POINT OF VIEW

Hepatic stellate cells and alcoholic liver disease

M. Vera and N. Nieto

Department of Medicine. Division of Liver Diseases. Mount Sinai School of Medicine. New York, USA

ABSTRACT

Liver fibrosis represents a significant health problem worldwide for which no effective therapy exists. A great deal of research has been carried out to understand the molecular mechanisms responsible for the development of liver fibrosis. Activated stellate cells are the primary cell type responsible for the production of collagen I, the key protein involved in the development of liver fibrosis. Excessive deposition of collagen I occurs along with impaired extracellular matrix remodeling. Following a fibrogenic stimulus stellate cells transform into an activated collagen type I-producing cell. Numerous changes in gene expression are associated with stellate cell activation, including the induction of several intracellular signaling cascades, which help maintain the activated phenotype and control the fibrogenic and proliferative state of the cell. Activation of stellate cells is mediated by factors released from hepatocytes and Kupffer cells as they produce reactive oxygen species, nitric oxide, cytokines, growth factors, and cyclooxygenase and lipoxygenase metabolites, which provide pivotal paracrine effects in the liver milieu. Inhibition of stellate cell activation, proliferation, and the increased production of extracellular matrix (i.e. collagen type I) are therefore crucial steps for intervention in hepatic fibrogenesis.

Key words: Hepatic stellate cells. Collagen I. Alcoholic liver disease. Fibrosis. Extracellular matrix. Reactive oxygen species.

Vera M, Nieto N. Hepatic stellate cells and alcoholic liver disease. *Rev Esp Enferm Dig* 2006; 98: 674-684.

Supported by US Public Health Service Grant 1RO1 DK 069286-01A1 from the National Institute of Diabetes and Digestive and Kidney Diseases.

Recibido: 06-09-06.

Aceptado: 06-09-06.

Correspondencia: Natalia Nieto. Department of Medicine. Division of Liver Diseases. Mount Sinai School of Medicine, Box 1123. 1425 Madison Avenue. Room 11-76. New York 10029. USA. Fax: 1-212-849-2574. e-mail: natalia.nieto@mssm.edu

ALCOHOLIC LIVER DISEASE

Liver fibrosis is the common consequence of chronic liver injury of wide etiology (1). Chronic alcohol abuse is the main reason for developing liver fibrosis and eventually cirrhosis, a major cause of death worldwide (1,2). Fibrosis is characterized by excessive accumulation of extracellular matrix (ECM) proteins with very little degradation (1,2). Advanced fibrosis disrupts the normal liver architecture, increases portal pressure, and impairs intercellular communication (3). In humans, collagen type I deposits are usually localized to the pericentral and perisinusoidal regions of the liver (2,4,5).

Prior to the onset of well established hepatic fibrosis, the liver usually becomes steatotic and undergoes steatohepatitis (6). Many of the metabolic and toxic effects of alcohol in the liver have been associated with its metabolism because it generates toxic metabolites and induces a state of oxidative stress (due to activation of CYP2E1, impairment of the mitochondrial function, and a decrease in glutathione stores) (7-10). Only about 2-10% of the ethanol absorbed in the gastrointestinal tract is eliminated in the kidney and in the lung, and the rest undergoes hepatic metabolism (8).

A major pathway for ethanol metabolism is alcohol dehydrogenase (ADH) (8). ADH catalyzes the oxidation of ethanol to acetaldehyde where hydrogen is transferred from ethanol to the cofactor nicotinamide adenine dinucleotide (NAD⁺), which is then converted into the reduced form (NADH) (8). Acetaldehyde is then reduced to acetate, most of which is then secreted to the bloodstream (8). A number of hepatic and metabolic effects of ethanol can be attributed to the redox change (increased NADH/NAD⁺ ratio) upon the oxidation of ethanol (8,9).

While most ethanol is oxidized by ADH when alcohol levels are low; cytochrome P4502E1 (CYP2E1), an ethanol inducible from in the microsomal compartment of hepatocytes and Kupffer cells, plays a more important

role in ethanol oxidation at high concentrations of ethanol (binge drinking) and in chronic alcohol consumption (chronic alcoholism) (8-15). CYP2E1 oxidizes ethanol to generate many toxic products, such as acetaldehyde, 1-hydroxyethyl radical, and other reactive oxygen species (ROS), such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^\cdot$); as well as lipid peroxidation-end products malondialdehyde (MDA) and 4-hydroxy nonenal (4-HNE) (12,15). There is a considerable interest in the role of oxidative stress and ethanol generation of ROS in the mechanism by which ethanol is hepatotoxic (13).

Among the noxious effects of alcohol are: a) damage to the mitochondria with the subsequent decrease in ATP levels (16,17); b) changes in membrane fluidity by interaction with either phospholipids or proteins (18,19); c) malnutrition (20); d) hypoxia in the pericentral zone of the liver acinus as oxygen is consumed in order to detoxify ethanol via oxidation (7); e) altered cytokine production (e.g. $TNF\alpha$ and $TGF\beta$) (21,22); f) induction of 'leaky gut' with translocation of bacterial-derived endotoxin (LPS), the consequent activation of Kupffer cells, and the release of other soluble mediators and ROS (23,24); and g) impairment of the antioxidant defense (17,25). All these effects enhance liver injury and contribute in one way or another to the activation of stellate cells (HSC).

