Commentary

Molecular Regulation of Hepatocyte Generation in Adult Animals

Joe W. Grisham and William B. Coleman

*From the Department of Pathology and Laboratory Medicine,
University of North Carolina at Chapel Hill, Chapel Hill,
North Carolina*

In this issue of *The American Journal of Pathology*, Bisgaard et al report the expression of the gp340/DMBT1 gene in oval cells proliferating in rat liver during the generation of new lineages of hepatocytes from stem/progenitor cells, but not in the new hepatocytes themselves.¹ Bisgaard et al cloned and sequenced the gp340/DMBT1 gene that is expressed in rat liver oval cells, demonstrating that it is a splice variant of a gene family that codes for a class of related proteins that includes the opsonin receptor, which participates in innate immunity, and the ductin/ebnerin/hensin group of proteins that modulate the differentiation and function of several types of epithelial cells. The gp340/DMBT1 gene, which may have a critical role in regulating the differentiation of hepatocytes from oval cells, joins several other immune modulating genes that participate in the molecular regulation of hepatocyte generation in adult animals. In this commentary we attempt to place this new information in the context of current insights into the molecular regulation of hepatocyte formation in livers of adult animals.

In terms of ongoing cell proliferation the liver is usually a static tissue in adult animals. Hepatocytes, responsible for essential hepatic functions, are long-lived in the absence of their unexpected demise as a result of physical trauma or toxicity. It is a measure of both the absolute necessity of maintaining adequate hepatic function for the sustenance of life and of the many agents and conditions in an animal's environment that can kill hepatocytes, that animals have evolved several cellular mechanisms to generate new hepatocytes and different regulatory pathways to control these mechanisms. New hepatocytes can be generated efficiently in adult animals by temporarily reactivating the proliferative cycle in fully differentiated, mitotically quiescent hepatocytes and by developing entirely new hepatocyte lineages from liver stem/progenitor cells.

A few hepatocytes may also be generated from bile duct epithelial cells² and putative bone marrow stem cells^{3,4} by direct phenotypic transition. Formation of hepatocytes by phenotypic transition from bile duct epithelial cells, variously termed modulation, transdifferentiation, or metaplasia, usually yields only sparse hepatocytes located in ducts among biliary epithelial cells, and may represent only a pathological curiosity of little functional significance. Transdifferentiation of hepatocytes from putative bone marrow stem cells has not yet been shown to result in significant regeneration of the hepatocyte population under most experimental conditions. Furthermore, the molecular mechanisms that regulate hepatocyte transdifferentiation from either bile duct epithelial or bone marrow cells are unknown.

In contrast, generation of new hepatocytes by amplifying the number of existing hepatocytes can significantly increase the functional capacity of the liver. The molecular regulation of hepatocyte formation differs radically depending on whether the new hepatocytes are added to an intact liver to meet increased functional demands in the absence of overt liver damage, or whether the production of new hepatocytes results from a deficit in liver function due to traumatic or toxic reduction in hepatocyte number and tissue mass. Formation of additional hepatocytes in an intact liver represents an important mechanism to augment hepatic function in response to both physiological and pathological demands for increased metabolic capacity. Physiological demands for increased hepatic function may reflect additional metabolic needs resulting from increased body mass or pregnancy, for example. Also, the hepatotoxic potential of some chemicals may be countered by adding new hepatocytes, thereby increasing the capacity of the liver to metabolically eliminate them before toxicity occurs. Some of these protoxic chemicals directly stimulate hepatocyte proliferation before toxicity causes hepatocyte necrosis. A common molecular pathway regulates the formation of new hepatocytes in intact, undamaged livers in both physiological and pathological circumstances, namely, by the binding of specific ligands (primary mitogens) to cognate nuclear receptors.⁵ Ligands for nuclear receptors include certain hormones (including thyroxine, sex steroids, and

Supported by NIH grant CA29323.

Accepted for publication August 1, 2002.

