Human Molecular Genetics, 1996, Vol. 5 Review 1397–1404

Applications of gene therapy to the CNS

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Received May 29, 1996

Gene therapy is a new method with potential for treating a broad range of acquired and inherited neurologic diseases, where the causative gene defect or deletion has been identified. In addition to gene replacement the application of gene products that reduce cellular dysfunction or death represent new therapeutic options. Gene transfer techniques to express novel proteins using different viral vectors *in vitro* and *in vivo*, as well as animal models and human trials will be reviewed in this article. We will focus on a new lentiviral vector as a recent gene transfer method and degenerative disorders of the CNS, and their related model systems.

INTRODUCTION

Genetic manipulation of the central nervous system (CNS) has progressed from molecular and cellular biology to a broad field of experiments in mammals and even into limited clinical trials. Current technology allows the expression of novel gene products or overexpression of endogenous proteins. Repair of the nervous system with its complex structures and various cell types presents a field that is extremely challenging but potentially amenable to current gene therapy strategies. Viral vectors, with their ability to introduce either DNA or RNA into the host cell using the cell machinery for survival and replication, are the preferred method of gene transfer into target cells of the CNS. Despite recent advances, technical problems remain, such as the need for specific targeting of the foreign gene to the appropriate tissues or cells, site-specific integration, long-term expression and the necessity to overcome the immune response related to some vectors.

Currently there are two main approaches for performing somatic gene therapy, the *ex vivo* and *in vivo* strategies. In the *ex vivo* approach the gene transfer is performed in cell culture (*in vitro*) and the cell is transplanted into the organism. In the *in vivo* approach the gene is delivered directly into an organism for *in situ* gene transfer into the cells (Fig. 1). Increasing knowledge about the host and donor cell, conditions for their maintenance in culture and transplantation techniques, has led to the realization that gene therapy not only applies to genetic diseases but also to many acquired disorders or trauma in the CNS.

VIRAL VECTORS

Viruses can be thought of as cell parasites that require the function of the host cell in order to live and duplicate. Depending on the viral family, DNA or RNA encodes a limited set of viral proteins, encased in a capsid that is either surrounded or not by a lipid coat. Viral proteins embedded in the outer layer interact with cellular receptors. The tropism of different viruses for specific target cells is due to differences in viral protein coatings. Viruses thus transfer their genes into host cells and use the cell machinery for replication and generation of progeny virus.

Viral vectors are modified viruses engineered to contain a gene of interest that is typically flanked by viral sequences that encode signals for packaging and expression. Typically viral vectors are replication defective and capable of a single round of host cell infection without viral spread. The gene transfer can be either transient, with the transgene staying as an episome, or stable, with integration of the viral genome into the host cell DNA. Upon infection by adeno and herpes simplex viral vectors (HSV), for example, the transgene is lost over time by dilution during cell division.

Retroviral and adeno-associated viral (AAV) vectors, however, are able to stably insert the viral sequences including the gene of interest into the host chromosome. The stable integration of foreign genes yields permanent alteration of the genome in the transduced cell and their progeny (Fig. 2). To integrate into the host cell genome, simple retroviral vectors, like Moloney murine leukemia virus-derived vectors, need the breakdown of the nuclear membrane that occurs during cell division. The major limitation of retroviral vectors lies in the exclusion of non-dividing terminally differentiated cells like neurons, liver and muscle cells. The AAV vector, although integrating into the host cell genome of dividing and non-dividing cells, suffers from limited efficiency and depends on helper viral function, provided by either the adeno or herpes simplex virus, to be efficient in transduction. However, lentiviruses, for example the HIV virus, a subclass of retroviruses, allow the stable transduction of non-dividing cells.

Transgene expression efficiency and persistence of viral vectors in general depend on the promoter driving the transgene and also the host immune response. The immune response may be directed against the transgene product itself and/or any viral protein synthesized in the transduced cell. Avoiding expression of viral genes may allow long-term survival of transduced cells.

