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Gene Transfer into the Central and Peripheral Nervous System: Applications for the Treatment of Neurodegenerative Diseases and Peripheral Neuropathies

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Introduction

The use of classical pharmacotherapy for the treatment of neurological disease is restricted by constraints specific to the nervous system. Potent therapeutic macromolecules, such as neurotrophic factors (Thorne and Frey, 2001), when they are systematically administered, penetrate the nervous system only inefficiently due to the presence of the blood–brain and –nerve barriers. This restriction arises from the presence of tight junctions (zonulae occludens) between adjacent endothelial cells and a relative paucity of pinocytotic vesicles within the endothelium of nervous system arterioles, capillaries, and venules. Furthermore, in many cases, proteins are rapidly degraded, and have a short half-life following systemic administration. Therefore, high doses must be administered in the hope of achieving therapeutic concentrations in the target neuronal cells, and such doses are likely to produce deleterious side effects. Although recent advances have shed light on the pathogenesis

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Abbreviations: AAV, adeno-associated virus; ALS, amyotrophic lateral sclerosis; BDNF, brain-derived neurotrophic factor; CNS, central nervous system; CNTF, ciliary neurotrophic factor; CSF, cerebrospinal fluid; DNA, deoxyribonucleic acid; DRG, dorsal root ganglia; GDNF, glial cell line-derived neurotrophic factor; HIV, human immunodeficiency virus; HSV, Herpes simplex virus; NGF, nerve growth factor; NRSE, neuron-restrictive silencer element; NSE, neuron-specific enolase; NT-3, neurotrophin-3; PD, Parkinson's disease; PNS, peripheral nervous system; RNA, ribonucleic acid; SOD, Cu/Zn superoxide dismutase; TH, tyrosine hydroxylase; VEGF, vascular endothelial growth factor.

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of many neurological diseases, leading to the discovery of new molecular targets, applications in therapy are dramatically hampered by delivery issues (Aebischer and Ridet, 2001). Gene transfer may at least partly solve this problem by allowing the sustained and prolonged production of therapeutic macromolecules within the body, possibly even within the diseased neuron itself.

Neurodegenerative diseases are a complex group of disorders sharing a common pathological event: neuronal death. There are four principal strategies of gene therapy in these diseases. The purpose of the first one, referred to as 'restorative strategy', is to compensate the loss of function of the neurons involved in the degenerative process. This objective is conceivable for diseases in which symptoms are related to the deficiency of a specific neurotransmitter. This is the case in Parkinson's disease (PD), which is characterized by the mostly selective death of dopamine-synthesizing neurons. Restorative gene therapy consists of the transfer of genes implicated in dopamine synthesis. However, other than PD, neurodegenerative diseases lead to dysfunctions of multiple and disseminated neurotransmitter systems, and are therefore not accessible to a restorative gene therapy strategy. A second strategy can potentially be applied to all neurodegenerative diseases, and consists of providing general support to neuronal survival with neuroprotective proteins, such as trophic factors, anti-oxidative enzymes, or anti-apoptotic proteins. A third strategy is to inhibit the deleterious expression of specific genes by antisense oligonucleotides designed to block translations of RNA species. Specifically, there is growing evidence that a broad range of neurodegenerative diseases are characterized by neuronal damage due to toxic, aggregation-prone, misfolded proteins (Wolozin and Behl, 2000; Taylor *et al.*, 2002). Thus, decreasing the production of these specific proteins would be an attractive strategy to prevent the progression of the disease. A fourth strategy, referred to as 'gene replacement therapy', consists of the replacement of missing or defective genes. This procedure is suitable only for inherited genetic diseases in which the genetic defect is responsible for the loss of a normal biochemical function.

Genes can be transferred either by direct injection of a vector system (*in vivo* gene transfer) or indirectly by grafting genetically modified cells (*ex vivo* gene transfer). Whereas *ex vivo* gene therapy is tailored to the delivery of secreted proteins, *in vivo* gene therapy can deliver both secreted and intracellular proteins. Whatever the strategy used, several issues have to be resolved: 1) targeting of the gene product to the appropriate cells; 2) extent of the transgene expression; 3) stability of transgene expression; 4) safety of the procedure; and 5) large-scale production of the gene therapy vector.

In the first part, we will discuss the general principles and the tools used for gene therapy in both the central nervous system (CNS) and the peripheral nervous system (PNS). In the second part, we will review the current experimental achievements in three categories of neurologic diseases which are particularly suitable for gene therapy: 1) a neurodegenerative disease confined to the CNS: Parkinson's disease; 2) a neurodegenerative disease affecting both the central and the PNS: amyotrophic lateral sclerosis (ALS); and 3) toxic and diabetic peripheral neuropathies.

Potent ways of targeting gene products in the central and peripheral nervous system

DELIVERY TO THE CENTRAL NERVOUS SYSTEM

Direct gene transfer to the CNS is an attractive strategy for the treatment of neurological diseases in which neurodegeneration involves a subset of neurons at specific locations, such as PD. Precisely localized injections are feasible by stereotactic surgery in order to target a cluster of cells involved in the pathogenic process, whilst preserving other areas of the CNS. Many cell types have been proposed for use as vehicles for *ex vivo* gene therapy in the CNS, including fibroblasts (Fisher *et al.*, 1991), astrocytes (Ridoux *et al.*, 1994; Ridet *et al.*, 1999), and human neural progenitors (Sabate *et al.*, 1995; Corti *et al.*, 1999a). The genetically engineered cells can be directly grafted in the nervous system in order to release the transgene locally. Cells can also be encapsulated, a technique developed by P. Aebischer's group: a non-degradable, synthetic polymer membrane surrounds the transplanted cells and protects them from the attacks by the host immune system (Aebischer *et al.*, 1991; Aebischer and Ridet, 2001).

In vivo direct gene delivery allows the delivery of a therapeutic protein directly into the injected area. Furthermore, spread of vectors to functionally related areas can be mediated by anterograde or retrograde transport of vectors within neurons projecting to the injection site. For example, dopaminergic neurons of the substantia nigra can be selectively infected and express a reporter gene after inoculation of the virus in the striatum. Numerous viral vectors have been shown to be effective for *in vivo* gene transfer in the CNS, including those derived from herpes viruses, adenoviruses, adeno-associated viruses (AAV), and lentiviral vectors.