Address reprint requests to Joe W. Grisham, M.D., Department of Pathology and Laboratory Medicine, CB # 7525, University of North Carolina at Chapel School of Medicine, Chapel Hill, NC 27599. E-mail: jwg@med.unc.edu.

adrenal corticoids) and various chemicals (including peroxisome proliferators, all-trans retinoic acid, 9-*cis*-retinoic acid, and vitamin D).⁶ Ligands have been identified for over 40 cognate nuclear receptors, and there are several orphan receptors for which specific ligands have not yet been discovered.⁶ Nuclear receptors with their bound ligands function as transcription factors that directly stimulate the expression of the combination of genes needed to enable quiescent hepatocytes to proliferate, without the participation of either activated surface receptors (including receptors for proinflammatory cytokines and mitogenic growth factors) or cytoplasmic signaling cascades.^{7,8} Ligand/nuclear receptor combinations directly up-regulate the expression of cyclin D to drive hepatocytes through the mitotic cycle.^{9,10}

The need for new hepatocytes in livers of adult animals is often triggered by loss of liver tissue as a consequence of trauma or toxicity, a situation involved in most acute and chronic hepatic diseases. Experimental models used to investigate the molecular regulation of hepatocyte formation following loss of liver tissue include surgical partial hepatectomy (PHx) or dosing with the potent hepatotoxin, carbon tetrachloride (CCl₄). Following either PHx or CCl₄, new hepatocytes are generated by the proliferation of the residual hepatocytes that remain after tissue loss.^{11,12} However, new hepatocytes also develop through the establishment of new lineages from liver stem/progenitor cells, rather than from already existing hepatocytes, when either PHx or CCl₄ is combined with treatments that prevent residual hepatocytes from completing a proliferative cycle.^{13,14} Exposure of experimental animals to 2-acetyl aminofluorene (AAF) or to the pyrrolizidine alkaloid, retrorsine (RET), before PHx or CCl₄ blocks the ability of residual hepatocytes to proliferate, apparently as a consequence of chemically cross-linking nuclear DNA.

Since Bisgaard et al¹ used both the AAF/PHx and RET/PHx models it may be useful to describe some of the differences in the new cell populations that characterize each model. When rats are first exposed to AAF or RET and subsequently subjected to PHx or CCl₄, new hepatocyte lineages are generated from small, poorly differentiated precursor cells that proliferate rapidly and ultimately acquire the differentiated properties of mature hepatocytes. The morphological forms of the small, poorly differentiated cells that ultimately acquire the phenotype of mature hepatocytes differ somewhat depending on whether AAF or RET is used. In the model using AAF, the new population is composed predominantly of small, rapidly proliferating oval cells (named after their ovoid nuclei), initially located near portal tracts, but later migrating into the lobular parenchyma among residual hepatocytes.^{13,14} Oval cells, probably derived from cells located in the terminal biliary ductules, form tangled duct-like structures that are continuous with both bile canaliculi and bile ducts in portal tracts.¹⁵ (The oval cell reaction is also termed the ductular reaction, as in the paper of Bisgaard et al¹) Foci of small, incompletely differentiated hepatocyte-like cells, sometimes called intermediate cells, develop among oval (ductular) cells and gradually

acquire hepatocyte differentiation as oval cells regress.^{16,17}

When RET is used only sparse oval cells appear around portal tracts, and they never accumulate in a large population as in AAF-exposed rats. The most prominent new cell population in the RET model is composed of small, incompletely differentiated hepatocyte-like cells, termed small hepatocyte precursor cells (SPHC), which form foci and nodules among residual hepatocytes, usually in the apparent absence of oval cells.¹⁸ SPHC proliferate rapidly, quickly acquire the fully differentiated hepatocyte phenotype, and efficiently restore hepatocyte numbers (and liver mass).¹⁹

