

Do Bone Marrow Cells Generate Neurons?

David C. Hess, MD; William D. Hill, PhD; James E. Carroll, MD; Cesar V. Borlongan, PhD

In the past 5 years, accumulating evidence has demonstrated plasticity of bone marrow–derived cells. Bone marrow–derived cells display the capacity to change their fate, differentiating into hepatocytes, endothelial cells, muscle cells, and cardiomyocytes, and even neurons.¹ The findings that bone marrow cells differentiate into neurons in vitro and in vivo challenge previous assumptions that tissue-specific stem cells give rise only to cells of their organ of origin and do not cross lineages.

Bone marrow contains a heterogeneous population of stem and progenitor cells. Two of the best defined are the hematopoietic stem cells and the bone marrow stromal cells or mesenchymal stem cells.¹ There have been a number of reports of in vitro differentiation of bone marrow stromal cells into neurons on exposure to various inducing regimens.²

Co-isolating with mesenchymal stem cells is the multipotent adult progenitor cell, a rare cell in the bone marrow that expands and exhibits remarkable plasticity in culture.³ Multipotent adult progenitor cells differentiate into endothelial cells, hepatocytes, and neurons in vitro and, after injection into a blastocyst, differentiate into cells from all germ layers including neurons.³ While originally isolated from bone marrow, multipotent adult progenitor cells have now been cultured from muscle and brain.⁴ It is not clear, however, whether the multipotent adult progenitor cell is an artifact of cell culture or whether it is a rare multipotent stem cell in vivo.

DIFFERENTIATION OF BONE MARROW CELLS INTO NEURONS: EVIDENCE FROM ANIMAL STUDIES

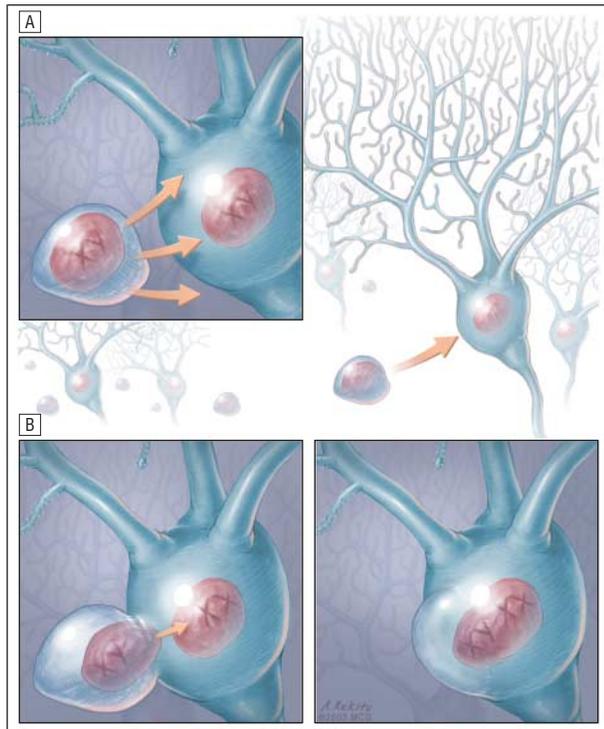
The common method for determining the fate of bone marrow cells involves trans-

planting genetically marked bone marrow in mice that have received lethal irradiation. This produces a “radiation chimera,” and the fate of the bone marrow cells is tracked over time by detecting the genetic tag in histologic sections. This tagging is often in the form of sex mismatch, in which male Y chromosome marrow is transplanted into female recipients or in which the donor’s bone marrow is genetically tagged, usually with lacZ or green fluorescent protein (GFP).

