TECHSIGHTING
TRANSPLANTATION

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Brain Transplants?

We have grown accustomed to the idea of human organ transplantation. Transplants of solid organs such as hearts, kidneys, and livers, as well as bone marrow, have become the life-saving treatments of choice for some diseases. Investigators are even looking at ways to successfully transplant organs from animals such as pigs into humans. But what about transplanting a human brain?

Although transplanting an entire human brain seems far-fetched, transplanting individual cell populations is not. In fact, the transfer of fetal donor neural grafts has been shown in repeated studies to correct some of the motor defects found in patients with Parkinson's disease. Turning to rodents as a study system, investigators have gone on to show that neural stem cells (NSCs) can also be isolated and used as donor cells. NSCs can be isolated from dissociated rodent brains and propagated in vitro by addition of extracellular growth factors (such as EGF or bFGF) or the introduction of growth-promoting genes (such as v-*myc* or large T-antigen).

As a natural follow-up to the rodent work, these authors set out to develop a graftable system of human NSCs. First, they isolated cell suspensions from the periventricular region of a 15-week-old human fetus, an area that is a rich source of NSCs in rodents. Next, they cultured these cells in an alternating mixture of EGF and bFGF for several months, screened the population for its ability to engraft, and then cloned out individual, stable cell lines. (They also introduced the growth-promoting v-*myc* gene in some experiments, but this proved unnecessary in the end.) By simply changing the culture medium to one containing serum, these NSC lines differentiated into neural and oligodendroglial cells in vitro. Coculture with primary murine central nervous system tissue was needed to drive differentiation most effectively down the astroglial pathway, another lineage that is naturally derived from the NSC in vivo and is the last cell type normally born during development; hence, the coculture may well have "recreated" the in vivo environment.

To determine how these NSC clones would react in situ, the authors transplanted them into the ventricles of newborn mice. In order to follow some of these clones in vivo, they were first transfected with a retrovirus that expresses the β-galactosidase gene that could serve as an unambiguous visual marker for human cells in a mouse brain. Following introduction, the human NSCs engrafted readily into the murine brain and migrated along pathways that have previously been demonstrated as natural routes for these cells in vivo. For example, microscopic examination of the introduced β-galactosidase tag showed that the cells moved to the mouse subcortical and cortical white matter, corpus callosum, and olfactory bulb. The human NSCs intercalated with native neurons and glia, and differentiated appropriately depending on the surrounding cell types and cues. They also integrated into the germinal zones at the opposite end of the brain and became appropriate neural cell types there. This proved that despite cell culture, cloning, and gene transfection, the NSCs retained their pluripotent status in vivo. In addition, the cells showed the ability to express a foreign gene seamlessly within the fabric of the brain.

The group went on to demonstrate the possibility of using NSCs in gene therapy applications in an in vitro setting. These cells express the alpha subunit of β-hexosaminidase, the gene that is defective in Tay-Sachs disease in humans. They then mixed in mouse brain cells that had the gene deleted to serve as a host for the cells. By enzyme analysis, they showed that significant quantities of the active β-hexosaminidase were produced by the cell mixture and resulted in diminished accumulation of pathologic material in the Tay-Sachs nerve cells.

As an in vivo test of the engrafting potential of the human NSC lines, they introduced NSC clones into the brains of another defective mouse strain, the meander tail (mea) strain. This particular mutant has a defect that prevents many granule neurons from developing or surviving in portions of the cerebellum. In mea mutants, after introduction into the external granule layer of the cerebellum, the human NSCs migrated to the proper layer of the cerebellum and differentiated into cells that appeared identical to normal mouse granule neurons. This feat is remarkable given the fact that the original cells used to create the NSC lines were derived from the human fetal periventricular region, not the postnatal cerebellum. This accomplishment demonstrates the plasticity of these cells and the retention of a response to normal differentiation cues in the animal.

Thus, human neural progenitor and stem cells can now be isolated and manipulated in vitro and in vivo. The amazing property of these cells to differentiate in situ in the recipient brain may eventually allow targeted introduction of cells into defined regions to correct specific defects, as well as to integrate in a more disseminated way for many types of neurologic diseases with widespread pathology.