Herpes simplex virus vectors

HSV is a large 150 kb DNA virus containing approximately 70 genes, which are not all required for growth in cell culture. Many HSV vectors used are recombination competent and basically

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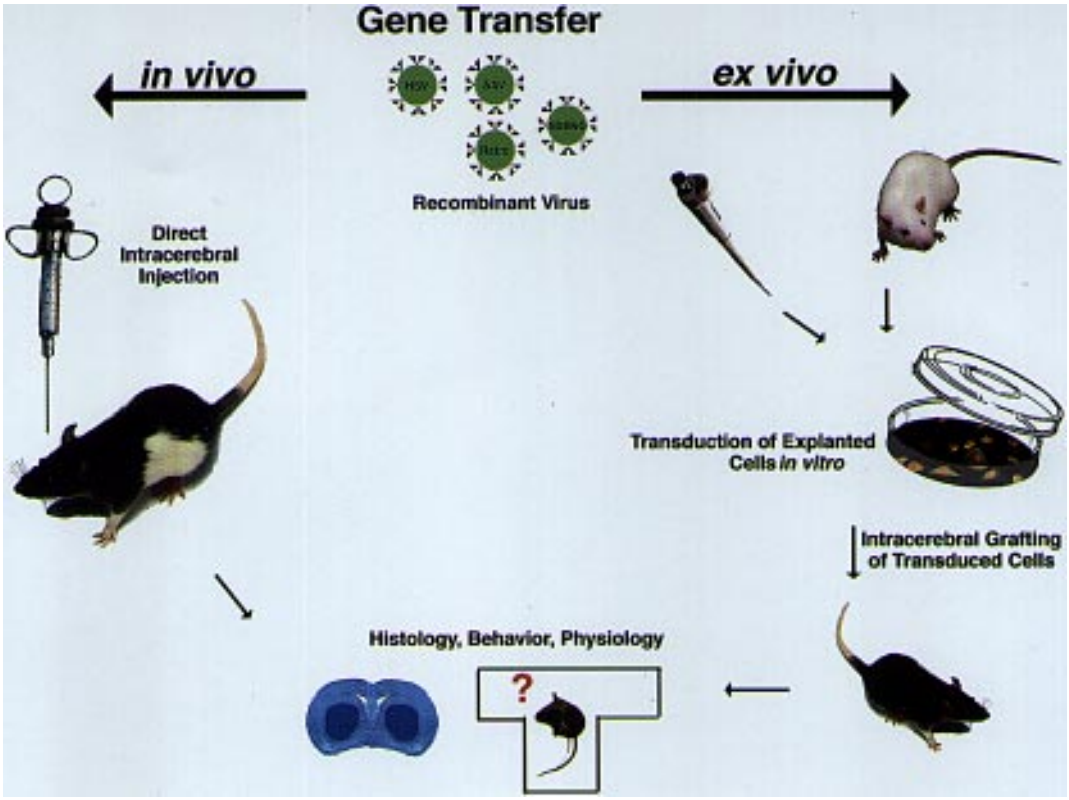

Figure 1. Schematic of the *in vivo* and *ex vivo* gene transfer strategies. The *in vivo* strategy uses viral vectors previously tested on cell cultures for direct injection into the central nervous system. Injected viral vectors integrate into the host cells (*in situ*) where the biochemical effect is assayed by biochemical, physiological, and/or behavioral testing. The *ex vivo* approach uses explanted cells, either clonal or heterogeneous populations, infected with viral vectors. Transduced cells are then grown to numbers sufficient for implantation into the target tissue. After transplantation, biochemical, physiological and behavioral testing are performed to determine the efficiency of the gene transfer.

concentrated units of the original plasmid, allowing a single insert of the gene of interest. In contrast, defective amplicon HSV vectors have multiple copies of the gene of interest which are packaged into HSV virions. Neurons and glial cells can be transduced, but HSV clearly has greater efficiency in transducing neurons (1). Once HSV virus infects the host cell, the capsid is released into the cytoplasm and transported to the nucleus, where the viral DNA enters through the nuclear pore. Progeny viral particles are produced and released by the infected cell and infect other cells, resulting in cell lysis or latency in the host cell (2–4).

