Hematopoietic stem cell trafficking in liver injury

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ABSTRACT **Bone marrow (BM) hematopoietic stem cells (HSCs) have been shown to facilitate regeneration in multiple nonhematopoietic tissues by either generating epithelial cells or altering the inflammatory response. Depending on injury type, the predominant mechanism of epithelial lineage regeneration occurs by spontaneous cell fusion or transdifferentiation. Irrespective of the mechanism, mobilization from the BM is a prerequisite. Mechanisms by which HSCs mobilize into damaged organs are currently under scrutiny. Murine and human studies have shown that the chemokine SDF-1 and its receptor CXCR4 participate in the mobilization of HSCs from BM and in the migration of HSCs to injured liver. SDF-1 is a potent HSC chemoattractant and is produced by the liver. Production is increased during liver injury leading to increased HSC migration to the liver, a finding diminished by neutralizing anti-CXCR4 antibodies. Additional factors have been implicated in the control of hepatic migration of HSCs such as IL-8, hepatocyte growth factor, and MMP-9. Matriceal remodeling is an essential component in HSC engraftment, and MMP-9 expression is increased in liver injury. This review focuses on the complex interaction of chemokines, adhesion molecules, and extracellular matrix factors required for successful migration and engraftment of HSCs into the liver.—Dalakas, E., Newsome, P. N., Harrison, D. J., Plevris, J. N. Hematopoietic stem cell trafficking in liver injury. *FASEB J.* 19, 1225–1231 (2005)**

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Bone marrow (BM) hematopoietic stem cells (HSCs) have long been known to possess the unique capacity for self-renewal and differentiation into hematopoietic and mesenchymal cell lineages (1). That this plasticity extended to nonhematopoietic lineages such as hepatic oval cells, hepatocytes, cholangiocytes (1–3), skeletal muscle cells (4), neurons (5), epithelial cells of the lung, GI tract, and skin (6) is a relatively new observation, and has raised hopes that such cells could in the future be used for the regeneration and reconstitution of damaged organ tissue. This process of epithelial lineage regeneration appears to occur via a mechanism of spontaneous cell fusion or transdifferentiation. Emerging data in the field of cardiac regeneration suggest that incoming stem cells can also contribute to tissue repair by promoting neoangiogenesis and minimizing cardiomyocyte apoptosis (7). Whatever the underlying mechanism by which the HSCs participate in tissue regeneration, it will still require the presence of HSCs to mobilize from the BM and reach their target organ. The aim of this review is to summarize current available information addressing the aspects of HSC mobilization and trafficking in response to liver injury.

ADULT STEM CELLS AND LIVER REGENERATION

While the liver is a mitotically quiescent organ in adult humans and animals (8), hepatocytes have a remarkable capacity to meet the replacement demands during cellular loss (9, 10). However, when either chronic/extensive damage is inflicted on the liver or when hepatocyte proliferation is inhibited, a facultative cellular compartment of hepatic oval cells (HOCs), located within the smallest branches of the intrahepatic biliary tree is activated and leads to liver repair (10, 11). More recently, several groups have demonstrated that BM-derived HSCs may contribute to liver repair (1–3, 12–14). The contribution of HSCs to liver repair has varied, but is generally related to the presence and severity of liver injury. Thus, the restitutive response of the liver to different injuries has been proposed to include three levels of proliferating cells: *1)* the hepatocyte, *2)* the endogenous ductular progenitor cell or HOC, and *3)* a pluripotent stem cell derived from circulating BM cells (9).

Controversy has recently arisen as to whether HSCs contribute to the hepatocyte lineage in liver injury via transdifferentiation alone or by adopting the phenotype of hepatocytes after spontaneous cell fusion (15). Recent reports in favor of the fusion hypothesis have demonstrated that adult cells can adopt the phenotype of other cell lines by fusing with embryonal stem cells (16, 17) as well as BM-derived hepatocytes generated by in vivo cell fusion (18). In support of transdifferentiation, several groups have demonstrated that HSCs can differentiate into hepatocytes (19, 20) and pancreatic endocrine cells (21) without any evidence of cell fusion. The mechanism of HSC hepatic regeneration

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remains unresolved; clearly, any future stem cell research will have to distinguish HSC transdifferentiation from fusion events. Whatever the mechanism of hepatic regeneration is, the trafficking of HSCs to the liver may play an important component of the reparative process in liver injury.

The contribution of HSCs to hepatocyte lineages in rodents and humans remains a controversial area with data both supporting (1–3, 13, 14, 22) and rebutting (23–25) findings. This may in part reflect the types of cells used, the injury models used, and the methods used to detect stem cell progeny. Nevertheless a therapeutic role of HSCs in liver injury has been described in rodents (13, 26), albeit with varying contributions of transdifferentiation and fusion. In other models, particularly in humans, the contribution that HSCs make to liver repair by transdifferentiation is lower, on the order of 0.011–20% (2, 3, 6, 27–29). To improve on this level of contribution will require greater understanding of the mechanisms by which stem cells mobilize from the BM and home to injured organs. There remains a pressing need for further studies to confirm or refute the claims that stem cells can lead to improved liver repair and hence survival in either a rodent or human setting.

Murine and human studies have shown that the chemokine stromal cell-derived factor-1 (SDF-1) and its receptor, CXCR4, are involved in recruiting inflammatory cells into injured livers as well as inducing proliferation of endogenous HOCs (30, 31). SDF-1/CXCR4 interactions participate in the mobilization of HSCs from BM and have been implicated in the migration of human HSCs to the liver during injury (32, 33). Other factors have been implicated in the regulation of hepatic migration of HSCs, including interleukin-8 (IL-8), hepatocyte growth factor (HGF), and matrix metalloproteinases (MMPs).

ADULT STEM CELL MOBILIZATION AND RECRUITMENT IN LIVER INJURY

Human studies have demonstrated increased levels of circulating HSCs in response to a systemic injury such as acute sickle cell crisis and surgical trauma (34, 35). A recent study by De Silvestro et al. demonstrated that peripheral blood HSC levels were elevated after extensive liver resection (36). Our group has demonstrated that in patients with alcoholic hepatitis there is an increase in circulating HSCs when compared with normal controls (37). The extent to which these peripheral blood HSCs are mobilized into the circulation of patients with liver injury and contribute to liver repair remains uncertain and is under investigation.