Alternatively, the goal of gene therapy may be to treat a wider population of neurons. This is the case for diseases such as Alzheimer disease or ALS, in which the neurons involved are disseminated within the CNS. Several routes of delivery have been proposed to target a disseminated population of cells in the nervous system. A well-studied method is the delivery to the cerebrospinal fluid (CSF) in order to reach the adjacent brain and spinal structures. A gene therapy *ex vivo* approach involves the implantation in the CSF of polymer-encapsulated cells. Viral vectors can also be directly injected into the CSF in order to infect ependymal and meningeal cells (Bajocchi *et al.*, 1993; Driesse *et al.*, 1999). Uptake of antisense oligonucleotides into the brain and spinal cord has also been achieved by intrathecal administration (Wahlestedt *et al.*, 1993; Yee *et al.*, 1994; Standifer *et al.*, 1995; Khasar *et al.*, 1996). Alternative ways to directly inject a viral vector into the CNS include nasal instillation (Draghia *et al.*, 1995), or injection into the carotid artery after blood–brain barrier disruption with the use of osmotic (Doran *et al.*, 1995; Muldoon *et al.*, 1995; Nilaver *et al.*, 1995) or pharmacologic agents (Rainov *et al.*, 1995, 1998; Barnett *et al.*, 1999).

Recently, an attractive means of widespread expression of an exogenous gene after intravenous administration has been described (Shi and Pardridge, 2000; Shi *et al.*, 2001). Brain capillary endothelial cells possess specific receptor-mediated transport mechanisms that can be exploited as a means to deliver therapeutics to the brain. Particularly, many exciting studies have been carried out using the transferrin receptor for drug delivery through the blood–brain barrier (Bickel *et al.*, 2001; Li *et*

al., 2002). Plasmid-DNA has been incorporated in a liposome conjugated with an anti-transferrin receptor antibody (Shi and Pardridge, 2000; Shi *et al.*, 2001). Intravenous injection of this vector system led to gene expression throughout the nervous system, including neurons, choroid plexus epithelium, and the brain microvasculature.

DELIVERY TO THE PERIPHERAL NERVOUS SYSTEM

Direct gene transfer in peripheral neurons can be achieved by direct injection of recombinant vectors into the dorsal root for sensory neurons (Glatzel *et al.*, 2000), or into the nerve fibres for both sensory and motor neurons (Sahenk *et al.*, 1993; Glatzel *et al.*, 2000; Joung *et al.*, 2000). A more attractive route for delivery, given its higher feasibility in clinics, is not to inject the nerve itself, but rather its peripheral target, taking advantage of the retrograde transport of some viral vectors. Infection of a motor neuron can be achieved by intra-muscular injection. Viral vectors, such as adenovirus (Finiels *et al.*, 1995; Ghadge *et al.*, 1995) and herpes virus (Keir *et al.*, 1995), undergo retrograde transport from muscular synapses to motor neuron cell bodies. Gene transfer can thus be specifically targeted to particular regions of the spinal cord by appropriate selection of the muscle injected. Infection of the sensory neurons can be obtained by subcutaneous injection of a recombinant herpes simplex virus into the skin, and its subsequent retrograde transport to the cell bodies localized in the dorsal root ganglia (DRG) (Goss *et al.*, 2001; Chattopadhyay *et al.*, 2002).

Intra-CSF delivery can also be used to target peripheral neurons. Cell bodies of the motor and sensory neurons are in close proximity to the CSF compartment. One *ex vivo* approach using encapsulated genetically engineered cells has been evaluated in a phase I study in ALS patients (Aebischer *et al.*, 1996b).

Sustained systemic delivery of a therapeutic protein is an attractive way to target a wide population of peripheral neurons. The peripheral distal parts of motor and sensory nerves are beyond the blood–nerve barrier, so it is possible for neurons to distally pick up the polypeptide and transfer it by retrograde transport to the cell body. In particular, such a mechanism has been demonstrated for neurotrophic factors (DiStefano *et al.*, 1992; Curtis *et al.*, 1993). Since there is no blood–nerve barrier in the DRG (Olsson, 1984), large molecules can readily access the cell bodies of the sensory neurons. The continuous delivery of low doses of recombinant neurotrophic factor by gene therapy resembles the physiologic condition, and therefore may be more effective and safer for therapeutic applications than repeated administrations of high doses (Gravel *et al.*, 1997; Haase *et al.*, 1997; Sendtner, 1997; Pradat *et al.*, 2001a,b). An *ex vivo* approach consists of the subcutaneous implantation of genetically modified cells that have been engineered *in vitro* to produce organ-like structures (Vandenburgh *et al.*, 1996), or encapsulated in a polymer envelope (Aebischer *et al.*, 1991; Aebischer and Ridet, 2001). Skeletal muscle, which is readily accessible for *in vivo* and *ex vivo* manipulation, is an attractive target for gene transfer aimed at such systemic protein delivery. The rich vascular supply of skeletal muscle provides efficient access to the systemic circulation of secreted proteins. In addition, myofibres are very long-lived cells, and therefore are potentially able to express exogenous genes for long periods of time. Several viral vectors, such as adenovirus, AAV, and lentivirus, and as well as non-viral vectors, have the capacity to transduce mature non-

dividing myotubes *in situ*. The grafting of *ex vivo* modified myoblasts has also been shown to result in the long-term secretion of recombinant protein (Yao *et al.*, 1994; Naffakh *et al.*, 1996).

Gene therapy vectors

In this section, we will review the main characteristics of the different vectors suitable for gene therapy in the nervous system. Because they naturally transfer their own genome into the cells that they infect, a modified virus is the most efficient biological vector system for therapeutic gene transfer. Adenovirus, AAV, herpes virus, and lentivirus are the most promising types of virus for the transfer of therapeutic genes. This part of the review will focus particularly on adenoviral vectors that have proved their great importance for addressing many disorders of the nervous system, and that are routinely used in our laboratory. Even though the efficiency of non-viral vectors for direct gene transfer in the nervous system remains insufficient, they are very good candidates for muscular-based gene therapy.

