patients. Other limitations of cell therapy derive from the scarcity of cells/tissues that are appropriate for transplantation. Although autografts can be performed in some cases, the most frequently used clinical protocols are based on allo- or even xenografts, thus requiring immunosuppression treatment. Allotransplants are also

rendered difficult because the use of tissue from human donors (i.e. human fetuses or embryos) raises numerous legal and ethical issues.

Despite the limitations described above, cell therapies have been successfully applied to the treatment of neurological diseases for over two decades. The pioneer studies, designed to treat advanced PD patients with excellent results in some cases, stimulated the development of the field and were followed by a great deal of experimental and clinical work. These studies have boosted the development of animal models of numerous CNS diseases and the appearance of well-defined clinical protocols to evaluate disease progression. Nevertheless, the initial enthusiasm derived from the results of open trials has been tempered by the less spectacular clinical outcomes of double-blind and placebo-controlled studies performed with large patient cohorts. Recently there has been, however, renewed interest in the cell therapy field due to the appearance of new cell sources, particularly stem cells, with potential clinical applicability. Currently, there are numerous ongoing experimental and clinical transplantation studies designed to test the therapeutic efficacy of several cell types in a variety of neurological diseases, such as PD, Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), stroke, and spinal cord injury (SCI), among others.

This chapter aims to provide a general overview of the current status of cell therapies applied to CNS disorders. After a concise update of the preclinical knowledge available, the studies and techniques that have already resulted in clinical application are discussed in more detail. In this respect, the chapter's main focus is PD, a prototypical neurodegenerative disease in which the most solid and promising conceptual and technological advances in neurologic cell therapy have been tested. In addition, recent developments related with cell therapy applied to other CNS disorders (i.e. HD, ALS, stroke, or SCI) are briefly addressed. The chapter ends with a concluding summary and look at future perspectives in the field.

Cell therapy in Parkinson's disease

Pathophysiology and novel therapeutic strategies in Parkinson's disease

Parkinson's disease (PD) is a progressive neurodegenerative disorder of unknown etiology. Although several genetic forms have been described (6) the majority of cases are sporadic and unrelated to familial traits. PD is a major health problem in developed countries, as it affects to 100-300 subjects per 100,000 inhabitants and up to 3% of people older than 65 (7, 8). The motor symptoms of PD (tremor; bradykinesia/hypokinesia, rigidity, and alterations of gait and posture) are due to the progressive loss of dopaminergic neurons in the substantia nigra (SN) and their projections to the striatum (Figure 1). The degenerative process also affects other areas of the CNS and the peripheral autonomic nervous system, thus causing several non-motor symptoms, such as depression, cognitive impairment, and autonomic dysregulation (9). Although the progressive neuronal loss is a common feature of all neurodegenerative diseases, a typical pa-

thological hallmark of PD is the appearance of cytoplasmic inclusions, called Lewy bodies, containing synuclein and ubiquitin among other proteins. The mechanism of neuronal death in PD is likely multi-factorial, involving a cascade of events among which cellular oxidative damage appears to have a prominent role (Figure 1). Selective decrease of reduced glutathione, mitochondrial complex I activity, and reactive oxygen species (ROS)-destroying enzymes, or elevated concentrations of iron, which can act as a catalyst for detrimental oxidative reactions, have been reported within the parkinsonian SN. Similarly, there is evidence of oxidative damage to lipids, proteins and DNA in the brains and leukocytes of PD patients. Inflammatory-related events, such as nitric oxide-derived reactive species produced by glial inducible nitric oxide synthase, appear also to participate in SN dopaminergic degeneration (10). Besides mitochondrial dysfunction and the oxidative or inflammatory stresses, alteration of protein-degrading mechanisms at the proteasome is an additional factor that participates in PD initiation and/or progression. For example, mutations in α -synuclein (a presynaptic protein of unknown function that is deposited in Lewy bodies) or in parkin (a ubiquitin ligase necessary for protein identification by the proteasome) are responsible for some forms of genetic PD (6).

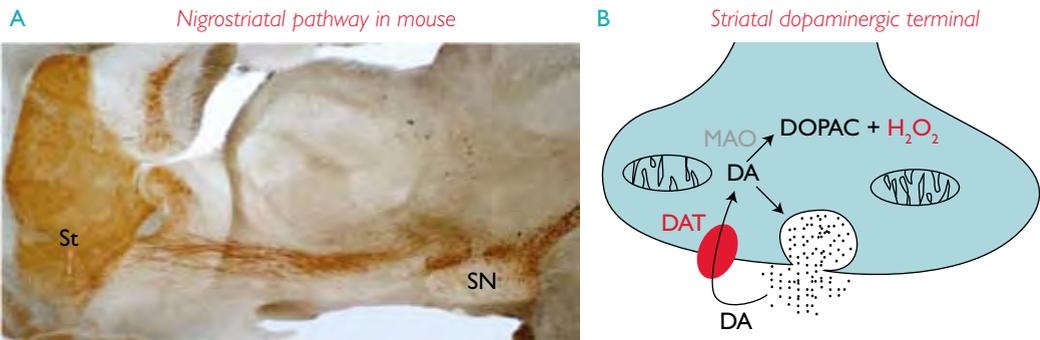


Figura 1. Dopaminérgico nigrostriatal pathway. A) Mouse dopaminérgico nigrostriatal neurons and fibers stained using antibodies anti tyrosine hydroxylase. SN, substantia nigra; St, Striatum. B) Schematic representation of a dopaminérgico presynaptic terminal. Uptake of dopamine (DA) and its metabolism to dihydroxyphenyl acetic acid (DOPAC) and hydrogen peroxide (H₂O₂) are illustrated. DAT, dopamine transporter; MAO, monoamine oxidase.

PD treatment relies mainly on L-dopa, a drug that is converted to dopamine in neuronal somata and presynaptic terminals. L-dopa is normally complemented with the administration of dopamine receptor agonists or inhibitors of dopamine-degrading enzymes (monoamine oxidase and catechol-o-methyl transferase). Patients who develop disabling motor complications (motor fluctuations and dyskinesias) or drug-resistant tremor can be candidates for surgical implantation of electrodes for electrical stimulation, these normally being placed at the subthalamic nuclei. This methodology, consisting of the application of high frequency (> 100 Hz) electrical pulses to inhibit neuronal activity, can correct the alterations of basal ganglia circuits in the parkinsonian brain. Although the current pharmacological and surgical therapies are symptomatically effective, their long-term utility is limited because they do not halt the disease progression (11). Therefore, there is a need for neuroprotective and/or neurorestorative therapies capable of arresting or reversing the neurodegenerative process. Dopamine cell replacement and the intracerebral administration of trophic factors are cell-based promising therapeutic approaches currently subjected to intense basic research and clinical evaluation.

Transplantation of dopamine-secreting cells. Preclinical studies

Mammalian models of Parkinson's disease

Development of research on CNS cell therapy depends on the existence of animal models of the neurological diseases, where the effectiveness, advantages, and limitations of the various therapeutic approaches can be tested experimentally. A brief description of the mammalian models of PD available is given below.

A commonly used PD animal model is the hemiparkinsonian rat, generated by unilateral stereotaxic injections of 6-hydroxydopamine (6-OHDA) either into the SN, the neighboring

medial forebrain bundle, or the striatum. In all cases the drug is metabolically converted to dopamine and generates H_2O_2 , which subsequently kills the dopaminergic nigrostriatal neurons due to oxidative stress (Figure 2). Unilateral destruction of the dopaminergic nigrostriatal neurons produces a spontaneous rotational behavior towards the side of the lesion (greatly exacerbated by administration of D-amphetamine) that is easily monitored with a rotameter. As the number of rotations is proportional to the degree of SN lesion (and to the extension of striatal dopaminergic denervation) this model permits the experimenter to test the recovery of the syndrome after striatal transplantation of dopamine (and/or trophic factor)-releasing cells (Figure 2) (12). The 6-OHDA model has, however, numerous limitations since it is generated by acute oxidative damage to SN dopaminergic neurons, a phenomenon quite different from the chronic and progressive death of this neuronal population characteristic of PD. Moreover, the direct mesencephalic injection of 6-OHDA makes it difficult to obtain animals with partial lesions, which are required to test neuroprotective therapies based on the trophic action of the transplanted cells on nigrostriatal neurons spared by the lesions or still unaffected by the ongoing neurodegenerative process. In the last few years there have been several attempts to generate chronic rat PD models, although their feasibility and reproducibility are still under scrutiny. Chronic intravenous or subcutaneous administration of rotenone (a membrane-permeable inhibitor of mitochondrial complex I) seems to produce bilateral destruction of dopaminergic SN neurons with Lewy body-like cytoplasmic inclusions. Similar results have also been reported after chronic administration of proteasome inhibitors. Unfortunately, the validity and reproducibility of this model (that initially raised great expectations) is being seriously questioned (10, 13).

The monkey model of PD is also broadly used owing, among other reasons, to the need for testing in non-human primates most of the

cell therapy procedures destined for clinical use. Monkeys are chronically treated by subcutaneous injections of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a drug converted in the primate brain to 1-methyl-4-phenylpyridinium ion (MPP⁺) which, in turn, is taken up selectively by dopaminergic neurons via the dopamine transporter (Figure 1). As MPP⁺ is a potent mitochondrial complex I inhibitor; its chronic application leads to progressive dopaminergic cell death. MPTP has similar actions in mice (see below) but is ineffective in rats since they do not express the enzymes required to convert MPTP into MPP⁺. In primates (monkey and human) MPTP produces a bilateral parkinsonian syndrome with motor features similar to those present in sporadic PD (10, 14). The objectives of experimental cell therapy in par-

kinsonian monkeys are similar to those described above for the rat. Dopamine- or trophic factor-releasing cells are normally deposited stereotactically in the striatum (normally at several locations in the putamen) and clinical recovery is monitored with *ad hoc* behavioral tests. The MPTP monkey is particularly useful to perform unilateral striatal transplants since the contralateral striatum (injected with saline solution) can be used as control. In these cases, the success of the procedure results in unilateral histological and clinical recovery, with the animals behaving in a hemiparkinsonian manner hence showing a typical rotational behavior towards the side contralateral to the transplant (15).

Systemic administration of MPTP in mice produces bilateral destruction of dopaminergic nigrostriatal neurons (10, 16, 17). The parkin-

Nigrostriatal denervation in the hemiparkinsonian rat

Striatal reinnervation after CB grafting

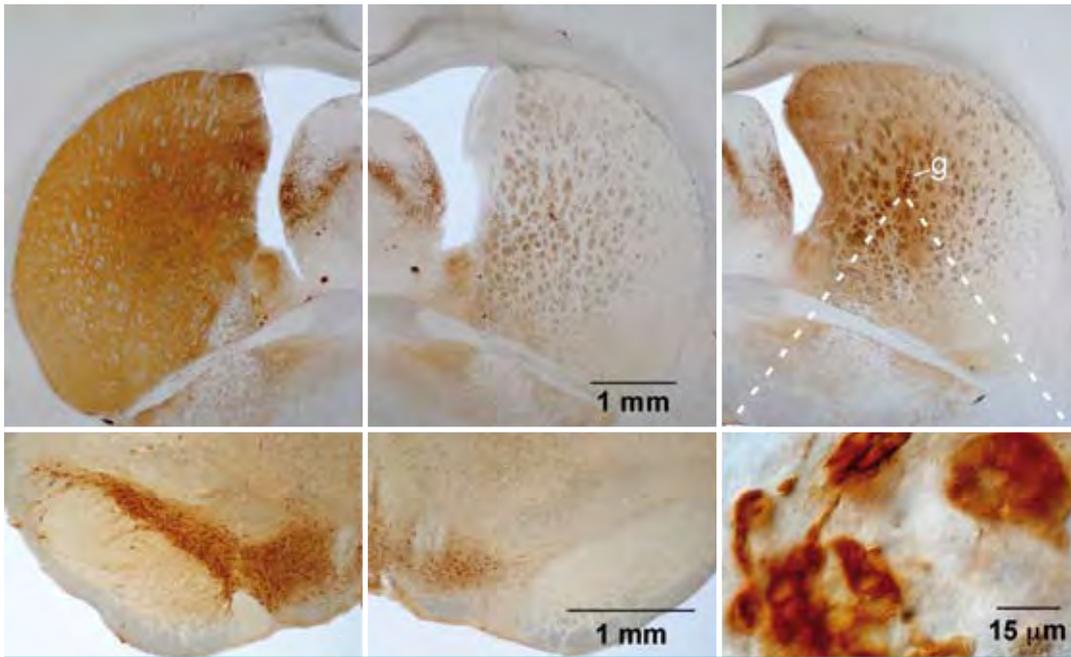


Figura 2. Hemiparkinsonian rat model and intrastriatal grafting of dopaminergic cells. A) Coronal sections at the level of the striatum (St) and the mesencephalon (SN, substantia nigra; VTA, ventral tegmental area) showing the normal (left) and denervated (right) nigrostriatal pathway. B) Striatal dopaminergic reinnervation after transplantation of carotid body glomus cells (g, graft).

sonian syndrome in mice is less amenable for behavioral analysis than the syndrome in the rat; however mice are sometimes chosen because MPTP administration requires no surgery and, as in the case of primates, if unilateral transplantation is performed the contralateral side can be used as an internal control. In addition, susceptibility to MPTP-derived toxicity can be studied in the numerous genetically modified mice strains available. An acute MPTP mice model is normally generated by subcutaneous injections (single or distributed over 12-24 h) of the drug. Chronic models, based on the continuous delivery of MPTP using subcutaneous pumps or repeated injections, are being assayed in several laboratories but results differ among the various groups and animals strains.

