TO THE EDITOR: Despite considerable effort over the past decade, no neuroprotective pharmacological therapy has been shown to be effective in attenuating the damage and disability associated with traumatic brain injury (TBI) in humans. Despite recent encouraging publications regarding transplantation of cultured human neuronal cells in patients with basal ganglia stroke and fixed motor deficits, surprisingly little attention has been given to the potential of cell transplant therapy to replace lost or damaged cells and provide potential protective and behavioral effects to the patient whose brain has been injured by traumatic insult. Traumatic injury to the adult brain is devastating, in part, because of the inability of central nervous system (CNS) neurons to regenerate and reform axonal and dendritic connections. Transplantation of progenitor cells that will become neurons or glial cells capable of providing a supportive environment for regeneration may be one novel strategy to promote recovery of function of the injured or damaged brain.

In this issue of Journal of Neurosurgery, Mahmood and colleagues (Mahmood A, Lu D, Li Y, et al: Intracranial bone marrow transplantation after traumatic brain injury improving functional outcome in adult rats. J. Neurosurg 94:589–595, April, 2001) tested the hypothesis that intracranial transplantation of bone marrow (BM) provides therapeutic benefit in rats subjected to experimental TBI. The authors describe a series of studies during which they harvested BM cells from the tibia and femur of normal adult rats, prelabeled these cells with bromodeoxyuridine (BrdU), and injected them into an area of cortex adjacent to the site of a traumatic injury, which had been induced by impacting the brain with a pneumatic piston. These cells appear to survive in the injured brain, proliferate, and even migrate toward the injury site. Phenotypic characterization revealed that the surviving cells had characteristics of both astrocytes and neurons. Perhaps even more striking was the observation of a statistically significant improvement in motor function in the animals receiving BM transplants at 14 and 28 days posttransplantation. These studies build on previous work by the same authors demonstrating that intrastriatal transplantation of BM nonhematopoietic cells improves functional recovery after embolic middle cerebral artery occlusion in adult mice. In this previous study, the authors used a modified neurological severity test score (including tests of motor, sensory, and reflex function) to demonstrate behavioral efficacy with their transplants. It is disappointing that similar tests were apparently not used in the present study, which would have provided more evidence for the neurobehavioral effects of these cells over the single rotarod test that was used. It is well known that tests such as the rotarod test can be greatly influenced by a number of variables, including body weight (gain or loss). Because these changes before and after brain injury and transplantation were not addressed in this paper, it is difficult to assess whether animals with weight loss (for example, brain-injured animals undergoing an inflammatory reaction to both injury and transplantation) may perform better than animals receiving vehicle (phosphate-buffered saline [PBS]) only. Although the authors state that they observed no evidence of secondary immune rejection, staining for markers of immunological or inflammatory response was not performed.

Several studies have previously reported the beneficial effects of fetal cell transplantation in experimental models of TBI. However, one limitation of fetal cell explants is that they represent a mixture of cell lines and, thus, it is difficult to tease out the specific properties of the cell type that appears responsible for the neuroprotective and behavioral effects of these mixed cell transplants. Unfortunately, many of these same issues concerning questions of purity, quantity, and quality of cell type(s) transplanted and responsible for the behavioral effects persist with the use of BM transplants. Further work with this cell system should include the characterization of the specific cell types that provide for the behavioral efficacy observed in the present study. Other more recent studies, not cited by Mahmood, et al., have demonstrated that transplants of cultured human neuronal cells can survive and integrate in the injured rodent brain for up to 4 weeks. It remains to be determined whether the transplantation strategy involving a single, isolated cell type (or progenitor) or progenitors of multiple cell phenotypes are the most efficacious in reducing cell damage and improving functional outcome.

In the paper by Mahmood, et al., the authors chose to compare their brain-injured BM–transplanted animals with brain-injured animals receiving injections of PBS vehicle. This is a somewhat risky strategy, because there is no control for simple mass effects of cellular transplantation. Future studies should likely include inert or nonfunctional cells as a control for any nonspecific behavioral effects due simply to the injection of a mass of cells into the injured brain.