ADENOVIRUS

The recombinant adenoviruses commonly used are derived from type 5 adenoviruses. The genome of the human adenovirus is large (36 kilobase DNA), and contains many genes that are classified into early (from E1 to E4) and late (L1 to L5) genes, according to whether they are expressed prior to or after viral DNA replication. Both ends of viral DNA bear a short sequence called 'inverted terminal repeat', which is essential for viral replication.

Adenoviral vectors have their E1 region deleted in order to render them replication-defective. The E3 region, which is not necessary for viral propagation in cell cultures, can also be deleted. Recombinant adenoviruses are amplified in a *trans*-complementing cell line established from human embryonic kidney cells, designed 293 (Graham *et al.*, 1977). These cells contain the E1 genes integrated in their genome so that, upon infection of this cell line, the E1-deleted viruses can replicate. Deletion in E1 and E3 can be combined in a single vector that should accommodate transgenes up to 8.3 kilobase in length, and can be obtained at titres as high as 10^{11}–10^{12} particles per ml (Bett *et al.*, 1994; Graham and Prevec, 1995). These replication-deficient adenoviruses have been referred to as 'first-generation' recombinant adenoviruses.

In vivo *gene transfer*

Recombinant adenovirus encoding for therapeutic agents can be delivered *in vivo* after direct intra-cerebral injection into particular brain areas. Le Gal La Salle *et al.* (1993) were the first to demonstrate the remarkable efficiency of adenoviral vectors for introducing foreign genetic material into rodent nervous system. The authors used a recombinant replicative adenovirus encoding for a reporter gene under the control of a strong virus promoter. Stereotactic intra-cerebral inoculation of this viral vector into the hippocampus and the substantia nigra allowed the extensive labelling of neurons, astrocytes, and microglial cells around the injection site. The intracellular expression of the marker gene was detected for about two months, with only minimal

neuropathological consequences. Several other groups confirmed at the same time the ability of an adenovirus to infect neuronal cells *in vivo* with a relatively long-term expression of the transgene and a limited toxicity (Akli *et al.*, 1993; Bajocchi *et al.*, 1993; Davidson *et al.*, 1993). Transgene expression is also observed in secondary sites due to the retrograde transport of the adenoviral vector.

Other routes for administration of an adenovirus into the CNS have been described: injection of the vector into the CSF (Bajocchi *et al.*, 1993; Driesse *et al.*, 1999), nasal instillation (Draghia *et al.*, 1995), and injection into the carotid artery after blood–brain barrier disruption with hyperosmotic mannitol (Doran *et al.*, 1995). Intra-muscular injections of a recombinant adenovirus can be used for infection of motor neurons by retrograde transport (Finiels *et al.*, 1995; Ghadge *et al.*, 1995). The efficiency of gene transfer is high, with 58 to 100 per cent of the motor neurons afferent to the injected muscle expressing the transgene (Finiels *et al.*, 1995). Transgene expression has been detected in motor neurons for at least 30 days after intra-muscular injection. Intra-muscular injection of modified adenoviruses is also an efficient means for systemic delivery of a recombinant protein (Tripathy *et al.*, 1994; Haase *et al.*, 1997; Pradat *et al.*, 2001b).

Ex vivo *gene transfer*

Adenoviral vectors are also powerful tools for *ex vivo* gene therapy. Cells originating from the periphery, such as fibroblasts, can be genetically modified by a recombinant adenovirus, and express the transgene *in situ* after grafting in the brain (Fisher *et al.*, 1991). However, CNS-derived cells are more attractive vehicles, since they naturally belong to the nervous system, which predicts a better integration over the long term. Astrocytes which can be expanded *in vitro* may be well suited for brain repair because of their role as neuronal support. Rat primary astrocytes, transduced with a recombinant adenovirus that has been grafted in the brain, survived well, and expressed the transgene for at least five months (Ridoux *et al.*, 1994).

Human neuronal progenitors offer great promise for intra-cerebral transplantation. Until recently, neurons have been refractory to genetic modifications because of their post-mitotic nature. Human neural progenitor cells present the great advantage that they provide a pool of dividing cells that can be genetically engineered *in vitro*. Furthermore, these cells are possibly multi-potential precursors of all neuronal and glial cells, and can show plasticity in new environments. Ideally, transplantation of these cells could allow a reconstruction of the altered neuronal pathway, including functional reconnection. Neural cells explanted from germinative zones of the CNS from 6- to 10-week-old human fetuses proliferate in serum-free culture medium containing basic fibroblast growth factor (Buc-Caron, 1995). A large number of progenitor cells can be obtained, and can be infected with a recombinant adenovirus. After transplantation to the rat brain, viable grafts containing cells expressing the transgene can survive up to 3 months (Sabate *et al.*, 1995; Corti *et al.*, 1999a).

Longevity, regulation and targeting of transgene expression

A limitation of first-generation adenoviruses, i.e. adenoviruses that lack E1 and E3 sequences, is the induction of an immune response when injected into immuno-

competent humans or animals (Yang *et al.*, 1994a). This leads to a decline in transgene expression due to the destruction of the host cells, and can cause deleterious side effects in patients. Although the CNS is considered to have poor immunological surveillance, the adenovirus vector elicits immune response in the brain (Wood *et al.*, 1996). The need for a long transgene expression is particularly important for gene delivery in the CNS because of the long evolution of neurodegenerative diseases, often over several years. Because neurosurgical procedures are complicated and potentially risky, repeated interventions are not desirable. The use of 'new generation' vectors, in which more of the viral genome has been deleted (E2 or E4 regions), limits the immune response after infection, thereby increasing the duration of transgene expression (Engelhardt *et al.*, 1994; Yang *et al.*, 1994b; Wang and Finer, 1996; Dedieu *et al.*, 1997; Wang *et al.*, 1997). High capacity, 'gutless' adenoviral vectors have also been developed. They possess only the sequences necessary for packaging and replication of the viral genome, in the absence of all structural genes (Fisher *et al.*, 1996; Kochanek *et al.*, 1996, 2001; Hardy *et al.*, 1997). In addition, these gutless vectors have the advantages of higher transgene capacity (up to 37 kilobase). *In vivo* studies have shown prolonged expression of transgenes delivered by these vectors with low inflammatory response (Lieber *et al.*, 1997; Kumar-Singh and Farber, 1998; Morsy *et al.*, 1998).