Using a radiation chimera model in which adult mice were subjected to lethal irradiation and then transplanted with marrow from mice that ubiquitously expressed GFP, Brazelton and colleagues⁵ reported, after flow cytometric analysis of the dissociated brain, that 20% of the cells did not express hematopoietic markers. Confocal microscopy demonstrated that individual cells in histologic sections coexpressed GFP and the neuron-specific proteins, NeuN, the 200-kDa isoform of neurofilament (NF-H), or class IIIB tubulin. Individual cells coexpressed not only GFP and NeuN but also pCREB (phosphorylated cyclic adenosine monophosphate–responsive element binding protein), a transcription factor activated by phosphorylation. The olfactory bulb, being an active site of neurogenesis, was selected for quantification; 0.2% to 0.3% of the neurons were bone marrow derived by 8 to 12 weeks after transplantation.

Mezey and colleagues⁶ transplanted Y chromosome marrow without irradiation

From the Departments of Neurology (Drs Hess, Hill, Carroll, and Borlongan) and Cell Biology and Anatomy (Dr Hill), and Institute of Molecular Medicine and Genetics (Drs Hess and Borlongan), Medical College of Georgia, and Veterans Affairs Medical Research Service (Drs Hess, Hill, and Borlongan), Augusta.



Generation of Purkinje cells from a bone marrow–derived cell may involve differentiation from bone marrow cell (A) or cell fusion (B).

tion of the recipient by using mice homozygous for a mutation in the PU.1 gene as recipients. These mice lack hematopoietic cells at birth and die within 48 hours unless they receive a bone marrow transplant. One to 4 months after transplantation, Y chromosome was present in 0.3% to 2.3% of NeuN immunoreactive nuclei. Confocal microscopy confirmed the colocalization of NeuN and the Y chromosome. Double-labeled cells were found in the cortex, hypothalamus, hippocampus, amygdala, periaqueductal gray matter, and striatum but absent from the spinal cord and brainstem.

An even more surprising finding was the generation of Purkinje cells from bone marrow. Priller and colleagues⁷ transplanted bone marrow cells retrovirally transduced with GFP into irradiated mice and found that after 12 to 15 months, 0.1% of Purkinje cells in the cerebellum were labeled with GFP. The GFP expression was detected in the perikaryon, axon, and dendritic branches. There was evidence of multiple synaptic contacts, and cells expressed calbindin-D28K and glutamic acid decarboxylase, strongly suggesting they were functional. Similar GFP-expressing Purkinje cells were detected in mice transplanted with bone marrow from mice that ubiquitously expressed GFP after 10 to 12 months, excluding the possibility that recombinant retrovirus was taken up by the Purkinje cells.

However, others have found little or no evidence of bone marrow–derived neurons *in vivo* in mice. In one study that failed to detect bone marrow–derived neurons even after a traumatic injury,⁸ lacZ was used to mark the bone marrow cells, leading to questions of sensitivity of lacZ.⁹ However, another study that transplanted GFP-marked bone marrow did not detect any bone mar-

row–derived neurons in uninjured brain and in brain mechanically injured.¹⁰

One of the limitations of the studies demonstrating bone marrow plasticity is the heterogeneity of bone marrow cells transplanted. As noted above, crude or unfractionated bone marrow contains hematopoietic stem cells and mesenchymal stem cells; they may also contain endothelial stem cells (angioblasts) or even neural and other progenitor cells. To establish the gold standard of differentiation of a hematopoietic cell to a nonhematopoietic cell, it is imperative to demonstrate that a single cell or clone of that single cell (clonal analysis) differentiates into functioning neurons. In the only study that has transplanted pure hematopoietic stem cells and tracked cells in the brain, a single Purkinje cell was seen in uninjured brain, indicating that the hematopoietic stem cell or its progeny are able to give rise to Purkinje cells.¹¹ To date, there is no evidence that bone marrow–derived cells expressing neuronal proteins possess the electrophysiologic characteristics of neurons; the rarity of these cells may pose a limiting factor for recording from these cells in cortical slices.

EVIDENCE OF BONE MARROW–DERIVED NEURONS IN HUMANS?

