Blood to liver and back again: seeds of understanding

Since the first reports of bone marrow-derived cells becoming liver tissue in rodents[1,2] and in humans,[3,4] an escalating number of confirmatory studies have been published, gradually expanding our understanding of the physiologic aspects of these events.[5-11] What is clear is that circulating cells, often if not always bone marrow-derived, can traffic to the liver, engrafting there as hepatocytes and cholangiocytes. While most studies have been performed with heterogeneous populations of cells, two studies report *in vivo* clonal expansion of single cells reconstituting all elements of marrow and blood as well as hepatocytes.[5,11] Thus, there is true plasticity for at least some hematopoietic stem cells to produce hepatocytes in addition to mature blood lineages. Thus, the categories of mesoderm and endoderm are not so exclusively defined as had been previously thought.

Our current understanding now suggests a three-tiered process of hepatic regeneration.[12] First, mature hepatocytes and cholangiocytes can regenerate themselves and probably do so for the bulk of normal cell turnover. Second, cells adjacent to and within the smallest branches of the biliary tree can generate both hepatocytes and cholangiocytes in response to moderate to severe injury.[13-16] Third, cells from the bone marrow can diffusely enter the liver and generate rare, isolated hepatocytes and cholangiocytes, in response to low level injury, and can enter the biliary or peribiliary compartment to lead to widespread epithelial reconstitution in the setting of moderate to severe injury.[12]

The details of these processes must now be worked out and newer studies such as that of Wulf *et al.*[17] in this current issue are beginning to do just this. In this study, the investigators have focused on the *side population* or *SP* fraction of cells exclusion from disaggregated livers in mice. These are small, blast-like cells, defined by Hoechst 33342 dye exclusion and their unique blue and red fluorescence by flow cytometry. *In vitro*, both $CD45^{pos}$ and $CD45^{neg}$ SP cells could give rise to mixed hematopoietic and hepatocytic populations. Hepatic SP cells transplanted by direct injection into liver contributed to both hepatocytes and cholangiocytes. These cells could also contribute to marrow reconstitution in lethally irradiated mice. Conversely, marrow-derived SP cells contributed to hepatic SP cells at 4 to 12 months post-bone marrow transplantation.

While most studies, including much of our own work, used whole bone marrow as a donor population, several of the studies of marrow-to-liver engraftment address which particular cell type, if any, is responsible for the phenomenon. Our own work highlighted the capacity of $CD34^{pos}lin^{neg}$ cells for hepatocyte engraftment.[2] The detailed study of Lagasse *et al.*,[6] in which they focused on the $kit^{pos}lin^{neg}Sca1^{pos}$ cell fraction of the marrow, clearly showed that this fraction was most ready to engraft and, in fact, accomplish metabolic rescue of the fumarylacetoacetate hydrolase.[6] In our own study[5] a single cell was derived by elutriation, lin exclusion (including antibody A44.1, which is perhaps of particular importance), marrow homing, and quiescent cell cycling, and was able to engraft multiple epithelial compartments including the liver. The recent paper by Wagers *et al.*,[12] which showed *little evidence* of cell plasticity, focused on the kit^{pos} $thy1^{pos}$ lin^{neg} $Sca1^{pos}$ population, which may be a more committed cell than the one we investigated. It differs from our isolated cell by the lack of antibody A44.1 as part of the lineage exclusion cocktail and by the inclusion in our approach of a 48-hour marrow-homing step in a primary recipient.[18] It is not, therefore, clear whether all these cell groups contain a cell of one single definable phenotype that is capable of hepatic epithelial engraftment. In particular, Lagasse's study also looked at the excluded populations, and the data showed that while the other excluded cell populations (kit^{neg}, $Sca1^{neg}$, lin^{pos}) did not accomplish metabolic rescue, they all engrafted as hepatocytes to some measurable quantity.[6] The difference lay in the efficiency of the engraftment, rather than in the ability to engraft as hepatocytes.

In Wulf's study[17] the SP fraction of marrow cells was able to reconstitute liver tissue. Do these cells map to any of the previously tested populations? It has been reported that the SP fraction contains kit^{pos}, $Sca1^{pos}$, and $CD45^{pos}$ cells, but is generally $CD34^{neg}$.[20-22] Thus, Wulf *et al.*[17] implicitly extend the concept that the marrow cell population which can contribute to liver parenchymal engraftment is a heterogeneous one.

Not only do they show that these cells can become hepatocytes and cholangiocytes, but they also show that they take the form of cells of an intermediate or earlier phenotype than these mature cell types. It has been noted by Petersen *et al.* that rat liver oval cells – blast-like in appearance, but with biliary-type cytokeratin expression – can be marrow-derived.[1] In our work in humans, we clearly demonstrated that cells in the ductular reaction in severe injury, the human equivalent of the rodent oval cells, were also sometimes marrow-derived.[3] In this study perhaps we are witnessing an *earlier* step in the marrow to hepatocyte/cholangiocyte transition. The SP cells of the liver, marrow-derived yet lacking in both CD45 and biliary cytokeratins, despite being adjacent to the bile ducts, perhaps correspond to the periductular cells described by Sell[15] and by Novikoff.[16] We have previously speculated that these periductular cells might represent cells in transit from the circulation into the liver where they will integrate with the epithelial cells.[13] This study supports that speculation.

Perhaps most intriguingly, the data of Wulf *et al.* are

applicable to another process that has been of great personal interest,[13] but for which the literature has so far been largely silent: extramedullary hematopoiesis (EMH) in the adult liver. The new occurrence of hepatic hematopoiesis in adult life is well documented, usually developing after marrow ablation caused by toxins, radiation, or neoplastic replacement or, more rarely and for more obscure reasons, with congestive heart failure.[23] It has always been assumed, primarily because of the dogma of lineage commitment of adult cells, that EMH is a result of latent hematopoietic stem cell *rests* in the adult liver. However, given the ability of circulating blood cells to become the smallest biliary epithelial cells, i.e. the intrahepatic stem cell compartment, we have suggested that the reverse might also be possible. Wulf *et al.*[17] now demonstrate that the SP fraction of liver cells, which can give rise to liver parenchyma, can also give rise to hematopoietic elements. While this has not yet been tested on the single cell level, it does suggest that EMH is hematopoietic activation of the same cells that can reconstitute hepatic epithelial cells. Their data therefore suggest that the arrow can go both ways.

The implications of these findings for physiologic and therapeutic organ regeneration and repair may be profound. They also raise the question of whether the liver is special in this regard. Can other epithelial organs' side populations perform the same trick? If so, are these organs actually sending out cells into the circulation, which can then return in response to injury? Do cells perhaps even traffic from one organ to another? What might the physiologic roles for such trafficking be? All of these questions, and more, await further elucidation. The answers will lead us to a tremendously revised, perhaps even fantastical understanding, by today's standards, of cell and tissue biology.[23,24]

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