HSV vector constructs have been used with viral and non-viral promoters and foreign gene inserts in mouse brain (5) and rat hippocampus (6). Transient expression peaks after inoculation and loss of expression have been reported after 2 weeks (7,8). The loss of transgene expression is due either to the promoter shut off or to the host's immune response. Current brain tumor strategies utilize HSV vector mutants that are attenuated for growth in non-dividing cells but replicate within growing tumor cells. Cell division allows the virus to enter one tumor cell, make multiple copies, kill the cell and spread to additional tumor cells. The surrounding brain tissue contains non-dividing cells and therefore is unable to support the replication (9). These studies in immune-compromised animals have shown promising results; however, the treatment has to be re-evaluated in the context of a competent immune system. Although HSV has a broad host range and gene transfer in many types of cultured cells is possible, widespread use of this transfer approach will be restricted until problems concerning the spread of the vector *in vivo* are solved. In addition it will be necessary to remove viral-induced cytotoxic functions, including those required for lytic replication (10).

Adenoviral vectors

Adenoviruses, which are linear double-stranded DNA viruses, contain approximately 36 000 base pairs encapsulated in a protein coat. Adenoviral vectors transiently transduce non-dividing cells with high expression of viral proteins, causing a pathogenic response by cytotoxic T lymphocytes (CTL) (11). Several reports have documented the expression of a transgene for up to 8 weeks after injection into the brain, and for over 6 months in fetal and immune compromised animals (12,13). Adenoviral vectors are available in two different forms that are replication deficient and reduced in their oncogenic potential. The first vector lacks two early viral genes (E1A, E1B), which are involved in the host cell cycle progression. In some adenoviral constructs the E3 region, which inhibits the cytolysis of the infected cell by CTL, and tumor necrosis factor (TGF-α) were deleted (14). Despite these manipulations increasing numbers of adenoviral vector experiments in the brain have revealed a significant immune response due to the remaining expression of viral proteins (12,15). The second generation of adenoviral vectors differs from the first generation in that the E3 region is only partially deleted. The

