

# Not the usual suspects: the unexpected sources of tissue regeneration

## Commentary

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It used to be simple. Regenerating liver was made only by liver cells, skin begat skin, endothelium begat endothelium, skeletal muscle came from skeletal muscle precursors, and brain and heart simply did not regenerate. Moreover, new cells made in regenerating tissues like endothelium and smooth muscle were derived from reserve cells in the vicinity of the damage within a given tissue.

In the last several years, virtually all of these long-held assumptions have come into question. There has been a plethora of reports that bone marrow-derived cells can differentiate *in vivo* into cells with properties characteristic of muscle, endothelium, liver, heart, and neuronal cells of the brain. Moreover, even cells derived from some non-bone marrow tissues appear to change their identities and to give rise to cells of other tissues. These transformations from one cell type into another suggest a novel level of complexity. Indeed, very little is known about the cells that serve as the “stem cells,” or precursors, for such a diverse range of tissues and functions. Two reports in this issue of the *JCI* (1, 2) furnish clues about the source of such cells and the tissues into which they might differentiate during regeneration.

The article by Jackson et al. (1) describes the isolation of cells from murine bone marrow that, when transplanted into lethally irradiated mice, were able to home to areas of damage in ischemic hearts and differentiate into both vascular endothelial cells and cardiac myocytes. This so-called side population (SP) had been previously identified as a CD34-negative subpopulation of bone marrow cells. SP cells actively extrude dyes and can be isolated by flow cytometry because of their faint staining relative to other cells. They are thought to represent a multipotent subset of hematopoietic stem cells (3), and indeed, Gussoni et al. (4) have found that these cells can become incorporated into differentiated muscle cells in a mouse model of Duchenne muscular dystro-

phy. These authors isolated SP cells from ROSA26 mice, which express the *lacZ* marker gene in most cells, and transplanted them into irradiated normal mice so that SP-derived bone marrow cells and their progeny could be identified by staining following incubation with the substrate X-gal. This powerful approach and similar genetic marking strategies have been used by several groups to follow the fate of bone marrow-derived cells that migrate to diverse tissues, including vascular endothelium, skeletal muscle, liver, and brain (5–12).

Jackson and colleagues (1) have now established that SP cells can help reconstitute the myocardium of recipient mice following ischemic tissue damage that mimics the consequences of a myocardial infarction. Under these conditions, SP-derived cells give rise to both vascular endothelial cells and cardiac myocytes within the ischemic hearts. No such engraftment occurs in control, nonischemic hearts. The SP-derived cells found in each of the two tissue types appear to have differentiated into the appropriate cell types, based on morphology and the expression of tissue-specific genes. This finding offers a tantalizing clue regarding the source of cells that participate in regeneration of these tissues and suggests potential clinical applications. It provides a significant extension of a recent remarkable report demonstrating that direct injection of a subset of bone marrow cells into infarcted myocardium can lead to substantial repopulation of that tissue (13).

Much work over the last several years has been devoted to identifying subpopulations of bone marrow and peripheral blood cells with varying degrees of differentiation capability, based on the presence or absence of markers such as CD34 (14). As an example, CD34-positive, Flk-1-positive cells isolated from peripheral blood have been identified as the population containing circulating endothelial progenitor cells (EPCs), which are capable of contributing to

growing vasculature (5). Interestingly, the SP bone marrow cells isolated by Jackson et al. (1) were negative for both CD34 and Flk-1, as well as Flt-1, suggesting that they are less mature than the circulating EPCs. Moreover, the authors found that upon isolation, the SP cells did not express markers of cardiac muscle or mature vascular endothelium. Thus, the SP cells underwent differentiation as they assumed the roles of cardiac and endothelial cells. However, as the authors point out, it remains unknown whether this differentiation took place before or after incorporation of the cells into those tissues.