There are several mouse genetic models in which some of the genes altered in familial PD (i.e. α -synuclein or parkin) have been transgenically overexpressed in an attempt to reproduce the disease. So far, none of these genetically modified animals exhibit clear histological, neurochemical or behavioral signs of parkinsonism. Another PD mouse model is the one obtained after genetic disruption of the glial cell line-derived neurotrophic factor (GDNF) signaling pathway. GDNF is the most representative member of a family of trophic factors (called dopaminotrophic factors) that promote the *in vivo* and *in vitro* survival of dopaminergic neurons (see below). GDNF null animals die at early neonatal stages due to kidney agenesis and alterations of the enteric nervous system, although in these animals both the SN and the striatum are normal. In adult life, the overall GDNF expression in the central nervous system decreases drastically, although high levels of GDNF are maintained in some striatal neurons and glial cells, possibly as a mechanism to aid trophic maintenance of the nigrostriatal pathway. Heterozygous GDNF $+/+$ animals develop normally, although it has been reported that they show a mild reduction of the dopaminergic neuronal pool at advanced age. Thus, it seems that abolition of GDNF expres-

sion (or function) in the adult mouse could serve as a good PD mouse model. Conflicting results have recently been reported regarding the effect of c-ret (a transmembrane component of the GDNF receptor) knock-out in adult life (18, 19). The conditional GDNF null mouse recently generated in our laboratory has demonstrated that striatal GDNF production is absolutely necessary for the survival of adult mesencephalic dopaminergic neurons. These animals show an extensive loss of cells in SN and VTA as well as in the Locus coeruleus. They also have a clear akinetic syndrome that ameliorates after L-dopa administration (19b).

Preclinical transplantation studies

The first cell therapy studies in animal models of PD were performed in the late 1970s in rats using fetal rat dopamine-containing neurons as donors with the aim of restoring striatal dopamine levels. In these investigations, improvement of the functional deficits in parkinsonian animals was paralleled by the survival of intrastriatal grafts and axonal outgrowth (20, 21). After this pioneer work, numerous studies have been performed in the last 25 years in an effort to address the following main objectives:

- I. To establish the effectiveness of different types of donor tissue on recovery from the parkinsonian syndrome.
- II. To test the survival of the transplanted tissue and its histological integration within the host striatal parenchyma.
- III. To evaluate electrophysiological and neurochemical activity in grafts and the establishment of functional connections (synapses) between the graft and the host brain.
- IV. To determine whether immunosuppression facilitates long-lasting cross-species transplantation of neural and non-neural tissues.

After the initial transplantation of mesencephalic tissue from rat embryo to rats, a step further was the transplantation of mesencepha-

lic dopaminergic neurons, taken from mouse embryos or human fetuses, to the dopaminergically denervated neostriatum of recipient rats, and the use of primates with MPTP-induced Parkinson-like syndrome as graft receptors (22). At the same time, adrenal chromaffin cells were used as an alternative dopamine source in PD animal models, from rats to primates, although with poorer results than those obtained using embryonic or fetal dopamine neurons (23). In parallel with these studies there have been several reports from groups using porcine mesencephalic neurons with good results (24). Similarly, tissues in the peripheral nervous system, other than the adrenal medulla, have been used. In those studies, transplants of sympathetic neurons from the superior cervical ganglion were performed in experimental animal models as well as in humans (25).

Among the most promising new experimental approaches developed within the last few years are the transplantation of retinal pigment epithelial cells and of carotid body tissue. In the first case, stereotaxic intrastriatal implantation of human retinal pigment epithelial cells attached to gelatin microcarriers in rodent and non-human primate models of PD produced long-term amelioration of motor and behavioral deficits, with histological and PET evidence of cell survival without immunosuppression (26). In the second case, the autotransplantation of dopaminergic carotid body (CB) cell aggregates was able to effect notable histological and functional recovery in parkinsonian rats (27, 28) and MPTP-treated monkeys (15). The CB is a tissue particularly attractive for antiparkinsonian cell therapy because it combines the properties necessary for dopamine cell replacement and for neuroprotection. The CB contains neural crest-derived dopaminergic glomus cells, which function as arterial oxygen sensors and release large amounts of dopamine in response to hypoxia. Long-term recovery of parkinsonian animals after intrastriatal CB grafting is induced not only by the release of dopamine, but also

through a trophic effect on nigrostriatal neurons. In fact, adult rodent CB cells express much higher GDNF levels than any other paraneural cells studied (adrenal medulla, superior cervical ganglion, Zuckerland's organ and PC12). Moreover, GDNF expression by CB glomus cells is maintained after intrastriatal grafting and in CBs of aged or MPTP-treated animals (29) (Figure 3). Thus, glomus cells appear to be miniaturized biological pumps useful for the local delivery of GDNF and possibly other trophic factors.

Besides the transplantation of dopamine-producing cells, other experimental strategies have been designed to directly increase the striatal concentration of trophic factors that could eventually induce regeneration of the dopaminergic nigrostriatal pathway. Among these studies Sertoli cells from the testis, amniotic epithelial cells or genetically modified cells overexpressing trophic factors have been used. Some of these trophic factor-producing cell types have been assayed in conjunction with dopamine releasing cells (i.e. cotransplantation of peripheral nerve plus adrenal medulla, and Sertoli or CB cells plus ventral mesencephalic neurons) (30).

After more than 20 years of preclinical research in antiparkinsonian cell therapy, the studies performed can be retrospectively classified into three major groups:

- I. Intrastriatal transplantation of heterologous dopaminergic neurons to compensate for the loss of intrinsic dopaminergic fibers. For these studies, fetal mesencephalic neurons were normally used and the establishment of synaptic connections between the graft and host neurons was considered a good indication of graft survival and integration in the recipient brain. More recently, fetal mesencephalic neurons are being substituted by stem cell-derived dopaminergic neurons (see Section 3.2).
- II. Intrastriatal transplantation of dopamine-releasing cells (adrenal chromaffin, retinal or

carotid body cells) with the sole objective of increasing the average level of dopamine in the striatal parenchyma. Functional connections between the graft and host tissues were not expected.

- III. Intrastratial delivery of cells releasing trophic factors (carotid body, retinal cells, Sertoli or amniotic epithelial cells or genetically modified cells), to encourage striatal reinnervation through sprouting of the intrinsic nigrostriatal neurons.

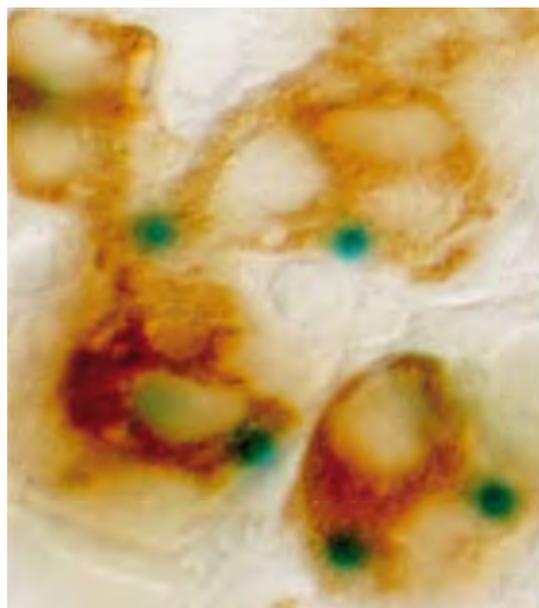
Over the years, interest has shifted from procedures that could be included in categories i/ii to those included in category iii. In the initial stages, the limiting factors in cell therapy research were graft survival and accessibility to an abundant dopaminergic neuronal source. Currently, it is considered more important to determine the mechanisms of action of the transplants to

help better define the type of patient that could eventually benefit from cell therapy.

Current developments using stem cells

Embryonic stem (ES) cells are generally thought to offer a promising source of dopaminergic neurons suitable for PD cell therapy. They are characterized by a high proliferation rate and the potential to give rise in vitro to any cell type of the adult organism (2). In recent years, the differentiation of mouse embryonic stem cells into dopaminergic neurons has been repeatedly obtained in different laboratories following two different approaches. One involves the co-culture of ES cells with stromal feeder cells (PA6, MS5) that facilitates the induction of the neural fate. Another approach implies a five-stage method based on the formation of a three-germ layer structure, the embryoid body, from which neural cells can be selected. In both protocols, neural progenitor cells

Intrastratially grafted carotid body cells



Carotid body cells in situ

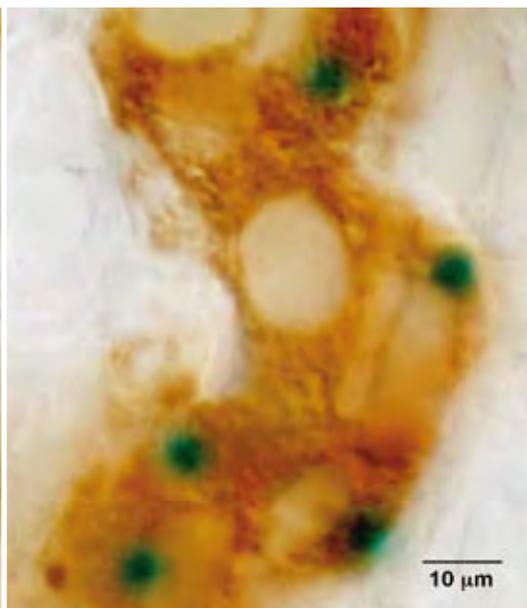


Figura 3. Maintenance of carotid body glomus cell phenotype *in situ* (right) and several weeks after intrastratial transplantation (left). Immunocytochemical staining using anti tyrosine hydroxylase antibodies (brown-yellow) and X-galactosidase (green). The X-gal signal indicates that the GDNF promoter is active in these dopaminergic cells. See reference (29) for further details.

can be expanded and induction to the midbrain dopamine fate can be favoured by exposing the cells to appropriate morphogenetic factors (31, 32). Neuronal differentiation is normally triggered by mitogen withdrawal, and the dopaminergic phenotype is normally demonstrated by identification of the cells with markers such as tyrosine hydroxylase and the dopamine transporter (Figure 4). Embryonic stem cell-derived dopaminergic neurons are normally obtained from established ES cell lines, however, dopaminergic neurons have also been derived from embryos obtained by nuclear transfer or from induced pluripotent stem cells (iPSC).

Striatal transplantation of previously specified midbrain ES cell-derived neural progenitors cells has been reported to provide surviving dopaminergic neurons in the host brain and render histological, neurochemical and behavioral recovery of hemiparkinsonian rats (33, 34) (Figure 4). If ES cells are genetically manipulated to overexpress the *nurr-1* gene, dopaminergic neuronal differentiation is markedly increased and, subsequently, a higher yield of surviving dopaminergic neurons is achieved upon grafting (33). One major drawback of ES cell-based therapy is tumor generation, however this possibility is greatly diminished with appropriate differentiation procedures that eliminate all proliferative ES cells from the transplanted preparation. Several recent studies performed in non-human primate and human ES cells have demonstrated the ability of these cells to differentiate into dopaminergic neurons. However, survival of transplanted dopaminergic neurons, an absence of proliferative cells in the grafts, and consistent behavioral recovery are issues that seriously compromise the clinical applicability of human ES cell-derived neurons. Identification of key factors determining the survival of human ES cell-derived dopaminergic neurons is an active field of research for regenerative medicine (35).