Inflammation is not the only factor responsible for the decrease of transgene expression over time. The gradual inactivation of the strong viral promoters is responsible for the decline in expression over time following adenovirus infection (Palmer *et al.*, 1991). Therefore, the use of promoters from housekeeping genes may allow the increase of the duration of transgene expression. Furthermore, neuron-specific promoters would allow precise targeting of expression to specific cell types (Morelli *et al.*, 1999). This has been achieved by using a recombinant adenoviral vector encoding a reporter gene under the control of the promoter of the neuron-specific enolase (NSE) (Navarro *et al.*, 1999). After injection of this vector into the rat hippocampus, neurons were preferentially transduced, and expressed the reporter gene for six months. Millecamps *et al.* (1999) constructed a recombinant adenovirus carrying neuron-restrictive silencer element (NRSE) upstream from an ubiquitous promoter. This system allowed the repression of the expression of the reporter gene in non-neuronal cells. After intra-muscular injection of this vector, a strong expression was obtained in motor neurons after retrograde transport of the adenoviral vector, whereas almost no expression was observed in muscular cells.

The regulation of the amount of protein produced by the transgene is an important factor for efficient gene therapy. Many therapeutic proteins may be toxic when doses are too high. For example, an excess of NGF can inhibit axonal growth of sensory neurons (Conti *et al.*, 1997). Ideally, a vector for clinical application should allow adaptation of the treatment to the needs of the patient and termination of the therapy, as necessary. The control of production of the transgene product can be achieved by using a regulatable promoter. The development of efficient gene regulatory systems based on specific transcription factors that respond to exogenous drugs has allowed the integration of inducible expression cassettes into gene delivery vectors (Clackson, 1997; Harvey and Caskey, 1998). Of these, the tetracycline-responsive promoters have been shown to be particularly versatile and efficient (Gossen and Bujard, 1992; Gossen *et al.*, 1995). In an exploratory study, Corti *et al.* (1996) showed that the

tetracycline-based regulatory system efficiently allows the regulation of luciferase-reporter gene expression in intra-cerebral grafts of a neural precursor cell line. The authors subsequently showed that an efficient and reversible control of TH expression may be obtained in a rat model of PD, both by an *ex vivo* gene transfer approach and by direct intra-cerebral injection of the regulatable adenovirus (Corti *et al.*, 1999a,b).

HERPES SIMPLEX VIRUS

Herpes simplex virus type-1 (HSV-1) naturally infects human neuronal cells to produce latent infection. HSV-1 produces a lytic infection of skin cells, and then migrates to the nerve processes, and is retrogradely transported to the sensory cell bodies in the dorsal root ganglia. The periodic reactivation vectors of the virus present in the sensory neurons and its re-migration to the periphery results in the characteristic pattern of recurrent cold sores. This ability to naturally produce a latent infection in neuronal cells made this vector a good candidate for gene therapy. Recombinant HSV-1 vectors contain the full viral genome mutated in one or more virus genes to reduce toxicity, prevent reactivation for latency, and provide space for transgene (30–50 kilobase) (Kennedy, 1997; Fink *et al.*, 2000; Latchman, 2001). These vectors retain the ability to enter an episomal state of latency in non-dividing host cells. The natural retrograde transport of the HSV vector can be used for targeting of transgene products in motor neurons after intra-muscular injection (Keir *et al.*, 1995), or in dorsal root ganglia after cutaneous injection (Goss *et al.*, 2001; Chattopadhyay *et al.*, 2002). Generally, the main limitations of herpes vectors are immune response against viral proteins, and cytotoxicity of the virions. A stripped-down version of the HSV, called an amplicon, has been developed, aiming to overcome these limitations (Spaete and Frenkel, 1982; Fraefel *et al.*, 1996; Saeki *et al.*, 1998; Stavropoulos and Strathdee, 1998).

LENTIVIRUS

Retroviral vectors present several characteristics that make them very suitable as gene therapy vectors: 1) they have a large cloning capacity (about 10 kilobase); 2) they integrate their genetic material in the chromosome of target cells, a condition favouring long-term expression of the transgene; and 3) they do not transfer viral sequences, avoiding immune reactions against viral proteins. However, most retroviral vectors, such as the Moloney murine leukaemia virus, cannot transfer genes into non-dividing cells, which hampers their use for gene therapy in the CNS (Roe *et al.*, 1993; Lewis and Emerman, 1994). To circumvent this problem, vector systems based on the lentivirus genus of retroviruses, which includes human immunodeficiency virus (HIV), have been developed (Buchschacher and Wong-Staal, 2000; Trono, 2000). In contrast to oncovirus, lentivirus possess viral components that enable them to infect non-dividing and terminally differentiated cells, including neurons (Lewis and Emerman, 1994). After intra-cerebral injection, lentiviral vectors have shown high efficiency to transduce cells of the nervous system, predominantly neurons, with long-term expression and no significant immune response (Naldini *et al.*, 1996; Blomer *et al.*, 1997). Recombinant lentivirus can also infect myofibres *in vivo*, and deliver a therapeutic protein in the circulation (Kafri *et al.*, 1997; Seppen *et al.*, 2001).