Sex-mismatched bone marrow transplants in which bone marrow derived from a male donor was transplanted into a female recipient affords an opportunity to track the fate of bone marrow cells in human brain. In 4 females with survival of 2 to 10 months, cells double labeled for Y chromosome and a neuronal marker (either NeuN or Kv2.1, a neuron-specific voltage-gated potassium channel) were noted, mostly in neocortex and hippocampus.¹² Estimates of the number of bone marrow–derived neurons were 0.025% to 0.05% in this short period.

Similar evidence has been found for bone marrow cells contributing to Purkinje cells in human brain.¹³ In female patients who received bone marrow transplants from males, 4 Purkinje cells were seen with Y chromosomes, indicating that these were derived from the male bone marrow. A total of 5860 Purkinje cells were examined, of which 4 showed a Y chromosome, indicating that bone marrow contributed to 0.1% of Purkinje cells. Interestingly, 2 Purkinje cells were seen that contained more than a diploid number of sex chromosomes, suggesting that cell fusion had occurred. Since the sections examined were 10 μm thick and a Purkinje cell nucleus is much larger than this, it is highly possible that sex chromosomes distributed in a large nucleus can be missed when thin sections are examined. Two possible mechanisms exist for appearance of bone marrow–derived Purkinje cells: either a change in cell fate or the fusion of bone marrow cells with host Purkinje cells (**Figure**). Since the period after transplantation was so short (3–15 months) and the Y chromosome containing Purkinje cells appeared to have full dendritic branching, cell fusion may be the most plausible mechanism.

FUSION

The first reports that cell fusion might be responsible for the apparent plasticity of bone marrow cells came from

in vitro studies in which bone marrow cells were cocultured with embryonic stem cells. Terada and colleagues¹⁴ cultured GFP-expressing bone marrow-derived cells with murine embryonic stem cells and found that, under selective pressure, GFP-expressing cells that developed the characteristics of embryonic stem cells (differentiated into cardiomyocytes in vitro and also formed teratomas after injection into nonobese diabetic-severe combined immunodeficiency mice) had double the amount of DNA, suggesting that the bone marrow cells had fused with the embryonic stem cells. These cell fusion events, however, were rare (1 in 10⁵), and a population of cells enriched for stem cell antigen 1-positive, lineage-negative cells did not show these hybrid events, making it unlikely that hematopoietic stem cells were involved in these fusion events, but that perhaps a more differentiated bone marrow cell such as a monocyte was involved.

Cell fusion has also been shown in vivo. In a murine model of tyrosinemia type I with mutations in the fumarylacetoacetate hydrolase gene (*Fah*^{-/-}), transplanted *Fah*^{+/+} bone marrow cells fused with recipient hepatocytes, expressed a hepatocyte gene profile and the wild-type *Fah* gene, and corrected the metabolic disorder.¹⁵ This observation demonstrates that these fused cells are functional and serve to rescue impaired recipient cells. However, while cell fusion may indeed account for some of the “plasticity” of bone marrow cells, particularly in the liver, it does not seem to account for all reports of bone marrow “plasticity.”¹

CONCLUSIONS

There is evidence in mice and humans that bone marrow cells contribute to neurons. However, most of this work has relied on immunocytochemistry, and there is concern over the specificity of the markers used. Still lacking is electrophysiologic evidence that the cells generated in vivo have the functional characteristics of neurons. There is strong evidence in mice and humans that bone marrow cells either fuse with or generate Purkinje cells. Cell fusion itself is a remarkable phenomenon, as evidence from the liver suggests that this mechanism may be important in repair and correction of a metabolic disorder. It is possible that bone marrow cells, either by direct generation or by cell fusion, could play a role in repair of central nervous system damage.

Since the acceptance of this manuscript, additional evidence has been published demonstrating that bone marrow cells fuse with Purkinje cells in the mouse.^{16,17}

Accepted for publication November 4, 2003.