Neural stem cells derived from fetal or adult brain have been shown to differentiate into dopaminergic neurons, although the yield is re-

latively poor due to their limited capacity for proliferation and because of their preferential glial differentiation. Different factors have been used to increase the dopaminergic differentiation of neural stem cells with relatively modest success, although good results are beginning to be reported by some laboratories (35b). Bone marrow stromal cells and umbilical stem cells are also attractive sources of multipotent stem/progenitor cells, currently subjected to intense research, that could eventually be used for autologous transplants. However, the ability for dopaminergic differentiation of these cells is quite limited. Neural crest-derived progenitors have also been recently described in the carotid body, which permit the adaptive growth of this organ in chronic hypoxia. *In vitro*, these progenitors can differentiate to dopamine and GDNF producing cells, hence they could be used for transplantation studies in PD (35c). In summary, several critical challenges need to be overcome before fetal, adult or autologous stem cells can be brought into the clinical field to treat PD (35).

Transplantation of dopamine-secreting cells. Clinical studies

General overview

Transplantation of dopamine-secreting cells in advanced PD patients was initiated in the mid 1980s. A summary of the transplantation procedures, with indication of the donor tissue and current status of the technique, is given in Table 1. Despite the highly variable clinical outcomes of these studies, with excellent results reported in selected patients but only modest effects in most cases, the overall benefit experienced by the patients stimulated further research and clinical tests. Clinical trials resulting in a higher impact have been those employing adrenal medulla or mesencephalic neurons as donor tissues.

The first stereotaxic intrastriatal implantation of autologous adrenal medulla on four patients was performed, with modest results, by the Lund

group in 1985 (36). In 1987, a Mexican group reported excellent clinical results (not confirmed by others) after transplantation by open surgery of adrenal tissue into a cavity made in the caudate nucleus (37). These pioneer trials were soon followed by numerous studies in several hospitals all over the world. These studies differed in the surgical approach (open versus stereotaxic)

as well as in the procedures followed for adrenalectomy and the treatment given to the cells prior to transplantation. All studies performed were open, with few patients (3 to 20) and without blind evaluation. In most cases the clinical efficacy of adrenal medulla autografts was very modest with a lack of neurochemical improvement based on positron emission tomography

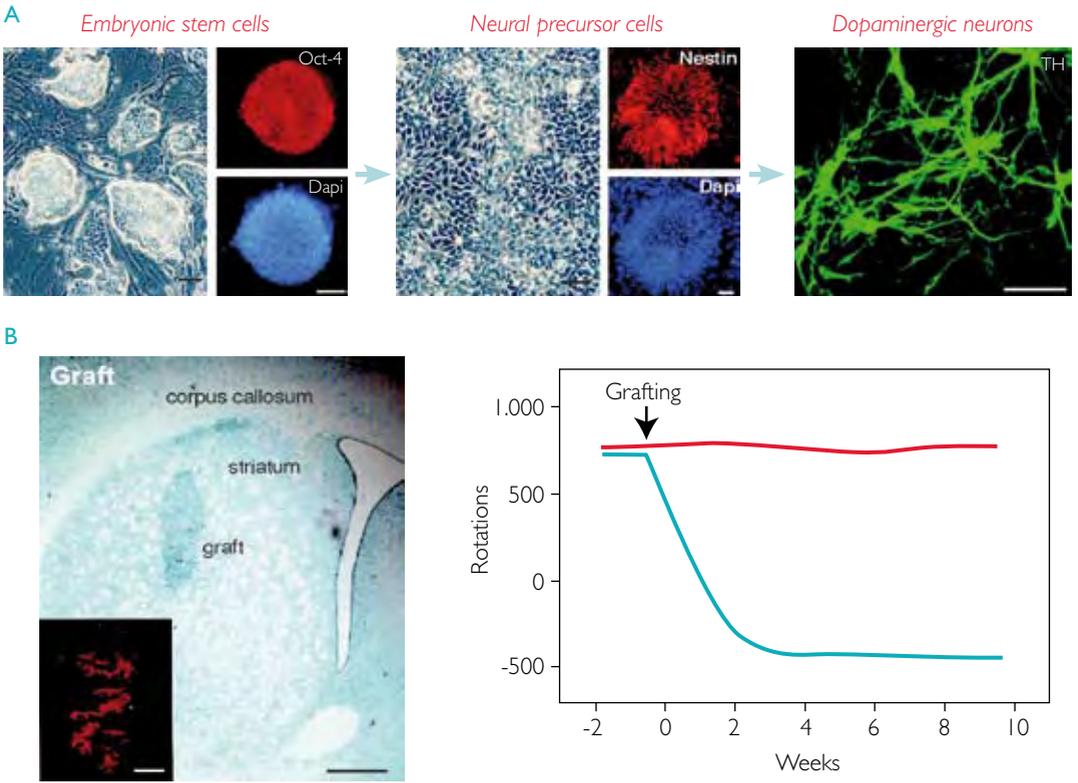


Figure 4. *In vitro* differentiation of dopaminergic neurons from embryonic stem (ES) cells. **A**) Left, Colonies of mouse ES cells. Undifferentiated state is proved by Oct-4 staining. Calibration bars: 100 μ m and 50 μ m, in phase contrast and immunostaining, respectively. Center, Monolayer of ES cells-derived neural precursors. Neural identity is proved by nestin staining on cells adopting typical rosette disposition. Calibration bars: 100 μ m and 50 μ m, in phase contrast and immunostaining, respectively. Right, ES cells-derived dopaminergic neurons (tyroxine hydroxylase, TH +). Calibration bar: 100 μ m. 4'-6-diamidino-2-phenylindole (Dapi) shows nuclear counterstaining. **B**) Left. Photograph of the transplanted rat brain illustrating the localization of the graft in the striatum. The inset shows the dopaminergic identity (TH+) of the ES cells-derived grafted neurons. Calibration bars: 500 μ m and 200 μ m (inset). Right. Schematic representation of the rotational behavior of hemiparkinsonian rats non-transplanted (red) and transplanted (green) with ES cells-derived dopaminergic neurons. See further details in the text and reference (33).

(PET). Postmortem analyses have demonstrated an almost complete absence of chromaffin cells at the transplanted sites. Several groups have reported that the viability of adrenal medulla grafts and their clinical efficacy increased if the cells were treated with trophic factors or co-transplanted with other tissues (i.e. peripheral nerve) (38). The transplantation of adrenal tissue to treat PD has, however, been abandoned due among other factors to the relatively high morbidity/mortality associated with the dual (abdominal and cranial) surgery and the development of other therapeutic approaches. The most compelling alternative to adrenal autografts is the transplantation of fetal mesencephalic neurons (see below), a technology that has dominated the clinical trials on cell therapy applied to PD patients for the last 15-20 years.

Transplantation of fetal mesencephalic neurons

The first intrastriatal transplants of human fetal mesencephalic tissue in PD patients were carried out by the Lund group between 1989 and 1991, stimulated by the good results obtained with the same methodology in preclinical studies. Since some patients treated with fetal cells showed long lasting clinical improvement, this methodology was extended to other laboratories. In most cases the technique used was the bilateral stereotaxic implantation of dispersed cells or tissue fragments in the caudate/putamen. It is believed that this technique has been applied in open trials to several hundred patients although only about 50 have been carefully evaluated in the scientific literature (see

Tabla I. Transplantation procedures in patients with Parkinson's disease

Tissue	Type of transplantation ¹	First report ²	Spread of the technique	Level of development & current status
Adrenal medulla	Autologous	(36)	Worldwide	Abandoned
	Homologous (human fetal)	(37)	Single or few centers	Abandoned
Adrenal medulla & peripheral nerve	Autologous	(38)	Single or few centers	Abandoned
Mesencephalic tissue	Homologous (human fetal)	(39)	Worldwide	Phase III studies ³ in progress
	Heterologous (porcine embryonic)	(40)	Single or few centers	Phase III study ³ in progress
Retinal pigment epithelial cells	Homologous (post-mortem eye donors)	(41)	Single or few centers	Phase III study ³ in progress
Carotid body cells	Autologous	(42)	Single or few centers	Phase II clinical and PET study completed
Sympathetic ganglion cells	Autologous	(43)	Single or few centers	Open-label series

¹ Type of transplantation. Autologous: transplantation of tissue obtained from the same patient. Homologous: donor tissue obtained from other individuals of the same species. Heterologous: donor tissue obtained from individuals of different species. ² First report. Refers to the first full publications of outcomes in patients. ³ Phase III studies refer to double-blind placebo-controlled trials.

summary in Table 2). Although the transplantation methodology differs in the age and number of donor fetuses, the preparation, storage and dissociation of the tissue, and the protocol of immunosuppression used, the clinical evaluation of these patients has been more homogeneous than in the case of adrenal transplants. Patient evaluation was generally done with the Unified

Parkinson's Disease Rating Scale (UPDRS) and most assays followed the Core Assessment Program for Intracerebral Transplantations (CAPIT) protocols (60). In the following sections we summarize the clinical results and other relevant features of the main open studies and of the two double-blind, placebo-controlled, trials completed in recent years.

Table 2. Methodology of the main open-label trials on human fetal mesencephalic transplants (see clinical outcomes in the text)

Country (city)	Ref.	n	Donors N.º per hemisphere	Age	Graft type/ technique	Location of implants	IMS	Clinical follow-up	PET ¹	Necropsy ²
Sweden (Lund)	(39, 44)	2	3-4	7-9 w	Cells Stereotaxic	C + P Unilateral	6 m	18 m	Yes (-)	No
	(45-47)	2	3-4	6-7 w	Cells Stereotaxic	P Unilateral	6 m	3 y	Yes (++)	No
	(48)	2 ³	3-4	6-8 w	Cells Stereotaxic	C + P Bilateral	12 m	2 y	Yes (++)	No
USA (Denver)	(49, 50)	7	1	7-8 w	Cells or strands of tissue/ Stereotaxic	C + P P Unilateral (2) Bilateral (5)	6 m (4) No (3)	4 y	Yes (+) (1) (-) (6)	No
USA (New Haven)	(51)	4	1	7-11 w	Tissue/ Stereotaxic	C Unilateral	6 m	18 m	Yes (+) (1)	Yes (+/-) (1)
USA (Tampa/ New York/ Chicago)	(52-54)	6	3-4	6-9 w	Tissue/ Stereotaxic	P Bilateral (6-8 tracks/side)	6 m	2 y	Yes (++)	Yes (+++) (2)
France (Créteil)	(55-57)	5	2-3	6-9 w	Cells Stereotaxic	C + P P Unilateral (4) Unilateral (1)	6 m	1-3 y	Yes (++)	No
Spain (Madrid)	(58)	10	1	6-15 w	Tissue/ Open surgery	C Unilateral	Chronic	5 y	No	No
USA (Los Angeles)	(59)	13	1 (6) ≥ 2 (7)	6-9 w	Tissue/ Stereotaxic	P Bilateral	18 m	6 m	Yes	No

Ref. (references): Publications where the results appeared. n: number of patients operated in the different trials. In other columns the number of patients is given between parentheses. Location of implants: C: caudate. P: putamen. IMS: immunosuppression.

Other notations: w: weeks. m: months. y: years.

¹ 18F-dopa PET. Yes: at least some patients were studied. (-): no significant changes; lesser (+) or greater (++) increase in 18F-dopa uptake. ² Necropsy. Yes: at least some patients were studied. Uncertain (+/-), minor (+), moderate (++) or major (+++) graft survival and integration. ³ Two patients with MPTP-induced parkinsonism.

