Journal of Cellular Biochemistry 45:252–257 (1991)

Cellular Replacement Therapy for Neurologic Disorders: Potential of Genetically Engineered Cells

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Abstract Neural transplantation, a mode of cellular replacement, has been used as a therapeutic trial for Parkinson's disease. Studies indicate that tonic release of the metabolites from the graft that can be utilized by the host brain, is likely to be the major mechanism responsible for the therapeutic effect. The use of fetal tissue is complicated by ethical controversy and immunological incompatibility. Autografting adult tissue has not been successful mainly due to poor survival. Genetically engineered cells are promising alternative sources of donor cells. We have investigated the potential of primary skin fibroblasts as donor cells for intracerebral grafting. Primary skin fibroblasts survive in the brain and remain in situ. A number of genes (nerve growth factor, tyrosine hydroxylase, glutamic acid decarboxylase, and choline acetyltransferase) have been successfully introduced and expressed in the primary fibroblasts. The L-dopa-secreting primary fibroblasts exhibited a behavioral effect in a rat model of Parkinson's disease up to 8 weeks after being grafted into denervated striatum. Factors that can maximize gene transfer, transgene expression, and fibroblast survival in the brain make up the future direction of investigation.

Key words: retrovirus, fibroblast, brain grafting, tyrosine hydroxylase, Parkinson's disease

Neural transplantation has attracted great interest in recent years for its potential as a mode of replacement therapy for the central nervous system (CNS) diseases, namely, grafting normal cells to replace or reactivate specific biochemical functions in the brain that have been lost as a consequence of diseases. Neural transplantation, in its simplest term, is to remove a piece of neural tissue from its normal site and place it in any site of the brain of the same or of another host animal. An increasing number of studies have demonstrated that developing nervous tissue from fetal brain can survive, develop normal properties in adult brain, and modify the function and behavior of the host animal. Fetal tissue grafted into the adult brain retains the capability to survive, differentiate, form synaptic contacts with host neurons, and exhibit normal neuronal function in synthesizing and releasing transmitters ([1] for review). There is evidence that continuously producing and releasing transmitters or growth factors may be sufficient to exert behavioral effect. In a search for candidate donor cells other than fetal tissue, neural transplantation has been extended to grafting of para-neural or non-neural cells (e.g., adrenal medulla tissue, cultured cells, or genetically engineered cells) into brain. As the factors that optimize the successful transplantation become recognized, intracerebral grafting has developed to the level of clinical trial in Parkinson's disease. In this communication, we review the conceptual development and practical application of the tissue replacement approach to CNS disease using Parkinson's disease as an example and discuss the role of genetically modified cells in this respect.

APPLICATION OF NEURAL TRANSPLANTATION IN PARKINSON'S DISEASE

Dopamine Replacement Therapy

Parkinson's disease is a degenerative disease characterized by a profound loss of neurons in the substantia nigra. Dopaminergic fibers from the substantia nigra supply dopamine to the striatum (caudate nucleus and globus pallidus) via a rich axonal network. The efferents from the striatum either go back to the substantia

Received September 7, 1990; accepted September 19, 1990.