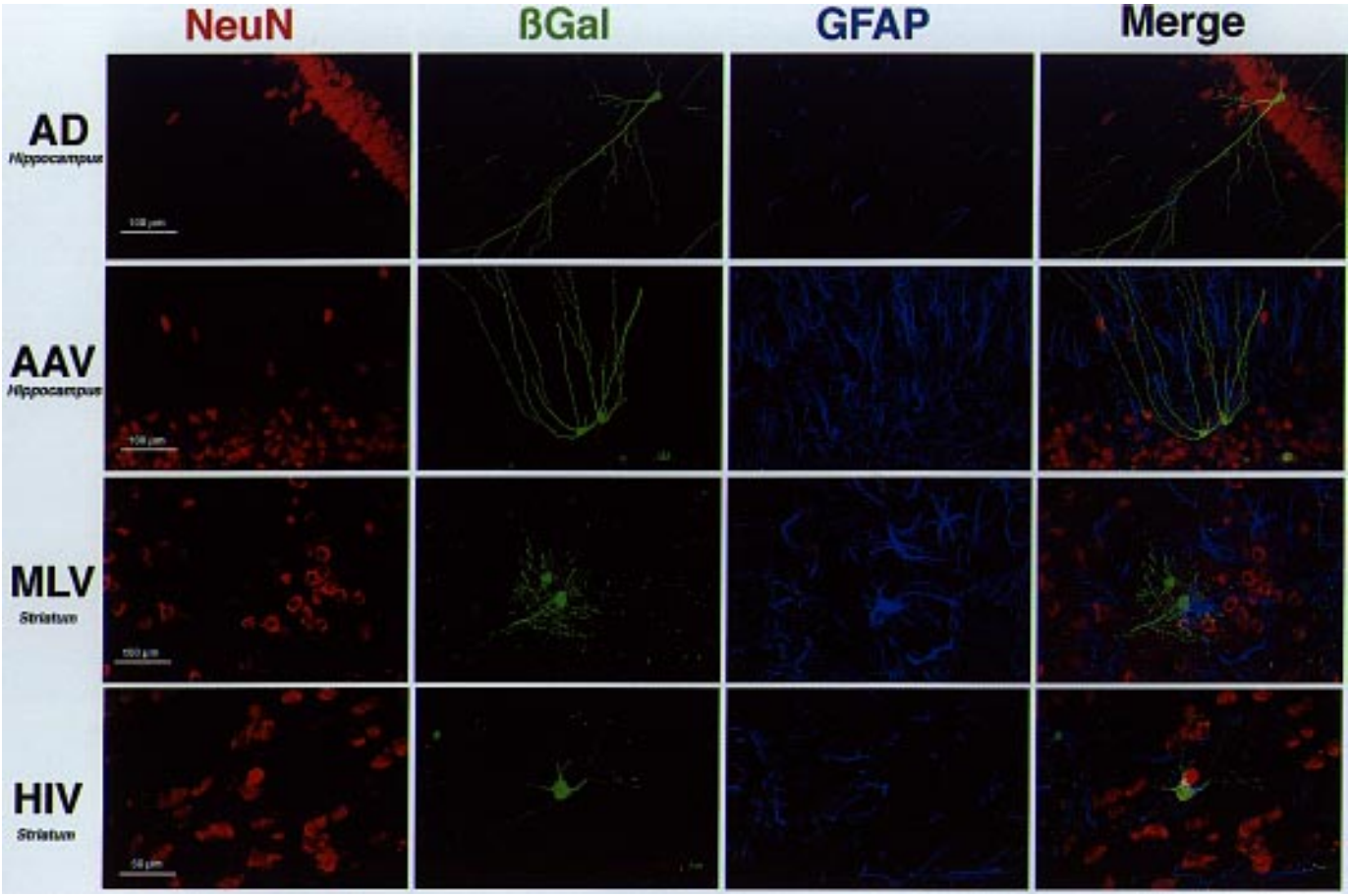

Figure 2. *In vivo* transduction of adult rat CNS cells. Confocal microscope images of sections of brains injected with adenoviral (AD), adeno-associated viral (AAV), Moloney murine leukemia virus (MLV) and human immune deficiency viral (HIV) vector are shown. The sections are stained by immunofluorescence forβ-gal (a reporter gene), NeuN (a neuron specific marker), and GFAP (glial fibrillary acidic protein). The images obtained from each individual staining as well as a merged image of all three stains are shown for each treatment as indicated.

vector retains the expression of the E3–19 kDa protein, responsible for the immune suppression ability of adenoviruses, and subsequently reduces the immune response (16). Still this vector continues to express viral genes at low levels and often leads to an inflammatory response (17), death of infected cells and rapid loss of transgene expression. In order to develop a third generation of adenoviral vectors, removal of the E4 region, which can likely cause oncogenic transformation in the host cell, is desired (18). However, elimination of E4 causes a drastic reduction of transgene expression (19).

Adeno-associated viral vectors

Adeno-associated viruses (AAV), which are single stranded DNA parvoviruses, are nonpathogenic for mammals. In AAV vectors all viral coding sequences, except the minimum AAV sequences required for transduction can be deleted, reducing the deleterious effects of viral protein expression. AAV vectors allow integration into the host cell genome, but the efficiency is very low. Studies of immortalized and primary cell cultures have shown that the vast majority of AAV vector genomes remain episomal and non-integrated (20,21). Helper viruses, either the adenovirus or HSV, provide proteins that are necessary for translation and transcription of the AAV, and perform a similar role during the transcription of the helper virus itself (21). A recent study has shown that the adenoviral E4 region is the limiting step in the AAV life cycle, specifically in the second strand DNA synthesis (22). For efficient transduction, however, the role of the helper viruses needs to be further elucidated. Helper viruses may have important implications for the use of AAV vectors in gene therapy protocols, because patients treated with recombinant viruses may subsequently be infected with wild type helper viruses and the interaction of recombinant viruses is only poorly understood (23). Low vector titers and transduction efficiency and the dependency on helper viruses seem to limit the use of AAV.

Retroviral vectors

Retroviruses were discovered as oncogenic agents, although the vast majority of retroviruses do not cause any pathology. These oncogenic viruses transform cells by expression of viral oncogenic sequences originally transduced from host cell genomes or by integration near cellular oncogenes with subsequent activation of the host oncogene (24). Their wide host range and ability to carry foreign genes and stably integrate into the host cell genome make them ideal vectors for gene transfer (24). Retroviral vector constructs, based on the Moloney murine leukemia virus (MLV), are significantly reduced in their viral genome and do not express

any viral protein that may evoke the host immune response. The gene of interest is flanked by minimal viral sequences acting as signals for packaging and retroviral transcription. The viral proteins, gag, pol and env, can be supplied in *trans* in packaging cell lines to generate retroviral vectors. The host cell specificity of retroviral vectors can be increased by replacing the ecotropic envelope gene with amphotropic envelope genes (25). Although retroviruses provide an efficient method for stable gene delivery, there are difficulties in obtaining high titers of vector without the risk of recombination and production of replication competent virus particles (26).

