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A hard sell for stem cells

Human stem cell studies have recently emerged as one of the most controversial areas of biomedical research. Not only are these cells derived from aborted fetuses or from the destruction of fertilized embryos, but their availability has also led to renewed concern about the possibility of human cloning. One of the main arguments advanced in favor of stem cell research is its potential to provide new therapies for otherwise intractable neurodegenerative diseases, and thus neuroscience should have a great deal to contribute to this debate. Given that strong feelings are involved on all sides—from anti-abortion activists to patient advocacy groups—it is important that the political and public discussion should be informed by a careful evaluation of both the potential and the limitations of neuronal stem cell therapy.

The stem cell controversy moved to center stage last November, when two groups reported the isolation of human embryonic stem cells (ES cells), in one case from embryos fertilized *in vitro*, in the other from the germ line of aborted fetuses. Both groups are associated with the California-based Geron Corporation, and a few days after their results were published, another biotechnology company, Advanced Cell Technology (ACT) in Massachusetts, raised the stakes still further by announcing the creation of hybrid embryos in which human nuclei had been transplanted into cow oocytes. It remains unclear how far such embryos can actually develop, but ACT claims to be pursuing the work in order to derive human ES cell lines while supposedly circumventing the ethical objections to the use of human embryos[1].

In mice, ES cells are known to be pluripotent—that is, capable of giving rise to all embryonic cell types, including germ line. It is likely that the same is true of human ES cells, although a definitive test (transplantation back into an embryo) would be illegal and unethical. It is also likely, although unproven, that ES cell lines can be created from adult human cells by transplanting a somatic cell nucleus into an enucleated egg and allowing it to develop to the blastocyst stage. This strategy, known as therapeutic cloning, has the potential to create donor cells of the same genotype as the recipient, thereby avoiding the problem of graft rejection.

The attraction of ES cells is that they can in principle give rise to any desired cell type. They cannot be transplanted directly into an adult organism, however, because rather than adopting a fate appropriate to the graft site, they tend to form teratomas. The ultimate goal of the field is to be able to generate isogenic ES cells that can be pushed in any desired developmental direction before transplantation; however, the methods for manipulating ES cells *in vitro* are still at a very early stage. An alternative strategy for transplantation, therefore, is to use multipotent rather than pluripotent stem cells. A multipotent stem cell has a more restricted capacity than a pluripotent cell, but is still is capable of self-renewal and differentiation into multiple cell types. Multipotent stem cells are thought to arise during the development of many organs, including the brain, but their existence is difficult to prove (it requires a complex series of cloning and differentiation assays), and in general there are no markers available to allow them to be identified prospectively, much less purified. Thus, although it seems very likely that self-renewing multipotential stem cells exist in the adult human brain, this has yet to be rigorously proved. For rodent brains, stem cells have been isolated that meet the definition—they can give rise to neurons, astrocytes and oligodendrocytes as well as to more stem cells—but it is not clear whether they can make all types of neurons. A plausible alternative is that there is no such thing as a universal neural stem cell, and that each might be committed with respect to brain structure, such that stem cells from (say) the hindbrain can give rise to hindbrain but not neocortical neurons.

If neuronal transplantation is to be a viable therapeutic strategy, the transplanted cells must be able to adopt fates and form synaptic connections that are appropriate to their new locations. So far, however, there is very little evidence that they can do either. There may be hundreds of neuronal cell types; the specific signals that trigger their differentiation are still largely unknown, but there is no reason to suppose that such signals must persist in the adult brain (except perhaps in a few areas, such as dentate gyrus or olfactory bulb, where neurons continue to be replenished throughout life). Even if persistent cues allow the right types of neurons to be formed in the right place, demonstrating that they can form appropriate connections and participate in a functional network will be very challenging.

Yet despite these uncertainties, clinical trials are already being conducted for a number of neurological conditions, including Parkinson's disease, Huntington's disease and even stroke. In most cases, the transplanted cells are heterogeneous populations of neural precursors from fetal brains, which are difficult to characterize but may not be directly comparable to purified stem cells. Nevertheless, the successes and limitations of these early studies illustrate the challenges that lie ahead if stem cells are to be useful for therapeutic purposes.

Consider Parkinson's disease (PD), often cited as the prototype for neural transplantation therapy. PD is caused by the

degeneration of dopaminergic neurons in the substantia nigra that project to the striatum, with the result that dopamine levels in the striatum are reduced. A number of animal studies, as well as a recent human clinical trial (which has been widely reported[2] but not yet published), have indicated that transplantation of embryonic dopaminergic neurons directly into the striatum can alleviate some of the symptoms of PD. Yet it is not obvious why this should work. The cells are being transplanted to an ectopic site, so it seems very unlikely they are restoring the spatiotemporal information that is normally conveyed by dopaminergic neurons. The transplanted cells may be releasing dopamine that can be taken up and re-released by the surviving nigrostriatal terminals, or they may simply be releasing trophic factors that enhance the survival of those terminals. Whatever the explanation, however, and whatever the clinical effects, it would be misleading to consider this to be restoration of a neural circuit, and as a general model for the feasibility of neural repair, these findings should be interpreted with great caution.

