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The Bone Marrow and Liver Fibrosis: Friend or Foe?

See "Negligible contribution of bone marrow-derived cells to collagen production during hepatic fibrogenesis in mice," by Higashiyama R, Moro T, Nakao S, et al, on page 1459.

Bone marrow-derived cells circulate frequently through the liver and can engraft it. There is considerable interest in the effects of these bone-marrow derived cells on liver fibrosis and regeneration. It is important to characterize these effects for 2 main reasons: to understand the pathogenesis of liver fibrosis with the aim of developing antifibrotic therapies, and because bone marrow-based cell therapy has been proposed as a clinical tool to promote liver regeneration and inhibit liver fibrosis. It has been reported that various components of the bone marrow can have antifibrotic effects on the liver.[1] Animal studies have shown that the bone marrow-derived scar-associated macrophage population can influence strongly the fibrotic response to liver injury, promoting liver scar production during injury and promoting scar resolution after the cessation of injury.[2] Bone marrow-derived endothelial progenitor cells have been used in rodent models of fibrosis to reduce liver damage[3] and bone marrow progenitors have been used to repair hepatic sinusoidal endothelium after liver injury.[4] Bringing immediacy to this matter is the fact that several groups are beginning to perform clinical studies of autologous bone marrow cell therapy for liver disease.[5–8] Because the aim of such bone marrow cell therapy is to reduce hepatic fibrosis and promote liver regeneration, one would not want to inject cells into the liver that could either directly make scar tissue or indirectly promote endogenous scar production. In this regard, there have been reports that bone marrow cells or their progeny can circulate into various damaged organs and differentiate into myofibroblasts or fibrocytes.[9] Several studies have suggested that bone marrow contributes to scar forming cells of various types in the liver.[10–18] In this month's issue of GASTROENTEROLOGY, however, Higashiyama et al[19] report their findings that the bone marrow contributes little to liver fibrosis or myofibroblasts in a mouse bone marrow transplantation model.

The bone marrow contains 2 main stem cell compartments, namely hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs) (Figure 1). Endothelial progenitor cells can also be derived from bone marrow. HSCs give rise to myeloid and lymphoid lineages, including macrophages, and are known to be both radio- and chemosensitive. Thus, after lethal irradiation and bone marrow transplantation, HSCs in recipient animals are of donor origin, which enables HSC transplantation-based lineage tracing studies. MSCs are less well defined and can give rise to bone, cartilage, and fat lineages, as well as to fibroblast cells. MSCs have been shown to remain of recipient origin after bone marrow transplantation because MSCs are radio- and chemoresistant.[20,21] Therefore, after lethal irradiation and bone marrow transplantation, a chimeric bone marrow is created where HSCs are of donor origin and MSCs are of recipient origin. Using bone marrow transplantation as a mechanism of lineage tracing, investigators can track transplantable bone marrow elements, that is, HSCs and their progeny.

Higashiyama et al[19] used a model whereby whole bone marrow from a constitutively green fluorescent protein (GFP)-expressing donor was transplanted into irradiated recipients. In the absence of details regarding the chimerism achieved in the bone marrow mesenchymal compartment, it is impossible to determine the relative proportions of donor and recipient MSCs, and it may be that the primary donor cell population studied was derived from HSCs and consisted principally of inflammatory and hematopoietic cells. Interestingly, the authors report little evidence of collagen transcription in the liver from these bone marrow-derived cells. This contradicts the work of Kisseleva et al,[15] in which bone marrow from