It is clear from the paper by Mahmood, et al., that transplanted BM cells survive in the injured brain, migrate, and have the potential to differentiate into neuronal and glial cells. The data also indicate that this transplantation strategy can improve motor recovery following experimental TBI. These observations are clearly exciting and suggest that transplantation may be a potentially new and viable form of treatment following TBI. The authors speculate that one mechanism of action is the release of trophic or growth factors from the transplanted cells. Measurement of growth factors following brain injury and transplantation was not performed, and it is somewhat disappointing that the authors do not demonstrate/speculate as to why a trophic factor should improve function without a histological correlate (reduced cell loss associated with injury). Previous studies have reported beneficial effects of trophic factor infusion into the brain following experimental brain injury and several have even provided evidence to support a link between improved functional outcome and anatomical/histological effects (for example, reduced apoptotic cell death in septohippocampal cholinergic neur...
The mechanism of action of these BM cells as well as other transplanted stem cells underlying observable neurobehavioral improvements should remain a significant topic of interest for years to come.

Last, the authors chose to use an experimental model of TBI in the rat that involved two craniotomies (one in each hemisphere). Although the rationale presented for this choice (the second craniotomy "allows for movement of cortical tissue laterally") is biomechanically sound, confirmation by this and other laboratories by using more conventional and clinically relevant models of TBI should be undertaken before this strategy can make the important move to the clinic.

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References

RESPONSE: I thank Dr. McIntosh for his comments on our article. Our report describes the results of experiments undertaken to evaluate the efficacy of intracerebral transplantation of BM cells in improving outcome after TBI. The reasons for choosing this therapeutic strategy are well elucidated by Dr. McIntosh (the lack of effective pharmacological therapy to ameliorate the damage associated with TBI). This was the first report related to this project and since then we have modified our experimental design and protocol in light of the preliminary results. We have already implemented many of Dr. McIntosh’s recommendations regarding future studies.

Our data indicate that transplanted BM cells survive, migrate, and exhibit the phenotype of intrinsic parenchymal cells. Most importantly, there is a statistically significant functional improvement following transplantation. We reported rotarod test results and not the modified neurological severity test scores as reported in stroke studies, because at that time we used only the rotarod test in both TBI and ischemia studies. Modified neurological severity test scores were used in subsequent experiments. However, the rotarod test is a reliable one and has been successfully used in assessing deficits after TBI. We understand that its results can be influenced by changes in body weight, and although we did not report the weight of animals before and after injury, the animals were weighed daily and there was no weight loss following injury.

We stated in our report that we observed no secondary immune rejection and this was based on histological analysis of sections stained by hematoxylin and eosin. We agree that we did not utilize specific markers for immunological or inflammatory response, because this was a preliminary study and we did not want to extend the breadth of experiments. However, in a comparable study performed in a stroke model, we demonstrated no obvious increase in inflammatory response to BM cells in brain tissue.

We utilized BM cells instead of fetal cells as a source of stem cells because the former are easily available and there are no ethical problems in using them, in contrast to fetal tissue. We agree with Dr. McIntosh that in this report, we used complete BM and it is not obvious which component of BM is actually responsible for the observed benefits. This was a preliminary study and since then we have been using BM stromal cells and not complete BM. Bone marrow stromal cells are separated from the hematopoietic progenitor cells by their ability to adhere to plastic. These BM stromal cells can differentiate into osteoblasts, chondroblasts, adipocytes, and myoblasts. There is also evidence that BM stromal cells are precursors of brain cells in TBI.

Dr. McIntosh has also recommended using inert and nonfunctional cells as an additional set of controls, in addition to PBS. We have used fibroblasts as inert cells in ischemia studies and we observed no beneficial effect from their transplantation. We did not perform parallel studies in TBI because of limitation of resources and time.

We observed an improvement in functional outcome following BM transplantation, but the mechanisms responsible for this improvement are unclear at the present time. We speculated on the release of trophic and growth factors following transplantation. Quantification of the growth factors and a clear link between them and histological changes were not provided because this was a preliminary study and we primarily focused on evaluating the outcome following BM transplantation. However, in our stroke studies, we demonstrated that marrow cells implanted in brain evoke proliferation and migration of cells within the subventricular zone.

We used an experimental model of controlled cortical impact developed by Dixon, et al. Though they originally described the model with one craniotomy, they subsequently modified it to include two craniotomies to allow for lateral movement of brain and to have the experimental condition more resemble reality. This model of two craniotomies is also utilized by other investigators. In short, two craniotomies is not an innovation developed by