One essential caveat that applies to any study showing infrequent transdifferentiation or stem cell conversion is that the biological relevance of such observations is uncertain. In this report (1), the authors estimate that 3% of the blood vessels (mostly capillaries) contained cells derived from donor SP cells, and 0.02% of all cardiomyocytes were derived from such cells. While the finding that SP cells are capable of differentiating into cardiac or endothelial cells is noteworthy, it remains to be determined whether or not these cells are a common source of cells for the regeneration of these tissues. As the authors note, more studies will be needed to determine if the efficiency of these processes can be increased. Historically, however, once evidence in support of a biological phenomenon is obtained, methods often soon appear to increase its efficiency.

A second article in this issue of the *JCI* (2) provides suggestive evidence that vascular smooth muscle cells (SMCs) can be derived from a circulating progenitor. Specifically, Hillebrands et al. demonstrate that the vascular SMCs responsible for neointimal formation in transplant arteriosclerosis, *i.e.*, the thickening of the intima of the artery, are predominantly derived from the transplant recipient. Previous studies by us using different methods and by

Hillebrands and coworkers reached the same conclusions (15, 16).

Transplant arteriosclerosis (TA), also called chronic vascular rejection or graft vascular disease, is the main cause of morbidity and mortality in long-term survivors of many types of organ transplants. The standard etiology of TA following organ transplantation is thought to be the local migration and proliferation of medial SMCs in response to inflammatory signals and/or growth factor expression with consequent thickening of the intima. Although this etiology of TA has never been demonstrated in its entirety, several steps have been documented in vessels with ongoing neointimal formation, including the expression of inflammatory cytokines and growth factors in both the media and neointima, as well as the proliferation of SMCs in the media (17).

To distinguish between donor and recipient origin of vascular SMCs and endothelial cells in allografted vessels, Hillebrands et al. (2) used a combination of immunohistochemical staining and PCR methods to identify an MHC class I haplotype. Their initial demonstration that irradiated aortic allografts developed TA to a degree similar to that of unirradiated allografts suggested that proliferation of medial SMC in the donor graft was not essential for TA. Subsequently, they found that most neointimal SMC nuclei were derived from the recipient, not the donor, confirming that the predominant SMCs were derived from infiltrating progenitors from the host.

Hillebrands et al. (2) also demonstrate that endothelial reseeded, which is well known to occur in artificial grafts as well as in normal vessels, correlates with the formation of a neointima. Cyclosporine therapy, which prevents neointimal formation, allows the donor endothelial cells to be maintained. Whether the retention of donor endothelial cells is merely due to a temporal association or plays a causal role in the etiology of TA remains to be determined. However, models of lung transplantation that involve obliteration

of the airway lumen also exhibit a concurrent loss of donor epithelium. Indeed, recently, several papers have demonstrated that maintenance of the epithelial barrier, even in the absence of immunosuppression, can prevent airway obliteration (18, 19). Thus, in chronic airway rejection, an intact epithelial layer may physically restrict migration of SMCs and myofibroblasts into the lumen or may act in a regulatory fashion to block proliferation of these cells in situ. Possibly, endothelial cells in allografted vessels perform analogous functions to those reported for epithelial cells in lung transplant models.

If smooth muscle progenitors enter into the neointima from the circulation, as both our research (15) and that of Hillebrands et al. (2) suggests, then presumably these cells leave the circulation and enter the tissue by a process similar to leukocyte-endothelial cell adhesion and diapedesis, the migration of cells through the vessel wall. This process involves sets of relatively well characterized adhesion molecules. Thus, strategies that interfere with the activity of these molecules may lead to the development of novel therapies for TA.

Thus, Jackson et al. (1) highlight and add to the growing body of literature that questions the identity of stem cells. Are these cells ubiquitous and present in all tissues? If not, how do they differ? What signals can induce a bone marrow-derived cell to migrate to the heart and assume a novel function in that tissue? If the cells need not be resident in the damaged tissue, are plasticity and trans-differentiation between cell types normal aspects of tissue repair throughout life? Hillebrands et al. (2) address another repair mechanism, that involved in SMC recruitment and neointimal proliferation following organ transplantation. Although neointimal thickening is not desirable, the finding that the cells involved are derived not only from the donor, but also from the host, is of fundamental interest. Thus, both reports demonstrate that cell identities and derivations can no longer be taken for granted.

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