Release of HSC from the bone marrow

In the adult BM, the release of HSCs into the peripheral circulation is regulated in part by the CXC chemokine SDF-1 and its receptor CXCR4 (32, 33, 38, 39). SDF-1 is a potent chemoattractant for HSCs and is produced by various BM stromal cell types and epithelial cells in a broad range of normal tissues, including the liver (40–46) (see **Table 1**). It plays a major role in the homing, migration, proliferation, differentiation, and survival of many cell types including human and murine hematopoietic stem/progenitor cells (32, 33, 38, 39, 47–50). Knockout mice deficient in SDF-1 exhibit disturbed hematopoiesis and knockout mice deficient in the CXCR4 receptor die in utero (51, 52), underlining their importance.

SDF-1 is highly conserved between mice and humans (53, 54), mediating its effect through the CXCR4 receptor that is expressed on $CD34^+$ HSCs, mononuclear leukocytes, and a variety of stromal cells (53). CXCR4 is a G-protein-coupled, 7-transmembrane receptor and is the only known receptor for SDF-1 (55). The interaction between SDF-1 and CXCR4 has been demonstrated to trigger multiple intracellular signals, including calcium mobilization and phosphorylation of adhesion components such as extracellular signal-regulated kinases 1 and 2 (ERK-1 and -2), proline-rich tyrosine kinase 2 (Pyk-2), focal adhesion kinase (FAK), and protein kinase C (PKC) (56, 57) In the adult BM, release of HSCs into the peripheral circulation is controlled in part by a concentration gradient of SDF-1 established within the BM microenvironment (39, 58, 59). Reduction of BM SDF-1 levels has been shown to result in release of HSC into the peripheral circulation, an effect mediated partly by granulocyte colony-stimulating factor (G-CSF), which induces the release and proliferation of neutrophil proteases such as elastase, cathepsin G, and MMPs (33). Increased expression of SDF-1 in the peripheral circulation facilitates further mobilization of HSCs down a concentration gradient (60).

Several reports demonstrate increased circulating plasma levels of SDF-1 in autoimmune and viral diseases, in conjunction with increased expression of SDF-1 in the parenchyma of rejecting liver transplants and viral/autoimmune liver diseases (31, 61). These observations have been reported in murine liver injury models (12, 30), suggesting that liver injury may, by the

TABLE 1. *SDF-1 expression in normal human tissue*

Tissue type	Cell line SDF-1 expression
Bone marrow	Stromal cell lines
Tonsil	Epithelial cells in tonsillar crypt
Spleen	Reticular cells
Fetal liver	Mesothelial cells, biliary epithelium, ductal plate
Adult liver	Biliary epithelium
Lung	Interstitial cells
Cardiac	Cardiac myocytes
Brain	Glial cells, cortical neuronal cells, astrocytes
Muscle	Skeletal myocytes
Skin	Epithelial cells of sweat glands, endothelial cells, pericytes, dendritic cells
Thymus	Stromal cells, medullary cells, epithelial cells

expression of SDF-1, produce a concentration gradient between liver and BM, which in turn facilitates the recruitment of inflammatory cells and HSCs from the BM into the circulation and then into the liver (12, 31) (see **Fig. 1*A–C***).

The mechanism by which SDF-1 influences HSC mobilization is unclear, although it is thought to involve specific changes to the adhesion of progenitor cells to the BM microenvironment via the modulation of adhesion molecules such as the integrin-dependent very late antigen-4 (VLA-4) (62). In vitro there is an increased trans-endothelial migration of human progenitor cells toward a gradient of SDF-1 (48, 63), and SDF-1 has been shown to promote the survival of circulating $CD34^+$ HSCs by counteracting apoptosis via the activation of the phosphotidyl inositol 3 kinase (PI3-K)/Akt pathway (64).

It has been speculated that the release of proteolytic enzymes and chemokines from injured liver into the circulation could also facilitate mobilization and recruitment of HSCs (12). Studies with G-CSF have revealed neutrophil proteolytic enzymes such as elastase, cathepsin G, and MMPs, including MMP-2 and MMP-9, result in the proteolytic degradation of SDF-1 in the BM, thus facilitating the release of stem cells (33, 65). MMPs degrade extracellular matrix proteins and are known to play important roles in tissue inflammation, tumor growth, and organ remodeling (66, 67). MMPs are secreted as zymogens (pro-MMPs) that are activated by a variety of proteinases and inhibited by tissue inhibitors of metalloproteinases (TIMPs) and α2-macroglobulin (66). In humans, MMP-9 is produced in a wide variety of cells types such as neutrophils, progenitor cells, endothelial cells, fibroblasts, connective tissue cells, tumor cells, and parenchymal cells, including the liver (66, 68). Human and animal studies have demonstrated that MMP-9 promotes the release of progenitor cells from the BM into the circulation by *1)* inducing the release of soluble kit-ligand (sKitL) from BM stromal cells, which accelerates the proliferation and migration of HSCs, *2)* cleaving the interaction of adhesion molecules VLA-4/vascular cell adhesion molecule-1 (VCAM-1) between stromal cells and HSCs in the BM, and *3)* enhancing the SDF-1 induced migration potential of HSCs across the subendothelial basement membrane (38, 69–71). In addition, MMP-9-induced recruitment of HSCs may occur via other mechanisms such as the shedding of membrane-bound stem cell factor (SCF) and the secretion of MMP-9 by progenitor cells in response to SDF-1 stimulation (70, 71). MMP-9 has been demonstrated to have an active involvement in liver remodeling in cirrhosis and inflammation as well as regulating hepatocyte regeneration after partial hepatectomy (72–74).