The small, incompletely differentiated hepatocyte-like cells that develop in both AAF and RET models may be derived from a common stem cell source with oval cells as a transitional population in both of these circumstances. In the AAF model oval cells may accumulate because their differentiation into small hepatocytes is slow relative to the RET model. This hypothesis of variation of cell flux between adjacent cellular compartments in the two models is supported by studies in the AAF model showing that the extent of oval cell accumulation and hepatocyte formation are reciprocally related to AAF dose: high AAF doses yield maximal accumulation of oval cells and less prominent foci of small hepatocytes, while fewer oval cells and more prominent foci of new hepatocytes develop at lower doses of AAF.²⁰ Although further study is required to validate or refute this kinetic hypothesis, it is consistent with the results reported by Bisgaard et al.¹

Current evidence suggests that regulation of hepatocyte formation from residual hepatocytes in the PHx or CCl₄ models centrally involves tumor necrosis factor- α (TNF- α), acting through the TNF-1 receptor (TNF-1R), and interleukin-6 (IL-6), acting through the binding of IL-6 to membrane-anchored binding glycoprotein, gp80 (IL-6R) and the association of IL-6/IL-6R with the signal transducer, gp130.^{12,21} Binding of TNF- α to TNF-R1 up-regulates the NF- κ B transcription factor,¹² activates the hepatic acute phase reaction,²² and stimulates the production of IL-6 by Kupffer cells.^{23,24} The IL-6/IL-6R/gp130 complex energizes the JAK-STAT cytoplasmic signaling pathway and phosphorylates STAT3.^{25,26} This pathway primes residual hepatocytes,¹² a step that moves them from G₀ to G₁ stages of the cell cycle and enables them to respond to secondary stimulation by mitogenic growth factors.^{11,12} Autocrine and paracrine stimulation of cognate receptors by tumor growth factor- α (TGF- α), epidermal growth factor (EGF), and hepatocyte growth factor (HGF), among others, activates the Ras-MAP kinase pathway and up-regulates *c-myc*, cyclins, and other genes to drive affected hepatocytes through the proliferative cycle.^{11,12} In mice lacking the ability to express TNF- α , TNF-1R, and/or IL-6 because of gene deletion (knockout), the ability of residual hepatocytes to respond to loss of liver tissue by generating new hepatocytes is greatly reduced and delayed.²³⁻²⁶ Replacing IL-6 in mice with deficiencies in TNF- α , TNF-R1 and/or IL-6 largely reverses the effects that deletion of each of these genes has on hepatocyte proliferation, suggesting that IL-6 is

located downstream from TNF- α in the regulatory pathway.^{23,25} This opinion is consistent with the observation that TNF- α stimulates the expression of IL-6.²² Nevertheless, this regulatory pathway appears to have redundant regulatory elements since deficits of hepatocytes are slowly replaced even in animals in which the TNF- α or IL-6 genes are deleted.

Other immunoregulatory elements are also involved in the regulation of hepatocyte proliferation after liver tissue loss. Liver regeneration is inhibited in germ-free and lipopolysaccharide-insensitive mice,²⁷ suggesting that LPS (endotoxin) from the gut may entrain the entire process of hepatocyte proliferation following PHx or CCl₄, perhaps by up-regulating TNF- α . Inflammatory cytokines in addition to TNF- α and IL-6 and proteins that are up-regulated in the liver during the acute phase reaction also take part in this complex regulatory pathway. Proliferation of residual hepatocytes is greatly impaired following liver necrosis induced by CCl₄ in mice that are deficient in complement factor 5 (C5) as the result of gene mutation.²⁸ Replacement of either C5 or its lytic product, C5a, corrects the defect in hepatocyte proliferation in C5-deficient mice.²⁸ Additionally, the action of IL-6 in priming residual hepatocytes is modulated by soluble IL-6R (sIL-6R).²⁹ IL-6R is clipped from its membrane-anchored sites by C-reactive protein, an acute phase protein synthesized by hepatocytes.³⁰ Binding of sIL-6R to IL-6 allows direct association of the complex with gp130 and results in an enhanced response to IL-6.³⁰ Intrahepatic natural killer T lymphocytes may also play a regulatory role in the generation of new hepatocytes following loss of liver tissue,³¹ perhaps by a mechanism involving Fas/FasR. Even though much is known about the regulation of hepatocyte generation from residual hepatocytes by proinflammatory cytokines and mitogenic growth factors, much information is still lacking and additional studies of these models are needed to further elucidate this complex molecular regulatory pathway.