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nigra, forming a closed-loop feedback circuit, or terminate in the globus pallidus. The pallidal efferent fibers then project to the thalamus, subthalamic nucleus, and reticular formation of brain stem. Those pallidal target areas have direct or indirect connections with the motor or pre-motor cortical field, from which the striatum receives excitatory inputs. The loss of dopamine neurons in the substantia nigra results in the degeneration of the nigrostriatal dopamine system. This upsets the operation of the reverberating neural circuits that regulate motor function. The core syndrome of Parkinson's disease is, indeed, motor dysfunction consisting of poverty and slowness of voluntary movements, tremor, stooped posture, rigidity, and gait disturbance. Ehringer and Hornykiewicz in 1960 found that dopamine was selectively decreased to a very low level in the striatum of patients suffering from Parkinson's disease and the major symptoms correlated with the depletion of dopamine. This observation has led to the breakthrough in treatment of Parkinson's disease, namely, supplementation of the dopamine precursor L-dopa. Administration of exogenous L-dopa increases dopamine concentrations in the Parkinsonian striatum and improves some of the motor symptoms. It is generally believed that the exogenous L-dopa is converted to dopamine in the surviving dopaminergic nerve terminals and/or in other non-dopaminergic dopa decarboxylase-containing compartments. Dopamine then activates postsynaptic dopaminergic receptors in the striatum and other areas and thus reestablishes the dopaminergic neurotransmission with consequent amelioration of motor symptoms. These advances in understanding the pathogenesis of and in the treatment of Parkinson's disease indicate that functional restoration can be achieved by replacing the deficient molecule locally in the damaged area. This discovery has inspired the attempt to replace dopamine by grafting the fetal tissue rich in dopaminergic neurons to the denervated striatum.

Grafting Fetal Nerve Cells

Degeneration of the nigrostriatal dopamine system can be modeled in animals by lesioning the substantia nigra with neurotoxic substances, e.g., 6-hydroxydopamine or MPTP (1-methyl-4-phenyl-1,2,3,6,-tetrahydropyridine). Animals with a unilateral substantia nigra lesion exhibit spontaneous or dopamine agonist-induced rotational asymmetry behavior, which has been widely used as a parameter to assess the effect of treatment [2]. In 1979, two independent groups reported significant reduction of rotational behaviors in lesioned adult rats after grafting a piece of embryonic ventral mesencephalon that contains the substantia nigra [3,4]. The reduction of rotation in rats with nigral lesion is clearly dependent on the survival of the graft and specific for dopamine-rich ventral mesencephalic tissue. The motor asymmetry returns immediately if the graft is removed at any time after transplantation. No functional recovery is seen if tissues other than dopaminergic neuron-containing mesencephalic tissue are grafted [1]. The degree of functional recovery is related to the extent of graft-derived reinnervation. Numerous subsequent animal studies including primates confirmed the behavioral effect of implanted fetal dopamine-rich mesencephalic tissue into denervated striatum [1]. These animal studies have led to clinical trials in the last 2 years [5–9]. Stereotaxic procedures are used to implant suspensions of nigral tissue obtained from first trimester fetuses to the striatum of Parkinson's patients who are poorly responsive to L-dopa treatment. The synthesis and storage of dopamine in the grafted area have been demonstrated in one patient at 5 months after receiving implantation [9]. However, the clinical results based on the limited number of patients are inconclusive.

Grafting Adrenal Medulla Tissue

The transplantation of human fetal dopamine cells for Parkinson's disease is complicated mainly by the ethical controversy of using aborted human fetus and the need for immunosuppression. Adult adrenal medulla cells have drawn great interest as an alternative source of catecholamine-producing cells for grafting. The idea is to transplant the patient's own dopamine-producing tissue into the damaged striatum. Adrenal medulla cells that primarily secret adrenaline and only low amounts of dopamine can be implanted into the lateral ventricle or striatum. Although it is conceptually attractive, the survival of grafts have been poor and behavioral effects have been transient in animal studies [1]. Furthermore, dopamine was not detected in the ventricular CSF or in the striatum by microdialysis methods [10]. With the obvious advantages of avoiding ethical and immunologic problems, the autotransplantation of adrenal

medulla using open microsurgery or stereotaxic procedures has been performed in patients with advanced Parkinson's disease. Since 1982, over 300 cases have been done. The conclusions drawn from these experiences are not encouraging. Adrenal medulla grafts do not survive, and the effects in improving motor function are inconspicuous and short-lasting [11]. Interestingly, the procedure itself seems to provoke some degree of regeneration of nigrostriatal terminals, which might account for modest effects observed in some patients.

SEARCH FOR ALTERNATIVE SOURCES OF DONOR CELLS FOR GRAFTING

Established Cell Lines

As more data indicate that neural grafting can be used as a means to correct neural dysfunction resulting from brain damage, investigations have been directed to identify practical sources of donor tissue. Research has been conducted to develop cultured cell lines with neuronal properties that are capable of releasing metabolites of therapeutical usefulness.

