RECONSTRUCTING THE BRAIN:

NEW STRATEGIES FOR BRAIN DISEASE

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Contents

The use of transplants to arrest and correct brain disease has attracted a great deal of professional and public interest. New methods in molecular biology are beginning to make a major impact on many areas of medicine. This paper discusses the nature and consequences of these new methods for brain transplant technology.

In the first and second sections two approaches to brain transplants will be presented.
* In the first approach either the patient's own tissue or primary tissue from another source is transplanted. The key features here are the methods for purification and manipulation of the cells to be transplanted.
* The central feature of the second approach to transplantation is the use of cell lines as a source of tissue in brain transplants. The technical and social advantages of these strategies using cell lines.

The third section focuses on the technical features of required in brain reconstruction. In particular we describe major benchmarks which can be used to monitor the progress of this technology from research lab to clinic. The major conclusion is that there are new methods which will provide effective, economic and socially acceptable therapy for several brain diseases.

[illegible] into the central nervous system has been pioneered as a potential therapy for Parkinson's disease. One very clear feature of this disease is the dramatic loss of cells in a particular region of the brain, the substantia nigra. The behavioral features of the disease are readily explained by loss of function in the substantia nigra. The neurotransmitter dopamine is present in high levels in this brain area. Oral doses of L-dopa, the precursor to dopamine, provide an effective therapy to the disease in some cases. With time this therapy often ceases to be effective, and in other cases the L-dopa is never effective. It is clear that treatment by other therapies is required. Grafting cells capable of making dopamine is a potential therapy which is recieving a great deal of attention.

Tyrosine hydroxylase is the enzyme that makes dopamine. This enzyme is present in other cells in the body, in addition to cells in the substantia nigra. One therapeutic strategy that is being explored is to transplant other cells that express tyrosine hydroxylase to the substantia nigra. We call this strategy Autologous Unmanipulated (AU). The major

source of tissue for AU transplants is the adrenal gland (Backlund, et al., 1985, Madrazo, et al., 1987). A patient undergoes double surgery: the adrenal gland removed, the dopaminergic cells separated and placed in the brain. Given the constraints of major surgery, it is difficult to isolate dopaminergic cells from the adrenal gland in sufficient numbers and purity for transplantation.

It would be advantageous to make a more abundant cell the basis for this operation. The gene coding for the tyrosine hydroxylase (TH) enzyme has been cloned and can express functional enzyme when replaced in cells. This technology allows cells expression of the enzyme in cells which do not normally express TH. The gene can be inserted into cells taken from a patient prior to transplantation. This strategy will be called Autologous Manipulated (AM), it has been successfully demonstrated in animal models for Alzheimer's disaese and may also be applicable to Parkinson's disease (Rosenberg et al., 1988). AM transplants offer a new route with important clinical applications.

The use of autologous transplants has the major advantage that the transplanted cells will be derived from the individual who will be the recipient of the transplant, minimizing possible complications resulting from an immune response to the transplant.

1b: HETEROLOGOUS TRANSPLANTS

An alternative to the use of the patient's own cells is to use cells from other sources. The heterologous sources can be separated into two classes either primary cells or cell lines. Primary cells are cells which have been acutely isolated from tissue of another individual or another species. Experimental data in animals show that tissue from the developing nervous system is capable of correcting behavioral deficits resulting from lesions which mimic Parkinson's and Alzheimer's diseases (Bjorklund, et al., 1988). The success of these experiments may be due to these transplanted cells reconstructing elaborate features of the brain. A consequence which contrasts sharply with the very simple single gene replacement in the AM transplants we have already discussed.

Heterologous transplants carried out with primary tissue, without further manipulation, will be called HU. HU transplants to Parkinsonian patients has been carried out using human embryonic substantia nigra. It is still unclear whether HU transplants are an effective neurosurgical tool. HU transplants bring several potential problems. These problems are of two kinds. The social problems of the regulation of tissue availability and the technical problems of quality control, long term survival and efficacy. One potential solution to both technical and social problems is to use customized cell lines.

[illegible] CELL LINES?

In the middle of this century techniques were developed to permit dissociated animal cells to grow outside the animal for long periods. The conditions for tissue culture of animal cells developed by experiment were simple. Tissue was taken from animals and the cells separated by methods that disrupt the specific proteins which hold cells together in a solid tissue. When the separated cells were placed on an appropriate surface and provided with a solution containing the right salts, vitamins, amino acids and growth factors, at the right temperature and pH, the cells very often grew. Using these methods cells from different tissues can be grown. In the tissue culture dish many specialized features of the cells were retained. Muscle cells could form aggregates capable of contracting. Neurons were capable of extending axons and forming synapses. One feature that was the same for all the different cultured cell types was that they had a limited life.