In contrast to other viral vectors that may have been attenuated but retain some ability to infect other cells, replication-deficient retroviral vectors infect only once and do not spread *in vivo*. Retroviral vectors have a broad host cell range, but their use is limited to dividing cells (27,28). As cell division is limited in the CNS, the application of this system is restricted mostly to *ex vivo* experiments.

To target non-dividing terminally differentiated cells, especially neurons of the CNS, a new vector system has been developed based on the human immune deficiency virus (HIV). Like other lentiviruses, HIV is able to infect dividing as well as quiescent cells, such as monocyte-derived macrophages and growth-arrested cells (29). Hijacking the nuclear import machinery, the HIV genome and its gene of interest are actively transported through the nuclear pore (30–33). The HIV-derived vector does not express the HIV virus envelope but uses the vesicular stomatits virus protein envelope (VSV G), which increases stability and allows high titers during the vector preparation. Gene delivery using this vector has been tested by intracerebral injection of highly concentrated vector (10^8 TU/ml) into the striatum and the hippocampus of adult rats. Three months after injection the reporter gene (β-galactosidase) was still detectable in every injection site and terminally differentiated neurons were transduced (34) (Fig. 2). Obvious pathological changes or signs of immune response were not detected in the rat brain tissue. In comparison control animals injected with a MLV-based retroviral vector did not express the β-galactosidase reporter gene after 6 weeks. Long-term transgene expression, stable integration and lack of expression of viral proteins associated with immune responses make this vector an attractive tool in CNS gene therapy.

EX VIVO GENE TRANSFER IN NEURONAL AND NON-NEURONAL CELLS

Many studies have explored somatic gene therapy focusing on the *ex vivo* approach (Fig. 1). Despite the complexity of the majority of human neurological disorders and the relative difficulty in accessing dysfunctional areas of the brain, intracerebral grafts of fetal and/or adult-derived cells are useful in somatic gene therapy. Cells of diverse origin survive transplantation into the brain and can replace or supplement deficient molecules. Behavioral abnormalities in animals models of CNS damage and in neurological diseases can be successfully reversed using cell transplantation (35,36). Although fetal tissue grafts are useful as a cell replacement source, genetically modified cells for intracerebral transplantation promise far greater benefits. For example, engineered cells can be autologous and therefore minimize the problems of cellular rejection. In addition, molecular biological methods allow the genetic modification of cells to produce a more controlled and broader range of desired factors than can be obtained with non-engineered cells. Following neuronal trauma, toxic compounds can be reduced or eliminated by cells engineered to produce free radical scavengers (37). In neurodegenerative disorders, cells that lose their vital source of trophic factors, e.g., nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), and neurotrophins (NT-3 and NT4–5), can be supported by transplantation of cells modified to produce these factors (38,39). The delivery of neurotransmitters and neuromodulators in models of neuronal degeneration has been found to restore neuronal function brain, although grafted cells are not able to mimic the normal dynamic functions of intercellular contact (35).

Engineered cells may also serve as a drug delivery system in cancer therapy, delivering suicide genes or toxic compounds to rapidly dividing tumor cells. Preferential incorporation of drug sensitive genes into tumor cells enables transduced cells to produce enzymes metabolizing drugs into toxic derivatives. These toxic derivatives result in the destruction of tumor cells following systematic administration of the appropriate drug, whereas the majority of healthy brain cells remain intact because they are quiescent (40–43).

Immortalized neuronal and non-neuronal cell lines (C6, neuroblastoma, AT20) have been used for gene therapy. However, the persistent growth leads to tumor formation and limits therapeutic applications.

Non-neuronal cells

Non-neuronal primary fibroblasts have been studied extensively because they are easily obtained, can be maintained in cell culture for weeks and can be genetically modified by various methods. Contact inhibition in high density cultures leads to decreased cell division and also prevents tumor-like growth (44,38) in the CNS as well as in peripheral tissue (45). The morphology of fibroblast grafts is similar to that of fibroblasts normally found in the skin, and viability has been demonstrated by collagen staining and abundant fibronectin production within the graft border (38,46). Genetically engineered fibroblasts producing neurotrophic factors have been successfully implanted into various rodent models, diminishing the neuronal loss following surgical and toxic lesion (39,44,47). Also primary myoblasts have been shown to survive well in the brain after transplantation (48,49).