Human studies have demonstrated elevated serum and plasma MMP-9 levels in various types of liver injury including acute allograft rejection (75), ischemic reperfusion injury (76, 77), chronic viral hepatitis (78, 79), and alcoholic liver cirrhosis (80), suggesting there is a correlation between disease severity/progression and MMP-9 expression. In these studies, 70–80% of the serum and plasma MMP-9 measured, appeared in the active complex form and could be detected in serum samples from as early as 30 min and >1 wk after an acute injury process. In chronic liver diseases such as alcoholic cirrhosis, persistently elevated plasma activities of MMP-9 have been demonstrated, suggesting its expression reflects a process of ongoing extracellular matrix remodeling (80). Carbon tetrachloride (CCl4) -induced liver injury studies in rats and NOD/SCID mice (in which bone marrow cells were seen to transdifferentiate into hepatocytes) demonstrated an increased expression and activation of MMP-9 in the liver, suggesting that this factor could potentially be involved

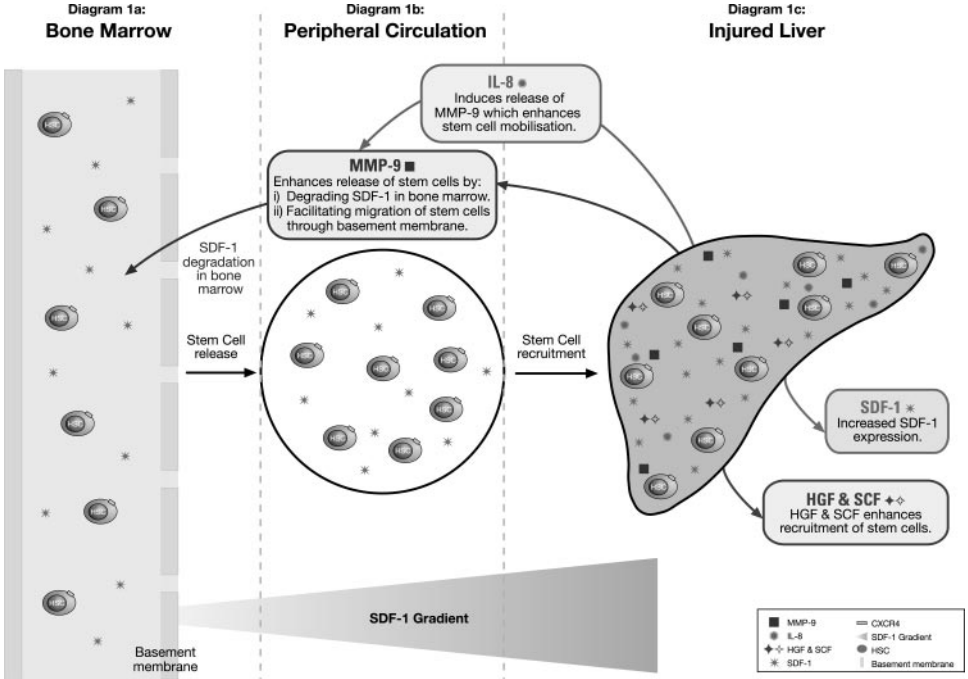

Figure 1. *A)* Release of HSCs from bone marrow into peripheral circulation mediated via SDF-1 concentration gradient. *B)* Release of HSCs into peripheral circulation enhanced via MMP-9 and IL-8. *C)* Recruitment of HSCs into injured liver mediated via SDF-1, HGF, and SCF.

in the stress-induced recruitment of HSCs from the BM to the injured liver (12, 81). A recent study by Hanumegowda et al. (82) has demonstrated an increased activation of MMP-9 in the livers of rats with monocrotaline-induced liver injury (which inhibits hepatocyte proliferation and promotes an HOC response). This increase in MMP-9 activity was produced from either the endothelial cells or from an activation or influx of inflammatory cells into the injured hepatic parenchyma (82). In a study by Watanabe et al, mice were injected with anti-Fas antibody (Jo2) to induce an acute hepatitis, demonstrating that MMP-9 expression in the circulation was elevated and accompanied by the recruitment of HSCs from the BM into the circulation (83).

Interactions between MMP-9 and other chemokines such as IL-8 have been demonstrated in mobilization studies whereby MMP-9 is rapidly induced in neutrophils after exposure to IL-8 and resulting in the release of HSCs into the peripheral circulation (84–87) (see Fig. 1*B*). Elevated IL-8 levels have been demonstrated in the circulation and hepatic parenchyma of many human liver conditions including alcoholic hepatitis, viral hepatitis, chronic alcoholic liver disease, and acute graft-vs.-host disease after liver transplantation (88–92). Thus IL-8, a known neutrophil chemoattractant in liver disease, also has the potential to induce the release of HSCs into the peripheral circulation via an indirect mechanism requiring the activation of circulating neutrophils and the release of MMP-9 (86).

Hepatic recruitment of HSCs in liver injury (Fig. 1C)

Kollet et al. have recently demonstrated the key role that SDF-1/CXCR4-mediated signaling plays in the migration of human progenitors to the murine liver. Neutralization of the CXCR4 receptor with an anti-CXCR4 antibody significantly inhibited the homing of human cord blood or mobilized peripheral blood $CD34^+$ stem cells to the liver of irradiated NOD/SCID mice (12). Furthermore, injection of human SDF-1 into the murine liver parenchyma further enhanced the hepatic migration of human stem cells. SDF-1 expression has been reported in a variety of liver and nonliver conditions such as liver allograft rejection (61), viral and autoimmune liver diseases (12, 31), ischemic brain injury (93), myocardial infarction (94), inflammatory skin conditions (44), and BM injury induced by total body irradiation or chemotherapy (95). It is unclear whether this expression is an attempt to recruit inflammatory cells or HSCs toward the damaged organ or is indeed entirely unrelated.

SDF-1 expression in rejecting liver transplants and viral/autoimmune liver diseases was seen to be confined to the biliary epithelium and other nonparenchymal cells, thus promoting the retention of $CXCR4^+$ lymphocytes and possibly HSCs in the portal tracts (12, 31, 61). Hatch et al. (30) were able to demonstrate that SDF-1 protein was up-regulated in the membrane fraction of the whole liver lysates. Notably, however, this was only the case in animals that had undergone HOC regeneration models [partial hepatectomy (PH) and 2-acetylaminofluorene (2-AAF) or 2-AAF and CCl4]. Animals that had undergone non-oval cell regeneration models of PH, CCl4 alone, and 2-AAF alone did not produce SDF-1 protein. Immunohistochemistry on the oval cell regeneration model liver sections revealed increased expression of SDF-1 in the hepatocytes adjacent to the proliferating oval cells and positive CXCR4 staining on these oval cells. These data argue for the defined production of SDF-1 in forms of liver injury that may be attempting to recruit HSCs to the reparative process.

