Further proof of the plasticity of adult stem cells and their role in tissue repair

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In this issue, De Bari et al. (2003) present elegant data to counter the recent claims that adult stem cells have a limited plasticity. Further, they provide evidence that adult stem cells can seek out damaged tissues and repair them.

De Bari et al. (2001) previously demonstrated that mesenchymal stem cells from human synovial membranes (hSM-MSCs)* are similar to the adult stem cells originally isolated from bone marrow and referred to as mesenchymal stem cells or marrow stromal cells (MSCs) (Friedenstein et al., 1976; Caplan, 1991; Prockop, 1997) and also to stem cells isolated from other tissues (for review see Sanchez-Ramos, 2002). hSM-MSCs, like other MSCs, can be expanded extensively without loss of multipotentiality. Hence, they have potential advantages for therapy over myoblasts that expand poorly in culture and rapidly undergo senescence.

In their current work, De Bari et al. (2003) infused hSM-MSCs into the tibialis anterior muscle of nude mice after muscle damage by infusion of cardiotoxin. They found that ~10% of the infused human cells were present in the muscle after 1 d and that about twice as many were present after 1 mo, demonstrating that the cells both engrafted and expanded in the tissue. They also showed that the engrafted human cells differentiated into muscle cells. Similar results were obtained when hSM-MSCs were infused intravenously; however, without prior damage to the muscle, the degree of engraftment was lower but still detectable. Some of the human cells engrafted as muscle satellite cells that, remarkably, 6 mo after their appearance, could be activated to differentiate into muscle by administration of cardiotoxin.

In experiments with the mdx mouse model for muscular dystrophy, infusion of the hSM-MSCs into muscle induced synthesis of human dystrophin. It also decreased the number of centronucleated fibers, a pathological feature of dystrophic mdx muscle. Most importantly, infusion of the hSM-MSCs restored the synthesis of mechano growth factor, a protein that controls repair of normal muscle and that undergoes deregulation in mdx muscle. In contrast, infusion of a plasmid containing the dystrophin gene produced synthesis of dystrophin but did not rescue expression of the mechano growth factor. Therefore, as the authors suggest, the results are encouraging for current efforts to develop hSM-MSCs or related cells for the potential therapy of muscular dystrophy.

One frequent criticism of papers describing plasticity of adult stem cells is that the preparations of cells were heterogeneous. Therefore, the apparent plasticity could be explained by selection for subpopulations already committed to differentiation to specific phenotypes. In practice, MSCs and similar cells from other tissues are among the easiest mammalian cells to clone. Since the earliest work of Friedenstein et al. (1976), a standard assay for MSCs from bone marrow has been to plate the cells at very low densities and count the single cell–generated colonies as a measure of the quality of the cells. De Bari et al. (2003) took care to demonstrate that the results they obtained with clonal hSM-MSCs were the same as those obtained with unfractionated cells.

Another criticism of reports on the plasticity of adult stem cells has been that many of the observations can be explained by cell fusion (Terada et al., 2002; Ying et al., 2002). As expected, De Bari et al. (2003) saw cell fusion in their experiments since myogenesis normally proceeds by fusion of myoblasts into multinucleated myotubes. However, they performed careful time course experiments to demonstrate that they did not detect muscle fibers containing human nuclei until day 7 after the infusions. After only 24 h, however, there was a sharp peak in expression of human Myf5, a factor that restricts undifferentiated cells to myogenesis. Therefore, they concluded that after infusion of the hSM-MSCs they were observing a multistep process in which the cells proliferated, became committed to a myogenic lineage, and then underwent final maturation and fusion.

This report comes in the midst of a current controversy over adult stem cells. The controversy has been fanned by the continuing ethical and political debate about restrictions on research with human embryonic stem cells. At the heart of the scientific controversy, however, is the difficulty of standardizing preparations of nonhematopoietic adult stem cells and of developing adequate in vivo assays. In effect, there is no animal test model comparable to the marrow-ablated mouse that has been the key to research on hemato-

poietic stem cells. Experiments in vivo are simple in concept but remarkably complex in practice. For example, recent observations (Terada et al., 2002; Ying et al., 2002) suggest that cell fusion may explain previous experiments in which stem cells from adult brain were used with embryonic stem cells to produce chimeric animals (Clarke et al., 2000). However, the observations on cell fusion by stem cells raise interesting questions about the role of cell fusion in differentiation and repair. We (Spees et al., 2003) recently observed that cell fusion was a relatively frequent event in an ex vivo model for tissue repair in which MSCs were cocultured with heat-shocked pulmonary epithelial cells.

The complexity of assaying nonhematopoietic adult stem cells in vivo is further illustrated by the surprising discrepancies in published data. In experiments in which marrow-derived cells were infused into mice, some investigators reported trace (Krause et al., 2001) or undetectable levels (Castro et al., 2002; Wagers et al., 2002) of engraftment into nonhematopoietic tissues. Others found relatively high levels of engraftment. For example, we (Pereira et al., 1998) reported that progeny of donor MSCs accounted for 5% or more of the cells in many tissues after infusion of the cells into young mice that were x ray irradiated. More recently, LaBarge and Blau (2002) found that bone marrow cells contributed to as many as 3.5% of muscle fibers after the muscles of mice were damaged by irradiation and exercise. Devine et al. (2002) reported that after GFP-transduced MSCs were infused into lethally irradiated baboons, progeny of the MSCs accounted for 0.1–2.7% of the cells in most nonhematopoietic tissues. Some of the discrepancies are probably explained by loss of the labels used to mark the cells as the cells differentiate and proliferate. However, the critical problems are clearly the cell populations used for the experiments and the status of the recipient animals in terms of variables such as age and the presence of tissue injury. For example, although clonal colonies of MSCs and related cells are readily prepared, the colonies rapidly become heterogeneous as they expand so that they contain subpopulations of early progenitors and more differentiated cells. The early progenitors are quickly lost during expansion unless the cells are maintained at very low densities and the culture conditions carefully monitored (Sekiya et al., 2002). It has not been established, however, which subpopulations provide the most robust engraftment and differentiation. Until these variables are defined, it is very likely that large discrepancies will continue to be observed in similar experiments performed by different investigators.

At the same time, the results of De Bari et al. (2003) add to the growing body of evidence demonstrating that stem-like cells can be obtained from a variety of tissues and that these cells have the remarkable ability to migrate to sites of tissue damage and stimulate repair either by differentiating into tissue-specific cells or, alternatively, by creating a milieu that enhances the repair of endogenous cells (Hofstetter et al., 2002; Li et al., 2002). The hypothesis that such stem-like cells take part in normal tissue repair is long-standing (Cohnheim, 1867; Prockop, 1997). The last vestiges of doubt about it have probably been erased by recent evidence from patients who have received sex-mismatched transplants of bone marrow or organs. In one study, 4–40% of the hepatocytes and cholangiocytes in liver were derived from donor bone marrow (Theise et al., 2000). In another study, 1–2% of the cardiomyocytes in transplanted donor hearts were host cells (Bayes-Genis et al., 2002). With better assays for adult stem cells, it should be possible to improve on the encouraging preliminary results that been obtained in clinical trials with MSCs in osteogenesis imperfecta (Horwitz et al., 2001) and in metachromatic leukodystrophy and Hurler syndrome (Koc et al., 2002).

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