Rat pheochromocytoma cells (PC12 cells) that synthesize dopamine and norepinephrine continue to proliferate slowly after they are transplanted into brain [12]. In contrast, the differentiated IMR-32 cells (from human neuroblastoma cells) were grafted into primates without evidence of neoplastic growth up to 9 months [13]. In theory, developed cultured tumorigenic cells can be sustained for infinitive period in an appropriate environment. However, the potential tumorigenicity is a negative feature for using established cell lines as donor cells for transplantation.

Genetically Engineered Cells

In parallel to the progress in developing neural grafting as a replacement therapy for CNS disease, a conceptually new view has been actively investigated and developed as a strategy of gene therapy by introducing functional genes into the appropriate target cells removed from the host to produce the missing substance and then implanting genetically modified cells back to the host [14,15]. In 1987, a combination of gene transfer and neural grafting was first proposed as a potential therapeutic approach to CNS disease [16]. The idea is to use genetically modified cells as a biologic pump to release the metabolites that can be utilized by the brain and, consequently, restore function.

Initial investigations have used immortalized fibroblast cell lines as donor cells and replicating-defective retrovirus as a gene-transfer vector to deliver a transgene under the control of a viral LTR (long terminal repeat) promoter. A nerve growth factor (NGF) gene was transferred into immortalized fibroblast [17]. These fibroblasts exhibited a biological effect within the brain by saving septal cholinergic neurons, which normally degenerate as a result of being deprived of NGF following axotomy. A behavioral effect of genetically modified cells grafted within the brain was demonstrated in a rat model of Parkinson's disease [18]. Tyrosine hydroxylase is the enzyme that converts tyrosine to L-dopa. The full length rat tyrosine hydroxylase cDNA was transferred into fibroblasts by a retroviral vector. The infected fibroblasts expressed tyrosine hydroxylase and secreted L-dopa in vitro. Rats with an unilateral nigral lesion were implanted with infected fibroblasts in the rostral caudate nucleus and showed a significant reduction in dopamine agonist-induced rotations 2 weeks after grafting. In contrast, there was no reduction in the rotational behavior of rats grafted with noninfected cells. While these results are promising, the possibility exists that immortalized fibroblast cells may transform and lead to tumor formation. Recently, studies have been undertaken to use primary skin fibroblasts as donor cells [19]. Primary fibroblasts obtained from inbred Fisher rats were able to express the tyrosine hydroxylase gene in vitro. The effect on reduction of rotation was tested in nigra-lesioned rats every 2 weeks after the implantation of fibroblast within the caudate nucleus. A pronounced reduction of drug-induced rotations was observed in rats grafted with tyrosine hydroxylase-expressing primary fibroblasts for 8 weeks after grafting. There was no change in the behavior of rats implanted with cells expressing *E. coli* beta-galactosidase. The findings derived from these experiments indicate that 1) fibroblasts can survive in the brain, 2) retroviral vector-transferred genes (NGF gene, tyrosine hydroxylase gene, and beta-galactosidase gene) can be expressed in fibroblasts, and 3) transgene products (NGF and L-dopa) can be secreted and utilized in the brain.

THE USE OF GENETICALLY ENGINEERED CELLS FOR INTRACEREBRAL GRAFTING

The basic steps of preparing genetically engineered cells for grafting include the following: 1) decide on a gene whose expression is therapeutic

to CNS disease, 2) choose donor cells suitable for intracerebral grafting, 3) selecting an efficient method for gene transfer, and 4) evaluate the transgene product.

Transgene Selection

Understanding the anatomic and biochemical mechanisms of the disease to be treated is a prerequisite in selecting the cloned gene for preparing genetically engineered cells. It is also mandatory to have a suitable animal model of the disease in which the biological or behavioral effect of implanted genetically modified cells can be monitored.