Similar caveats apply in the case of Huntington's disease, which involves degeneration of striatal GABAergic neurons. As with PD, there is evidence from animal models that some symptoms can be alleviated by transplantation of embryonic neuronal precursors. In one sense, the rationale seems more attractive than for PD, in that embryonic striatal neurons are being transplanted into normal rather than ectopic sites. However, the animal models (which involve excitotoxic lesions or systemic administration of a mitochondrial inhibitor) do not mimic either the cause or the detailed cellular pathology of the human disease, so any functional recovery is difficult to interpret. In particular, despite the presence of anatomical projections to and from the grafts, it is unclear exactly what causes the recovery, or whether the transplanted neurons integrate into normal functional circuits.

Perhaps the most dramatic example of the gap between basic science and clinical application is the use of teratocarcinoma cells as a putative therapy for stroke. Layton Bioscience, a California-based company, has begun a phase I trial of a radical procedure in which so-called 'clinical grade' neuronal cells, derived from a human teratocarcinoma, are transplanted into the brains of stroke patients. This is based on the finding that ischemic rats injected with these cells show recovery on several behavioral tasks[3,4]. Yet the reason for this recovery is unknown, the biology of the teratocarcinoma cells remains only poorly understood, and the prospect that they will lead to restoration of damaged circuits will strike many as fairly remote.

It seems clear that there is an urgent need for more basic research if the field is to progress beyond the level of clinical phenomenology. There are three main challenges. First, it will be necessary to learn much more about neuronal development, in order to define cell types that can be cultured in sufficient quantities and that can adopt appropriate fates when transplanted to different sites *in vivo*. This would free the field from the constraints associated with the need for large quantities of human fetal tissue. Second, it will be necessary to establish better animal models—perhaps including genetically modified primates—in order to perform more realistic tests of cognitive recovery after transplantation. Finally, it will be important to develop methods for testing whether transplanted neurons can become functionally integrated into brain circuitry, in other words, whether they can actually contribute to the restoration of normal information processing in the damaged brain. This last challenge may prove the most difficult, in that it will probably require the identification and electrophysiological characterization of transplanted neurons *in vivo*.

This research program will require the full arsenal of biological techniques, including stem cells. Much of it can and should be done in animals, but certain questions can only be answered by research on human cells. Opponents of human stem cell research—including, for instance, the former US surgeon general, C. Everett Koop—have argued that it is unnecessary to use pluripotent cells for these experiments, and that other cells with more restricted potential (whose derivation does not require the destruction of human embryos) will suffice. Most experts, however, would argue that the current state of knowledge is insufficient to support such a strong conclusion, and the NIH has taken the position that both avenues should be explored.

Obviously the decision whether to allow stem cell research to proceed is a political one, which will be made separately in each country. In the USA, for instance, the NIH is prohibited by law from funding research that involves the destruction of human embryos, but no such restrictions apply to the privately funded companies that have been responsible for much of the recent progress in the field. NIH has now concluded that it is free to support work on ES cells obtained from private or foreign sources, and it intends to do so as soon as appropriate guidelines have been established. A draft of these guidelines will be posted for public comment within the next few weeks; it is already clear that they will draw fierce criticism from the abortion opponents in Congress. In Britain, the government has taken a more cautious approach, deciding very recently to postpone any decision on whether the law should be changed to permit therapeutic cloning, pending further examination of the possible benefits and risks. Clearly the ethical qualms many people feel about interfering with human embryology will have to be weighed against the potential payoff, not just in neuroscience but also in many other areas. If neuroscientists are to participate effectively in this debate, however, it will be important to represent the field accurately, neither exaggerating the modest results that have been achieved so far nor underselling the enormous potential that may lie ahead. Most importantly, they must make it clear how little we know, and how much needs to be done before transplantation therapy becomes a routine option for the treatment of neurological disease.

As a final thought, the prospect of neural repair raises another ethical conundrum, one that has not yet been widely discussed. A person receiving a heterologous cell transplant is in some sense a chimera, and although this may not be a concern in the case of heart or pancreas, it surely raises some deep questions if one is considering the restoration of cognitive functions using neurons from another individual. A philosophical purist might even make the same argument for testing human neural stem cells in a monkey brain. As will be clear from the above discussion, the prospect of creating a chimeric mind is still fairly distant, but it is not absurd. At least it should give the next generation of bioethicists something to chew on.

1. Alper, J. *Science* **283**, 1432–1435 (1999).
2. Hints of success in fetal cell transplants. *New York Times*, April 22, 1999.
3. Borlongan, C. V. *et al. Exp. Neurol.* **149**, 310–321 (1998).
4. Borlongan, C. V. *et al. Neuroreport* **9**, 2837–2842 (1998).