The limited life span of a cell in culture can be overcome by introducing a particular kind of gene into the cell. The cells carrying these additional or altered genes will grow indefinitely and are known as cell lines. The genes which turn a primary cell into a cell line form part of a rapidly growing group of genes called oncogenes. The study of oncogenes is now extremely sophisticated. Many different oncogenes have been identified and the specific mechanisms leading to continued cell growth are increasingly well understood. Although oncogenes were initially isolated from RNA and DNA tumor viruses, one of the major insights to come from recent work is that many viral oncogenes are very similar to genes which normally function in cell growth. These genes, whether viral or cellular in origin, can be used to overcome the limited life span of primary cells. The current state of this field is that dozens of genes are known which influence the growth of cells in different ways - some subtle, some dramatic. As our understanding of oncogenes has developed it has become possible to manipulate the growth of cells more precisely. It is now possible to grow cells indefinitely as cell lines which retain useful functions in culture and after transplantation.

good but little tech.

2b: MAKING CELL LINES FROM THE BRAIN

The ability to make cell lines has become a major technical tool in biology. For example, the widespread use of monoclonal antibodies in

are often present in small numbers, for a short time, during the development of the brain. Because of this cellular complexity, powerful methods for making cell lines would be particularly useful in neurobiology.

In addition to having an efficient method for making cell lines, another important feature of these new techniques is that cell lines can be generated which can differentiate in the animal. Cells are not static objects, they normally exist in different states. The cells that carry out most of the major tasks in the body have a limited life and are constantly replenished from specific precursors. The precursor cell gains the differentiated functions when it changes state in response to particular signals.

We have already learned that the discovery of oncogenes was a crucial step in developing technology for making cell lines. In order to make cell lines that differentiate it is important to be able to regulate oncogene function. Growing cells at different temperatures offers a very simple way to regulate oncogene function. We have used a gene derived from a temperature sensitive oncogene from a monkey virus to make cell lines from the rat brain. The oncogene was introduced into cells from the central nervous system of embryonic rats. From these we can derive cells which can grow indefinitely when the oncogene is active. When the cells were grown at elevated temperature they differentiate (Frederiksen, et al., 1988). Cell lines which retain the ability to change state - to differentiate - are potentially particularly important in brain transplants because of the success in transplanting embryonic brain tissue in animal models. These methods should be directly applicable to cells from the human brain.

2c: TRANSPLANTING CELL LINES

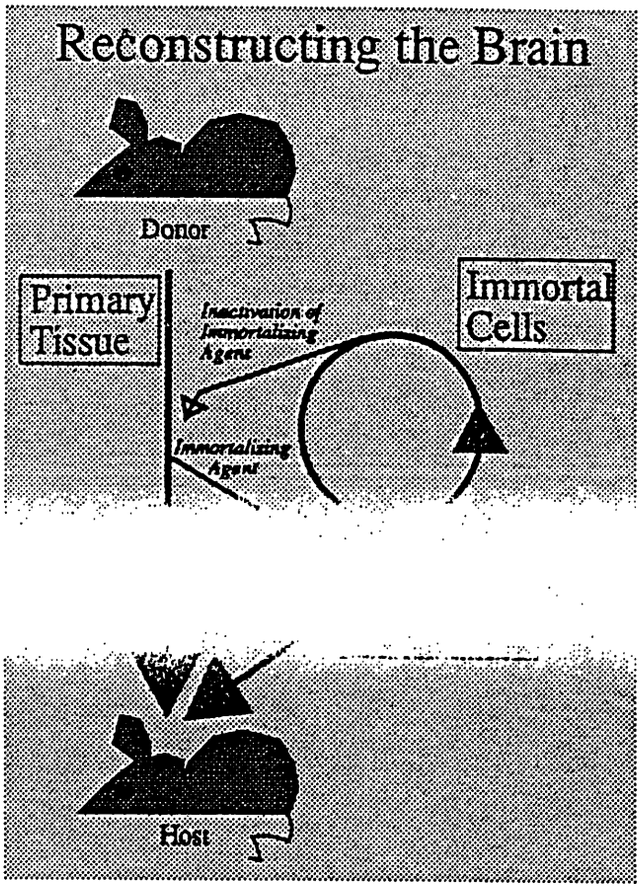
Two recent cell line transplant experiments have important implications for neurosurgical approaches to brain disease. A set of experiments carried out by group centered at the University of California, San Diego, used a fibroblast cell line carrying the nerve growth factor gene in an animal model of Alzheimer's disease (Rosenberg, et al., 1988). Fibroblast cell lines are commonly available and the fibroblast is a vehicle

in the same animal model of Alzheimer's Disease (Whittemore, et al., 1988). Currently we are transplanting a cell line which differentiates into oligodendrocytes. These experiments have importance for understanding and treating demyelinating diseases such as Multiple Sclerosis.