Astrocytes and oliogodendrocytes are very attractive cells for grafting studies, due to their intrinsic supportive role in the CNS (50). However, their use has been limited to fetal or neonatal tissue and has been slowed by insufficient growth *in vitro* and the related low transduction rates with retroviral vectors (51). In addition Schwann cells have been used *in vitro* to produce tyrosine hydroxylase (52).

Chromaffin cells

Chromaffin cells from adrenal medulla have been used as graft donor cells and showed only poor survival and low basal production of catecholamines (53,54). Increased survival of chromaffin cells transplanted together with peripheral nerve fragments is the result of NGF supplementation. *In vitro* studies demonstrated that chromaffin cells convert to sympathetic neurons when NGF is included in the medium (55,54). Co-grafting of NGF-producing fibroblasts with chromaffin cells enhances both survival and transdifferentiation (56–58).

Neuronal progenitor cells

Immature neuronal progenitor cells isolated from the adult and fetal brain have been successfully cultured and characterized (59–65). These cells are found early in development and can survive *in vitro* in growth factor-enriched media over many passages, expressing glial and neuronal markers (63). Immortalized rodent progenitor cell lines have been successfully transplanted in various regions of the brain, with subsequent migration and integration into the host system (62,63). These cells are accessible to *ex vivo* gene therapy, because they grow quickly *ex vivo* and allow retroviral vector modification. Also their pluripotentiality allows them to assume different cell phenotypes in different regions of the brain depending on the local cues (64). The ease of culturing and manipulation of neuronal progenitor cells, their integration into the host system without uncontrolled proliferation and their potential to differentiate into mature neurons makes these cells a promising tool for *ex vivo* gene therapy (65,67). However, to achieve an unlimited supply of well-characterized uniform cells, the biological properties of immortalized progenitor cells require further research.

Immortalized and regulatable neuronal cells

Oncogenes (e.g., *v-myc*, *r-ras*) have been used for immortalization of slowly dividing cells. These genes maintain cells in a highly mitotic undifferentiated state for as long as 22 months (68). Transplantation of oncogene-expressing cells has revealed chromosomal damage and various cell morphologies (69,70). Oncogene-expressing cells can also exhibit uncontrolled growth with resulting tumor formation (71).

To obtain regulatable expression of transgenes, the temperature-sensitive mutant of SV40 large T antigen (TsA58) has been used (7). SV40 regulates the expression of oncogenes at 25°C and leaves cells in an undifferentiated, rapidly dividing state (73). Downregulation of oncogene expression and differentiation of these cells into neurons occur at 37°C. To externally regulate transgene expression, a regulatable retroviral vector in which the oncogene *v-myc* is driven by a tetracycline-controlled transactivator has also been used for conditional immortalization of adult progenitor cells (74). The suppression of the *v-myc* oncogene expression was sufficient to make proliferating cells exit from the cell cycles and induce terminal differentiation.

GENE THERAPY MODELS

The identification of mutant genes and mechanisms responsible for neurological disorders provides an opportunity to consider new approaches to their treatment. The identification of gene products and delineation of the cellular dysfunction and cell death in animal models may suggest new therapeutic options. In this review we will focus on neurodegenerative diseases. Therefore, a number of intriguing topics, like Huntington's disease, Lesch-Nyhan's disease and lysosomal storage disorders, must unfortunately be excluded.

Parkinson's disease

With 0.1–1% prevalence, Parkinson's disease (PD) is one of the most widespread neurodegenerative disorders. The majority of cases are acquired and its biological cause is generally unknown but may be related to oxidative stress, lack of neurotrophic support, or exposure to toxins. The disease is characterized by a loss of dopamine-producing neurons, specifically, dopaminergic neurons of the substantia nigra that project to the striatum. Tremor, rigidity and movement disorder result from the loss of inhibitory input on the extrapyramidal system. The current treatment, oral L-Dopa therapy, becomes less effective with progression of the disease, and the number of side effects increases.

The effect of oral L-Dopa indicates that the restoration of the neuronal circuitry is not necessary for improvement, but local delivery of L-Dopa is an alternative therapy. The enzyme tyrosine hydroxylase (TH) is responsible for the biosynthesis of L-Dopa from tyrosine. A single gene introducing TH to cells in regions of terminal loss can therefore increase the local supply of L-Dopa (75,76).