Clinical outcome. Open studies

The main methodological features of the open clinical studies performed with heterologous implantation of mesencephalic neurons in PD patients are summarized in Table 2. In most trials the patients (between 2 and 13) suffered advanced PD with motor complications, and one study included two cases of MPTP-induced

parkinsonism. The surgical procedures are variable, with uni- or bilateral caudate and /or putamenal implantations. The clinical follow-up of the patients varied between 6 months and 5 years and in most cases included neurochemical evaluation with [¹⁸F]-dopa PET analyses. Despite the methodological differences, all studies reported some degree of motor improvement in most

Tabla 3. Methodology of the two double-blind placebo-controlled studies on human fetal mesencephalic transplants (see clinical outcomes in the text)

Double-blind placebo-controlled trials	Denver/New York Group (61)	New York/Chicago/Tampa Group (62)
Patients	Transplant group: 20 (≤ 60 yrs: 10; > 60: 10) Placebo group: 20 (≤ 60 yrs.: 11; > 60: 9)	Transplant group: 23 (1 donor: 11; 4 donors: 12) Placebo group: 11
Follow-up	1 year	2 years
Loss of follow-up	1 (transplant group)	3 (group not specified)
Primary outcome	Subjective global self-rating of change (scale from -3 to +3)	Change in UPDRS III
Donors:		
• N.º/hemisphere	2	1 (11 patients); 4 (12 patients)
• Age	7-8 weeks	6-9 weeks
Graft type	Strands of tissue (cultured up to 4 weeks)	Fragments of tissue (stored in cool hibernation medium up to 2 days)
Surgical technique	Stereotaxic (bilateral in one stage) 2 twist-drill holes per side	Stereotaxic (bilateral in two stages) 1 burr hole per side
Location of implants	Putamen bilateral (4 tracks per side; tissue deposited continuously)	Posterior putamen bilateral (8 tracks per side; 4 deposits per track)
Immunosuppression	No	Cyclosporine during 6 months
¹⁸F-dopa PET	One year after surgery: <ul style="list-style-type: none"> • mean increase in uptake (– in 3 patients) • difference to placebo group • no difference in ≤ 60 / > 60 years groups 	One and two years after surgery: <ul style="list-style-type: none"> • mean increase in uptake • difference to placebo group • trend to a greater increase in the 4 donors group
Necropsy^l	2 patients (> 60 years): ++	2 patients (1 donor/side): ++ 2 patients (4 donors/side): +++

III: motor sub-scale of the Unified Parkinson's Disease Rating Scale. ^l Necropsy. See footnote of Table 2.

patients. In general, patients experienced an increase in the «on» period and improvement of symptoms, particularly rigidity and bradykinesia, in the «off» period. «On» dyskinesias were reduced in some patients but increased in others (see «Complications» below). The clinical benefits of the transplants, perceived either immediately or around 3-6 months after the surgery, reached a peak at approximately 1-2 years after surgery and then progressively disappeared. There are, however, reports of persistent clinical improvements 5 to 10 years after transplantation. In the few open studies that included controls the results reported are also quite distinct. In the Los Angeles group study (Table 2) the blinded evaluation of motor benefits was higher in patients randomly assigned to receive more tissue than a control group. However, the New Haven group (Table 2) failed to see differences between transplanted patients and a control group of similar characteristics that received only pharmacological treatment. In conclusion, the open studies summarized in this section represent hallmarks in the development of cell therapy strategies to fight PD, although their scientific value is limited because of the lack of uniform analytical methodologies.

Clinical outcome. Double-blind studies

To progress in the evaluation of the clinical applicability of fetal mesencephalic cell transplantation in PD, two double-blind, placebo-controlled studies have been performed in recent years. These are the first controlled phase III clinical trials ever done to test a cell therapy strategy applied to a CNS disease. The major methodological features of these studies are summarized in Table 3. Patients were subjected to stereotaxic bilateral implantation of fetal tissue in the putamen. In the two trials a randomly selected placebo group was subjected to sham surgery consisting of craniectomy without duramater perforation. Both the patients and the clinical evaluators were unaware of the type of treatment (either «placebo» or «transplant») applied to each case. All patients were evalua-

ted pre- and post-surgically with [¹⁸F]-dopa PET scans. The major objective of the studies was the precise evaluation of «actual» symptomatic improvement of advanced PD patients after transplantation. The trial of the Denver/New York group (61) placed special emphasis on the effect of patient age on the clinical outcome, whereas the New York/Chicago/Tampa group (62) focused on the clinical effects of transplantation of different amounts of tissue.

In the Denver/New York trial transplanted patients showed a statistically significant improvement of 18% in the UPDRS III scale in «off» with respect to the placebo group one year after surgery. This improvement, affecting mainly rigidity and bradykinesia, was due to the marked effect of the transplants on patients below 60 years of age (34% average improvement). The clinical benefit appeared during the first 4 months and was maintained in open evaluations for up to 3 years. More recent analysis of this patient cohort has suggested that the best prognostic factor was responsiveness to levodopa rather than patient's age. Fetal cell transplantation did not induce significant changes in the diary of fluctuations, levodopa dose or cognitive function. In the New York/Chicago/Tampa trial no statistically significant clinical differences in the UPDRS III scale in «off» were observed between patients of the transplanted and placebo groups two years after surgery. There was, however, a trend towards a clinical amelioration proportional to the amount of tissue transplanted. Patients with UPDRS III < 49 who received a larger amount of tissue showed a statistically significant clinical recovery with respect to the placebo controls. In this last subgroup, the clinical effects were perceived around three months after the surgery and reached a peak around 6-12 months. There were no significant changes in the diary of fluctuations or the levodopa dose.

Complications

In most of the transplantation trials done with fetal mesencephalic tissue, the procedure was, in general, well tolerated although some complica-

tions associated with the cranial surgery were reported. Intracerebral hemorrhages, epileptic seizures, or postsurgical confusional syndromes were the most frequent complications, normally resolved after a few days of treatment. In one case, patient death by hydrocephaly was reported due to migration of the transplanted tissue to the fourth ventricle. In some patients transplanted with homologous fetal tissue, the administration of immunosuppressants has been associated with renal dysfunction or nosocomial infections. Besides these complications, the placebo-controlled studies have reported the appearance in some transplanted patients of severe and persistent dyskinesias (characterized by stereotyped abnormal movements of the limbs) during the «off» periods. In the Denver/New York study this complication affected 15% of the patients (younger than 65 years) that had experienced clinical improvement. In the New York/Chicago/Tampa trial, dyskinesias appeared in more than 50% of the patients and were particularly severe in three cases. The «off» dyskinesias have been suggested to originate from an excess of dopamine released from the transplant. However, no correlation between the severity of the dyskinesias and the amount of tissue transplanted or [^{18}F]-dopa uptake in PET studies (see below) has been reported. It has been suggested that the dyskinesias reflect the anomalous innervation of striatal cells by the transplanted neurons, however it cannot be discounted that they are generated by other variables (i.e. tissue preparation) different from those in previous open studies.

Neurochemical and histological data

The open trials testing the effect of fetal neuronal transplants have generally included the monitoring of striatal dopamine content using [^{18}F]-dopa PET scans (see Tables 2 and 3). Dopa (a dopamine precursor) is taken up by the dopamine transporter in the nigrostriatal presynaptic terminals (Figure 1), as well as in the soma and neurites of the transplanted dopaminergic neurons. Therefore accumula-

tion of the marker (which results in a higher amount of emitted radiation) provides an indication of striatal dopaminergic innervation. Practically all the studies performed have reported an increase of [^{18}F]-dopa uptake in the transplanted area at one-year follow-up. In one study synaptic release of dopamine in the transplant was demonstrated by neuroimaging using [^{11}C]-raclopride, a drug that competes with dopamine for binding to postsynaptic D2 receptors (63). In the two double blind-studies [^{18}F]-dopa uptake one year after the surgery was significantly higher in the transplanted patients in comparison with the placebo group. Thus, the neuroimaging data suggest that fetal mesencephalic grafts survive for several years after transplantation and remain neurochemically and functionally active.

In patients that died during the studies (generally for causes not related to the transplantation procedure) and were subjected to necropsy, the direct histological examination of the striatum normally confirmed the *in vivo* PET data. These *postmortem* histological analyses have also demonstrated that the surviving dopaminergic neurons account for less than 5% of the several hundreds or millions initially transplanted, which suggests a marked mortality of donor cells during the transplantation procedure.

Current status of the methodology and future developments

After almost 20 years of intense research, the applicability of fetal mesencephalic tissue for treatment of advanced PD is being seriously questioned. Besides the limitations of this methodology already recognized in the open studies (scarcity of donor tissue and poor cell viability), the double-blind studies have suggested that the successful implantation of dopaminergic cells in the striatum is not sufficient to induce significant clinical amelioration of symptoms. The scarcity of donor tissue (several fetuses per patient) is heightened by ethical and legal concerns regarding the use of human embryonic material. Additionally, the dissection

and manipulation of fetal mesencephalic tissue is technically difficult and results in a mixture of different neural populations with a high incidence of cell death. The numerous attempts to minimize these limitations have included the treatment of cells with neurotrophic factors or antioxidants as well as the use of porcine mesencephalic tissue. Although both approaches have been tested in experimental animal models as well as in open clinical studies with some positive results, they have not provided clear improvement with respect to the trials utilizing human tissue. Porcine xenographs (40) have been virtually abandoned because of the danger of interspecies infections, although a phase III clinical trial is in progress.

A major limitation of fetal mesencephalic tissue transplantation is the relatively poor clinical effect of this procedure despite the fact that the grafted dopaminergic cells remain neurochemically active. Therefore, it seems that the *de novo* formation of an ectopic SN in the striatum (the result of intrastriatal dopaminergic neuronal grafts) is not sufficient to ameliorate PD patient symptoms. In contrast, it seems that an excess of dopamine or the establishment of aberrant synaptic connections between the transplanted cells and striatal neurons, might lead to severe motor complications that, in terms of patient's life quality can be even worse than the manifestation of the disease.

The limitations and complications derived from the use of fetal mesencephalic tissue have led to the conclusion that, in its current state, this procedure, although conceptually pioneering in the field, is not an advisable therapeutic option for PD. Moreover, the double-blind placebo-controlled studies on fetal mesencephalic transplantation suggest that dopamine cell replacement in the striatum is not the ideal approach to compensate for the progressive nigrostriatal neuronal loss. Given this scenario, the clinical applicability of other transplantation procedures based on a similar rationale (e.g., intrastriatal grafting of porcine mesencephalic neurons or stem cell-derived dopaminergic

neurons) is, for the moment, uncertain. It is generally believed that other cell types and methodologies, capable of delivering locally the proper cocktail of trophic factors, might be more effective in protecting the dopaminergic nigrostriatal neurons from whatever insults cause PD and to arrest or even reverse the neurodegenerative process.

Recent pilot studies with other tissues

In recent years there have been numerous attempts to evaluate the potential applicability of dopamine-producing cells other than fetal mesencephalic neurons for transplantation in PD. In the context of this chapter the studies done with carotid body (CB) and retinal cells are particularly relevant since they have been transferred from animal models to PD patients.

Transplantation of carotid body tissue

As described above, the CB is an organ composed of highly dopaminergic glomus cells whose efficacy for antiparkinsonian cell therapy has been tested in animal models of PD (15, 27). *A priori*, a major advantage of the CB with respect to fetal mesencephalic neurons is that the former can be used for autotransplantation since its unilateral surgical resection has no significant side effects (42), thus circumventing most of the limitations of fetal transplants. Intrastriatal CB grafts can induce notable behavioral amelioration in parkinsonian rats and monkeys due not only to increase of the intrastriatal dopamine level but also to a trophic effect of the transplant on the dopaminergic nigrostriatal neurons (28). In fact, CB glomus cells are among those with the highest GDNF content in adult rodents (29). The favorable results in preclinical studies stimulated the execution of two pilot phase I/II open trials to test the feasibility, safety and clinical efficacy of CB autotransplantation in PD. The experimental protocol in these studies consisted of the unilateral removal of the CB and the preparation of cell aggregates, which were bilaterally deposited at the putamen through a stereotaxic needle (42) (Figure 5). Typically,

about 150-200 aggregates, with approximately 200 cells each, were obtained from a human CB and, therefore the total number of cells transplanted was around 15,000-20,000 on each side of the brain.

