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Commentary

Bone Marrow-Derived Hepatocytes

Rare but Promising

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In the past 3 years, several reports have demonstrated the capacity of bone marrow stem cells to transdifferentiate into hepatocytes.[1–5] These findings created enormous interest because they uncovered a new property of bone marrow stem cells and opened the possibility that these cells could be used in the treatment of liver injury and acute or chronic liver failure. Even though it is now widely accepted that the transdifferentiation phenomenon is real, many questions still remain. There has been a great divergence among the reports concerning the efficiency of hepatocyte replacement after bone marrow transplantation. Moreover, it was uncertain whether the transdifferentiation events were dependent on liver damage. In this issue of *The American Journal of Pathology*, Wang et al[6] address these questions and advance our understanding of the intriguing and fascinating phenomenon of the generation of hepatocytes by bone marrow stem cells. They conclude that hepatocyte replacement by bone marrow cells is a slow and rare event that can occur independently of liver injury.

The first demonstration of transdifferentiation of bone marrow cells into liver cells was obtained in 1999 by Petersen et al.[3] In this study, irradiated female rats received bone marrow cells from congenic male rats and their livers were subsequently injured by a treatment of carbon tetrachloride and 2-acetyl-aminofluorene, which prevented hepatocyte division and stimulated oval cell proliferation. The results obtained by Petersen et al[3] demonstrated that oval cells arose from hematopoietic precursors and gave rise to Y-chromosome-positive hepatocytes.

Subsequently, other groups reported differentiation of bone marrow cells into hepatocytes in mice[4] and in humans.[1,5] Although these reports confirmed and broadened the findings from the original paper, the reported frequency of transdifferentiation was quite variable. Also left unsolved was the question of whether transdifferentiation could occur in the absence of liver damage.

The first characterization of the bone marrow cells that are capable of differentiating into hepatocytes was presented by Lagasse et al.[2] They transplanted highly purified hematopoietic stem cells (HSC) from metabolic competent donor mice into fumarylacetoacetate hydrolase (FAH)-deficient mice. These mice suffer from a permanent liver injury as a consequence of accumulation of the hepatotoxic metabolites fumarylacetoacetate and its precursor maleylacetoacetate.[7] FAH−/− mice cannot survive unless they are treated with the drug 2-(2-nitro-4-trifluoro-methylbenzyol)-1,3-cyclohexanedione (NTBC) that prevents the production of the toxic metabolites.[8] Lagasse et al[2] showed that HSC purified by flow cytometry and cell sorting (c-kit^{high} Thy^{low} Lin^{-}Sca-1^{+} fraction) could differentiate into hepatocytes. Thirty percent of the transplanted FAH−/− mice survived after NTBC withdrawal, demonstrating that HSC-derived hepatocytes did restore liver function in this model.

In the present paper, Wang et al[6] used the FAH−/− mouse model to establish the kinetics of hepatocyte replacement after bone marrow transplantation. Irradiated FAH−/− mice received bone marrow cells from congenic wild-type donors and were maintained on NTBC treatment during the period of hematopoietic engraftment. They determined the time-course necessary for formation of hepatocyte clones derived from transplanted cells and performed a quantitative analysis of hepatocyte replacement. Two months after bone marrow transplantation, they detected the presence of about 1 of 150,000 bone marrow-derived hepatocytes, suggesting that the transdifferentiation of bone marrow stem cells into hepatocytes is a slow and rare event. Only when strong selective pressure was applied (ie, NTBC withdrawal), could they observe a significant level of liver repopulation. Moreover, the authors showed that transdifferentiation from donor cells only occurs after bone marrow reconstitution.

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These findings are in agreement with a recently published paper showing that bone marrow reconstitution and the presence of selective pressure are both necessary to obtain detectable levels of hepatocyte replacement.[9]

Wang et al[6] also demonstrate that liver injury is not a requirement for the transdifferentiation of HSC into hepatocytes. This finding is of major significance for the understanding of the mechanisms driving the transdifferentiation process. It suggests that differentiation from bone marrow cells and from other hepatic stem cells (eg, oval cells) may differ in significant ways. While oval cells differentiate into hepatocytes only when the proliferation of the latter is impaired, Wang et al[6] indicate that differentiation from bone marrow cells may occur in undamaged livers. This raises the fundamental issue of the role of HSC transdifferentiation in liver physiopathological processes.

Even if the stimuli that promote bone marrow cells to differentiate into hepatocyte remain to be defined, the data presented here and in other recent reports strongly indicate that the frequency of this phenomenon is extremely low. Although some studies reported a much higher level of hepatic replacement, it is possible that such results are related to technical artifacts. The techniques used consisted of detection of the Y-chromosome by *in situ* hybridization in gender mismatched transplants. The major difficulty with this approach is that, unless confocal microscopy is used to verify co-localization of hepatocyte markers and Y-chromosome, non-parenchymal cells could be easily mistaken for hepatocytes. Indeed, it has been shown that a significant percentage of donor-derived endothelial and Kupffer cells can be detected in the liver after bone marrow transplantation.[10,11] It is thus very probable that non-parenchymal cells rather than hepatocytes may account for the results of studies showing a high degree of bone marrow-derived hepatocytes.

The present study, as well as that of Mallet et al,[9] highlights the need for selection procedures to achieve significant hepatocyte replacement by HSC. It is clear that a strong selective pressure is required to achieve therapeutic levels of liver repopulation by bone marrow-derived hepatocytes. Whenever the precise stimuli that lead to transdifferentiation are defined, it should be possible to improve the engraftment of these cells into the liver parenchyma and consequently enhance the levels and speed of repopulation. However, even if HSC are a potential alternative source of liver cells, having a major advantage over hepatocytes because of their availability, for the time being, hepatocytes are still the best option for cell-based therapies. As demonstrated by Wang et al, it takes only 3 weeks to achieve around 50% repopulation of FAH−/− mice after transplantation of normal adult hepatocytes. In contrast, the same degree of repopulation is not achieved until 22 weeks after bone marrow cell transplantation. Despite the efficiency of the transplantation of adult hepatocytes, significant levels of repopulation are achieved only when a method of selection for the transplanted cells is applied. Perhaps, the transplantation of fetal hepatocytes may be a useful alternative. In contrast to adult hepatocytes, these cells proliferate for long periods after transplantation and can repopulate a normal liver without selective pressure.[12] Nevertheless, like adult hepatocytes, these cells are not so easily obtained.

Regardless of the type of cell to be used, the development of strategies for liver cell therapy that could in the not too distant future serve as an alternative to liver transplantation is of extreme relevance. Therefore, efforts to uncover the mechanisms that regulate the differentiation of bone marrow cells into hepatocytes are greatly welcome. The promise of bone marrow to liver still stands; it is up to us to make it clinically useful. To accomplish this goal, the efficiency of the process needs to be greatly enhanced.

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