In summary, the ability to make transplantable cell lines with specific functions will certainly provide a simple and reproducible transplant therapy with attractive features from both clinical and social perspectives.

3. Benchmarks for a new technology

U.S. government support of academic research in the life sciences is the basis of dramatic advances in biotechnology. The translation of this pure research into products is a major trend in the pharmaceutical industry. As in any new technology it is crucial to identify markers which chart progress towards commercially viable and socially useful products. The purpose of this section of this report is to identify a set of major technical benchmarks in the development a new transplant technology.



This figure illustrates the major steps in transplantation technology. The first two sections of this report discuss the differences between primary and immortal cells. The major advantages of immortal cells are reproducibility, availability in unlimited quantities and the possibility of systematic genetic engineering. Transplanting primary tissue is also a useful strategy and here too, cells can be engineered. These two strategies interact and these bench marks apply to both. In addition a third strategy of direct gene transfer is introduced this section. The main purpose of this section is to show it is realistic to plan major therapeutic advances based on new techniques for reconstructing the brain.

3.1 Identification of brain precursor cells.

One of the most exciting results in this area is the ability of embryonic tissue to regenerate almost complete brain structures after transplantation into adult tissue. Another important recent finding is that many of the different cell types in the brain are derived from a single, multipotential precursor (Holt, et al., 1988, Price and Thurlow, 1988, Turner and Cepko, 1987, Wetts and Fraser, 1988). These findings suggest that the the transplantation of this single cell type would allow dramatic restoration of brain function.

We have identified a gene, Rat 401, which is selectively expressed in neuronal stem cells in the rat. The methods we have developed for measuring the number and types of cells in the CNS *in vivo* have been applied to the developing spinal cord and cerebrum (Frederiksen and McKay, 1988). The protein coded by this gene is abundantly expressed in the immediate stem cells and is not expressed in neurons themselves. The expression of this gene is a characteristic of the cell type that can regenerate complex features of the brain. The isolation of this gene in the rat can now be used to identify the homologous gene and cell type in the human brain.

regulate these critical cells.

3.2 Characterization of stem cells in different brain regions

We use the properties of cells *in vivo* as the standard for defining stem cells. We have concentrated on the cerebellum, hippocampus and spinal cord on our preliminary work. In each of these regions we can identify cells which express the Rat 401 gene but it is clear that cells of that stem cells in other regions of the brain that are clinically most significant must now be pursued. The striatum, mesencephalon and basal

forebrain are of particular interest to Alzheimer's, Parkinson's and Huntington's diseases.

3.3 Survival and functional properties of stem cells.

We have already shown that Rat 401 positive stem cells, from both cerebellum and hippocampus, survive in primary culture and differentiate to become both neurons and astrocytes. We can measure the number of these cells present on different days in culture to establish if the proliferation and differentiation of these cells in vivo parallels their behaviour in culture. The exciting outcome of these experiments is that we can directly determine the survival, proliferative potential, and antigenic profile of cells from different brain regions. This information is critical to ant transplant strategy.

These first three goals form the basis of a systematic understanding of the cells of the early brain which must underly any transplant technology. The next three sections deal, directly, with methods for therapeutic intervention. I concentrate on the use of immortal cell lines as this is the technology we have developed at MIT but it is important to understand the close relations with technologies being developed by others. Together these approaches form a powerful new way of addressing brain disease.

3.4 Engineering primary cells

An initial therapeutic goal is to take primary cells from a patient, introduce a therapeutic gene into these cells and transplant these cells into the patient. This autologous transplantation has many attractions. There should be little difficulty with immune rejection. The cells should come under the normal contral mechanisms which regulate cell proliferation. Ofcourse these two questions must be directly investigated in both rodent and primate models prior .to the therapeutic use of this approach. The introduction of therapeutic genes of different kinds into autologous cells forms the key to this method. What genes should be used? How should they be altered? What transcriptional regulators should be used? How many cells should transplanted? At what sites? How can recovery be monitored?

Huntington's

3.5 Direct transfer of therapeutic genes

Down syndrome.