In the first CB trial, 5 of 6 PD patients showed amelioration of the UPDRS III scale in «off» periods when blindly evaluated by an independent neurologist using masked video recordings (42). Clinical improvement was between 26-74% and 13-52% at 6 and 12 months of follow-up, respectively. The patient that did not receive any benefit from the implantation had a fibrous carotid body with major absence of dopaminergic

glomus cells. In the long term a trend towards the presurgical clinical status was observed, although 3 patients still maintained a measurable motor improvement (15-48%) 36 months after transplantation. A patient of this sub-study who, for technical reasons, only received cells in one side of the brain (although the needle tracts were done bilaterally), showed clinical improvement only in the side contralateral to the transplant. The clinical evolution of the patients in the second sub-study performed by the same group was essentially similar to that described above (64). Patients of the second sub-study were subjected to [¹⁸F]-dopa PET scans before

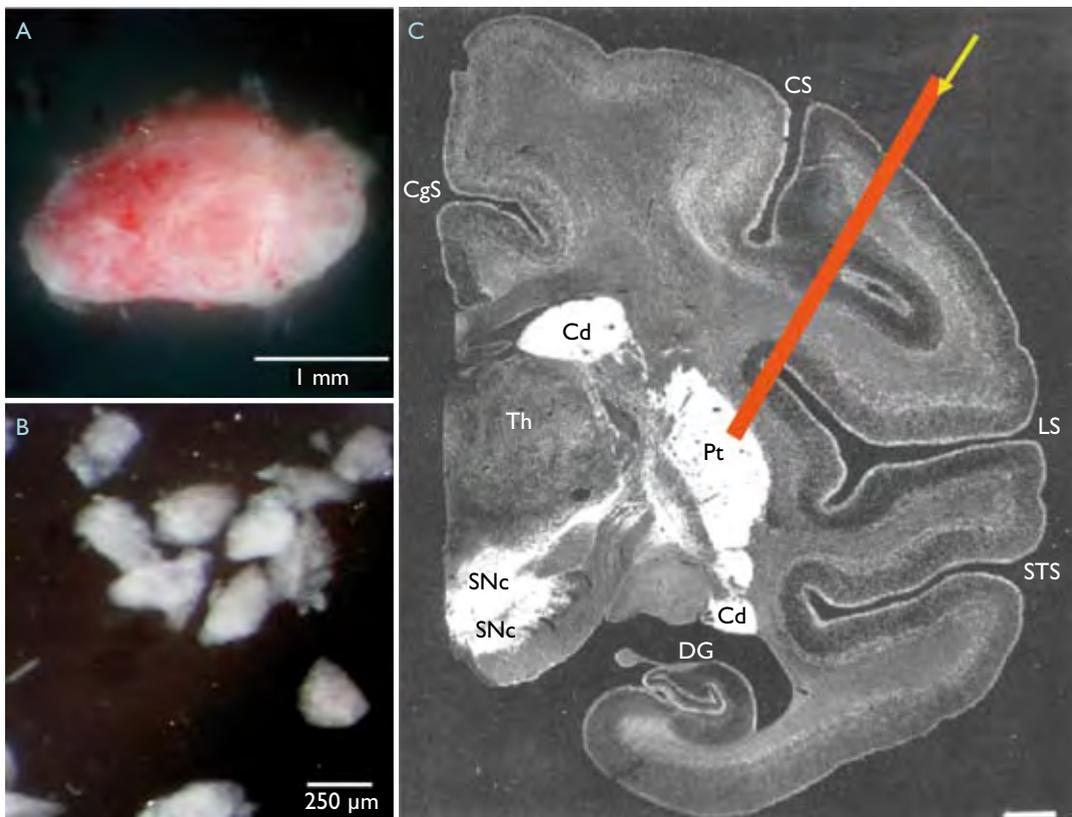


Figura 5. Intraputamenal stereotaxic transplantation of dopaminergic cells in Parkinson disease patients. A) Carotid body resected from a parkinsonian patient after it was cleaned of surrounding connective tissue. B) Carotid body cells aggregates transplanted through a stereotaxic needle as illustrated in C. Pt, putamen; Cd, caudate nucleus; Th, thalamus; SNc, substantia nigra *pars compacta*; SNr, substantia nigra *pars reticulata*. See reference (42) for further details.

and 1 year after transplantation. This neurochemical analysis showed a non-significant 5% increase in mean putaminal [^{18}F]-dopa uptake but there was a significant inverse relationship between clinical amelioration and annual decline in putaminal [^{18}F]-dopa uptake. Complications of CB grafting are similar to those reported for other intracerebral transplantation procedures. However, carotid body grafted patients, unlike those subjected to fetal transplantation, do not seem to develop off-period dyskinesias, which might be related to the fact that they received a much lower number of dopaminergic cells and that the main effect of CB transplants is to stimulate intrinsic dopaminergic striatal reinnervation (42, 64).

The pilot studies performed have indicated that CB autotransplantation is a feasible and safe procedure with potential clinical applicability to treat PD patients. In addition, CB grafting has demonstrated similar symptomatic efficacy as the placebo-controlled mesencephalic grafts. The beneficial effect of CB grafts is higher in young patients with a well-preserved CB and in individuals with a less severe disease. Altogether, these studies suggest that the clinical improvement observed after CB transplantation, although modest, derive from a true neuroprotective effect of the transplanted glomus cells on the nigrostriatal pathway. Nevertheless, at present, CB autotransplantation is not a methodology ready to be evaluated in large controlled phase III trials until the characteristics of suitable candidates and the objectives of the therapy are clearly defined, and the origin and quantity of donor tissue are determined. Although autotransplantation is conceptually attractive, the amount of tissue obtained from a single CB appears to be smaller than that needed to obtain significant clinical benefit consistently. The *in vitro* expansion of CB tissue using either conditionally immortalized glomus cells or their differentiation from multipotent precursors (35c), provides possible therapeutic strategies that will surely be explored in future work.

Transplantation of retinal cells

The retinal pigment epithelium contains dopaminergic cells whose suitability for transplantation studies has recently been tested in animal models of PD (26, 41). Good results in preclinical experiments led to the execution of an open pilot study in six patients with advanced PD. About 300,000 human pigment cells attached to gelatine microcarriers were grafted, without immunosuppression, into the most affected putamen of each patient. All subjects were reported to have a clear improvement in the UPDRS III motor scale in «off» periods that was initiated during the first month of the surgery and improved subsequently. Average reduction of the UPDRS III score with respect to the basal value was 48% at 12 months. No major complications or dyskinesias have been reported (65). Therefore, allografts of retinal pigment epithelial cells seem to be well tolerated and clinically effective in PD patients. The mechanism of action of these transplants is unknown, although a recent study has proposed that, as for CB cells, retinal cells could also release trophic factors to support nigrostriatal neurons (66). This methodology is being evaluated in a double blind, placebo-controlled study currently in progress (Table I).

Administration of trophic factors (GDNF delivery)

Neurotrophic factors are substances (generally proteins or peptides) that, among other functions, regulate the differentiation and maintenance of neuronal phenotype. These factors also protect neurons against cell death by apoptosis and/or exogenous pathological insults. Numerous studies both *in vivo* and *in vitro* have shown that mesencephalic dopaminergic neurons are sensitive to a variety of trophic factors including the glial cell line-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor, neurotrophin 4, insulin-like growth factor, fibroblast growth factor and platelet-derived

growth factor, among others. GDNF has consistently shown to exert the strongest and most selective *in vitro* and *in vivo* trophic actions on nigrostriatal dopaminergic cells (67). In animal models of PD, GDNF can prevent neuronal degeneration and restore the striatal dopaminergic function (19b, 68-70). Depletion of GDNF has been found in the SN of human PD brains, which may constitute an additional pathogenic mechanism of the disease.

Although all the precedents summarized in the previous paragraph suggest that GDNF administration might be a valuable neuroprotective and neurorestorative therapy for PD, the method of GDNF delivery into the striatum has become a critical issue, since GDNF does not cross the blood-brain barrier, diffuses poorly in the extracellular environment and is easily destroyed by proteases. Different methods of direct administration have been experimentally tested, including intermittent injections, continuous infusion, and implantation of GDNF-releasing biodegradable microspheres (71). Intraventricular or intraputamenal GDNF administration has been reported to promote structural and functional recovery in advanced parkinsonian monkeys (70). However, in a controlled clinical trial, monthly intraventricular administration of GDNF failed to provide clinical benefit in advanced PD patients but resulted in frequent adverse events (72). A *post-mortem* examination in one patient suggested that GDNF did not reach the target cells via this route. The safety and clinical effects of a continuous intraputamenal GDNF infusion have been evaluated in two open-label trials. An initial trial of a British group on five PD patients reported encouraging clinical outcomes at one year, while [^{18}F]-dopa PET studies showed an increase in putamenal uptake around the tip of each catheter (73). A second American study on 10 patients using a different delivery protocol also reported positive results at six months (74). However, a randomized placebo-controlled trial involving 34 PD patients showed no significant clinical differences bet-

ween groups at six months, despite increased [^{18}F]-dopa uptake in the recombinant GDNF-treated group (75). The open-label extension of this study was halted due to safety concerns: three patients developed neutralizing antibodies, which could potentially cross-react with endogenous GDNF, while in a parallel toxicology study some monkeys developed cerebellar damage.

In this context, research interest has focused on other indirect methods of GDNF delivery, such as cell therapy, i.e. the intrastriatal grafting of GDNF-producing cells (CB or genetically modified cells), and *in vivo* gene therapy using GDNF-encoding viral vectors (71). CB cells have already been tested in PD patients with good results in less-affected and younger patients, this finding being compatible with the trophic action of the grafted tissue. Besides GDNF, CB glomus cells also produce BDNF and other factors, so they could supply damaged neurons with the appropriate cocktail of trophic factors to encourage nigrostriatal regeneration. Despite these stimulating results, the use of CB or other cell types to deliver trophic factors in PD patients is still under debate and experimental evaluation.

Cell therapy in other CNS disorders

Huntington's disease

Huntington's disease (HD) is a dominantly inherited neurodegenerative disorder caused by a polyglutamine expansion in the gene encoding the huntingtin protein. Its pathological hallmark is the preferential loss of medium size spiny GABAergic neurons in the striatum, although degeneration progressively extends to the cortex and other brain regions. The function of huntingtin is unknown and the pathogenesis of HD remains obscure. The disease is clinically

characterized by movement disorders (mainly chorea), dementia and behavioral disturbances, leading to progressive disability and death. The relatively selective striatal damage at early stages of HD made this disease a potential target for cell therapy shortly after the initial experimental work on PD. Animal (normally rodents) models of HD are obtained by striatal neural death-induction either with excitotoxic drugs or after metabolic poisoning. There also transgenic mouse models of HD expressing polyglutamine repeat expansions. Numerous preclinical studies have demonstrated that transplants of fetal striatal cells survive in the host striatum and induce reversion of the motor and behavioral abnormalities. More recently, other donor tissues, such as neural progenitor cells, pre-differentiated GABAergic neurons obtained from a neural stem cell line, or several types of trophic factor-producing cells, have also been assayed in rodent HD models (76).

Transplantation of human fetal striatal tissue has been undertaken in a few open clinical trials each involving each 4-7 mild to moderate HD patients. A Core Assessment Program for Intracerebral Transplantation in HD (CAPIT-HD) has been developed in order to standardize the study protocols. Some bilaterally transplanted patients had small improvements in clinical measures at one-year, which correlated with an increase in the striatal and cortical metabolic activity measured by PET. One autopsy study performed 18 months after transplantation demonstrated surviving grafted cells innervated by host-derived dopaminergic fibers (77). However, in the long-term follow-up, a progressive clinical and PET decline has been consistently observed (78). Porcine xenografts have also been tried in 12 HD patients, with no change in functional capacity one year after unilateral transplantation (79). Although improvements in transplantation procedures and patient selection could lead to better outcomes, striatal cell replacement alone seems unlikely to induce a long-lasting benefit taking into account the progressive and exten-

sive nature of neurodegeneration in HD. In this context, other strategies aiming to exert a neuroprotective effect on damaged neurons should be further developed.

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a devastating disease characterized by progressive upper (cortical) and lower (spinal) motor neuron degeneration. The absence of disease-modifying therapies has prompted experimental and clinical research on cell therapy. Although the etiopathogeny of ALS is unknown it seems that alteration of redox regulation both in neurons and astrocytes is a major cause of the disease. In a minority of cases the disease is familial due to a mutation (G93A) in the Cu-Zn superoxide dismutase (SOD1), and overexpression of the mutant human SOD1 in transgenic mice results in a syndrome that resembles human ALS. Using this animal model, several cell-replacement and/or neuroprotective strategies have been tested (80). Potential benefits have been advocated after transplantation into the spinal cord of hNT cells (derived from a human teratocarcinoma cell line), bone marrow cells, Sertoli cells, neural precursor cells, astrocytes derived from embryonic stem cells, or different genetically modified cells to produce trophic factors. Intravenous administration of human umbilical cord blood mononuclear cells has also been tried in rodents with promising results.

