DEVELOPMENT

Failure of Bone Marrow Cells to Transdifferentiate into Neural Cells in Vivo

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Reports have suggested that adult mouse bone marrow cells (BMCs) are capable of transdifferentiating into cells with neural characteristics in the central nervous system (CNS) (*1*, *2*). Because side-population (SP) cells within whole bone marrow are hematopoietic stem cells that can reconstitute the BMC population and are capable of differentiating into other types of cells such as cardiac myocytes and endothelial cells (*3*–*5*), we surmised that they too would transdifferentiate into neural cells.

To test this, 8 C57Bl/6 (B6) mice were treated with a lethal dose of irradiation and transplanted with 2×10^3 SP cells derived from the Rosa26 mouse. The Rosa26 mouse carries the LacZ gene that is constitutively expressed in most cells including neural and SP cells and is an unambiguous marker for donor-derived cells (*6*). Ten to 12 weeks after transplantation, 80 to 95% of the recipient blood cells were LacZ positive. Four months after transplantation, the CNS of two of the recipient mice were inspected for cells derived from the Rosa26 donor with standard X-gal cytohistochemistry. In coronal sections (50 to 100 sections per brain representing more than 10^6 cells per brain) taken throughout the full extent of the brain, including the olfactory bulbs and cervical spinal cord, the only β-galactosidase (β-Gal)–positive cells detected were a few cells (<5) that were associated with blood vessels. These β-Gal–positive cells had a globular morphology and no processes that would suggest that they were neural cells. Their close association with blood vessels and the lack of morphological features of neural cells suggested that they were hematopoietic. The brains from Rosa26 control mice had robust β-Gal staining in neural as well as blood cells.

Because injury increases SP transdifferentiation in other tissue systems (*5*), we tested whether neural injury would cause donor cells to transdifferentiate into neural-like cells. Four mice with SP transplants underwent cortical stab injury, and two underwent cortical contusion injury (*7*) 4 months after the transplant. Reconstitution of the hematopoietic system is considered stable and complete around 4 months after BMC or SP transplantation. Therefore, cells capable of transdifferentiation should be in place at that time. One month after cortical stab injury and 4 months after contusion injury, no β-Gal–positive cells were observed in brains (fig. S1) or cervical spinal cords except a rare few associated with blood vessels. We concluded that adult bone marrow SP cells or cells derived from them were incapable of transdifferentiating into neural cells in this experimental system.

Because SP cells will reconstitute the BMC population in irradiated mice (*4*), we tested whether unfractionated BMCs would transdifferentiate into neural-type cells in our system. We transplanted Rosa26 BMCs (2×10^6 cells) into 12 irradiated B6 mice. Donor cell engraftment in the blood was 80 to 90% at 10 weeks after transplantation. To test whether injury would cause cells derived from the transplanted BMCs to transdifferentiate into neural-type cells, we injured 7 of the 12 recipients with a cortical stab lesion 4 months after transplantation. At 1, 2, and 5 months after injury, the recipient brains and cervical spinal cords were analyzed for donor-derived cells with standard X-gal cytohistochemical methods. In 50 to 100 coronal sections from each animal, none of the recipients had Rosa26 donor-derived neural-like cells in the brains and cervical spinal cords whether or not they had been injured.

To confirm that transdifferentiation was possible in other tissues in our experimental system, we injected cardiotoxin into the tibialis anterior muscle of 10 recipients of Rosa26 BMC and 10 recipients of Rosa26 SP cells. Four weeks after injury, the tibialis anterior muscles of the recipients were analyzed with standard X-gal cytohistochemistry. In both the BMC and SP recipients, we observed β-Gal–positive cells in the tibialis anterior muscle indicating that the BMC and SP cells had contributed to the muscle tissue.

Hence, exhaustive analysis of a total of 8 mice that received SP transplants and 12 that received BMC transplants revealed no neural-like cells in the CNS that could have been derived from ROSA26 BMCs (Table 1). These data suggest that "bone to brain" transdifferentiation may not be a general phenomenon but may depend on the experimental system in which the hypothesis is tested.

Table 1. Summary of experimental manipulations and results. ND, not done.

Type of cells transplanted	Type and number of mice receiving injury to the brain			Brains with donor-derived neural cells/brains analyzed
	Contusion injury	Stab injury	No injury	
SP cells	2	4	2	0/8*
BMCs	ND	7	5	0/12*

*50 to 100 coronal sections containing more than 10^6 neural cells were analyzed per brain.

References and Notes

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Supporting Online Material

www.sciencemag.org/cgi/content/full/297/5585/1299/DC1
Material and Methods
Fig. S1

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