Author contributions: Study concept and design (Drs Hess, Hill, and Carroll); analysis and interpretation of data (Drs Hill and Borlongan); drafting of the manuscript (Dr Hess); critical revision of the manuscript for important in-

tellectual content (Drs Hess, Hill, Carroll, and Borlongan); obtained funding (Dr Hess); administrative, technical, and material support (Drs Hill and Borlongan); study supervision (Drs Hess and Hill).

This work was supported by the Veterans Affairs Medical Research Service, Washington, DC, and grants R21 NS 43487-01 and R21 NS43439-01 from the National Institute of Neurological Disorders and Stroke, Bethesda, Md.

We acknowledge Andy Rekito, MS, Departments of Neurosurgery and Neurology, Medical College of Georgia, who composed the Figure.

Corresponding author: David C. Hess, MD, Department of Neurology, Medical College of Georgia, 112015th St, Augusta, GA 30912 (e-mail: dhess@mail.mcg.edu).

REFERENCES

- Herzog EL, Chai L, Krause DS. Plasticity of marrow-derived stem cells. *Blood*. 2003;102:3483-3493.
- Munoz-Elias G, Woodbury D, Black IB. Marrow stromal cells, mitosis, and neuronal differentiation: stem cell and precursor function. *Stem Cells*. 2003;21:437-448.
- Jiang Y, Jahagirdar BN, Reinhardt RL, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature*. 2002;418:41-49.
- Jiang Y, Vaessen B, Lenvik T, Blackstad M, Reyes M, Verfaillie CM. Multipotent progenitor cells can be isolated from postnatal murine bone marrow, muscle, and brain. *Exp Hematol*. 2002;30:896-904.
- Brazelton TR, Rossi FM, Keshet GI, Blau HM. From marrow to brain: expression of neuronal phenotypes in adult mice. *Science*. 2000;290:1775-1779.
- Mezey E, Chandross KJ, Harta G, Maki RA, McKecher SR. Turning blood into brain: cells bearing neuronal antigens generated in vivo from bone marrow. *Science*. 2000;290:1779-1782.
- Priller J, Persons DA, Klett FF, Kempermann G, Kreutzberg GW, Dirnagl U. Neogenesis of cerebellar Purkinje neurons from gene-marked bone marrow cells in vivo. *J Cell Biol*. 2001;155:733-738.
- Castro RF, Jackson KA, Goodell MA, Robertson CS, Liu H, Shine HD. Failure of bone marrow cells to transdifferentiate into neural cells in vivo. *Science*. 2002; 297:1299.
- Blau H, Brazelton T, Keshet G, Rossi F. Something in the eye of the beholder. *Science*. 2002;298:361-362.
- Vallieres L, Sawchenko PE. Bone marrow-derived cells that populate the adult mouse brain preserve their hematopoietic identity. *J Neurosci*. 2003;23:5197-5207.
- Wagers AJ, Sherwood RI, Christensen JL, Weissman IL. Little evidence for developmental plasticity of adult hematopoietic stem cells. *Science*. 2002;297: 2256-2259.
- Mezey E, Key S, Vogelsang G, Szalayova I, Lange GD, Crain B. Transplanted bone marrow generates new neurons in human brains. *Proc Natl Acad Sci U S A*. 2003; 100:1364-1369.
- Weimann JM, Charlton CA, Brazelton TR, Hackman RC, Blau HM. Contribution of transplanted bone marrow cells to Purkinje neurons in human adult brains. *Proc Natl Acad Sci U S A*. 2003;100:2088-2093.
- Terada N, Hamazaki T, Oka M, et al. Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. *Nature*. 2002;416:542-545.
- Vassilopoulos G, Wang PR, Russell DW. Transplanted bone marrow regenerates liver by cell fusion. *Nature*. 2003;422:901-904.
- Weimann JM, Johansson CB, Trejo A, Blau HM. Stable reprogrammed heterokaryons form spontaneously in Purkinje neurons after bone marrow transplant. *Nat Cell Biol*. 2003;5:959-566.
- Alvarez-Dolado M, Pardoll R, Garcia-Verdugo JM, et al. Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. *Nature*. 2003;425:968-973.