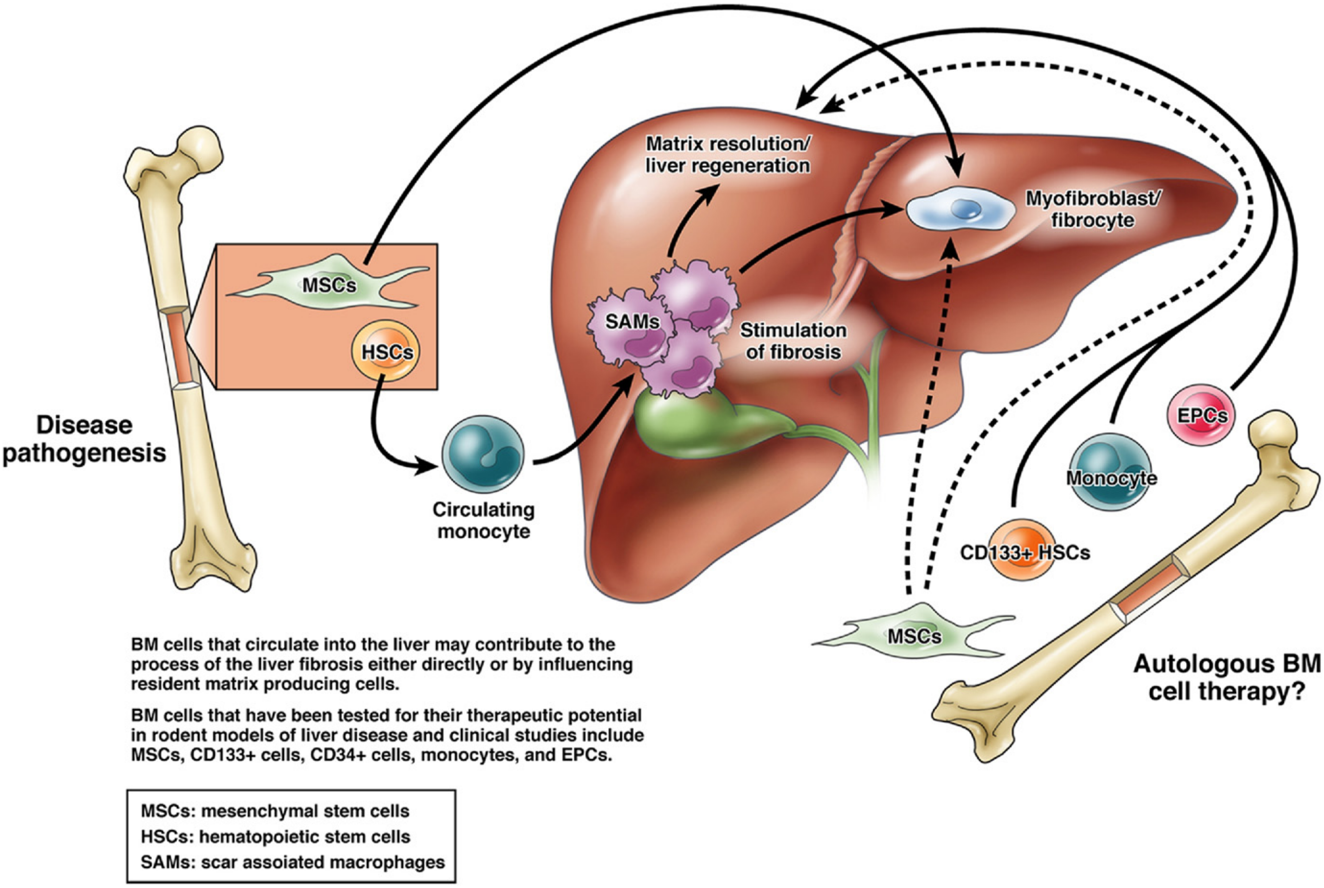

Figure 1. The potential interaction between the bone marrow (BM) and the liver either during liver injury or as a potential therapy.

collagen $\alpha 1$(I)-GFP reporter mice was transplanted into wild-type recipients before bile duct ligation liver injury, revealing a population of bone marrow-derived CD45$^+$ fibrocytes that were transcriptionally active for collagen. Two previous studies have attempted to analyze the relative contribution of the hematopoietic and mesenchymal compartments to the hepatic scar forming population in the liver. Russo et al[14] found that HSCs supplied few, if any, myofibroblasts to the damaged liver and that MSCs were a more likely source of liver myofibroblasts. Li et al[22] recently replicated these findings and took these observations further. In an interesting paper, they suggested that MSCs migrated to the liver from the bone marrow along a sphingosine 1-phosphate (S1P) gradient. By using suramin, a selective S1P$_3$ receptor antagonist, they showed potent inhibition of MSC migration to S1P in vitro. Furthermore when the antagonist was administered in vivo, fewer GFP-positive myofibroblasts engrafted the liver, implying that the drug had prevented migration of these cells from the bone marrow to the damaged liver. What requires further clarification is whether in these studies the MSCs engrafted the liver directly after injection, or whether this truly represented pathophysiologic homing from the bone marrow. Further experiments are thus required to give a full picture of the role of the bone marrow in liver fibrosis.

Higashiyama et al[19] found few bone marrow-derived fibrotic cells in the livers of transplanted animals, although they did observe a population of GFP-positive/α-smooth muscle antibody (SMA)-positive cells in recipient livers (Figure 3). It would have been informative had they provided a more extensive characterization of these bone marrow-derived myofibroblasts. This could have been performed in situ in the liver (using dual staining for glial fibrillary acidic protein [GFAP], desmin, vimentin, etc), or by isolating the GFP-positive cells and examining them in detail ex vivo. This second method was carried out originally by Baba et al in 2004,[10] who reported isolating hepatic stellate cells from the livers of mice that had received transplants of GFP-positive bone marrow; a proportion of these stellate cells were found to be strongly GFP positive. The findings of Higashiyama et al[19] contradict several recent studies that identified bone marrow-derived fibrotic cells in the injured liver. In these studies, a number of different techniques to trace cell lineage were used, including bone marrow transplantation into wild-type mice from donors with constitutive reporter gene expression, and gender mismatched bone marrow transplantation with sex chromosome tracking techniques. This allowed the detection of a range of bone marrow-derived cells in the liver using a variety of markers (vimentin, α-SMA, desmin, GFAP, collagen-1; Table 1). Some investigators have carefully isolated hepatic stellate cells and found bone marrow markers (GFP and sex chromosomes).[10,14] Higashiyama et al[19] suggest tissue autofluorescence as a cause for the discrepancy between their findings and those of other groups. Modern confocal microscopy should allow this distinction to be made and, interestingly, autofluorescence was considered by Miyata et al, who performed anti-GFP immunostaining to confirm the specificity of their eGFP tracking technique.[16]