ADENO-ASSOCIATED VIRUS

AAV are small, encapsulated viruses, containing a single-stranded DNA genome. They have 96 per cent of the viral genome removed, leaving only two short, inverted terminal repeats that promote extrachromosomal replication and genomic integration of the transgene. Transgenes delivered by AAV vectors integrate into the host cell genome, either randomly or in a specific site in chromosome 19 (Kotin *et al.*, 1992; Weitzman *et al.*, 1994; Balague *et al.*, 1997; Walker *et al.*, 1997; Wu *et al.*, 1998). Long-term expression is favoured by the integration of the transgene, and by the absence of viral genes, which minimizes the risk of an immune reaction (Muzyczka, 1992). An important inconvenience of AAV vector is their small 4.5 kilobase transgene capacity (Muzyczka, 1992), and the difficulty to produce viral particles in large quantity. However, recent improvements in recombinant AAV vector production allow obtaining higher titres of infectious particles (Xiao *et al.*, 1998; Zolotukhin *et al.*, 1999). AAV-based vectors have been shown to produce high levels of transgene expression after injection in the CNS (Kaplitt *et al.*, 1994b; Peel *et al.*, 1997; Bartlett *et al.*, 1998; Klein *et al.*, 1998; Mandel *et al.*, 1998). However, not all types of neurons are equally good targets, and the transduction efficiency varies greatly between brain regions (Kaplitt *et al.*, 1994b; McCown *et al.*, 1996; Klein *et al.*, 1998; Mandel *et al.*, 1998). As adenoviral and lentiviral vectors, AAV are suitable for the systemic delivery of a therapeutic protein after intra-muscular injection (Herzog *et al.*, 1997; Snyder *et al.*, 1997; Hagstrom *et al.*, 2000).

BACULOVIRUS

Baculovirus-derived vectors have emerged as a possible tool for gene transfer into mammalian cells (Hofmann *et al.*, 1995; Boyce and Bucher, 1996; Pieroni and La Monica, 2001). Baculovirus is an insect virus with a large, double-stranded, circular DNA genome packaged into a rod-shaped capsid, which is itself enveloped by a unit membrane. Baculovirus-derived vectors are particularly well suited for gene therapy of non-dividing cells because their DNA genome remains episomal, and their promoters are silent in mammalian cells, making them naturally non-replicative in these cells (Brusca *et al.*, 1986; Hartig *et al.*, 1991). Furthermore, the structure of the baculovirus allows it to carry very large transgenes. Sarkis *et al.* (2000) showed that baculovirus vectors are promising tools for gene therapy of the CNS. They developed a baculovirus-derived vector containing a reporter gene that efficiently transduced neuroblastoma cell lines and primary neural cultures. Furthermore, after direct intra-cerebral injection of the vector, the baculovirus transduced neural cells, mostly glial.

NON-VIRAL VECTORS

Non-viral gene vectors include naked plasmid DNA, cationic lipids, and polycationic polymers. Compared to viral vectors, the major advantage of these vectors is their safety, and the ease of manufacturing. However, an important limitation for gene transfer in the nervous system is their very low efficiency, as compared with viral vectors (Costantini *et al.*, 2000; Hsich *et al.*, 2002). Non-viral vectors are promising tools for gene delivery into muscle for the systemic delivery of a therapeutic

compound (Davis *et al.*, 1993; Raz *et al.*, 1993; Tripathy *et al.*, 1996; Anwer *et al.*, 1998; MacColl *et al.*, 1999, 2000;). However, only a few muscular fibres (1–2 per cent) are transformed after intra-muscular injection of a naked DNA (Wolff *et al.*, 1990; Danko and Wolff, 1994; Levy *et al.*, 1996), and many authors have strengthened the great inter- and intra-individual variability of transfer efficacy (Wolff *et al.*, 1990, 1992; Jiao *et al.*, 1992; Manthorpe *et al.*, 1993; Danko and Wolff, 1994; Levy *et al.*, 1996). For these reasons, techniques have been developed to increase the efficiency of plasmid DNA uptake by muscle cells (MacColl *et al.*, 1999). One of the most promising methods is *in vivo* electroporation, involving a set of electric pulses delivered through a pair of needle electrodes inserted into the DNA injection site. This technique has been shown to dramatically increase both the number of muscle fibres taking up DNA, and the plasmid copy number within these fibres (Aihara and Miyazaki, 1998; Mir *et al.*, 1999)

Current achievements of gene therapy in diseases of the central and peripheral nervous system

PARKINSON'S DISEASE

PD is a progressive, neurodegenerative movement disorder whose prevalence increases exponentially with age between 65 and 90 years. Approximately 0.3 per cent of the general population and 3 per cent of people over the age of 65 have PD (Zhang and Roman, 1993). Five to 10 per cent of patients have symptoms prior to the age of 40, and are classified as suffering from 'young-onset PD' (Moghal *et al.*, 1994). The classic triad of major signs is composed of tremor, rigidity, and akinesia (Lang and Lozano, 1998a). Neuropathological and functional neuroimaging studies have shown a progressive loss of nigral dopamine neurons, and a concomitant decline of striatal dopamine function at a rate of 5–10 per cent per year (Fearnley and Lees, 1991; Brooks, 1998). Symptoms start to appear when about 70–80 per cent of the nigral neurons have degenerated (Pakkenberg *et al.*, 1991). Neuropathology shows a predominant loss of dopaminergic neurons in the substantia nigra with the presence of extracellular inclusion, called Lewy bodies. These structures contain α-synuclein and ubiquitin, and a number of other lipids and proteins (Gai *et al.*, 2000). Etiologically, PD is likely the result of the cumulative effects of genetic and environmental factors in a given patient. Three genes, α-synuclein (Polymeropoulos *et al.*, 1997), Parkin (Kitada *et al.*, 1998), and ubiquitin C terminal hydrolase L1 (Leroy *et al.*, 1998), have been identified in rare inherited forms.

Treatment is only symptomatic and based on L-dopa, a precursor of dopamine that crosses the blood–brain barrier, and on agonists of dopamine (Lang and Lozano, 1998b). The treatment has an important benefit for only five to seven years, but thereafter patients lose their response to the drug as severe side effects outweigh its benefits. A greater understanding of the pathophysiologic correlates of Parkinsonism has led to the resurgence of functional neurosurgical procedures, such as pallidotomy (Lang and Lozano, 1998b) and electrical stimulation through deep-brain electrodes implanted in the subthalamus (Lang and Lozano, 1998b; Limousin *et al.*, 1998), that have shown favourable results in a selected population of patients. Some encouraging results have been obtained from small and non-controlled studies of engraftment of

fetal dopamine-producing neurons into the striatum of patients (Freed *et al.*, 1992a,b; Lindvall *et al.*, 1992; Spencer *et al.*, 1992; Piccini *et al.*, 2000). However, a recent study in 40 PD patients who received either a transplantation of fetal dopaminergic neurons or a sham surgery showed only a small improvement that was limited to younger patients (Freed *et al.*, 2001). This study has suggested that this strategy must be improved to demonstrate its usefulness in the treatment of PD.

