Have the bloody cells gone to our heads?

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Recent studies have shown that cells expressing neuronal antigens can be derived from a bone marrow transplant. A new report lends support to and extends these previous results by presenting compelling morphological evidence for the generation and integration of highly differentiated bone marrow–derived neurons.

Stem cells in adult mammals have lately started showing off some unexpected talents. Stem cells from several adult organs, such as brain and bone marrow, seem to ignore cell lineage restrictions and may not be as rigid in their fate choices as we previously thought. Several studies have shown that stem cells feature a previously unknown plasticity to adapt to the microenvironment regardless of germ layer origin (Bjornson et al., 1999; Clarke et al., 2000; Clarke and Frisén., 2001; Krause et al., 2001; Morrison, 2001).

Bone marrow off the beaten path

Over the last few years, several groups have presented surprising and sometimes challenging results on the ability of adult bone marrow cells to adopt different cell fates. These results defy our classical view of a linear relationship between stem cells and the cell progenitors and mature cells to which they give rise. Several reports have presented evidence for bone marrow cells contributing to the generation of hepatic cells (Petersen et al., 1999; Alison et al., 2000; Lagasse et al., 2000; Theise et al., 2000), to skeletal muscle myocytes (Ferrari et al., 1998; Gussoni et al., 1999), and to myocardium (Jackson et al., 2001; Orlic et al., 2001a,b). However, the bone marrow harbors a multitude of cell types, including at least two distinct stem cell lineages: hematopoietic and mesenchymal stem cells (Morrison et al., 1995; Prockop, 1997). In only a few of the above studies, prospectively identified stem cells have been isolated and studied (Lagasse et al., 2000; Orlic et al., 2001a), whereas in the majority of these studies unfractionated whole bone marrow was used. A recent study further pushed the boundaries of conventional wisdom by showing that a single hematopoietic stem cell could differentiate into epithelial cells of liver, skin, lung, and gastrointestinal tract and convincingly does so to a significant degree (Krause et al., 2001). Current evidence thus suggests that stem cells residing in the adult bone marrow are capable of adopting cell fates outside of the expected hematopoietic and mesenchymal lineages.

Brainy marrow

The main cell types of the central nervous system are neurons, astrocytes, oligodendrocytes, and microglia. Whereas microglia derive from hematopoietic stem cells and are specialized macrophages that populate the nervous system during development, the other cell types constitute the neural lineage, which derives from the ectoderm. A study by Eglitis and Mezey (1997) pioneered the field of bone marrow–derived cells contributing to the neural lineage by studying adult mice that had been injected systemically with bone marrow cells. This study presented evidence for graft-derived cells turning into astrocytes by virtue of their GFAP expression. The total amount of bone marrow–derived cells increased with time (500 cells/brain after 3 d and up to 10,000 after 4 wk), but still only 10% of these cells could be identified as astrocytes or microglia. This study did not address whether bone marrow cells could generate neurons, although the early time points of analysis that were chosen probably would not reveal such events. A large fraction of the bone marrow–derived cells could be found in the ependymal layer of the ventricle walls and in the subventricular zone, regions that accommodate neural stem cells in the adult (Doetsch et al., 1999; Johansson et al., 1999), but one can only speculate as to the importance of this interaction.

A new effort was undertaken to study phenotypic conversions of bone marrow cells, and this time preliminary evidence pointed to neurons (Mezey and Chandross, 2000). Mice homozygous for a mutation in the PU.1 gene were irradiated and used as bone marrow recipients. When analyzing the brains of these mice after transplantation, unexpectedly, 5% of the total amount of brain cells and 1–2% of all cells expressing the neuronal marker NeuN were derived from the grafted bone marrow. However amazing and hard to grasp, these results have been corroborated by two more thorough studies (Brazelton et al., 2000; Mezey et al., 2000). Mezey and colleagues found that 2.3–4.6% of the transplant recipients brains were bone marrow–derived, and that 0.3–2.3% of these cells expressed neuronal markers (NeuN or neuron-specific enolase) after grafting. Most of the presumptive bone marrow–derived neurons were found in the cerebral cortex, and they were also present in the hypothalamus, hippocampus, amygdala, periaqueductal gray, and striatum.

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The study by Helen Blau's group reached the same conclusion and reported that ∼0.2–0.3% of the total number of neurons in the olfactory bulb were derived from the bone marrow by 8–12 wk after transplantation. In the latter study, three neuronal markers were used, NeuN, type III β-tubulin, and neurofilament, as well as the phosphorylated form of CREB as support for a neuronal identity of the cells.