—ROBERT SIKORSKI AND RICHARD PETERS

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TECHSIGHTING
CELL BIOLOGY

Mailbox: www.sciencemag.org/cgi/dmail?53972b

Gentle Slam

Cell biologists are often interested in manipulating the environment of a single cell. When they want to affect the internal environment of a cell, they often use a technique called single-cell microinjection. Often referred to as "stab" microinjection, the approach consists of rapid insertion of the tip of a glass micropipette into the cell's cytosol under high pressures (100 to 200 mbar) to introduce sufficient material from the pipette. The injection pressure must be carefully controlled: too high a pressure will burst the cell, whereas too low a pressure results in delivery of too little material. The technique works best in large cells (because there is less chance of damaging intracellular organelles such as the nucleus) and in cells that are adherent, because they do not move away from the tip as they are being punctured. Stab injection is used to deliver synthetic molecules, nucleic acids, peptides, and proteins into the cytoplasm of these cells.

In small cells (2 to 15 μm in diameter), however, the approach becomes problematic because the nucleus-to-cytoplasm ratio of these cells increases, leading to a greater likelihood of damaging the nucleus during the stab. A report in last month's issue of the *Biophysical Journal* describes an interesting modification of the stab approach for injection of solutes into smaller and more mobile cells (*1*). The researchers refer to their technique as "slam" (simple lipid-assisted microinjection). This gentler approach consists of coating the tip of the micropipette with a lipid layer and letting this layer fuse with the plasma membrane of the cell to form a lipid bridge with an aqueous channel between the pipette and the cytosol. Through this channel, the solute gently diffuses inside the cell.

The glass micropipette is filled with injection medium, and the tip of the pipette is dipped in a 1 mM solution of phosphatidylcholine-oleyl-palmitoyl dissolved in chloroform and kept on ice. Next, the chloroform is allowed to evaporate, so that the synthetic lipid ends up coating the

pipette. The pipette is then connected to a pressure control device, and the tip is soaked in aqueous medium to allow the dried lipid to swell and form a bilayer. The pressure inside the pipette is subsequently increased to 10 mbar. Absence of leakage of lucifer yellow, a fluorescent dye added to the injection medium, indicates that an effective lipid seal has been formed.

The authors tested their method on neutrophils, because these cells have features that are challenging obstacles to any microinjection strategy: small size, large nucleus-to-cytoplasm ratio, and loose adherence. The neutrophils were allowed to sediment onto a glass cover slip, and then the micropipette was brought to the surface of single cells with a motorized, microprocessor-controlled micromanipulator. Gentle contact with the cell surface led to transfer of the lipid and aqueous contents of the pipette to the cell. The pressure in the pipette was held constant at 10 mbar to ensure diffusion of its contents inside the cell. To monitor the transfer of the aqueous content, the motion of the lucifer yellow dye was monitored by fluorescence microscopy. The dye could clearly be seen to diffuse inside the neutrophil in a matter of seconds. To monitor the fate of the lipid bilayer, the authors used the fluorochrome 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbo-cyanine perchlorate; this dye is weakly fluorescent in water, but strongly fluorescent in lipid bilayers. Upon touching the cell, this dye was transferred from the micropipette to the cell membrane, where the fluorescence became uniform over time. The authors suggest that the lipid bilayer merges with the cell membrane, while the solutes are transferred to the cytosol. After several seconds, the pipette was retracted and the cells left in place for further observation.

The authors monitored cell damage using the trypan blue exclusion technique. Less than 5% of neutrophils survive the stab injection technique intact, whereas almost all survive the slam approach when the injection is at a pressure of 10 mbar. Higher injection pressures leads to cell damage or even complete cell rupture.

The method described appears simple and could be useful when cells need to be handled very gently. It appears not to damage cells and delivers a reasonable amount of material (about 1% of the cell volume). In addition, the approach could potentially be used to deliver material to the cell membrane by exploiting its fusion with the lipid bilayer. For example, the approach could be used to deliver membrane-soluble receptors to specific cell types. Of course, the method will need to be reproduced in other laboratories and with other cell types before the stab technique can be retired.