Some cell therapy strategies have been assayed on ALS patients in small phase I-II clinical trials: twelve subjects were subjected to intrathecal implantation of encapsulated genetically engineered baby hamster kidney cells releasing human ciliary neurotrophic factor (81), and seven patients participated in a study on intraspinal cord implantation of autologous mesenchymal stem cells (82). Although preliminary results of the later study might be encouraging, the widespread nature of cell death in ALS (involving the spinal cord, brainstem and cortex) is a daunting

challenge for focal cell replacement strategies. On the other hand, diffuse trophic factor delivery from glial cells or combined approaches (cell replacement plus growth factor delivery) may show promise in modifying the course of the disease.

Currently, numerous cell-based therapies for ALS are being performed in institutions outside the academic environment without sufficient scientific knowledge or medical control. The clinical outcome of these operations is not well documented and therefore there is urgent need of phase III (double-blind and placebo-controlled) studies to clarify the actual efficacy of cell therapy in ALS.

Spinal cord injury

In recent years cell-based therapies have been intensely studied to ascertain whether they exert favorable effects on traumatic spinal cord injury (SCI). Several groups have reported excellent results in animal models and some pilot clinical studies are being performed. The experimental strategies designed for cell therapy in SCI vary depending on the nature of the lesion (complete or incomplete), and whether the treatment is applied few hours/days (acute) or weeks/months (chronic) after the lesion. In the case of complete spinal cord lesions the ultimate goal is to induce neuronal regeneration, a challenging but as yet unreachable scenario that is worth pursuing since it is expected that a significant functional recovery could be obtained even if only 1% of the severed axons are replaced or regenerated. In incomplete spinal cord lesions the functional failure is derived in part from axons partially damaged by contusion, edema, or ischemia. In these cases neuroprotective measures can help to obtain axonal re-myelination and sprouting.

A variety of cell types tested in animal models of SCI have shown considerable therapeutic potential after intraspinal transplantation or following administration by other routes, such as

intravascular, intraventricular or intrathecal (83). Schwann cells have been transplanted into spinal cord animal models based on the regenerative capacity of this cell type observed in the peripheral nervous system. Similarly, olfactory ensheathing glia, a specialized form of glial cell able to promote regeneration of axons in the olfactory nerve, have been tested, with some success, for regeneration in spinal cord injuries (84). Neural progenitor cells have been implanted in spinal cord animal models yielding behavioral recovery presumably due to neurogenesis and synaptogenesis from the transplanted cell preparation or from donor-host humoral interactions that may support regeneration of host axons. Most of the effects exerted by either neural or stem cell-derived progenitors appear to be due to their preferential differentiation to oligodendrocytes or astrocytes. In fact oligodendrocytes derived from human embryonic stem cells have also been reported to myelinate axon in foci of contused spinal cords. An entirely different approach relies on the capacity of endogenous mesenchymal or immune cells to provide axonal regeneration. With this objective appropriately activated macrophages have been shown to provide functional recovery.

Regarding the clinical application of cell therapy in SCI, several small phase I-II pilot studies using various cells types have shown promising but inconclusive results. Twenty patients with acute ($n = 7$) or chronic ($n = 13$) SCI received autologous bone marrow transplantation by intra-arterial (via catheterization of a vertebralis) or intravenous route, with promising results in the acute phase (up to 1 month after injury) (85). Eight patients were subjected to intraspinal injection of incubated autologous macrophages within 14 days of injury, three of whom achieved neurological improvement (86). For the purpose of clinical applicability a number of groups have demonstrated the production of olfactory ensheathing glial cells (OECs) by tissue culture of the olfactory epithelium obtained from biopsy samples of the

upper nasal lining. Others have used OECs obtained from fetuses or cadavers. An initial published clinical trial has shown no adverse effects one year after transplanting autologous cultured mucosal OECs into spinal injuries (87). Two other neurosurgical teams (one in Beijing and another in Lisbon), have adopted transplantation of olfactory tissue as a clinical procedure for treating patients with SCI. The results from these groups are uncertain, not well documented, and criticized by part of the scientific community (84, 88). Apart from press commentaries, no real breakthroughs in clinical achievements have been proved. Therefore, validation of these and other procedures must await the outcomes of independent randomized double-blind controlled trials that will surely be performed in the near future.

Stroke

Among neurological disorders, stroke represents a leading cause of death and disability in developed countries and despite intense research only a few options exist for the treatment of stroke-related infarction of brain tissue. Numerous preclinical experimental studies on cell therapy have been performed in rodent models of stroke (endothelin-induced transient middle cerebral artery occlusion or collagenase-induced intracerebral hemorrhage), which include direct grafting of cells in the infarcted area or surroundings as well as intraventricular, subarachnoidal, intra-arterial or intravenous cell administration. Among the cells tested are adult stem cells from bone marrow, umbilical cord and neural or adipose tissue, as well as embryonic stem cell-derived neural (neurons and glial) cells. Some studies have used engineered neural stem cells to produce angiogenic factors (VEGF and others) as well as the GDNF-secreting CB cells. In experimental stroke, cell therapy can partly reverse some behavioral deficits. However, the underlying mechanisms remain unknown as most studies

reveal little, if any, evidence for neuronal replacement and the observed behavioral improvements appeared to be related more to a graft-derived induction of a positive response in the remaining host tissue than to cell replacement by the graft itself (89-91).

From a clinical standpoint the information available pertains only to a few pilot studies performed on a small patient cohort. The group of Pittsburgh pioneered the clinical application of cell therapy for stroke by performing stereotaxic implantation of human neuronal cell lines with promising results. However, a controlled randomized trial by the same group in patients with ischemic or hemorrhagic subcortical motor strokes (treatment $n = 14$; control $n = 4$) failed to show significant benefit in the primary outcome measure (92). A study based on transplantation of fetal porcine cells was terminated by the USA Food and Drug Administration after the inclusion of five patients. Autologous mesenchymal stem cell transplantation by intravenous infusion, which appear to migrate to the site of the lesion, has also been tested in some clinical trials. One of the most representative studies is that performed by Bang et al. (93) on five stroke patients and 25 controls. In this trial the treated group achieved a better functional outcome at six months compared with controls. As indicated above, both the mechanisms of action of transplanted cells and their actual clinical efficacy are still undetermined. Cell therapy in stroke is a promising experimental approach but for the moment not a realistic therapeutic option.

Other diseases

Besides the preclinical and clinical studies already discussed, cell-based neuroregenerative or protective therapies have been assayed in several other neurological disorders. Neural precursor cell transplantation has been shown to attenuate the severity of experimental autoimmune encephalomyelitis, an animal model

of multiple sclerosis. Intraventricular transplanted cells migrated along white matter tracts and seemed to down-regulate the acute inflammatory process and reduce axonal injury (94). A different procedure, autologous hematopoietic stem cell transplantation, has been used to treat patients with severe multiple sclerosis. In a series of 178 patients, 63% were reported to have neurological improvement or stabilization at a median of 41.7 months after transplantation (95). However, a recent autopsy study on five patients who received this transplant found evidence for ongoing demyelination and neurodegeneration.

Hematopoietic stem cell transplantation, including umbilical cord blood transplantation, is being currently under consideration as a possible therapy in young patients with storage diseases such as mucopolysaccharidosis, mucopolipidosis, leukodystrophies, Krabbe disease, Tay-Sachs disease or neuronal ceroid lipofuscinosis. Intracranial transplantation of neural stem cells has recently shown striking beneficial effects in a mouse model of Sandhoff disease, a lethal gangliosidosis (96). However, further basic research in these areas is needed before the experimental data warrant the translation to patients of safe procedures with reasonable and well-demonstrated clinical efficacy.

Conclusions and perspectives

Although neuroregeneration and neurorepair are concepts and goals intimately associated with the development of modern neuroscience, it is only in the last 25 years that experimental and clinical studies have systematically addressed the possibility of neural reparation by means of cell therapy. Diseases of the brain and spinal cord represent especially daunting challenges for cell-based strategies of repair, given the multiplicity of cell types within the adult central nervous

system, and the precision with which they must interact in both space and time. Nonetheless, a number of diseases are, in principle, especially appropriate for cell-based therapy, in particular those in which single phenotypes are lost, and in which the re-establishment of vectorially specific connections is not entirely requisite for therapeutic benefit. In recent years, the preclinical and clinical studies on Parkinson's disease initiated in the 1980s have been extended to other neurological diseases, such as Huntington's disease, amyotrophic lateral sclerosis, stroke, and spinal cord injury, among others.

Originally, the goal of cell therapy was the restoration of function by replacement of dead cells with healthy ones. However, in most recent studies the primary interest has shifted from cell replacement to neuroprotection, in the hope that application of the proper cocktail of trophic factors released from cells grafted at the injured sites or administered systemically would either prevent neuronal damage or activate intrinsic regenerative mechanisms leading to restoration of the destroyed neurons or synaptic connections. The discovery of neurogenic centers in the adult brain (in the subventricular zone of the lateral ventricles and the dentate gyrus of the hippocampus) has stimulated research on the potential therapeutic applicability of pre-existing neuronal precursors, which under appropriate conditions could migrate and differentiate to replace destroyed cells in other parts of the brain. This possibility is, however, speculative and only based on preliminary experimental observations. Recently, much attention has been focused on the relevance of hippocampal neurogenesis to the pathophysiology and treatment of mood disorders. Indeed all major pharmacological and non-pharmacological treatments for depression enhance hippocampal neurogenesis and suppressing hippocampal neurogenesis in mice blocks behavioral responses in some antidepressant-sensitive tests.

Neurologic cell-based therapies have been also much influenced by the development of

embryonic stem (ES) cell research, as these cells have the potential to give rise *in vitro* to any cell type of the adult organism. ES or iPS cells are generally thought to offer a promising source of neurons suitable for cell replacement therapy in Parkinson's disease and other disorders. Most investigators in the field are, however, aware that translation of ES/iPS cells to the clinical setting is confronted with numerous limitations and unsolved problems. Differentiation of ES/iPS cells to mature neurons is still not well controlled and therefore the possibility of tumor generation is high. In addition, the short lasting viability of neurons derived from human ES/iPS cells compromises their use in cell therapy. ES/iPS cell-derived neuronal types may not possess the complete set of phenotypic features of their *in vivo* counterparts, which may contribute to the limited success of these cells in repairing injured or diseased brain and spinal cord in animal models. Hence, efficient generation of neural subtypes with correct phenotype remains a challenge. Consequently, major hurdles still lie ahead in applying human ES/iPS cell-derived neural cells clinically.

Although cell therapy constitutes an actively progressing research field, it is also the subject of numerous criticisms and concerns. The scientific and technical advances being made are quite heterogeneous and erratic, as in many cases transplantation studies are performed without a precise knowledge of the putative therapeutic products released by the cells, their mechanisms of action, or even the type of cell transplanted. Moreover, together with well-controlled pilot clinical trials being performed in academic research environments, there are institutions offering cell-based therapies to heavily deteriorated patients without having performed the necessary scientific preclinical studies and methodological (safety and feasibility) tests. In conclusion, neurologic cell-based therapies still remain a promise rather than a reality, and effective clinical translation in this area will necessarily require a period of sustained and solid basic research.