The objective of restorative gene therapy is to provide sustained levels of dopamine directly in the striatum. Because tyrosine hydroxylase (TH) is the rate-limiting enzyme in the synthesis of dopamine, most experiments have focused on the transfer of this enzyme in the striatum. This goal can be achieved by transplanting cells which have been transduced *ex vivo* to express TH (Horellou *et al.*, 1990; Fisher *et al.*, 1991; Kang *et al.*, 1993; Lundberg *et al.*, 1996; Fitoussi *et al.*, 1998; Corti *et al.*, 1999b; Ridet *et al.*, 1999; Trejo *et al.*, 1999). In this context, the control of transgene expression levels is particularly important. Indeed, the amount of dopamine needed varies between patients, and over time for an individual patient. Therefore, the control of dopamine release is highly desirable for clinical application. Astrocytes derived from human adult cerebral cortex have been efficiently infected with an adenoviral vector encoding TH under the negative control of a tetracycline-regulated system. These cells synthesized large amounts of active TH, released L-dopa, and responded to tetracycline (Ridet *et al.*, 1999). More recently, human neural progenitor cells have been transformed by a recombinant adenovirus to synthesize TH in a conditional manner (Corti *et al.*, 1999a). After transplantation to the striatum of immunosuppressed rats, the infected neuroblasts expressed the transgene in a tetracycline-dependent manner. Grafts containing TH-immunolabelled cells were present in all animals for up to three months after transplantation.

Delivery of TH can also be achieved by direct transfer to striatal cells via recombinant viral vectors (During *et al.*, 1994, 1998; Horellou *et al.*, 1994; Kaplitt *et al.*, 1994a; Jin *et al.*, 1996) and DNA–liposome complexes encoding TH (Cao *et al.*, 1995; Imaoka *et al.*, 1998; Segovia *et al.*, 1998). The conversion of part of the striatal cell population to TH-synthesizing cells obtained by direct infection with a recombinant adenovirus encoding the human TH can lead to significant recovery in 6-hydroxydopamine-lesioned rats, a model of PD (Horellou *et al.*, 1994). The recombinant adenovirus was stereotactically inoculated in the striatum three to four weeks after the 6-hydroxydopamine lesion. The contra-lateral rotational behaviour induced by apomorphine in this rat model was significantly decreased one and two weeks after injection of the recombinant adenovirus encoding TH, as compared with controls (rats injected with a recombinant adenovirus encoding the non-therapeutic gene, α-galactosidase).

More recently, the restorative gene therapy approach has included the additional transfer of genes encoding other enzymes of the dopamine pathway synthesis in order to maximize production of dopamine by the striatum. Experiments have been conducted to co-transfer the synthetic enzyme GTP-cyclohydrolase to generate the TH co-factor, tetrahydrobiopterin (Bencsics *et al.*, 1996; Mandel *et al.*, 1998; Kirik *et al.*, 2002; Muramatsu *et al.*, 2002), or the aromatic amino acid decarboxylase to facilitate conversion of L-dopa to dopamine (Fan *et al.*, 1998; Lee *et al.*, 1999; Leff *et al.*, 1999; Muramatsu *et al.*, 2002).

A second important approach of gene therapy in PD is neuroprotection. Gene therapy can be used to transfer neuroprotective proteins, such as antioxidants, anti-apoptotic molecules, and trophic factors. Barkats *et al.* (1997) constructed an adenovirus encoding the human Cu/Zn superoxide dismutase (SOD1), a major anti-oxidant enzyme. This vector was used for transducing embryonic mesencephalic rat cells *in vitro*, before grafting them into the denervated striatum of 6-hydroxydopamine-lesioned rats. The production of SOD1 in the grafts persisted for at least five weeks. Five weeks after grafting, functional recovery was more extensive in the SOD1 group than in the controls. Direct injection of an HSV-1 vector encoding the anti-apoptotic protein Bcl-2 into the striatum of rats 1 week before injection of 6-hydroxydopamine into the ipsilateral striatum increased neuronal survival by 50 per cent (Yamada *et al.*, 1999).

Using *in vivo* or *ex vivo* gene therapy, local delivery of neurotrophic protein, such as brain-derived neurotrophic factor (BDNF) (Frim *et al.*, 1994; Levivier *et al.*, 1995; Yoshimoto *et al.*, 1995; Galpern *et al.*, 1996) and glial cell line-derived neurotrophic factor (GDNF) (Lindner *et al.*, 1995; Bilang-Bleuel *et al.*, 1997; Choi-Lundberg *et al.*, 1997; Lapchak *et al.*, 1997; Mandel *et al.*, 1997), in the striatum has shown promising results in rodent models of PD. The efficacy of direct injection of a lentiviral vector encoding GDNF in the stiatum has been demonstrated in a primate model of PD (Kordower *et al.*, 2000). The combination of fetal tissue transplantation with growth factor delivery might allow to enhance the survival of the grafted cells and reduce the amount of fetal tissue required, two important limitations of transplantation in PD (Bjorklund and Lindvall, 2000). The striatal placement of a capsule loaded with GDNF-secreting cells one week before transplantation promoted a significant increase in the survival and fibre extension of fetal ventral mesencephalic grafted cells (Sautter *et al.*, 1998).