An established animal model in rodents allows testing of the efficiency of gene therapy in Parkinson's disease. The injection of a neurotoxin, 6-hydroxydopamine, destroys nigro-striatal dopaminergic neurons and results in elimination of nigral dopaminergic input and upregulation of dopamine receptors in the lesioned striatum, while the striatal dopamine receptor density in the unlesioned side remains unchanged. The asymmetry caused by the resulting differential postsynaptic receptor sensitivities between denervated and intact striatum results in rotational behavior after application of apomorphine.

Direct gene delivery of the TH gene into the denervated striatum has been achieved with several viral vectors. During and colleagues (77) used defective HSV vector encoding TH and Kaplitt *et al*. (78) showed long term expression *in vivo* in lesioned animals using the AAV vector. Previous reports mostly using adenoviral vectors were not able to retain long-term transgene expression (79–82).

Although fetal tissue has been effective in experimental models and partially effective in applications in humans, access to tissue and characterization prior to transplantation are problematic. In addition, transplantation of adrenal chromaffin cells has proven unsuccessful in preclinical and clinical trials (83,84). Currently, the use of genetically modified cells that produce TH is one of the major interests in gene therapy. Fibroblasts, retrovirally transfected with the TH gene and implanted into the striatum, are able to reduce experimentally induced rotational behavior in 6-hydroxydopamine lesioned rats (85). These data have shown that a small number of TH-producing graft cells are capable of inducing behavior improvements in this model. Despite graft cell survival for at least 2 months after injection, however, the number of TH expressing cells decreases with increasing time (38). Methods which extend the duration of *in vivo* transgene expression remain to be developed.

Cell death in PD has been related to oxidative stress, lack of neurotrophic support and exposure to toxins. The hypothesis that oxidative stress causes the loss of transplanted cells was tested in transplantation studies with transgenic mice, overexpressing Cu/Zn superoxide dismutase (Cu/Zn–SOD). This enzyme is crucial in the detoxification of free radicals. Transgenic mice producing Cu/Zn–SOD have been shown to be more resistant to neuronal damage induced by oxidative stress. The transplantation of neurons of Cu/Zn–SOD mice into immune-suppressed animals showed four times higher cell survival of genetically engineered neurons with concomitant functional recovery after 6-dihydroxydopamine lesion (86).

Alzheimer's disease

Alzheimer's disease (AD) is a common dementia (0.02–5% prevalence) of older patients and belongs in a large group of degenerative brain disorders. Only a small number of cases are inherited compared to the large number of acquired cases. The neuropathology is characterized by progressive dementia caused by cortical atrophy, neuronal loss, neurofibrillary tangles, senile plaques and vascular deposits of β-amyloid in various regions of the cerebral cortex and the hippocampus. β-Amyloid and its precursor play a crucial role in the pathogenesis of AD (87,88). The degeneration of forebrain cholinergic neurons responsible for memory acquisition and retention is well known, but the cause of the cell loss is not. A well-established model for degeneration of cholinergic neurons in rodents is created by the fimbria fornix lesion. This lesion disconnects the cholinergic neurons of the medial septum to their NGF supply. Exogenous replacement of NGF in this model can prevent cholinergic neurons from degeneration and ameliorates some forms of memory deficit (89,90). Direct intraventricular infusion of NGF into adult rats from the time of fimbria fornix lesion onward prevents the death of most of the axotomized cholinergic neurons. In the fimbria fornix model even non-cholinergic septal neurons are destined to die and probably not to be saved by NGF administration (89,91,92). Based on this observation NGF infusions into aged, cognitively impaired rats demonstrated improvement in learning tasks, compared to non-infused, cognitively impaired rats (93). Additional studies extended the findings, showing the age range and magnitude of the deficits that can be ameliorated by NGF infusions in aged, cognitively impaired rats (94,95). Based on these results primary fibroblasts, genetically modified to produce NGF, were implanted in the nucleus basalis magnocellularis (NBM) of aged impaired rats (96). Amelioration of learning and memory was observed, associated with significant increases in the size and number of NGF receptor-positive neurons in the basal forebrain.