References

1. Dunnett SB, Bjorklund A. Prospects for new restorative and neuroprotective treatments in Parkinson's disease. *Nature*. 1999; 399(6738 Suppl):A32-9.
2. Tai YT, Svendsen CN. Stem cells as a potential treatment of neurological disorders. *Curr Opin Pharmacol*. 2004; 4:98-104.
3. Goldman SA, Windrem MS. Cell replacement therapy in neurological disease. *Philos Trans R Soc Lond B Biol Sci*. 2006; 361:1463-75.
4. Arvidsson A, Collin T, Kirik D, Kokaia Z, Lindvall O. Neuronal replacement from endogenous precursors in the adult brain after stroke. *Nat Med*. 2002; 8:963-70.
5. Kuo CT, Mirzadeh Z, Soriano-Navarro M, Rasin M, Wang D, Shen J, Sestan N, Garcia-Verdugo J, Alvarez-Buylla A, Jan LY, Jan YN. Postnatal deletion of Numb/Numbl reveals repair and remodeling capacity in the subventricular neurogenic niche. *Cell*. 2006; 127:1253-64.
6. Hardy J, Cai H, Cookson MR, Gwinn-Hardy K, Singleton A. Genetics of Parkinson's disease and parkinsonism. *Ann Neurol*. 2006; 60:389-98.
7. de Rijk MC, Tzourio C, Breteler MM, Dartigues JF, Amaducci L, Lopez-Pousa S, Manubens-Bertran JM, Alperovitch A, Rocca W. Prevalence of parkinsonism and Parkinson's disease in Europe: the EUROPARKINSON Collaborative Study. European Community Concerted Action on the Epidemiology of Parkinson's disease. *J Neurol Neurosurg Psychiatry*. 1997; 62:10-5.
8. de Lau LM, Breteler MM. Epidemiology of Parkinson's disease. *Lancet Neurol*. 2006; 5:525-35.
9. Braak H, DelTredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging*. 2003; 24:197-211.
10. Dauer W, Przedborski S. Parkinson's disease: mechanisms and models. *Neuron*. 2003; 39:889-909.
11. Lang AE, Lozano AM. Parkinson's disease. Second of two parts. *N Engl J Med*. 1998; 339:1130-43.
12. Schwarting RK, Huston JR. The unilateral 6-hydroxydopamine lesion model in behavioral brain research. Analysis of functional deficits, recovery and treatments. *Prog Neurobiol*. 1996; 50:275-331.
13. Beal F, Lang A. The proteasomal inhibition model of Parkinson's disease: "Boon or bust"? *Ann Neurol*. 2006; 60:158-61.
14. Burns RS, Chiu CC, Markey SP, Ebert MH, Jacobowitz DM, Kopin JJ. A primate model of parkinsonism: selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Proc Natl Acad Sci U S A*. 1983; 80:4546-50.
15. Luquin MR, Montoro RJ, Guillen J, Saldise L, Insausti R, Del Rio J, Lopez-Barneo J. Recovery of chronic parkinsonian monkeys by autotransplants of carotid body cell aggregates into putamen. *Neuron*. 1999; 22:743-50.

16. Heikkilä RE, Hess A, Duvoisin RC. Dopaminergic neurotoxicity of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine in mice. *Science*. 1984; 224:1451-3.
17. Mejias R, Villadiego J, Pintado CO, Vime PJ, Gao L, Toledo-Aral JJ, Echevarria M, Lopez-Barneo J. Neuroprotection by transgenic expression of glucose-6-phosphate dehydrogenase in dopaminergic nigrostriatal neurons of mice. *J Neurosci*. 2006; 26:4500-8.
18. Jain S, Golden JF, Wozniak D, Pehek E, Johnson EM Jr, Milbrandt J. RET is dispensable for maintenance of midbrain dopaminergic neurons in adult mice. *J Neurosci*. 2006; 26:11230-8.
19. Kramer ER, Aron L, Ramakers GM, Seitz S, Zhuang X, Beyer K, Smidt MP, Klein R. Absence of Ret Signaling in Mice Causes Progressive and Late Degeneration of the Nigrostriatal System. *PLoS Biol*. 2007; 5:e39.
- 19b. Pascual A, Hidalgo-Figueroa M, Piruat JJ, Pintado CO, Gómez-Díaz R, López-Barneo J. Absolute requirement of GDNF for adult catecholaminergic neuron survival. *Nature Neurosci*. 2008; 11:755-761
20. Perlow MJ, Freed WJ, Hoffer BJ, Seiger A, Olson L, Wyatt RJ. Brain grafts reduce motor abnormalities produced by destruction of nigrostriatal dopamine system. *Science*. 1979; 204:643-7.
21. Bjorklund A, Stenevi U. Reconstruction of the nigrostriatal dopamine pathway by intracerebral nigral transplants. *Brain Res*. 1979; 177:555-60.
22. Redmond DE, Sladek JR Jr, Roth RH, Collier TJ, Elsworth JD, Deutch AY, Haber S. Fetal neuronal grafts in monkeys given methylphenyltetrahydropyridine. *Lancet*. 1986; 1:1125-7.
23. Freed WJ, Morihisa JM, Spoor E, Hoffer BJ, Olson L, Seiger A, Wyatt RJ. Transplanted adrenal chromaffin cells in rat brain reduce lesion-induced rotational behaviour. *Nature*. 1981; 292:351-2.
24. Galpern WR, Burns LH, Deacon TW, Dinsmore J, Isacson O. Xenotransplantation of porcine fetal ventral mesencephalon in a rat model of Parkinson's disease: functional recovery and graft morphology. *Exp Neurol*. 1996; 140:1-13.
25. Nakao N, Kakishita K, Uematsu Y, Yoshimasu T, Besho T, Nakai K, Naito Y, Itakura T. Enhancement of the response to levodopa therapy after intrastriatal transplantation of autologous sympathetic neurons in patients with Parkinson disease. *J Neurosurg*. 2001; 95:275-84.
26. Subramanian T, Marchionini D, Potter EM, Cornfeldt ML. Striatal xenotransplantation of human retinal pigment epithelial cells attached to microcarriers in hemiparkinsonian rats ameliorates behavioral deficits without provoking a host immune response. *Cell Transplant*. 2002; 11:207-14.
27. Espejo EF, Montoro RJ, Armengol JA, Lopez-Barneo J. Cellular and functional recovery of Parkinsonian rats after intrastriatal transplantation of carotid body cell aggregates. *Neuron*. 1998; 20:197-206.
28. Toledo-Aral JJ, Mendez-Ferrer S, Pardal R, Echevarria M, Lopez-Barneo J. Trophic restoration of the nigrostriatal dopaminergic pathway in long-term carotid body-grafted parkinsonian rats. *J Neurosci*. 2003; 23:141-8
29. Villadiego J, Mendez-Ferrer S, Valdes-Sanchez T, Silos-Santiago I, Farinas I, Lopez-Barneo J, Toledo-Aral JJ. Selective glial cell line-derived neurotrophic factor production in adult dopaminergic carotid body cells in situ and after intrastriatal transplantation. *J Neurosci*. 2005; 25:4091-8.
30. Minguez-Castellanos A, Escamilla-Sevilla F. [Cell therapy and other neuroregenerative strategies in Parkinson's disease (I and II)]. *Rev Neurol*. 2005; 41:604-14 and 684-93.
31. Kawasaki H, Mizuseki K, Nishikawa S, Kaneko S, Kuwana Y, Nakanishi S, Nishikawa SI, Sasai Y. Induction of midbrain dopaminergic neurons from ES cells by stromal cell-derived inducing activity. *Neuron*. 2000; 28:31-40.
32. Lee SH, Lumelsky N, Studer L, Auerbach JM, McKay RD. Efficient generation of midbrain and hindbrain neurons from mouse embryonic stem cells. *Nat Biotechnol*. 2000; 18:675-9.
33. Kim JH, Auerbach JM, Rodriguez-Gomez JA, Velasco I, Gavin D, Lumelsky N, Lee SH, Nguyen J, Sanchez-Pernaute R, Bankiewicz K, McKay R. Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease. *Nature* 2002; 418:50-6.
34. Rodríguez-Gómez JA, Lu J-Q, Velasco I, Rivera S, Zoghbi SS, Liow JS, Musachio JL, Chin FT, Toyama H, Seidel J, Green MV, Thanos PK, Ichise M, Pike VW, Innis RB, McKay RD. Persistent dopamine functions of neurons derived from embryonic stem cells in a rodent model of Parkinson's disease. *Stem Cells*. 2007; 25:918-28.
35. Parson A. The long journey from stem cells to medical product. *Cell*. 2006; 125:9-11.
- 35b. Lonardo E, Parish CL, Ponticelli S, Marasco D, Ribeiro D, Ruvo M, De Falco S, Arenas E, Minchiotti G. A small synthetic cripto blocking peptide improves neural induction, dopaminergic differentiation, and functional integration of mouse embryonic stem cells in a rat model of Parkinson's disease. *Stem Cells*. 2010; 28:1326-1337.
- 35c. Pardal R, Ortega-Sáenz P, Duran R, López-Barneo J. Glia-like stem cells sustain physiologic neurogenesis in the adult carotid body. *Cell* 2007; 131:364-377
36. Backlund EO, Granberg PO, Hamberger B, Knutsson E, Martensson A, Sedvall G, Seiger A, Olson L. Transplantation of adrenal medullary tissue to striatum in parkinsonism. First clinical trials. *J Neurosurg*. 1985; 62:169-73.
37. Madrazo I, Drucker-Colin R, Diaz V, Martinez-Mata J, Torres C, Becerra JJ. Open microsurgical autograft of adrenal medulla to the right caudate nucleus in two patients with intractable Parkinson's disease. *N Engl J Med*. 1987; 316:831-4.
38. Lopez-Lozano JJ, Bravo G, Abascal J, Brera B, Millan I. Clinical outcome of cotransplantation of peripheral

- nerve and adrenal medulla in patients with Parkinson's disease. *Clinica Puerta de Hierro Neural Transplantation Group. J Neurosurg.* 1999; 90:875-82.
39. Lindvall O, Rehnström S, Brundin P, Gustavii B, Astedt B, Widner H, Lindholm T, Björklund A, Leenders KL, Rothwell JC, et al. Human fetal dopamine neurons grafted into the striatum in two patients with severe Parkinson's disease. A detailed account of methodology and a 6-month follow-up. *Arch Neurol.* 1989; 46:615-31.
 40. Schumacher JM, Ellis SA, Palmer EP, Kott HS, Dinsmore J, Dempsey PK, Fischman AJ, Thomas C, Feldman RG, Kassisi S, Raineri R, Manhart C, Penney D, Fink JS, Isacson O. Transplantation of embryonic porcine mesencephalic tissue in patients with PD. *Neurology.* 2000 14; 54:1042-50.
 41. Watts RL, Raiser CD, Stover NP, Cornfeldt ML, Schweikert AW, Allen RC, Subramanian T, Doudet D, Honey CR, Bakay RA. Stereotaxic intrastriatal implantation of human retinal pigment epithelial (hRPE) cells attached to gelatin microcarriers: a potential new cell therapy for Parkinson's disease. *J Neural Transm Suppl.* 2003; 65:215-27.
 42. Arjona V, Minguéz-Castellanos A, Montoro RJ, Ortega A, Escamilla F, Toledo-Aral JJ, Pardal R, Mendez-Ferrer S, Martin JM, Perez M, Katati MJ, Valencia E, Garcia T, Lopez-Barneo J. Autotransplantation of human carotid body cell aggregates for treatment of Parkinson's disease. *Neurosurgery.* 2003; 53:321-8.
 43. Horvath M, Pasztor E, Palkovits M, Solyom A, Tarczy M, Lekka N, Csanda E. Autotransplantation of superior cervical ganglion to the caudate nucleus in three patients with Parkinson's disease (preliminary report). *Neurosurg Rev.* 1990; 13:119-22.
 44. Hoffer BJ, Leenders KL, Young D, Gerhardt G, Zerbe GO, Bygdeman M, Seiger A, Olson L, Stromberg I, Freedman R. Eighteen-month course of two patients with grafts of fetal dopamine neurons for severe Parkinson's disease. *Exp Neurol.* 1992; 118:243-52.
 45. Lindvall O, Brundin P, Widner H, Rehnström S, Gustavii B, Frackowiak R, Leenders KL, Sawle G, Rothwell JC, Marsden CD, et al. Grafts of fetal dopamine neurons survive and improve motor function in Parkinson's disease. *Science.* 1990; 247:574-7.
 46. Lindvall O, Sawle G, Widner H, Rothwell JC, Björklund A, Brooks D, Brundin P, Frackowiak R, Marsden CD, Odin P, et al. Evidence for long-term survival and function of dopaminergic grafts in progressive Parkinson's disease. *Ann Neurol.* 1994; 35:172-80.
 47. Sawle GV, Bloomfield PM, Björklund A, Brooks DJ, Brundin P, Leenders KL, Lindvall O, Marsden CD, Rehnström S, Widner H, et al. Transplantation of fetal dopamine neurons in Parkinson's disease: PET [18F]6-L-fluorodopa studies in two patients with putaminal implants. *Ann Neurol.* 1992; 31:166-73.
 48. Widner H, Tetrad J, Rehnström S, Snow B, Brundin P, Gustavii B, Björklund A, Lindvall O, Langston JW. Bilateral fetal mesencephalic grafting in two patients with parkinsonism induced by 1-methyl-4-phenyl-1-,2,3,6-tetrahydropyridine (MPTP). *N Engl J Med.* 1992; 327:1556-63.
 49. Freed CR, Breeze RE, Rosenberg NL, Schneck SA, Wells TH, Barrett JN, Grafton ST, Huang SC, Eidelberg D, Rottenberg DA. Transplantation of human fetal dopamine cells for Parkinson's disease. Results at 1 year. *Arch Neurol.* 1990; 47:505-12.
 50. Freed CR, Breeze RE, Rosenberg NL, Schneck SA, Kriek E, Qi JX, Lone T, Zhang YB, Snyder JA, Wells TH, et al. Survival of implanted fetal dopamine cells and neurologic improvement 12 to 46 months after transplantation for Parkinson's disease. *N Engl J Med.* 1992; 327:1549-55.
 51. Spencer DD, Robbins RJ, Naftolin F, Marek KL, Vollmer T, Leranath C, Roth RH, Price LH, Gjedde A, Bunney BS, et al. Unilateral transplantation of human fetal mesencephalic tissue into the caudate nucleus of patients with Parkinson's disease. *N Engl J Med.* 1992; 327:1541-8.
 52. Freeman TB, Olanow CW, Hauser RA, Nauert GM, Smith DA, Borlongan CV, Sanberg PR, Holt DA, Kordower JH, Vingerhoets FJ, et al. Bilateral fetal nigral transplantation into the postcommissural putamen in Parkinson's disease. *Ann Neurol.* 1995; 38:379-88.
 53. Kordower JH, Freeman TB, Snow BJ, Vingerhoets FJ, Mufson EJ, Sanberg PR, Hauser RA, Smith DA, Nauert GM, Perl DP, et al. Neuropathological evidence of graft survival and striatal reinnervation after the transplantation of fetal mesencephalic tissue in a patient with Parkinson's disease. *N Engl J Med.* 1995; 332:1118-24.
 54. Hauser RA, Freeman TB, Snow BJ, Nauert M, Gauger L, Kordower JH, Olanow CW. Long-term evaluation of bilateral fetal nigral transplantation in Parkinson disease. *Arch Neurol.* 1999; 56:179-87.
 55. Peschanski M, Defer G, N'Guyen JP, Ricolfi F, Monfort JC, Remy P, Geny C, Samson Y, Hantraye P, Jeny R, et al. Bilateral motor improvement and alteration of L-dopa effect in two patients with Parkinson's disease following intrastriatal transplantation of foetal ventral mesencephalon. *Brain.* 1994; 117:487-99.
 56. Remy P, Samson Y, Hantraye P, Fontaine A, Defer G, Mangin JF, Fenelon G, Geny C, Ricolfi F, Frouin V, et al. Clinical correlates of [18F]fluorodopa uptake in five grafted parkinsonian patients. *Ann Neurol.* 1995; 38:580-8.
 57. Defer GL, Geny C, Ricolfi F, Fenelon G, Monfort JC, Remy P, Villafane G, Jeny R, Samson Y, Keravel Y, Gaston A, Degos JD, Peschanski M, Cesaro P, Nguyen JP. Long-term outcome of unilaterally transplanted parkinsonian patients. I. Clinical approach. *Brain.* 1996; 119:41-50.
 58. Lopez-Lozano JJ, Bravo G, Brera B, Millan I, Dargallo J, Salméan J, Uria J, Insausti J. Long-term improvement in patients with severe Parkinson's disease after implantation of fetal ventral mesencephalic tissue in a cavity of the caudate nucleus: 5-year follow up in 10 patients. *Clinica Puerta de Hierro Neural Transplantation Group. J Neurosurg.* 1997; 86:931-42.