Genetically modified cells may be applied to some degenerative diseases. Parkinson's disease, in which the pathophysiological mechanism is well recognized, is a most suitable model. It is obvious that the tyrosine hydroxylase gene is of choice for tyrosine hydroxylase, the synthetic enzyme converting tyrosine to L-dopa. One of the possible mechanisms accounting for the resistance to L-dopa treatment, which is commonly observed in advanced cases of Parkinson's disease, is the degeneration of dopaminergic fibers to the point that remaining fibers are unable to synthesize sufficient dopamine from L-dopa. With the availability of multiple selection markers for gene transfer, co-transfection of two genes is feasible. Therefore, cells releasing dopamine by co-transferring the tyrosine hydroxylase gene and dopa decarboxylase gene may be more effective for advanced cases. Other degenerative disease such as Alzheimer's disease or Huntington's disease may be the disease models of choice for genetically engineered cells. Chronic infusion of NGF can increase the size of NGF receptor positive cells (most are cholinergic neurons) in septal nuclei and nucleus basalis with correlated improvement of learning acquisition in aged rats [20]. This indicates that supplement of NGF, which is the direct product of the NGF gene in those areas, might have a therapeutic effect on Alzheimer's disease in which degeneration of cholinergic neurons is one of the pathologic features. Huntington's disease, a degenerative disorder characterized by motor dysfunction and intellectual deterioration, is another possible disease model for genetically modified cells. A rat model of Huntington's disease has been produced by infusing ibotenic acid to the striatum, which results in the profound loss of cells in the striatum and its target areas (substantia nigra and globus pallidus) associated with motor and memory impairment, a pathology similar to that of Huntington's disease. Fetal striatal tissue that is rich in GABAergic (gamma-aminobutyric acid) and cholinergic neurons grafted into rats with ibotenic acid lesioned striatum significantly improve motor function [21,22]. Chronic infusion of GABA receptor agonist saved the neurons in the substantia nigra that normally degenerated as the result of an ibotenic acid lesion of the striatum [23]. We propose that grafting genetically modified cells secreting GABA into the striatum target area (substantia nigra and globus pallidus) might have a behavioral effect in the Huntington's disease model. Glutamic acid decarboxylase whose gene has been cloned is the enzyme responsible for converting glutamic acid to GABA. Our preliminary data demonstrated that the glutamic acid decarboxylase gene can be transferred by retroviral vector and expressed in the fibroblast [24].

Non-degenerative CNS disease with localized pathology such as focal seizure disorder probably can be benefited from this therapeutic approach. It has been demonstrated that chronic infusion of GABA, the major inhibitory transmitter in the brain, could suppress seizure in an animal model of epilepsy [25]. Other neurologic diseases in which genetically modified cells may offer a therapeutic effect are neuroendocrine disorders.

Donor Cells

The brain is an organ confined in a limited space, therefore it is poorly tolerant to space-occupying mass. Donor cells chosen for intracerebral grafting should not risk the potential neoplastic growth. However, the donor cells have been restricted to actively growing cells, for most current available methods of gene transfer require cell division for the transgene to be integrated. Recently, there has been increased investigation into developing a vector derived from several classes of nonintegrating viruses, such as herpes viruses [26]. The success of using such a vector will extend the donor cell candidates to non-replicating cells. The possible donor cell candidates include primary fibroblasts, some immortalized cell lines, embryonic neuronal cells, and developing or reactive astrocytes. Our ultimate strategy for cellular replacement therapy is autografting, that is, to obtain cells from the patient himself, modify them in vitro, and implant them back to donor. Therefore, we have

focused on primary skin fibroblasts because they are readily available and easily cultured and manipulated in vitro. Our preliminary histological studies at the light microscopic level showed that a significant number of fibroblasts survived in the brain for 6 months. Abundant blood vessels were also seen in the graft, indicating vascularization. Electron microscopic studies were conducted to examine the structural organization of primary fibroblasts at 8 weeks after intracerebral implantation [27]. The grafts were composed primarily of fibroblasts and bundles of collagen. At the periphery, the graft was surrounded by reactive astrocytic processes. These histology data indicated that primary skin fibroblasts can survive in the brain and remain in situ.