Direct introduction of a therapeutic gene is an alternative to using engineered cells as a vehicle. This strategy may be particularly useful in some diseases. For example; if a single gene product is therapeutic, a retrovirus may be used to introduce these genes directly into the cells of the brain. Here the host cells are minimally altered. Additional questions to be answered include: what viruses are most effective? many of the issues in section 3.4...

3.6 Strategies for the immortalization of brain cells.

One of the most ambitous goals of this field is to generate immortal stem cell lines. Immortal, or established, cell lines which are still capable of differentiation offer detailed answers to outstanding problems in both clinical and basic neurobiology. To apply molecular genetic methods to the analysis of brain organization we have developed a novel method for immortalizing cell lines using the tsA 58 thermolabile form of SV40 T antigen incorporated into a pZipNeo retroviral vector. Immortalization with a conditional oncogene is a simple method for generating precursor cell lines which will differentiate under controlled conditions in vitro. This method may help solve a wide range of problems in vertebrate developmental biology. Our preliminary experience with this vector has been very encouraging as we have obtained cells lines which differentiate into both neurons and glia.

The immortalization of early neural cells may be strongly influenced by three parameters: the mechanism of oncogene immortalisation, the regulatory sequences which control oncogene expression, and the target cell types used for immortalization.

* We have made extensive use of an oncogene (T-antigen) from the simian virus, SV40. The reason for our focus on SV40 T-antigen is that the gene is available in a temperature sensitive form. The temperature sensitive feature is important because it allows the function of the oncogene to be removed from the cell simply by raising the temperature. The method we currently use to immortalize cells is to infect the cells with a defective retrovirus carrying the oncogene and a dominant selectable marker(Frederiksen, et al., 1988). The advantages of immortal cell lines are that the cells can be obtained in unlimited quantities allowing a crucial standardization of transplant procedures. A second and equally important advantage of this method is that the immortal cell lines can be genetically manipulated. Almost all of modern biology centers around methods to manipulate the genome and the immortal cell lines can be used to bring to transplants the enormous power of modern molecular biology.

* It is quite clear from work with transgenic mice that the properties of cells expressing an oncogene are importantly influenced by the sequences controlling transcription of the oncogene. For example, Hanahan placed the SV40 wild type T antigen under the control of the [illegible]

[illegible] it is clear that the regulatory sequence, as well as the structural sequence, offers another key intervention point to control oncogene function.

* The differentiation of cell lines is one of the most interesting aspects of our preliminary results. It is obviously important to test whether this strategy can be used with all precursor cell types. An important criterion for choosing new cellular candidates for immortalization must be how well characterized the primary cells are. In this respect the 02-A oligodendrocyte precursor cell is clearly a suitable target for immortalization.

The 02-A precursor is the one precursor cell in the CNS which proliferates and differentiates under defined primary culture conditions. The development of oligodendrogilal cells in the rat optic nerve has been carefully studies by Raff and his colleagues providing the background information necessary for the detailed characterization of immortal cell lines. The question of interest here is: when the oncogene is inactivated

by raising the temperature, does the cell line differentiate like primary 02-A progenitors?

Primary cells from the rat optic nerve were infected with a recombinant retrovirus transducing temperature sensitive SV40 T-antigen. Immortal cell lines were derived from these infected cultures. When T-antigen was inactivated the cells differentiated into oligodendrocytes by both biochemical and morphological criteria(Almazan and McKay, 1988). These oligoprecursors cells are potentially important to the diagnosis and treatment of demyelinating diseases.

We have already generated several cell lines from the rat which differentiate into cells with neuronal properties. Characterizing different cell lines further is clearly an important goal of this overall strategy. These experiments cover many different aspects of cellular function: proliferation, survival, neurite extension, axon navigation, and cell-body migration. The development of assays for different cell functions is a crucial step towards a systematic molecular biology of the mammalian nervous system. However a great deal of information can be obtained rapidly by transplanting immortal cells into the brain. Additional cell lines must also be generated, particularly from the brain regions which are most damaged in neurodegenerative diseases.

[illegible] to deal neurodegenerative diseases is based on two general assumptions. First, that we have devised methods to define the number, types and functions of cells present in the brain. Second, that the cell types of the brain can not only be accurately mapped, but reproducibly manipulated. Recent work shows that both of these conditions are true. In addition there are a series of bench marks which define the steps towards the succesfull application of these new techniques. With appropriate support a systematic and powerful new technology will develop. This technology will allow new therapies for many damaging brain diseases. Our group at MIT has particularly focussed on the basic molecular biology which permits immortal cell lines to be made from the brain. One measure of the rapid advance of this field is that these immortal cells may become dramatically powerful tools in brain reconstruction.

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