Many studies have investigated genes and proteins that are expressed during the generation of new hepatocyte lineages from stem/progenitor cells in the AAF/PHx and RET/PHx models, but a coherent regulatory pathway has not yet been developed. As one might expect, many of the same immunoregulatory molecules and acute phase proteins that participate in the regulation of hepatocyte generation from residual hepatocytes in the PHx and CCl₄ models also appear to be involved in the related models for generating new hepatocyte lineages from stem/progenitor cells. Indirect evidence suggests that IL-6 is required for proliferation of both oval cells and SHPCs. Treatment of animals with dexamethasone, which blocks the expression of IL-6, simultaneously delays or prevents the proliferation of oval cells, blocks the emergence of small hepatocytes, and slows the replacement of residual hepatocytes.^{32,33}

Oval cells express several genes/proteins that are not expressed by differentiated hepatocytes and vice versa, some of which may be involved in regulating hepatocyte differentiation. For example, among molecules that are expressed only in oval cells but not in hepatocytes is stem cell factor and its cognate receptor, c-kit,³⁴ both of

which are universal features of classic stem cells and their immediate progeny in other tissues, such as bone marrow. Preliminary studies also suggest that oval cells do not express TNF-1R, which is first detected in intermediate cells or small hepatocytes (SS Thorgeirsson, personal communication). Thorgeirsson has proposed that an expression switch simultaneously down-regulating c-kit and up-regulating TNF-1R may be important in controlling the hepatocytic differentiation of oval cells (SS Thorgeirsson, personal communication). The finding that gp340/DMBT1 is highly expressed in oval cells and that expression of this gene is eclipsed in small hepatocytes may provide another key to ultimately unraveling the molecular pathway that regulates the generation of hepatocytes from stem/progenitor cells. This possibility is made more attractive by data provided by Bisgaard et al, derived from cloning and sequencing the gp340/DMBT1 gene expressed in rat oval cells.¹ Rat gp340/DMBT1 is homologous to opsonin receptor, providing a connection with other immunoregulatory genes/proteins that are also involved in regulating the formation of new hepatocytes from residual hepatocytes and stem/progenitor cells. The gp340/DMBT1 gene family also codes for proteins that regulate the differentiation of other types of epithelial cells. Further investigation of the role of gp340/DMBT1 in regulating the proliferation and differentiation of liver stem/progenitor cells may be critical to understanding how this cellular process is regulated.

Since the loss of liver tissue from trauma or toxicity stimulates the hepatic acute phase reaction,²² it is perhaps not too surprising that the molecular pathways that regulate the formation of new hepatocytes in the PHx and CCl₄ models, with or without AAF or RET, use proinflammatory cytokines and the proteins that are up-regulated in the liver as a part of the acute phase reaction. Indeed, repair of the liver after loss of tissue is an acute phase response in which Kupffer cells, stellate cells, sinusoidal endothelial cells, and, perhaps, inflammatory cells, interact with residual hepatocytes to generate new hepatocytes. Future studies on the molecular pathways that regulate the formation of hepatocytes after cell loss will do well to continue to investigate other elements that are shared with the regulation of the immune system.