The bloody neurons show their true colors

In a study in this issue, Priller et al. (2001) injected unsorted bone marrow cells into lethally irradiated adult mice and searched for brain integration at different time points. To trace the transplanted cells and their progeny, they were prelabeled with a retrovirus-expressing eGFP or isolated from a mouse line ubiquitously expressing eGFP. The genetically labeled cells could be identified and localized in the brain as early as 2 wk after intravenous injection and the number of cells colonizing different brain regions increased with time in accordance with previous studies. However, in sharp contrast to the studies mentioned above, the authors found that up to 4 mo posttransplantation all graft-derived cells in the brain expressed monocyte/macrophage-specific markers and that no cells displayed astrocyte or neuronal characteristics. On rare occasions, Priller et al. (2001) found eGFP+ cells in the olfactory bulb that coexpressed the neuronal marker NeuN, but these cells lacked neuronal morphology.

In spite of these somewhat discouraging findings, Priller and colleagues went on to analyze other mice at much longer survival times after the bone marrow transplantation. Astonishingly, they then found highly differentiated bone marrow–derived neurons in the cerebellum. These neurons had the typical morphology and marker profile of Purkinje cells. At 12–15 mo after transplantation, up to 0.1% of the Purkinje cells derived from bone marrow cells by virtue of their eGFP expression. This neuronal type is characterized by its extremely elaborate processes, and the generation of one of the more complex neuronal morphologies by a bone marrow–derived cell is a true tour de force. Although there is a continuous generation of neurons in certain parts of the adult mammalian brain, the genesis of this neuronal type has never been described in adulthood. However, the methods used to study adult neurogenesis would be unlikely to detect such a rare event. Interestingly, adult-generated neurons are typically interneurons with short processes, which may be related to the great challenge it must be to grow and extend long processes to a distant target within the adult brain. In light of this challenge, it is truly astonishing that Purkinje cells, with their long processes, can be generated in adulthood. The authors also provide ultrastructural evidence for synaptic integration of the bone marrow–derived Purkinje cells, suggesting that they may become functionally integrated in the circuitry of the adult brain.

How many neurons derive from the bone marrow?

The new study by Priller et al. (2001) supports recent findings of cells from bone marrow being capable of entering brain and differentiating to neurons. However, some of the new data appear contradictory to the previous reports. Whereas the previous studies indicated that a very substantial number of neurons were generated from bone marrow–

derived cells within a short time, the new report points to a much smaller number of neurons and at very late time points after the transplantation.

How can these data then be reconciled? Differences in the experimental design between the studies may, at least in part, account for the discrepancies. Another possible explanation, supported by data from the new report, is that the bone marrow–derived cells, shortly after the transplantation, may start differentiating toward a neuronal phenotype but die before reaching full maturation, perhaps due to limited functional integration and trophic support.

It is also important to consider that the conclusions of the previous studies have rested on the detection of neuron-specific antigens in bone marrow–derived cells. Some of these markers may be less reliable than we previously thought and can occasionally be found on cells that lack other neuronal features, such as for example synapses, typical electrophysio-
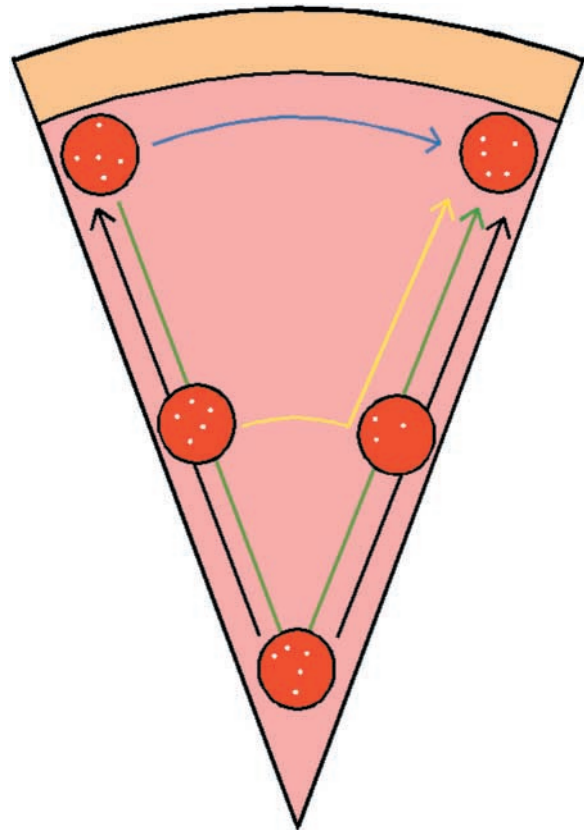