The cytokine HGF, which is produced in the nonparenchymal perisinusoidal cells of the liver and induces hepatocyte proliferation, may also be involved in the migration and differentiation of HSC into the injured liver (12, 96). Increased expression of HGF has been demonstrated in CCl4-induced liver injury and in rodent HOC regeneration models, suggesting it is involved in stem cell proliferation, migration, and differentiation (22, 97). Kollet et al. recently demonstrated that after liver injury, levels of HGF were increased and contributed to the recruitment of human $CD34^+$ stem cells to the injured liver (12) by increasing the motility of human progenitors and in synergy with SCF potentiated both CXCR4- and SDF-1-induced directional migration.

CONCLUSIONS

Many concepts regarding stem cell migration and plasticity come from studies of multipotent hematopoietic stem cells and the molecular pathways of hematopoiesis (98). There is now increasing evidence to suggest that liver injury induces the expression and secretion of signaling mediators such as SDF-1, IL-8, MMPs, HGF, and SCF, which facilitate the homing and engraftment of HSCs to the liver (12, 30, 31). Factors regulating long-term engraftment and differentiation of HSCs into hepatocytes are yet to be defined, although chemokines, adhesion molecules, and extracellular matrix factors would appear to have an important role to play. A better understanding of the factors regulating HSC homing, subsequent engraftment into the liver, and finally differentiation into hepatocytes are essential if the potential therapeutic manipulation of HSCs to treat liver disease is to be realized. FJ

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REFERENCES

1. Petersen, B. E., Bowen, W. C., Patrene, K. D., Mars, W. M., Sullivan, A. K., Murase, N., Boggs, S. S., Greenberger, J. S., and