References

1. Bisgaard HC, Holmskov U, Santoni-Rugiu E, Nagy P, Nielsen O, Ott P, Hage E, Dalhoff K, Rasmussen LJ, Tygstrup N: Heterogeneity of ductular reactions in adult rat and human liver revealed by novel expression of deleted in malignant brain tumor 1. *Am J Pathol* 2002, 161:1187–1198
2. Sirica AE: Ductular hepatocytes. *Histol Histopathol* 1995, 10:433–456
3. Petersen BF, Bowen WC, Mars WM, Sullivan AK, Murase N, Boggs SS, Greenberger JS, Goff JP: Bone marrow as a potential source of hepatic oval cells. *Science* 1999, 284:1168–1170
4. Thiese ND, Badve S, Saxena R, Henegariu O, Sell S, Crawford JM, Krause DS: Derivation of hepatocytes from bone marrow cells in mice after irradiation-induced myeloablation. *Hepatology* 2000, 31:235–240
5. Columbano A, Shinozuka H: Liver regeneration versus direct hyperplasia. *EMBO J* 1996, 15:1118–1128
6. Mangelsdorf DJ, Thummel C, Beato M, Herlich P, Schutz G, Umesano K, Blumberg B, Kastner P, Mark M, Chambon P, Evans RM: The