Therapeutic strategies for AD have also targeted the replacement or replenishment of deficient neurotransmitters, for example acetylcholine (ACh). In one assessment of graft effect on cognitive impairments, cholinergic-rich tissue derived from the septum was implanted into the hippocampus of aged impaired rats (97). When compared to non-grafted impaired rats, the septal grafted rats showed significant improvement in spatial tasks. Fibroblasts, retrovirally transduced to produce ACh, survived within the brain and released ACh at least 10 days post-implantation (98). ACh-producing fibroblasts implanted into the frontal and parietal cortices of rats with bilateral lesions of the NBM could also ameliorate cognitive dysfunction in a rat model of AD (99). In addition to the basal forebrain, the entorhinal cortex (EC) is one of the first regions affected by neuropathological changes associated with AD. Lesions of the perforant pathway, which connects the EC with the hippocampus, result in selective loss of glutaminergic neurons (100). Transplants of fibroblast growth factor-producing fibroblasts prevented cell death of glutaminergic neurons of the EC. The rat models of cell death similar to that seen in AD provide a background for ongoing experiments in non-human primates, which are essential to evaluate the clinical potential of these approaches to gene therapy of AD (101).

Brain tumors

Brain tumors have become a major target of novel gene transfer during the last decade, probably presenting the best model of an acquired genetic disease. The growth rate of malignant tumor cells is different from that of mature brain cells, which are mostly quiescent. Rapidly dividing cells are theoretically ideal targets for gene transfer methods, without transfection of the surrounding brain tissue. Current therapeutic strategies include the direct killing of tumor cells, the production of new tumor antigens on the cell surface to induce tumor rejection and transfer of drug sensitivity genes to tumor cells.

A large number of animal models and lately even clinical trials have taken advantage of the thymidine kinase model (TK) (43,102–104). The transfection of cells with the TK gene enables the transfected cell to metabolize the anti-viral drug ganciclovir (GCV) into ganciclovir-triphosphate, which is cytotoxic and causes cell death. Only cells transduced with the TK gene are sensitive to GCV treatment. The poor efficiency in the beginning by direct injection of viral vectors carrying the TK gene into the tumor bed was overcome by implantation of producer cells to continuously supply the vector, which has only a short half-life time (2–4 h) (105). Culver *et al*. (41) demonstrated this approach by injecting inoculated 9L glioma tumors with fibroblasts producing HSV thymidine kinase recombinant retroviruses. Using retroviral vectors, only rapidly dividing cells (e.g. tumor cells) are infected and killed; the majority of quiescent cells of the brain do not adopt the foreign gene. In order to achieve a cure, it was originally thought that 100% of tumor cells would have to be transfected with TK and subsequently killed by GCV. In rodent studies several groups have seen tumor regression even with rates of 70% and less, due to the bystander effect (106,107). The bystander effect is based on the observation that HSV–TK containing tumor cells in the presence of GCV are directly toxic to unmodified adjacent tumor cells.

To stimulate the immune response and increase the tumor rejection, the delivery of interleukins and granulocytes-macrophages stimulating factors has been investigated (108,109). In addition, several studies successfully used the increasing immune response against tumors after vaccination strategies with irradiated tumor cells (110, Barba in prep.). In clinical trials, patients with primary or metastatic brain tumors have been treated in pilot studies with HSV–TK producing cells and GCV, but solid conclusions from these trials are not yet available.

FUTURE CHALLENGES

The determination of the specific biochemical deficits responsible for neurological disorders has provided direction for the development of gene therapy strategies. In addition, the increasing knowledge of basic cell biology, *ex vivo* cell maintenance and transplantation techniques will provide more specific vehicles for treatment of neurological diseases. Technical problems in the use of viral vector systems need to be solved. Transduction and infection efficiency and long-term regulatable expression are two of the major goals for the future. The development of high titer lentiviral vector systems that transduce neuronal cells adds to a growing sense of optimism that neurological disorders and defects may become accessible to gene therapy.

ACKNOWLEDGMENTS

We thank Dr P.J. Horner, Dr H.K. Raymon and Dr S.T. Suhr for critical comments on the review and M.L. Gage for her help in preparation of the manuscript. U.B. is a fellow of the Deutsche

Forschungsgemeinschaft on Grant BL389 (1/2). The research was supported by ISRT, the Hollfelder Foundation, NPF and NIH.

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