—RICHARD PETERS AND ROBERT SIKORSKI

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TECHSIGHTING
PLANT GENOMICS

Mailbox: www.sciencemag.org/cgi/dmail?53972c

Plant Genotoxicity

Several organisms have been used to monitor and test the effect of radiation on the genome, including *Drosophila melanogaster* and transgenic mice. In the case of both *Drosophila* and transgenic mice, testing of the effect of ionizing radiation is performed by monitoring the reversal of a specific gene mutation: the reversion of a recessive eye color mutation in *Drosophila* and the restoration of the *lacZ* gene in germ cells in transgenic mice.

A plant model is also available for testing radiation's effect on the genome. As with animal models, this model is based on the premise that exposure to radiation results in an increased frequency of homologous recombination. In this case, transgenic *Arabidopsis thaliana* plants carry two overlapping, nonfunctional truncated versions of a chimeric β-glucuronidase marker gene in inverted orientation (*1*). Homologous recombination at this locus restores a functional β-glucuronidase gene that can be detected by histochemical staining.

A recent report in *Nature Biotechnology* describes the use of this plant model to monitor the genotoxic effect of the radiation fallout from the Chernobyl, Ukraine, accident (*2*), which occurred on 26 April 1986, and contaminated a large region of the former Soviet Union, as well as Scandinavia and Eastern Europe. Following the accident, numerous studies reported genotoxicity in humans, animals, and plants around Chernobyl. Around the reactor itself, a 600-km^2 zone has been closed to agriculture since the accident, because the average soil contamination is too high. This disaster, although very unfortunate, offers the opportunity to study the effect of ionizing radiation on living organisms and to compare data collected in a laboratory setting with data obtained from the field.

The authors collected soil from seven locations around Chernobyl in which they seeded *A. thaliana* in small pots in the laboratory. In parallel, they seeded plants on location in plots of 1 m^2. For each experiment at the different geographical sites, they planted 200 plants and repeated the experiments at each site six times. Soil samples were assayed for various isotopes and were found to contain ^{137}Cs, ^{90}Sr, ^{241}Am, as well as other radionuclides. The density of soil pollution in the soil samples ranged from 20 to 6000 Ci/km^2, with gamma dose rates of 97 and 8500 microroentgen per hour, respectively. The plants were harvested 5 weeks after germination and vacuum infiltrated for 15 min in staining buffer containing 5-bromo-4-chloro-3-indolyl glucuronide substrate. The plants were then incubated for 48 hours at 37°C before scores were assigned under the binocular scope.

Germination rates of the seeds decreased with increasing density of soil pollution, and there was a high degree of correlation between laboratory and open-field experiments. Moreover, an increase in soil contamination was linked to an increase in the average number of recombinations (up to five per plant) in both open-field and laboratory experiments. The number of recombinations peaked at a soil contamination of about 1000 Ci/km^2 and decreased at higher levels. The authors suggest that under higher radiation doses, the precision of the recombination process may decrease. Another explanation is that higher doses lead to more double-strand breaks that may be repaired more often through end-to-end joining (often referred to as illegitimate recombination). Cytological studies in other plants indicated a correlation between homologous recombination frequencies in *A. thaliana* and in the frequency of chromosomal bridges and fragments. Of note, the authors found that the dose-response curves they observed tended to be left-shifted for the field experiments compared with those for the laboratory experiments, that is, although the trends were identical, there was more damage in the field. They explain the differences by indicating that the plants in pots were surrounded by much less contaminated soil than the plants in the field.

This plant model has several advantages over animal-based models for studying the effects of environmental radiation: it is easy and sensitive, and reasonably fast, taking only 5 weeks before harvesting of plants. The method is also simple to perform—histochemical staining of the plant appears to be the most complicated step. Finally, the use of plants has economical and ethical advantages over the use of animals.

—RICHARD PETERS AND ROBERT SIKORSKI

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