Goff, J. P. (1999) Bone marrow as a potential source of hepatic oval cells. *Science* **284,** 1168–1170
2. Alison, M. R., Poulsom, R., Jeffery, R., Dhillon, A. P., Quaglia, A., Jacob, J., Novelli, M., Prentice, G., Williamson, J., and Wright, N. A. (2000) Hepatocytes from non-hepatic adult stem cells. *Nature (London)* **406,** 257
3. Theise, N. D., Nimmakayalu, M., Gardner, R., Illei, P. B., Morgan, G., Teperman, L., Henegariu, O., and Krause, D. S. (2000) Liver from bone marrow in humans. *Hepatology* **32,** 11–16
4. Ferrari, G., Cusella-De Angelis, G., Coletta, M., Paolucci, E., Stornaiuolo, A., Cossu, G., and Mavilio, F. (1998) Muscle regeneration by bone marrow-derived myogenic progenitors. *Science* **279,** 1528–1530
5. Li, Y., Chen, J., Wang, L., Lu, M., and Chopp, M. (2001) Treatment of stroke in rat with intracarotid administration of marrow stromal cells. *Neurology* **56,** 1666–1672
6. Korbling, M., Katz, R. L., Khanna, A., Ruifrok, A. C., Rondon, G., Albitar, M., Champlin, R. E., and Estrov, Z. (2002) Hepatocytes and epithelial cells of donor origin in recipients of peripheral-blood stem cells. *N. Engl. J. Med.* **346,** 738–746
7. Jackson, K. A., Majka, S. M., Wang, H., Pocius, J., Hartley, C. J., Majesky, M. W., Entman, M. L., Michael, L. H., Hirschi, K. K., and Goodell, M. A. (2001) Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. *J. Clin. Invest.* **107,** 1395–1402
8. Alison, M. R., Poulsom, R., and Forbes, S. J. (2001) Update on hepatic stem cells. *Liver* **21,** 367–373
9. Alison, M. (1998) Liver stem cells: a two compartment system. *Curr. Opin. Cell Biol.* **10,** 710–715
10. Thorgeirsson, S. S. (1996) Hepatic stem cells in liver regeneration. *FASEB J.* **10,** 1249–1256
11. Sell, S. (2001) Heterogeneity and plasticity of hepatocyte lineage cells. *Hepatology* **33,** 738–750
12. Kollet, O., Shivtiel, S., Chen, Y. Q., Suriawinata, J., Thung, S. N., Dabeva, M. D., Kahn, J., Spiegel, A., Dar, A., Samira, S., et al. (2003) HGF, SDF-1, and MMP-9 are involved in stress-induced human CD34+ stem cell recruitment to the liver. *J. Clin. Invest.* **112,** 160–169
13. Lagasse, E., Connors, H., Al Dhalimy, M., Reitsma, M., Dohse, M., Osborne, L., Wang, X., Finegold, M., Weissman, I. L., and Grompe, M. (2000) Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. *Nat. Med.* **6,** 1229–1234
14. Theise, N. D., Badve, S., Saxena, R., Henegariu, O., Sell, S., Crawford, J. M., and Krause, D. S. (2000) Derivation of hepatocytes from bone marrow cells in mice after radiation-induced myeloablation. *Hepatology* **31,** 235–240
15. Austin, T. W., and Lagasse, E. (2003) Hepatic regeneration from hematopoietic stem cells. *Mech. Dev.* **120,** 131–135
16. Terada, N., Hamazaki, T., Oka, M., Hoki, M., Mastalerz, D. M., Nakano, Y., Meyer, E. M., Morel, L., Petersen, B. E., and Scott, E. W. (2002) Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. *Nature (London)* **416,** 542–545
17. Ying, Q. L., Nichols, J., Evans, E. P., and Smith, A. G. (2002) Changing potency by spontaneous fusion. *Nature (London)* **416,** 545–548
18. Wang, X., Willenbring, H., Akkari, Y., Torimaru, Y., Foster, M., Al Dhalimy, M., Lagasse, E., Finegold, M., Olson, S., and Grompe, M. (2003) Cell fusion is the principal source of bone-marrow-derived hepatocytes. *Nature (London)* **422,** 897–901
19. Ishikawa, F., Drake, C. J., Yang, S., Fleming, P., Minamiguchi, H., Visconti, R. P., Crosby, C. V., Argraves, W. S., Harada, M., Key, L. L., Jr., et al. (2003) Transplanted human cord blood cells give rise to hepatocytes in engrafted mice. *Ann. N.Y. Acad. Sci.* **996,** 174–185
20. Newsome, P. N., Johannessen, I., Boyle, S., Dalakas, E., McAulay, K. A., Samuel, K., Rae, F., Forrester, L., Turner, M. L., Hayes, P. C., et al. (2003) Human cord blood-derived cells can differentiate into hepatocytes in the mouse liver with no evidence of cellular fusion. *Gastroenterology* **124,** 1891–1900
21. Ianus, A., Holz, G. G., Theise, N. D., and Hussain, M. A. (2003) In vivo derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. *J. Clin. Invest.* **111,** 843–850
22. Wang, X., Ge, S., McNamara, G., Hao, Q. L., Crooks, G. M., and Nolta, J. A. (2003) Albumin-expressing hepatocyte-like cells develop in the livers of immune-deficient mice that received transplants of highly purified human hematopoietic stem cells. *Blood* **101,** 4201–4208
23. Kanazawa, Y., and Verma, I. M. (2003) Little evidence of bone marrow-derived hepatocytes in the replacement of injured liver. *Proc. Natl. Acad. Sci. USA* **100, Suppl. 1,** 11850–11853
24. Dahlke, M. H., Popp, F. C., Bahlmann, F. H., Aselmann, H., Jager, M. D., Neipp, M., Piso, P., Klempnauer, J., and Schlitt, H. J. (2003) Liver regeneration in a retrorsine/CCl4-induced acute liver failure model: do bone marrow-derived cells contribute? *J. Hepatol.* **39,** 365–373
25. Wu, T., Cieply, K., Nalesnik, M. A., Randhawa, P. S., Sonzogni, A., Bellamy, C., Abu-Elmagd, K., Michalopolous, G. K., Jaffe, R., Kormos, R. L., et al. (2003) Minimal evidence of transdifferentiation from recipient bone marrow to parenchymal cells in regenerating and long-surviving human allografts. *Am. J. Transplant.* **3,** 1173–1181
26. Sakaida, I., Terai, S., Yamamoto, N., Aoyama, K., Ishikawa, T., Nishina, H., and Okita, K. (2004) Transplantation of bone marrow cells reduces CCl4-induced liver fibrosis in mice. *Hepatology* **40,** 1304–1311
27. Hove, W. R., van Hoek, B., Bajema, I. M., Ringers, J., van Krieken, J. H., and Lagaaij, E. L. (2003) Extensive chimerism in liver transplants: vascular endothelium, bile duct epithelium, and hepatocytes. *Liver Transpl.* **9,** 552–556
28. Idilman, R., Erden, E., Kuzu, I., Ersoz, S., Karasu, Z., Karayalcin, K., Yuce, G., Tokat, Y., Sahin, Y., Tukun, A., et al. (2004) Recipient-derived hepatocytes in sex-mismatched liver allografts after liver transplantation: early versus late transplant biopsies. *Transplantation* **78,** 1647–1652
29. Ng, I. O., Chan, K. L., Shek, W. H., Lee, J. M., Fong, D. Y., Lo, C. M., and Fan, S. T. (2003) High frequency of chimerism in transplanted livers. *Hepatology* **38,** 989–998
30. Hatch, H. M., Zheng, D., Jorgensen, M. L., and Petersen, B. E. (2002) SDF-1alpha/CXCR4: a mechanism for hepatic oval cell activation and bone marrow stem cell recruitment to the injured liver of rats. *Cloning Stem Cells* **4,** 339–351
31. Terada, R., Yamamoto, K., Hakoda, T., Shimada, N., Okano, N., Baba, N., Ninomiya, Y., Gershwin, M. E., and Shiratori, Y. (2003) Stromal cell-derived factor-1 from biliary epithelial cells recruits CXCR4-positive cells: implications for inflammatory liver diseases. *Lab. Invest.* **83,** 665–672
32. Lapidot, T., and Petit, I. (2002) Current understanding of stem cell mobilization: the roles of chemokines, proteolytic enzymes, adhesion molecules, cytokines, and stromal cells. *Exp. Hematol.* **30,** 973–981
33. Petit, I., Szyper-Kravitz, M., Nagler, A., Lahav, M., Peled, A., Habler, L., Ponomaryov, T., Taichman, R. S., Arenzana-Seisdedos, F., Fujii, N., et al. (2002) G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. *Nat. Immunol.* **3,** 687–694
34. Grzelak, I., Olszewski, W. L., Zaleska, M., Ziolkowska, A., Durlik, M., Lagiewska, B., Muszynski, M., and Rowinski, W. (1998) Surgical trauma evokes a rise in the frequency of hematopoietic progenitor cells and cytokine levels in blood circulation. *Eur. Surg. Res.* **30,** 198–204
35. Lamming, C. E., Augustin, L., Blackstad, M., Lund, T. C., Hebbel, R. P., and Verfaillie, C. M. (2003) Spontaneous circulation of myeloid-lymphoid-initiating cells and SCID-repopulating cells in sickle cell crisis. *J. Clin. Invest.* **111,** 811–819
36. De Silvestro, G., Vicarioto, M., Donadel, C., Menegazzo, M., Marson, P., and Corsini, A. (2004) Mobilization of peripheral blood hematopoietic stem cells following liver resection surgery. *Hepatogastroenterology* **51,** 805–810
37. Dalakas, E., Newsome, P. N., Qing, L., Brown, R., McCall, S., Hayes, P. C., Harrison, D. J., and Plevris, J. N. (2003) Mobilisation of pluripotent haematopoietic stem cells occurs in alcoholic hepatitis and is associated with an improved clinical outcome. *Hepatology* **38, Suppl. 1,** 284A
38. Aiuti, A., Webb, I. J., Bleul, C., Springer, T., and Gutierrez-Ramos, J. C. (1997) The chemokine SDF-1 is a chemoattractant for human CD34+ hematopoietic progenitor cells and provides a new mechanism to explain the mobilization of CD34+ progenitors to peripheral blood. *J. Exp. Med.* **185,** 111–120
39. Kim, C. H., and Broxmeyer, H. E. (1998) In vitro behavior of hematopoietic progenitor cells under the influence of chemoattractants: stromal cell-derived factor-1, steel factor, and the bone marrow environment. *Blood* **91,** 100–110