- nuclear receptor superfamily: the second decade. *Cell* 1995, 83:835–839
7. Columbano A, Ledda-Columbano GM, Pibiri M, Piga R, Shinozuka H, DeLuca V, Cerignol F, Tripodi M: Increased expression of c-fos, c-jun, and LRF-1 is not required for in vivo priming of hepatocytes by the mitogen TCPBOP. *Oncogene* 1997, 14:857–863
8. Menegazzi M, Carcereri-DePrati A, Suzuki H, Shinozki H, Pibiri M, Piga R, Columbano A, Ledda-Columbano GM: Liver cell proliferation induced by nafenopin and cyproterone acetate is not associated with increases in activation of transcription factors NF- κ B and AP-1 or with activation of tumor necrosis factor α . *Hepatology* 1997, 25:585–592
9. Ledda-Columbano GM, Pibiri M, Loi R, Perra A, Shinozuka H, Columbano A: Early increase in cyclin D-1 expression and accelerated entry of mouse hepatocytes into S phase after administration of the mitogen 1, 4-bis[2-(3, 5-dichloropyridyloxy)] benzene. *Am J Pathol* 2000, 156: 91–97
10. Pibiri M, Ledda-Columbano GM, Cossu C, Simbula G, Menegazzi M, Shinozuka H, Columbano A: Cyclin D1 is an early target in hepatocyte proliferation induced by thyroid hormone (T3). *EMBO J* 2001, 15: 1006–1013
11. Michalopoulos GK, DeFrances MC: Liver regeneration. *Science* 1997, 276:60–66
12. Fausto N: Liver regeneration. *J Hepatol* 2000, 32(Suppl 1):19–31
13. Grisham JW, Thorgeirsson SS: Liver stem cells. *Stem Cells*. Edited by CS Potten. London, Academic, 1997, pp 233–282
14. Alison M, Golding M, Lalani E-N, Sarraf C: Wound healing in the liver with particular reference to stem cells. *Phil Trans R Soc Lond B* 1998, 353:877–894
15. Paku S, Schur J, Nagy P, Thorgeirsson SS: Origin and structural evolution of the early proliferating oval cells in rat liver. *Am J Pathol* 2001, 158:1313–1323
16. Evarts RP, Nagy P, Nakatsukasa H, Marsden E, Thorgeirsson SS: In vivo differentiation of rat liver oval cells into hepatocytes. *Cancer Res* 1989, 49:1541–1547
17. Alison M, Golding M, Lalani E-N, Nagy P, Thorgeirsson SS, Sarraf C: Wholesale hepatocytic differentiation in the rat from ductular oval cells, the progeny of biliary stem cells. *J Hepatol* 1997, 26:343–352
18. Gordon GJ, Coleman WB, Hixson DC, Grisham JW: Liver regeneration in rats with retrorsine-hepatocellular injury proceeds through a novel hepatocellular response. *Am J Pathol* 2000, 156: 607–619
19. Gordon GJ, Coleman WB, Grisham JW: Temporal analysis of hepatocyte differentiation by small hepatocyte-like progenitor cells during liver regeneration in retrorsine-exposed rats. *Am J Pathol* 2000, 157: 771–786
20. Alison MR, Golding M, Sarraf CE, Edwards RJ, Lalani E-N: Liver damage in the rat induces hepatocyte stem cells from biliary epithelium. *Gastroenterology* 1996, 110:1182–1190
21. Diehl AM, Rai RM: Regulation of signal transduction during liver regeneration. *EMBO J* 1996, 10:215–227
22. Morshage H: Cytokines and the hepatic acute phase reaction. *J Pathol* 1997, 181:257–266
23. Yamada Y, Kirillova I, Peschon JJ, Fausto N: Initiation of liver growth by tumor necrosis factor: deficient liver regeneration in mice lacking type I tumor necrosis factor receptor. *Proc Natl Acad Sci USA* 1997, 94:1441–1446
24. Yamada Y, Webber EM, Kirillova I, Peschon JJ, Fausto N: Analysis of liver regeneration in mice lacking type I or type 2 tumor necrosis factor receptor: requirement for type 1 but not type 2 receptor. *Hepatology* 1998, 28:959–970
25. Cressman DE, Greenbaum LE, DeAngelis RA, Ciliberto G, Furth EE, Poli V, Taub R: Liver failure and defective hepatocyte regeneration in interleukin-6-deficient mice. *Science* 1996, 274:1379–1383
26. Sakamoto T, Liu Z, Murase N, Ezure T, Yokomuro S, Poli V, Demetris AJ: Mitosis and apoptosis in the livers of interleukin-6-deficient mice after partial hepatectomy. *Hepatology* 1999, 29:403–411
27. Cornell RP, Lilequist BL, Bartizal KF: Depressed liver regeneration after partial hepatectomy in germ-free, athymic and lipopolysaccharide-resistant mice. *Hepatology* 1990, 11:916–922
28. Mastellos D, Papadimitriou JC, Franchini S, Tsonis PA, Lambris JD: A novel role of complement: mice deficient in the fifth component of complement (C5) exhibit impaired liver regeneration. *J Immunol* 2001, 166:2479–2486
29. Peters M, Blinn G, Jostock T, Schirmacher P, Meyer zum Büschenfeld K-H, Galle P, Rose-Jahn S: Combined interleukin 6 and soluble interleukin 6 receptor accelerates murine liver regeneration. *Gastroenterology* 2000, 119:1663–1671
30. Jones SA, Novick DA, Horiuchi S, Yamamoto N, Szalai J, Fuller GM: C-reactive protein: a physiological activator of interleukin-6 receptor shedding. *J Exp Med* 1999, 189:599–604
31. Mingawa M, Oya H, Yamamoto S, Shimizu T, Bannai M, Kawamura H, Hatakeyama K, Abo T: Intensive expansion of natural killer T cells in the early phase of hepatocyte regeneration after partial hepatectomy in mice and its association with sympathetic nerve stimulation. *Hepatology* 2000, 31:907–915
32. Nagy P, Kiss A, Schnur J, Thorgeirsson SS: Dexamethasone inhibits the proliferation of hepatocytes and oval cells but not bile duct epithelial cells in rat liver. *Hepatology* 1998, 28:423–429
33. Butz GM, Grisham JW, Coleman WB: Cytokine dependency of small hepatocyte progenitor cell activation after partial hepatectomy in retrorsine-exposed rats. *EMBO J* 2002, 10:A365 (Abstract)
34. Fujio K, Evarts RP, Hu Z, Marsden ER, Thorgeirsson SS: Expression of stem cell factor and its receptor, c-kit, during liver regeneration from stem cells in the adult rat. *Lab Invest* 1994, 70:511–516