MOTOR NEURON DISEASES

ALS is the most frequent motor neuron disease. Its incidence is 1 to 2.5 per 100 000 worldwide (Kurtzke, 1982; Norris *et al.*, 1993). Disease onset usually occurs between 50 and 70 years of age. ALS involves the loss of both upper and lower motor neurons, leading to muscle weakness, wasting, weight loss, and respiratory failure. The disease is very disabling, and invariably fatal. Death generally occurs between two and five years after the appearance of the first symptoms. The majority of ALS cases, about 90 per cent, are sporadic, with no known cause. Many hypotheses have been put forward to explain the etiology of ALS: protein aggregation, oxidative stress, excitotoxicity, cytoskeletal defects, autoimmune processes, environmental toxins, and unusual viral infections (Cleveland, 1999; Brown and Robberecht, 2001; Julien, 2001; Rowland and Shneider, 2001). In most cases of familial ALS (FALS), the genetic defect is not identified. However, in about 20 per cent of FALS cases, point mutations in the gene encoding SOD1, which is located on chromosome 21q, are responsible for an autosomal dominant transmission (Rosen *et al.*, 1993). Recently, mutations in a putative GTPase regulator have been identified in a rare recessive juvenile FALS (Hadano *et al.*, 2001). No curative treatment is available, and symptomatic treatment remains the main focus of patient care. One drug, riluzole, has been made available for treating ALS following two placebo-controlled trials demonstrating that this drug

offers a modest, but significant, survival benefit (Bensimon *et al.*, 1994; Lacomblez *et al.*, 1996).

Delivery of neurotrophic factors by *ex vivo* and *in vivo* gene therapy has been successfully tested in animal models of motor neuron degeneration. Systemic delivery of neurotrophic factors has been achieved by subcutaneous implantation of polymer-encapsulated, genetically engineered cells. Using this method, continuous delivery of ciliary neurotrophic factor (CNTF) partially prevented facial motor neuron death after axotomy in neonatal rats (Tan *et al.*, 1996). This strategy is also effective in *pmn* mice, a mouse mutant with recessive progressive motor neuropathy (Sagot *et al.*, 1995). In general, homozygotes develop neurogenic atrophy and paralysis of their hind limb and pelvic girdle muscles during the third week of life, and they die at 6 to 7 weeks of age (Schmalbruch *et al.*, 1991). Axonal degeneration starts at the endplate and is restricted to motor neurons. CNTF-secreting, polymer-encapsulated cells were implanted subcutaneously in affected mice as soon as the disease was detected, i.e. between 16 and 20 days (Sagot *et al.*, 1995). Compared to controls, treated mice exhibited longer survival, better behavioural motor performances, and a less marked axonal loss in the phrenic nerves. Another *ex vivo* gene therapy strategy used intra-muscular grafts of genetically modified myoblasts to supply motor neuron terminals with GDNF (Mohajeri *et al.*, 1999). This treatment was tested in transgenic mice which carry a human-SOD1 gene with a mutation associated with FALS (mutation G93A) (Gurney *et al.*, 1994). It has been considered to be the best animal model of ALS, since transgenic mice show clinical and pathological abnormalities similar to those in humans. Mice with G93A SOD1 mutation are outwardly normal until about 3 months of age (Chiu *et al.*, 1995). They survive until about five months of age, at which time complete paralysis, with inability to forage for food and water, necessitates that they be killed. Grafting of GDNF-secreting myoblasts prolonged the onset of disease, delayed the deterioration of performances, and slowed muscle atrophy (Mohajeri *et al.*, 1999).

Ex vivo gene therapy has also been used in the context of motor neuron diseases to deliver a neurotrophic protein in the CSF. A phase I/II clinical trial has been performed in 12 ALS patients to evaluate the safety and tolerability of intrathecal implants of encapsulated, genetically engineered cells releasing human CNTF (Aebischer *et al.*, 1996a,b; Zurn *et al.*, 2000). Encapsulated xenogenic cells, implanted intrathecally, survived for up to 20 weeks, with minor biological signs of intrathecal immunological response (Zurn *et al.*, 2000). Treated patients experienced none of the adverse effects previously reported with systemic administration of CNTF (Miller *et al.*, 1996). The disease course was not modified by treatment, but the small number of patients and the short observation period prevented accurate assessment of a potential therapeutic effect.

An *in vivo* gene therapy strategy consists of intra-muscular injection of a recombinant adenoviral vector encoding a neurotrophic factor. It was first tested in *pmn* mice using an adenovirus encoding NT-3 (Haase *et al.*, 1997, 1998). Treated mice showed a 50 per cent increase in life span, reduced loss of motor axons, and improved neuromuscular function, as assessed by electromyography. The therapeutic effect was very probably due to the high-level production of NT-3 in injected muscles, and its liberation into the bloodstream, with the neurotrophic factor acting peripherally, or after its retrograde axonal transport by motor neurons, or both. Indeed, the neuro-

trophic factor was detected in the blood, and the effects of treatment were bilateral in both the injected and contra-lateral, uninjected muscle, and shown to be present at some distance from the injection site. Further work has demonstrated the effectiveness of this strategy using other neurotrophic factors: cardiotrophin-1 in *pmn* (Bordet *et al.*, 1999) and SOD1 transgenic mice (Bordet *et al.*, 2001), or GDNF in SOD1 transgenic mice (Acsadi *et al.*, 2002).

Gene therapy using direct gene transfer in the medulla has been tested in SOD1 transgenic mice. The authors injected an AAV virus encoding the anti-apoptotic protein Bcl-2 directly in the medulla of affected mice (Azzouz *et al.*, 2000). Treatment resulted in sustained Bcl-2 expression in the motor neurons surrounding the injection site, increased the number of surviving motor neurons, and improved electrophysiological markers of motor neuron function.

PERIPHERAL NEUROPATHIES

Peripheral neuropathies are common neurologic diseases due to multiple causes (genetic, inflammatory, infectious, metabolic, toxic...). Their prevalence is about 2400 per 100 000 (2.4 per cent), rising with age to 8 per cent (Martyn and Hughes, 1997). Patients suffering from peripheral neuropathies present altered sensation, pain, weakness, or autonomic symptoms (Hughes, 2002). In most cases, evolution is chronic, and symptoms usually develop over several months, frequently leading to a distressing and disabling condition. Although some causes are curable by specific treatment, such as antibiotics or anti-inflammatory drugs, the treatment is often only symptomatic.