40. Bajetto, A., Bonavia, R., Barbero, S., Piccioli, P., Costa, A., Florio, T., and Schettini, G. (1999) Glial and neuronal cells express functional chemokine receptor CXCR4 and its natural ligand stromal cell-derived factor 1. *J. Neurochem.* **73,** 2348–2357
41. Casamayor-Palleja, M., Mondiere, P., Amara, A., Bella, C., Dieu-Nosjean, M. C., Caux, C., and Defrance, T. (2001) Expression of macrophage inflammatory protein-3alpha, stromal cell-derived factor-1, and B-cell-attracting chemokine-1 identifies the tonsil crypt as an attractive site for B cells. *Blood* **97,** 3992–3994
42. Godiska, R., Chantry, D., Dietsch, G. N., and Gray, P. W. (1995) Chemokine expression in murine experimental allergic encephalomyelitis. *J. Neuroimmunol.* **58,** 167–176
43. Jiang, W., Zhou, P., Kahn, S. M., Tomita, N., Johnson, M. D., and Weinstein, I. B. (1994) Molecular cloning of TPAR1, a gene whose expression is repressed by the tumor promoter 12-O-tetradecanoylphorbol 13-acetate (TPA). *Exp. Cell Res.* **215,** 284–293
44. Pablos, J. L., Amara, A., Bouloc, A., Santiago, B., Caruz, A., Galindo, M., Delaunay, T., Virelizier, J. L., and Arenzana-Seisdedos, F. (1999) Stromal-cell-derived factor is expressed by dendritic cells and endothelium in human skin. *Am. J. Pathol.* **155,** 1577–1586
45. Tashiro, K., Tada, H., Heilker, R., Shirozu, M., Nakano, T., and Honjo, T. (1993) Signal sequence trap: a cloning strategy for secreted proteins and type I membrane proteins. *Science* **261,** 600–603
46. Zaitseva, M., Kawamura, T., Loomis, R., Goldstein, H., Blauvelt, A., and Golding, H. (2002) Stromal-derived factor 1 expression in the human thymus. *J. Immunol.* **168,** 2609–2617
47. D'Apuzzo, M., Rolink, A., Loetscher, M., Hoxie, J. A., Clark-Lewis, I., Melchers, F., Baggiolini, M., and Moser, B. (1997) The chemokine SDF-1, stromal cell-derived factor 1, attracts early stage B cell precursors via the chemokine receptor CXCR4. *Eur. J. Immunol.* **27,** 1788–1793
48. Mohle, R., Bautz, F., Denzlinger, C., and Kanz, L. (2001) Transendothelial migration of hematopoietic progenitor cells. Role of chemotactic factors. *Ann. N.Y. Acad. Sci.* **938,** 26–34
49. Whetton, A. D., and Graham, G. J. (1999) Homing and mobilization in the stem cell niche. *Trends Cell Biol.* **9,** 233–238
50. Bleul, C. C., Schultze, J. L., and Springer, T. A. (1998) B lymphocyte chemotaxis regulated in association with microanatomic localization, differentiation state, and B cell receptor engagement. *J. Exp. Med.* **187,** 753–762
51. Kawabata, K., Ujikawa, M., Egawa, T., Kawamoto, H., Tachibana, K., Iizasa, H., Katsura, Y., Kishimoto, T., and Nagasawa, T. (1999) A cell-autonomous requirement for CXCR4 in long-term lymphoid and myeloid reconstitution. *Proc. Natl. Acad. Sci. USA* **96,** 5663–5667
52. Nagasawa, T., Hirota, S., Tachibana, K., Takakura, N., Nishikawa, S., Kitamura, Y., Yoshida, N., Kikutani, H., and Kishimoto, T. (1996) Defects of B cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. *Nature (London)* **382,** 635–638
53. Kollet, O., Petit, I., Kahn, J., Samira, S., Dar, A., Peled, A., Deutsch, V., Gunetti, M., Piacibello, W., Nagler, A., et al. (2002) Human CD34(+)CXCR4(–) sorted cells harbor intracellular CXCR4, which can be functionally expressed and provide NOD/SCID repopulation. *Blood* **100,** 2778–2786
54. Sweeney, E. A., and Papayannopoulou, T. (2001) Increase in circulating SDF-1 after treatment with sulfated glycans. The role of SDF-1 in mobilization. *Ann. N.Y. Acad. Sci.* **938,** 48–52
55. Nagasawa, T., Tachibana, K., and Kawabata, K. (1999) A CXC chemokine SDF-1/PBSF: a ligand for a HIV coreceptor, CXCR4. *Adv. Immunol.* **71,** 211–228
56. Roland, J., Murphy, B. J., Ahr, B., Robert-Hebmann, V., Delauzun, V., Nye, K. E., Devaux, C., and Biard-Piechaczyk, M. (2003) Role of the intracellular domains of CXCR4 in SDF-1-mediated signaling. *Blood* **101,** 399–406
57. Wang, J. F., Park, I. W., and Groopman, J. E. (2000) Stromal cell-derived factor-1alpha stimulates tyrosine phosphorylation of multiple focal adhesion proteins and induces migration of hematopoietic progenitor cells: roles of phosphoinositide-3 kinase and protein kinase C. *Blood* **95,** 2505–2513
58. Bleul, C. C., Fuhlbrigge, R. C., Casasnovas, J. M., Aiuti, A., and Springer, T. A. (1996) A highly efficacious lymphocyte chemoattractant, stromal cell-derived factor 1 (SDF-1). *J. Exp. Med.* **184,** 1101–1109