It is important to know which cells are capable of either secreting collagen in the liver or inducing collagen secretion from endogenous cells. Indeed, MSCs can be coaxed into a hepatocyte-like phenotype and have some limited degree of hepatocytic function in vitro. For clinical use, MSC-derived hepatocyte-like cells would need to remain hepatocyte-like within the recipient liver, even in the context of ongoing inflammation and injury; reversion to a mesenchymal phenotype would be highly undesirable. Exogenously derived MSCs have been proposed to enhance liver regeneration[23] and reduce liver fibrosis in some reports[24]; however, other reports have been less positive. di Bonzo et al[11] showed that exogenous MSCs were much more likely to adopt a myofibroblast phenotype (α-SMA positive) than a hepatocyte phenotype in the chronically injured liver. Although there have been several reports of the beneficial effect of MSCs on liver fibrosis in rodent models, other studies have found no benefit.[25] Time will tell whether this potentially promising therapy can be translated into the clinic. Factors that need to be considered include whether the MSCs themselves produce scar or matrix-degrading substances, and whether they have a net positive or negative effect on the surrounding cell population. If MSCs are found to be beneficial, it will be important to know how they compare with other putative therapeutic bone marrow-derived cells such as CD34$^+$ cells and monocytes.

The virtual absence of collagen production from bone marrow-derived cells, probably primarily HSCs, in the injured liver observed by Higashiyama et al[19] encourages the further testing of autologous HSCs (such as CD34$^+$ and CD133$^+$ cells and derivatives such as monocytes) for liver therapy. To date, small, uncontrolled studies have suggested an overall benefit.[5–7] There are obviously 2 main ways to address this particular issue—larger, randomized clinical studies or additional mechanistic studies in rodent and other model systems to define the optimal cells for therapy. At this stage, it seems reasonable that both approaches be undertaken with care.

Table 1. Papers Reporting Bone Marrow Cells Engrafting the Liver and Adopting a Scar-Forming Cell Phenotype

Authors	Irradiation and liver injury method	BM population transferred	Cell tracking method	Demonstration of fibrotic cell type of BM origin	Functional effect of BM cells in liver
Baba et al[10]	1200 cGy CCl_4	Whole BM	GFP into WT	In vivo colocalization of GFP/GFAP, GFP/desmin. In vitro analysis of nonparenchymal cells isolated from BM transplanted mice-colocalization of GFAP/GFP, desmin/GFP, α-SMA/GFP, reverse transcriptase PCR of isolated NPCs GFP/α-SMA/desmin positive	
Forbes et al[13]	Hepatitis B, C, B+delta	N/A	Clinical tissue from gender mismatched liver and BM transplants	Y chromosome tracking plus immunodetection of α-SMA, vimentin, desmin, fibulin-2	
Russo et al[14]	1000 cGy CCl_4 Thioacetamide	Whole BM MSCs and HSCs	Male BM into female recipients	FISH for Y chromosome, immunodetection of α-SMA, GFAP, desmin, vimentin, collagen gene reporter mice, transplants from mice with MMP resistant collagen. In vitro analysis of NPCs isolated from BM transplanted mice; Y chromosome detection	Increased fibrosis seen after BM transplantation from mice with MMP-resistant collagen
Kisselva et al[15]	1050 cGy BDL	Whole BM	Col(α1)I-GFP into WT recipients	GFP expression	
Asawa et al[18]	1200 cGy BDL	Whole BM	GFP into WT recipients	Colocalization of GFP/α-SMA and GFP/FSP-1	
Di Bonzo et al[11]	350 cGy CCl_4	Human MSCs	Human MSCs into NOD/SCID mice	Colocalization of HLA-I antigens/GFAP	
Miyata et al[16]	950 cGy CCl_4	Clonal cultures from a single HSC	eGFP mice (C57BL/6-Ly5.2 back ground) into C57BL/6-Ly 5.1 WT recipients	In vivo detection of dual eGFP/vimentin, eGFP/α-SMA, eGFP/ADAMTS13 in liver; in vitro analysis of NPCs isolated from BM transplanted mice; colocalization of eGFP/collagen-1, eGFP/ADAMTS13	
Li C et al[12]	800 cGy CCl_4 BDL	Whole BM MSCs	eGFP into WT	In vivo colocalization of eGFP/α-SMA	Blocking of BM MSC migration to liver reduced fibrosis
Fujimiya et al[17]	900 cGy ethanol	Whole BM	GFP into WT and ROSA	In vivo colocalization of GFP/α-SMA and GFP/GFAP	

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Conflicts of interest

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