Figure 1. **Going to the side or back and forward?** We use the shape of a pizza slice to illustrate how cells may switch lineage. In this slice the totipotent zygote is represented at the tip and a more differentiated phenotype is placed toward the crust following two different lineages. The classical view of differentiation/lineage restriction involves the totipotent cell moving forward along a single lineage to reach the crust, as indicated by the black arrows. Dedifferentiation is defined as a reversal of this process toward the tip of the slice, representing acquisition of a more potent and unrestricted phenotype. This is then followed by differentiation to another lineage (green arrow). Transdifferentiation, illustrated by the blue arrow, is when a differentiated cell takes on another differentiated phenotype. The yellow arrow outlines transcommitment, which means that a stem or progenitor cell which is committed to a certain lineage switches to another lineage and then generates differentiated progeny.

logical properties or a mature neuronal morphology. Thus, the expression of certain proteins does not necessarily reflect a functional conversion to a mature neuronal phenotype. Another possibility, which has not yet been fully ruled out, is that localization of neuronal markers to bone marrow–derived cells could be a result of bone marrow–derived cells phagocytosing neurons (which may be dying due to the irradiation preceding the transplantation), and in that way transiently acquire neuronal proteins.

The present work does support the idea that a subpopulation of bone marrow cells actually can generate new neurons by providing persuasive morphological evidence. This means that at least some bone marrow–derived cells that are identified as neurons by the expression of a few neuronal proteins indeed exhibit all the morphological characteristics of a mature neuron, such as an axon, dendrites, and synapses, giving very strong support to the concept of bone marrow–derived neurons.

How and why?

There are several outstanding issues that will be important to address to understand the unexpected versatility of bone marrow cells. First, the identity of the cells bearing this capability for lineage crossover amongst the heterogeneous bone marrow population has to be established. The hematopoietic stem cell may be the prime suspect. These cells are in constant flux from the bone marrow and they are especially abundant in brain. Moreover, neural stem cells can reconstitute the hematopoietic system, perhaps indicating the possibility of bilateral interchange between the neural and hematopoietic lineages. Marrow stromal cells, on the other hand, may express some neuronal markers in vitro, and are thus another strong candidate.

Second, do bone marrow cells generate neurons under physiological conditions, or is this a result of the contrived experimental situations that have been used to study and establish this phenomenon? In all these studies the bone marrow transplant recipient mice have been compromised either by irradiation or by a genetic defect, which is necessary for the bone marrow transplant to take. Methods to label endogenous bone marrow cells in a minimally traumatic way will be necessary to elucidate whether this cellular exchange is an innate physiological process.

Third, what is the functional significance of the process? It is difficult to imagine that the exchange of 0.1% of Purkinje cells may have a discernable impact on brain function. However, the much higher numbers indicated by the previous studies may no doubt have a significant impact. Loss-of-function experiments will be needed to establish the physiological role and to answer whether the ablation of this process leads to any functional deficit.

The cell biology of lineage infidelity

How can we explain the phenomenon of cells crossing what were thought to be impenetrable lineage boundaries? There are a few conceptually different ways one may envisage how a cell may switch lineage (Fig. 1). First, one possibility is that mammalian cells under extraordinary conditions can, as seen in amphibians, dedifferentiate. This would mean a liberation from the epigenetic program that keeps them restricted,

allowing them to go back to a less differentiated state and then allowing them to differentiate again along another lineage. The cloning of adult mammals by nuclear transfer demonstrates that the differentiated state is reversible. However, it appears a much larger challenge to induce reprogramming with extra cellular stimuli compared with changing the intracellular milieu, as in nuclear transfer experiments.

A second possible route for a cell to switch lineage is by a differentiated cell directly jumping to a different cell lineage without cell division by directly establishing a new transcriptional program. This event is called transdifferentiation, and occurs in response to injury in amphibians (Brockes, 1997) and can be reconstituted artificially in mammalian heterokaryons (Baron and Maniatis, 1986; Pavlath and Blau, 1986).

A third possibility is that a committed stem or progenitor cell, in a new environment and influenced by local cues, takes on the identity of a stem or progenitor cell of that tissue. This would not, strictly speaking, be a case of transdifferentiation, which requires the cell swapping phenotype to be differentiated. A stem or progenitor cell switching lineage could perhaps be thought of as transcommitment. In this model, stem cells in different tissues would be able to adapt to a new niche in an unrelated tissue and generate the type of differentiated cells appropriate for the new context.

The question of how the differentiated state is maintained and reversed is a key question in biology today, the elucidation of which promises to bring insight into physiology and may teach us how to control cell differentiation in therapeutic situations.

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