Response to Comment on "Failure of Bone Marrow Cells to Transdifferentiate into Neural Cells in Vivo"

Mezey *et al*. (*1*) question the validity of our conclusion that bone marrow cells (BMCs) cannot transdifferentiate into neural cells (*2*), because we did not observe large numbers of donor-derived microglia in the brains of mouse BMC transplant recipients. They suggest that the LacZ transgene may not have been expressed in the brains of these animals, which would preclude detection of donor-derived cells. However, as stated in (*2, 3*) we observed LacZ-positive, donor-derived cells in the central nervous system (CNS) of transplanted recipients. These cells were in perivascular locations and had a globular morphology that was not consistent with a neuronal phenotype. Furthermore, we readily detected LacZ-positive hematopoietic cells in the CNS (Fig. 1) when Rosa26 BMCs were transplanted systemically into mice after contusion injury of the spinal cord—a demonstration that our technique detects LacZ-positive cells in the CNS if they are present. Mezey *et al*. (*1*) present a figure showing absence of enhanced green fluorescent protein (EGFP) positive cells, but presence of Y chromosome–positive cells in the CNS as an example of down-regulation of a marker gene in the CNS. However, they did not consider the possibility that the Y chromosome staining may be artifactual and that the reason that no EGFP-positive cells were present was because there were no, or very few, donor-derived cells present.

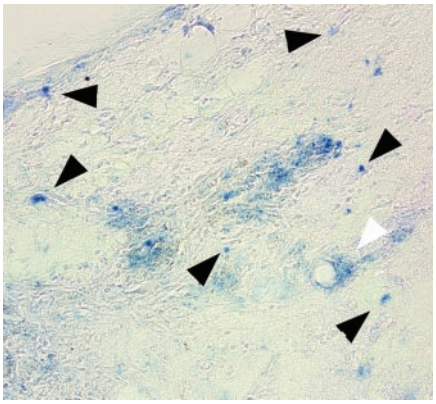

Fig. 1. LacZ-positive hematopoietic cells present in the mouse CNS. Rosa26 BMCs were transplanted by retro-orbital sinus injection into a wild-type mouse that had received a contusion injury to the spinal cord 10 days earlier. Seven days later, the mouse was perfused with saline and 4% paraformaldehyde. Tissue sections (50 μm) from the contusion site were stained for β-galactosidase (β-gal) activity by standard X-gal histochemistry. Many β-gal–positive cells were localized in the CNS parenchyma (black arrowheads) and some were associated with blood vessels (white arrowhead). This photomicrograph confirms that standard X-gal histochemistry readily detects the presence of Rosa26 cells in the CNS if they are present.

The number of donor-derived cells that we observed was not unreasonably low compared with the reports cited in (*1*), because there are significant differences between those experimental systems and our own. For example, Priller *et al*. (*4*) and Nakano *et al*. (*5*) stimulated BMCs with interleukins in vitro for 48 hours prior to transplantation to mark them with retroviral vectors. This may have selected for a subpopulation of cells that targeted the CNS more efficiently. Wagers *et al*. (*6*) provided no quantitative data on the number of hematopoietic cells in the brain and showed a microphotograph of only one CD45-positive cell in the brain. Mezey *et al*. seem to have overstated the number of donor-derived microglia that should be present in the brains of BMC transplant recipients. Based upon the only cited report that provides quantitative data (*4*), we calculate that at 15 weeks, an average of 280 donor-derived microglia would be detected per brain—not 500 to 1000 per section, as stated in (*1*). Finally, it is hard to compare our data with that of Flügel *et al*. (*7*), who used fetal liver cells to reconstitute the hematopoietic system of rats with experimental autoimmune encephalitis and facial nerve axotomy.

We agree with Mezey *et al.* that research to test whether BMCs are able to reconstitute non-hematopoietic tissues should continue. We suggest that proponents of bone-to-brain transdifferentiation unequivocally demonstrate that BMCs can change into neural cells by isolating these cells from the CNS of transplant recipients and demonstrating in vitro that they function as authentic neural cells.

Raymond F. Castro
Department of Pediatrics
Baylor College of Medicine
Houston, TX 77030, USA

Kathyjo A. Jackson
Department of Pediatrics
and *Center for Cell and Gene Therapy*
Baylor College of Medicine

Margaret A. Goodell
Departments of Pediatrics, Immunology
and *Center for Cell and Gene Therapy*
Baylor College of Medicine

Claudia S. Robertson
Hao Liu
Department of Neurosurgery
Baylor College of Medicine

H. David Shine
Departments of Neurosurgery, Neuroscience, Molecular and Cellular Biology
and *Center for Cell and Gene Therapy*
Baylor College of Medicine
E-mail: hshine@bcm.tmc.edu

References

1. E. Mezey *et al.*, *Science* **299**, 1184 (2003); www.sciencemag.org/cgi/content/full/299/5610/1184b.
2. R. F. Castro *et al.*, *Science* **297**, 1299 (2002).
3. R. F. Castro *et al.*, *Science* **298**, 362 (2002).
4. J. Priller *et al.*, *Nature Med.* **7**, 1356 (2001).
5. K. Nakano, M. Migita, H. Mochizuki, T. Shimada, *Transplantation* **71**, 1735 (2001).
6. A. J. Wagers, R. I. Sherwood, J. L. Christensen, I. L. Weissman, *Science* **297**, 2256 (2002).
7. A. Flügel, M. Bradl, G. W. Kreutzberg, M. B. Graeber, *J. Neurosci. Res.* **66**, 74 (2001).

19 November 2002; accepted 7 January 2003

Response to Comment on "Failure of Bone Marrow Cells to Transdifferentiate into Neural Cells in Vivo"

Raymond F. Castro, Kathyjo A. Jackson, Margaret A. Goodell, Claudia S. Robertson, Hao Liu and H. David Shine

Science **299** (5610), 1184.
DOI: 10.1126/science.1080631