59. Kim, C. H., Pelus, L. M., White, J. R., and Broxmeyer, H. E. (1998) Differential chemotactic behavior of developing T cells in response to thymic chemokines. *Blood* **91,** 4434–4443
60. Hattori, K., Heissig, B., Tashiro, K., Honjo, T., Tateno, M., Shieh, J. H., Hackett, N. R., Quitoriano, M. S., Crystal, R. G., Rafii, S., et al. (2001) Plasma elevation of stromal cell-derived factor-1 induces mobilization of mature and immature hematopoietic progenitor and stem cells. *Blood* **97,** 3354–3360
61. Goddard, S., Williams, A., Morland, C., Qin, S., Gladue, R., Hubscher, S. G., and Adams, D. H. (2001) Differential expression of chemokines and chemokine receptors shapes the inflammatory response in rejecting human liver transplants. *Transplantation* **72,** 1957–1967
62. Peled, A., Kollet, O., Ponomaryov, T., Petit, I., Franitza, S., Grabovsky, V., Slav, M. M., Nagler, A., Lider, O., Alon, R., et al. (2000) The chemokine SDF-1 activates the integrins LFA-1, VLA-4, and VLA-5 on immature human CD34 (+) cells: role in transendothelial/stromal migration and engraftment of NOD/SCID mice. *Blood* **95,** 3289–3296
63. Netelenbos, T., Zuijderduijn, S., Van Den, B. J., Kessler, F. L., Zweegman, S., Huijgens, P. C., and Drager, A. M. (2002) Proteoglycans guide SDF-1-induced migration of hematopoietic progenitor cells. *J. Leukoc. Biol.* **72,** 353–362
64. Lataillade, J. J., Clay, D., Bourin, P., Herodin, F., Dupuy, C., Jasmin, C., and Bousse-Kerdiles, M. C. (2002) Stromal cell-derived factor 1 regulates primitive hematopoiesis by suppressing apoptosis and by promoting G(0)/G(1) transition in CD34(+) cells: evidence for an autocrine/paracrine mechanism. *Blood* **99,** 1117–1129
65. Levesque, J. P., Hendy, J., Takamatsu, Y., Simmons, P. J., and Bendall, L. J. (2003) Disruption of the CXCR4/CXCL12 chemotactic interaction during hematopoietic stem cell mobilization induced by GCSF or cyclophosphamide. *J. Clin. Invest.* **111,** 187–196
66. Birkedal-Hansen, H., Moore, W. G., Bodden, M. K., Windsor, L. J., Birkedal-Hansen, B., DeCarlo, A., and Engler, J. A. (1993) Matrix metalloproteinases: a review. *Crit. Rev. Oral Biol. Med.* **4,** 197–250
67. Visse, R., and Nagase, H. (2003) Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. *Circ. Res.* **92,** 827–839
68. Geisler, S., Lichtinghagen, R., Boker, K. H., and Veh, R. W. (1997) Differential distribution of five members of the matrix metalloproteinase family and one inhibitor (TIMP-1) in human liver and skin. *Cell Tissue Res.* **289,** 173–183
69. Hattori, K., Heissig, B., Wu, Y., Dias, S., Tejada, R., Ferris, B., Hicklin, D. J., Zhu, Z., Bohlen, P., Witte, L., et al. (2002) Placental growth factor reconstitutes hematopoiesis by recruiting VEGFR1(+) stem cells from bone-marrow microenvironment. *Nat. Med.* **8,** 841–849
70. Heissig, B., Hattori, K., Dias, S., Friedrich, M., Ferris, B., Hackett, N. R., Crystal, R. G., Besmer, P., Lyden, D., Moore, M. A., et al. (2002) Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand. *Cell* **109,** 625–637
71. Janowska-Wieczorek, A., Marquez, L. A., Dobrowsky, A., Ratajczak, M. Z., and Cabuhat, M. L. (2000) Differential MMP and TIMP production by human marrow and peripheral blood CD34(+) cells in response to chemokines. *Exp. Hematol.* **28,** 1274–1285
72. Haruyama, T., Ajioka, I., Akaike, T., and Watanabe, Y. (2000) Regulation and significance of hepatocyte-derived matrix metalloproteinases in liver remodeling. *Biochem. Biophys. Res. Commun.* **272,** 681–686
73. Kuyvenhoven, J. P., Verspaget, H. W., Gao, Q., Ringers, J., Smit, V. T., Lamers, C. B., and van Hoek, B. (2004) Assessment of serum matrix metalloproteinases MMP-2 and MMP-9 after human liver transplantation: increased serum MMP-9 level in acute rejection. *Transplantation* **77,** 1646–1652
74. Lichtinghagen, R., Bahr, M. J., Wehmeier, M., Michels, D., Haberkorn, C. I., Arndt, B., Flemming, P., Manns, M. P., and Boeker, K. H. (2003) Expression and coordinated regulation of matrix metalloproteinases in chronic hepatitis C and hepatitis C virus-induced liver cirrhosis. *Clin. Sci. (London)* **105,** 373–382
75. Kuyvenhoven, J. P., Verspaget, H. W., Gao, Q., Ringers, J., Smit, V. T., Lamers, C. B., and van Hoek, B. (2004) Assessment of serum matrix metalloproteinases MMP-2 and MMP-9 after hu-