59. Kopyov OV, Jacques DS, Lieberman A, Duma CM, Rogers RL. Outcome following intrastriatal fetal mesencephalic grafts for Parkinson's patients is directly related to the volume of grafted tissue. *Exp Neurol*. 1997; 146:536-45.
60. Langston JW, Widner H, Goetz CG, Brooks D, Fahn S, Freeman T, Watts R. Core assessment program for intracerebral transplantations (CAPIT). *Mov Disord*. 1992; 7:2-13.
61. Freed CR, Greene PE, Breeze RE, Tsai WY, DuMouchel W, Kao R, Dillon S, Winfield H, Culver S, Trojanowski JQ, Eidelberg D, Fahn S. Transplantation of embryonic dopamine neurons for severe Parkinson's disease. *N Engl J Med*. 2001; 344:710-9.
62. Olanow CW, Goetz CG, Kordower JH, Stoessl AJ, Sossi V, Brin MF, Shannon KM, Nauert GM, Perl DP, Godbold J, Freeman TB. A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease. *Ann Neurol*. 2003; 54:403-14.
63. Piccini P, Brooks DJ, Bjorklund A, Gunn RN, Grasby PM, Rimoldi O, Brundin P, Hagell P, Rehncrona S, Widner H, Lindvall O. Dopamine release from nigral transplants visualized in vivo in a Parkinson's patient. *Nat Neurosci*. 1999; 2:1137-40.
64. Minguez-Castellanos A, Escamilla-Sevilla F, Hotton GR, Toledo-Aral JJ, Ortega-Moreno A, Mendez-Ferrer S, Martin-Linares JM, Katati MJ, Mir P, Villadiego J, Meersmans M, Perez-Garcia M, Brooks DJ, Arjona V, Lopez-Barneo J. Carotid body autotransplantation in Parkinson disease: A clinical and PET study. *J Neurol Neurosurg Psychiatry*. 2007; [Epub ahead of print].
65. Stover NP, Bakay RAE, Subramanian T et al. Intrastriatal implantation of human retinal pigment epithelial cells attached to microcarriers in advanced Parkinson disease. *Arch Neurol* 2005; 62:1833-1837.
66. McKay BS, Goodman B, Falk T, Sherman SJ. Retinal epithelial cell transplantation could provide trophic support in Parkinson's disease: results from an in vitro model system. *Exp Neurol* 2006; 201:234-243.
67. Lin LF, Doherty DH, Lile JD, Bektesh S, Collins F. GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. *Science*. 1993; 260:1130-2.
68. Tomac A, Lindqvist E, Lin LF, Ogren SO, Young D, Hoffer BJ, Olson L. Protection and repair of the nigrostriatal dopaminergic system by GDNF in vivo. *Nature*. 1995; 373:335-9.
69. Gash DM, Zhang Z, Ovadia A, Cass WA, Yi A, Simmerman L, Russell D, Martin D, Lapchak PA, Collins F, Hoffer BJ, Gerhardt GA. Functional recovery in parkinsonian monkeys treated with GDNF. *Nature*. 1996; 380:252-5.
70. Grondin R, Zhang Z, Yi A, Cass WA, Maswood N, Andersen AH, Elsberry DD, Klein MC, Gerhardt GA, Gash DM. Chronic, controlled GDNF infusion promotes structural and functional recovery in advanced parkinsonian monkeys. *Brain*. 2002; 125:2191-201.
71. Kirik D, Georgievska B, Bjorklund A. Localized striatal delivery of GDNF as a treatment for Parkinson disease. *Nat Neurosci*. 2004; 7:105-10.
72. Nutt JG, Burchiel KJ, Comella CL, Jankovic J, Lang AE, Laws ER Jr, Lozano AM, Penn RD, Simpson RK Jr, Stacy M, Wooten GF; ICV GDNF Study Group. Implanted intracerebroventricular. Glial cell line-derived neurotrophic factor: Randomized, double-blind trial of glial cell line-derived neurotrophic factor (GDNF) in PD. *Neurology*. 2003; 60:69-73.
73. Gill SS, Patel NK, Hotton GR, O'Sullivan K, McCarter R, Bunnage M, Brooks DJ, Svendsen CN, Heywood P. Direct brain infusion of glial cell line-derived neurotrophic factor in Parkinson disease. *Nat Med*. 2003; 9:589-95.
74. Slevin JT, Gerhardt GA, Smith CD et al. Improvement of bilateral motor functions in patients with Parkinson disease through the unilateral intraputamenal infusion of glial cell line-derived neurotrophic factor. *J Neurosurg* 2005; 102:216-22.
75. Lang AE, Gill S, Patel NK et al. Randomized control trial of intraputamenal glial cell line-derived neurotrophic factor infusion in Parkinson disease. *Ann Neurol*. 2006; 59:459-66.
76. Rosser AE, Dunnett SB. Cell transplantation for Huntington's disease. *Lancet Neurol*. 2006; 5:284-5.
77. Hauser RA, Furtado S, Cimino CR et al. Bilateral human fetal striatal transplantation in Huntington's disease. *Neurology* 2002; 58:687-95.
78. Bachoud-Levi AC, Gaura V, Brugieres P et al. Effect of fetal neural transplants in patients with Huntington's disease 6 years after surgery: a long-term follow-up study. *Lancet Neurol*. 2006; 5:303-309.
79. Fink JS, Schumacher JM, Elias SL et al. Porcine xenografts in Parkinson's disease and Huntington's disease patients: preliminary results. *Cell Transplant*. 2000; 9:273-278.
80. Veldink JH, Van den Berg LH, Wokke JH. The future of motor neuron disease: the challenge is in the genes. *J Neurol*. 2004; 251:491-500.
81. Zurn AD, Henry H, Schlupe M et al. Evaluation of an intrathecal immune response in amyotrophic lateral sclerosis patients implanted with encapsulated genetically engineered xenogeneic cells. *Cell Transplant*. 2000; 9:471-484.
82. Mazzini L, Mareschi K, Ferrero I et al. Autologous mesenchymal stem cells: clinical applications in amyotrophic lateral sclerosis. *Neurol Res*. 2006; 28:523-526.
83. Pearse DD, Bunge MB. Designing cell- and gene-based regeneration strategies to repair the injured spinal cord. *J Neurotrauma*. 2006; 23:438-52.
84. Raisman G, Li Y. Repair of neural pathways by olfactory ensheathing cells. *Nat Rev Neurosci*. 2007; 8:312-9.
85. Sykova E, Homola A, Mazanec R et al. Autologous bone marrow transplantation in patients with subacute and chronic spinal cord injury. *Cell Transplant*. 2006; 15:675-87.
86. Knoller N, Auerbach G, Fulga V et al. Clinical experience using incubated autologous macrophages as a treatment for complete spinal cord injury: phase I study results. *J Neurosurg Spine* 2005; 3:173-81.
87. Feron F, Perry C, Cochrane J, Licina P, Nowitzke A, Urquhart S, Geraghty T, Mackay-Sim A. Autologous

- olfactory ensheathing cell transplantation in human spinal cord injury. *Brain*. 2005; 128: 2951-60.
88. Cyranoski, D. Warned about unproven spinal surgery, *Nature* 2006; 440:850-1.
89. Haas S, Weidner N, Winkler J. et al., 2005. Adult stem cell therapy in stroke. *Current Opin. Neurol*. 2005; 18: 59-64.
90. Yu G, Fournier C, Hess DC, Borlongan CV. Transplantation of carotid body cells in the treatment of neurological disorders. *Neurosci Biobehav Rev*. 2005; 28:803-10.
91. Buhemann C, Scholz A, Bernreuther C, Malik CY, Braun H, Schachner M, Reymann KG, Dihne M. Neuronal differentiation of transplanted embryonic stem cell-derived precursors in stroke lesions of adult rats. *Brain*. 2006; 129:3238-48.
92. Kondziolka D, Steinberg GK, Wechsler L et al. Neurotransplantation for patients with subcortical motor stroke: a phase 2 randomized trial. *J Neurosurg*. 2005; 103:38-45.
93. Bang OY, Lee JS, Lee PH, Lee G. Autologous mesenchymal stem cell transplantation in stroke patients. *Ann Neurol*. 2005; 57:874-82.
94. Einstein O, Grigoriadis N, Mizrachi-Kol R et al. Transplanted neural precursor cells reduce brain inflammation to attenuate chronic experimental autoimmune encephalomyelitis. *Exp Neurol*. 2006; 198:275-84.
95. Saccardi R, Kozak T, Bocelli-Tyndall C et al. Autologous stem cell transplantation for progressive multiple sclerosis: update of the European Group for Blood and Marrow Transplantation autoimmune diseases working party database. *Mult Scler*. 2006; 12:814-23.
96. Lee JP, Jeyakumar M, Gonzalez R et al. Stem cells act through multiple mechanisms to benefit mice with neurodegenerative metabolic disease. *Nat Med*. 2007; 13:439-47.