Gene Transfer and Expression

The success of genetic modification relies heavily on 1) efficiently introducing cloned genes into the cell and their eventual integration into the cellular genome of particular cell types and 2) stable and continued expression of the transgene ([28] for review). Murine and avian retroviruses have proven to be an efficient vector to introduce foreign genes into target mammalian cells. A continuing problem with the use of a defective retroviral vector has been the production of wild-type virus in producer cell lines, presumably through recombination between the endogenous retroviral sequences and transfected vector plasmid [29]. This problem may eventually hinder the clinical application despite the fact that it occurs in a relatively low frequency. A number of chemical and physical methods have been used to deliver functional genes into mammalian cells. These methods include coprecipitation with calcium phosphate, encapsidation of DNA into liposomes or erythrocyte ghosts, electroporation, and direct microinjection [28]. Recently, a liposome formed from cationic lipid was developed. The liposome, because of its positive charge, can interact and trap negatively charged DNA, fuse with membrane, and thus deliver DNA into the cell [30]. This technique, called lipofection, is simple and highly reproducible. Our preliminary work showed that the efficiency of lipofection is sufficiently high enough to transfect primary fibroblasts. It is believed that the development of viral vector-mediated methods devoid of wild-type virus contamination or highly efficient non-viral methods is an important step toward the clinical application of genetically modified cells.

The long-term stability of retroviral promoter-controlled transgene expression in engineered cells after being grafted in the brain is not clear. Cultured mouse cerebellar cells expressed the chloramphenicol acetyltransferase gene transferred by retroviral vector up to 3 weeks but not to 3 months after being grafted in the adult mice brain [31]. We have grafted primary fibroblasts expressing the glutamic acid decarboxylase gene under the control of retroviral promoter into rat brain and dissected out the grafts at 8 weeks. Glutamic acid decarboxylase activity assayed by the $^{14}CO_2$ trapping method could be detected in the dissected graft. The expression of transgene is dependent on promoter activity. Some types of cells contain cell-specific regulatory factors that can interact with viral enhancer sequences [32]. With the availability of a non-viral gene transfer method, the transgene's own promoter or housekeeping gene promoter from mammalian cells can be evaluated. The increasing recognition of factors that regulate transcription will assist in designing the engineered cells with maximal expression of the transgene.

Transgene Product Monitoring

The functional effect of genetically modified cells is dependent on the capability of these cells to release the metabolic product of the transgene that can be utilized by the brain. The transgene product should be measured using enzyme assay and immunohistochemistry. High performance liquid chromatography can measure most neurotransmitters. Using the microdialysis technique, the in vivo release of the metabolic product of the transgene can be monitored and correlated with the functional effect. The quantitative assay is also important in evaluating factors that may increase the functional activity of the transgene product, e.g., enzyme cofactors.

SUMMARY

Studies of neural transplantation, a mode of cellular replacement, have demonstrated that replacing the deficient molecules (neurotransmitters or growth factors) locally in the damaged brain area by grafting specific cells can restore the neurologic function that is lost as a consequence of disease. The therapeutic potential of genetically modified cells in animal mod-

els of neurologic disease is being actively investigated. Primary fibroblasts that are easy to obtain and to manipulate in vitro can be used as donor cells for autografting. Primary fibroblasts can survive in the brain and express a number of genes including NGF, tyrosine hydroxylase, glutamic acid decarboxylase, and choline acetyltransferase transferred by retroviral vectors. The NGF and L-dopa-secreting fibroblast exhibited biological and behavioral effects after being grafted into the brain. Future investigation should be directed at identifying factors that can optimize the survival of fibroblast, maximize the efficiency of gene delivery, and enhance transgene expression.

ACKNOWLEDGMENT

This work was supported by NIH grant NS01411.

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