man liver transplantation: increased serum MMP-9 level in acute rejection. *Transplantation* **77,** 1646–1652
76. Kuyvenhoven, J. P., Molenaar, I. Q., Verspaget, H. W., Veldman, M. G., Palareti, G., Legnani, C., Moolenburgh, S. E., Terpstra, O. T., Lamers, C. B., van Hoek, B., et al. (2004) Plasma MMP-2 and MMP-9 and their inhibitors TIMP-1 and TIMP-2 during human orthotopic liver transplantation. The effect of aprotinin and the relation to ischemia/reperfusion injury. *Thromb. Haemost.* **91,** 506–513
77. Kuyvenhoven, J. P., Ringers, J., Verspaget, H. W., Lamers, C. B., and van Hoek, B. (2003) Serum matrix metalloproteinase MMP-2 and MMP-9 in the late phase of ischemia and reperfusion injury in human orthotopic liver transplantation. *Transplant. Proc.* **35,** 2967–2969
78. Leroy, V., Monier, F., Bottari, S., Trocme, C., Sturm, N., Hilleret, M. N., Morel, F., and Zarski, J. P. (2004) Circulating matrix metalloproteinases 1, 2, 9 and their inhibitors TIMP-1 and TIMP-2 as serum markers of liver fibrosis in patients with chronic hepatitis C: comparison with PIIINP and hyaluronic acid. *Am. J. Gastroenterol.* **99,** 271–279
79. Chung, T. W., Kim, J. R., Suh, J. I., Lee, Y. C., Chang, Y. C., Chung, T. H., and Kim, C. H. (2004) Correlation between plasma levels of matrix metalloproteinase (MMP) -9 /MMP-2 ratio and alpha-fetoproteins in chronic hepatitis carrying hepatitis B virus. *J. Gastroenterol. Hepatol.* **19,** 565–571
80. Kwon, O. S., Lim, d. Y., Kwon, K. A., Chung, M. G., Park, D. K., Kim, S. S., Kim, Y. S., Kwon, S. Y., Koo, Y. S., Kim, Y. K., Choi, D. J., Kim, J. H., Hwang, Y. J., Byun, K. S., and Lee, C. H. (2003) [Clinical usefulness of plasma activities of gelatinase (matrix metalloproteinase-2 and 9) in chronic liver disease]. *Taehan Kan Hakhoe. Chi* **9,** 222–230
81. Knittel, T., Mehde, M., Grundmann, A., Saile, B., Scharf, J. G., and Ramadori, G. (2000) Expression of matrix metalloproteinases and their inhibitors during hepatic tissue repair in the rat. *Histochem. Cell Biol.* **113,** 443–453
82. Hanumegowda, U. M., Copple, B. L., Shibuya, M., Malle, E., Ganey, P. E., and Roth, R. A. (2003) Basement membrane and matrix metalloproteinases in monocrotaline-induced liver injury. *Toxicol. Sci.* **76,** 237–246
83. Watanabe, Y., Haruyama, T., and Akaike, T. (2003) Liver-derived matrix metalloproteinase-9 (gelatinase B) recruits progenitor cells from bone marrow into the blood circulation. *Biol. Pharm. Bull.* **26,** 564–568
84. Carion, A., Benboubker, L., Herault, O., Roingeard, F., Degenne, M., Senecal, D., Desbois, I., Colombat, P., Charbord, P., Binet, C., et al. (2003) Stromal-derived factor 1 and matrix metalloproteinase 9 levels in bone marrow and peripheral blood of patients mobilized by granulocyte colony-stimulating factor and chemotherapy. Relationship with mobilizing capacity of haematopoietic progenitor cells. *Br. J. Haematol.* **122,** 918–926
85. Fibbe, W. E., Pruijt, J. F., Velders, G. A., Opdenakker, G., van Kooyk, Y., Figdor, C. G., and Willemze, R. (1999) Biology of IL-8-induced stem cell mobilization. *Ann. N.Y. Acad. Sci.* **872,** 71–82
86. Pruijt, J. F., Verzaal, P., van Os, R., de Kruijf, E. J., van Schie, M. L., Mantovani, A., Vecchi, A., Lindley, I. J., Willemze, R., Starckx, S., et al. (2002) Neutrophils are indispensable for hematopoietic stem cell mobilization induced by interleukin-8 in mice. *Proc. Natl. Acad. Sci. USA* **99,** 6228–6233
87. Van Zee, K. J., Fischer, E., Hawes, A. S., Hebert, C. A., Terrell, T. G., Baker, J. B., Lowry, S. F., and Moldawer, L. L. (1992) Effects of intravenous IL-8 administration in nonhuman primates. *J. Immunol.* **148,** 1746–1752
88. Hill, D. B., Marsano, L. S., and McClain, C. J. (1993) Increased plasma interleukin-8 concentrations in alcoholic hepatitis. *Hepatology* **18,** 576–580
89. Huang, Y. S., Chan, C. Y., Wu, J. C., Pai, C. H., Chao, Y., and Lee, S. D. (1996) Serum levels of interleukin-8 in alcoholic liver disease: relationship with disease stage, biochemical parameters and survival. *J. Hepatol.* **24,** 377–384
90. Sheron, N., Bird, G., Koskinas, J., Portmann, B., Ceska, M., Lindley, I., and Williams, R. (1993) Circulating and tissue levels of the neutrophil chemotaxin interleukin-8 are elevated in severe acute alcoholic hepatitis, and tissue levels correlate with neutrophil infiltration. *Hepatology* **18,** 41–46
91. Shimoda, K., Begum, N. A., Shibuta, K., Mori, M., Bonkovsky, H. L., Banner, B. F., and Barnard, G. F. (1998) Interleukin-8 and hIRH (SDF1-alpha/PBSF) mRNA expression and histological activity index in patients with chronic hepatitis C. *Hepatology* **28,** 108–115
92. Tilg, H., Ceska, M., Vogel, W., Herold, M., Margreiter, R., and Huber, C. (1992) Interleukin-8 serum concentrations after liver transplantation. *Transplantation* **53,** 800–803
93. Stumm, R. K., Rummel, J., Junker, V., Culmsee, C., Pfeiffer, M., Krieglstein, J., Hollt, V., and Schulz, S. (2002) A dual role for the SDF-1/CXCR4 chemokine receptor system in adult brain: isoform-selective regulation of SDF-1 expression modulates CXCR4-dependent neuronal plasticity and cerebral leukocyte recruitment after focal ischemia. *J. Neurosci.* **22,** 5865–5878
94. Pillarisetti, K., and Gupta, S. K. (2001) Cloning and relative expression analysis of rat stromal cell derived factor-1 (SDF-1)1: SDF-1 alpha mRNA is selectively induced in rat model of myocardial infarction. *Inflammation* **25,** 293–300
95. Ponomaryov, T., Peled, A., Petit, I., Taichman, R. S., Habler, L., Sandbank, J., Arenzana-Seisdedos, F., Magerus, A., Caruz, A., Fujii, N., et al. (2000) Induction of the chemokine stromal-derived factor-1 following DNA damage improves human stem cell function. *J. Clin. Invest.* **106,** 1331–1339
96. Kiss, A., Schnur, J., Szabo, Z., and Nagy, P. (2001) Immunohistochemical analysis of atypical ductular reaction in the human liver, with special emphasis on the presence of growth factors and their receptors. *Liver* **21,** 237–246
97. Alison, M. R., Poulsom, R., Jeffery, R., Anilkumar, T. V., Jagoe, R., and Sarraf, C. E. (1993) Expression of hepatocyte growth factor mRNA during oval cell activation in the rat liver. *J. Pathol.* **171,** 291–299
98. Fuchs, E., and Segre, J. A. (2000) Stem cells: a new lease on life. *Cell* **100,** 143–155

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