Peripheral neuropathies are a frequent complication of chemotherapeutic agents, and are a major issue in oncological practice (Corbo and Balmaceda, 2001). Particularly, the use of cisplatin, one of the most effective anti-neoplastic agents, is greatly hampered by its neurotoxicity. Cisplatin causes a dose-dependent and dose-limiting sensory neuropathy, which is often disabling, and from which recovery is often slow and incomplete (Roelofs *et al.*, 1984; Thompson *et al.*, 1984; Mollman, 1990; van der Hoop *et al.*, 1990). Cisplatin toxicity induces selective damage or loss of large myelinated sensory fibres. Neuropathy leads to withdrawal, and may reduce the quality of life of affected patients. There is no available treatment to prevent or cure cisplatin-induced peripheral neuropathy. Finding a treatment able to prevent cisplatin neuropathy may allow the cumulative dose to be increased, and consequently the antitumour effect of cisplatin. NT-3 is a promising neuroprotectant since it promotes the survival of the large fibre sensory neurones (DiStefano *et al.*, 1992) affected in cases of cisplatin-induced neuropathy (Thompson *et al.*, 1984). A strategy of continuous delivery of physiological amounts of NT-3 by a gene transfer technology has recently been shown to partially prevent neuropathy in cisplatin-treated mice (Pradat *et al.*, 2001a; Pradat *et al.*, 2002). Delivery of sustained low levels of NT-3 was achieved by intra-muscular injection of a recombinant vector encoding NT-3. The vector used was either non-viral, a plasmid DNA associated with *in vivo* electroporation, or a recombinant adenovirus. Both techniques significantly prevented electrophysiological abnormalities related to sensory nerve dysfunction with minimal muscle toxicity. Subcutaneous injection of a recombinant herpes virus encoding NT-3 has been shown to prevent pyridoxin-induced neuropathy in rats, a

model of pure sensory neuropathy (Chattopadhyay *et al.*, 2002). The neuroprotective effect was related to the retrograde transport of the viral vector and the expression of NT-3 in the DRG.

Diabetic neuropathy is the most common cause of peripheral neuropathies in developed countries. Diabetes affects 15 to 16 million individuals in the United States, of whom about 30 per cent suffer from a symptomatic neuropathy (Harris *et al.*, 1993; Young *et al.*, 1993; Tesfaye *et al.*, 1996). The incidence of this complication increases with the duration of diabetes, and it has been estimated that about 50 per cent of individuals who have had diabetes for 25 years suffer symptomatic peripheral neuropathy (Pirart, 1978). The most common type of neuropathy is symmetrical polyneuropathy that is primarily sensorimotor, and often includes the autonomic system (Thomas and Tomlinson, 1993; Said, 1996). Therapeutic agents to combat diabetic neuropathy would obviously have profound effects on neurological symptoms, but also on complications associated with neuropathy, such as trophic troubles and foot ulcerations, which can lead to amputation.

The effect of NT-3 delivery using intra-muscular injection of a recombinant adenovirus encoding NT-3 has been investigated in two animal models of diabetic neuropathy (Pradat *et al.*, 2001b): 1) streptozotocin-induced diabetes, which is a classic model of early human diabetic neuropathy; and 2) acrylamide experimental neuropathy, which mimics axonal late-onset human diabetic neuropathy. Treatment with gene therapy partially prevented sensory and motor neuropathy in both models as assessed by electrophysiology, behavioural measurement, and a biological index of motor innervation. These encouraging results suggest that gene therapy could be effective to prevent the appearance of neuropathy in diabetic patients, as well as slow the progression of symptoms in patients already affected by this complication.

The intra-muscular gene transfer of plasmid DNA encoding vascular endothelial growth factor (VEGF) has been investigated in diabetic rats (Schratzberger *et al.*, 2001). Microvascular disease with impaired blood flow (Tuck *et al.*, 1984; Cameron *et al.*, 1991; Tesfaye *et al.*, 1993) and ischaemia (Dyck, 1989; Stevens *et al.*, 1994) in peripheral nerves has been implicated in the pathogenesis of diabetic neuropathy. VEGF is an endothelial cell-specific mitogen that plays a critical role in regulating neovascularization in response to tissue ischaemia (Senger *et al.*, 1983; Keck *et al.*, 1989; Leung *et al.*, 1989; Banai *et al.*, 1994). Intra-muscular injection of plasmid DNA encoding VEGF resulted in restoration of vascularity, blood flow, and conduction velocities in nerves of treated animals. The clinical usefulness of VEGF delivery by intra-muscular gene transfer is highlighted by the results of a prospective study of 24 patients undergoing this treatment for critical limb ischaemia (Simovic *et al.*, 2001). Peripheral neuropathy, which is a classic complication related to ischaemia of peripheral nerves (Eames and Lange, 1967; Farinon *et al.*, 1984; Pasini *et al.*, 1996), was improved by treatment as assessed by clinical examination and electrophysiological measurements (Simovic *et al.*, 2001).

Conclusion

Over the past few years, considerable progress has been made to improve the efficiency and safety of gene therapy vectors. During the same time, experiments in animal models have demonstrated the high potential of gene therapy in a wide variety

of neurological diseases, such as PD, ALS, and more recently, peripheral neuropathies. The clinical need for gene therapy of neurologic diseases is considerable. With the exception of riluzole, which slows the progression of ALS, there is no neuroprotective drug available. New neurosurgical approaches, such as deep-brain stimulation for the treatment of PD, are only symptomatic, and do not prevent the progression of the disease. Many potent neuroprotective proteins have been identified, but delivery issues hamper their administration by classical pharmacotherapy. For these reasons, gene therapy offers great hopes for the emergence of neuroprotection strategies by delivering therapeutic proteins in specific regions of the nervous system in a regulatable fashion. Furthermore, gene therapy can improve the efficiency of the emerging cell therapy procedures by delivering trophic factors to increase the survival of the grafts. The choice of the vector, of a regulation system to control the level of transgene expression, and of the route of delivery will need to be tailored accordingly to meet the particular features of each disease, particularly the region of the nervous system involved, and the natural course of the disease. For these reasons, future clinical trials will necessitate a multidisciplinary approach, with close collaboration between basic researchers and clinicians.

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