STELLATE CELL ACTIVATION

The normal liver contains an epithelial component (hepatocytes), an endothelial lining (endothelial cells), tissue macrophages (Kupffer cells), natural killer cells (NK), and perivascular mesenchymal cell, HSC, which are the key fibrogenic cells (26). The cellular elements of the liver are organized within the sinusoid, with the subendothelial *space of Disse* separating the epithelium from the sinusoidal endothelium. In normal liver this space contains a non electron-dense basement membrane-like matrix which is essential for maintaining the differentiated function of all resident liver cells (27). As the liver becomes fibrotic, the total content of collagens and non-collagenous components increases and it is accompanied by a shift in the type of ECM in the subendothelial space from the normal low density basement membrane-like matrix to interstitial type matrix containing fibril-forming collagens (mostly collagens I and III) (26). HSC activation is a key feature in excessive ECM deposition (27,28).

Under physiological conditions, HSC comprise about 15% of the total number of resident liver cells (28,29). Their main function is storage and homeostasis of vitamin A and other retinoids, which are stored in cytoplasmic lipid droplets as retinyl esters (29,30). HSC regulate the sinusoidal blood flow, produce apolipoproteins, prostaglandins, growth factors, and cytokines all of which contribute to ECM homeostasis (29,30). Synthesis

and degradation of normal hepatic ECM is essential for the integrity of the space of Disse and for the intra- and intercellular communication among neighboring cells (29,30).

Following a fibrogenic stimulus, HSC undergo a complex process of activation in which they become transformed from quiescent to activated myofibroblast-like cells (8,26). Phenotypic changes include stretching, nuclear and cellular enlargement, cytoplasmic spreading, elongation of processes to establish contacts among cells, and loss of lipid droplets (26,29,31). As they become activated, HSC increase α -smooth muscle actin (α -sma) and collagen type I protein expression (26-29,31), they proliferate, and migrate to the site of injury where ECM builds up and scarring occurs (26).

The phenomenon of HSC activation takes place as a sequence of well interrelated events (26,28-31). The first steps encompass rapid changes in gene expression, associated with transcriptional events (e.g. *COL1A1* and *COL1A2* up-regulation), and induction of immediate early genes, which render the cells responsive to cytokines and other local stimuli from paracrine or autocrine origin (26). The subsequent steps incorporate the cellular events that amplify the activated phenotype through enhanced cytokine expression and responsiveness (26).

The perpetuation of HSC activation involves key phenotypic responses mediated by increased cytokine production and remodeling of ECM. These phenotypic responses of HSC include:

1. *Proliferation*: increased numbers of HSC in injured liver develop at least in part as a response to local growth factors, most of which signal through receptor tyrosine kinases (32,33). Among these factors, platelet-derived growth factor (PDGF) and $TGF\beta$ are the best characterized and most powerful (34,35).

2. *Contractility*: represents an important mechanism underlying increased portal resistance during liver injury. The key contractile stimulus toward HSC is endothelin-1 (ET-1), likely autocrine derived (36).

3. *Fibrogenesis*: up-regulation of collagen type I synthesis is among the most striking molecular responses of HSC to injury and is regulated at the transcriptional and translational levels (37-40).

4. *Matrix degradation*: changes in matrix proteolytic activity lead to remodeling of ECM during liver injury, which accelerate HSC activation (37). A family of matrix-metalloproteinases (MMPs) contributes to either pathologic or restorative matrix degradation (37,41,42). MMPs can be either activated through proteolytic cleavage (43), or inhibited by binding to specific inhibitors known as tissue inhibitors of metalloproteinases (TIMPs) (43). MMP-2, MMP-3, MMP-9 and MT-MMP1 are involved in the degradation of the normal subendothelial ECM which is replaced by fibril-forming collagens, which can stimulate HSC growth (44-46). Furthermore, expression of TIMP-1 in activated HSC inhibits MMP-1, the main metalloproteinase responsible for col-

lagen type I degradation in humans (46,47). Sustained TIMP-1 expression is emerging as a key reason why fibrosis progresses (48, 49). MMPs and TIMPs are sensitive to ROS, reactive nitrogen species, cytokines, and growth factors (44-46,48,49).

5. *Chemotaxis*: directed migration of activated HSC enhances their accumulation in areas of injury (34,50). PDGF and monocyte chemotactic protein (MCP-1) are chemoattractants toward activated but not quiescent cells (34,51).

6. *Retinoids*: loss of intracellular vitamin A is remarkable during HSC activation (52), yet it remains unknown whether it is required for HSC to activate, and also which retinoids might accelerate or prevent activation *in vivo* (52). Retinoids may be directly linked to fibrogenesis as they stimulate the activation of latent TGF β 1, thereby increasing its fibrogenic activity (53).

7. *Cytokine release*: increased production and activity of cytokines is critical for perpetuation of HSC activation (22,50). ECM is also an important reservoir for growth factors including TGF β 1, PDGF, VEGF, fibroblast growth factor (FGF), hepatocyte growth factor (HGF), and platelet activating factor (PAF) (22,32,50). Finally, HSC also release neutrophil and monocyte chemoattractants that can amplify inflammation during liver injury, including colony stimulating factor (CSF), monocyte chemotactic protein-1 (MCP-1), interleukin-6, and cytokine-induced neutrophil chemoattractant/IL-8 (CINC) (54-57). Anti-fibrogenic cytokines produced by HSC have also been identified, in particular IL-10 (58) and TNF α (59).

ALCOHOL AND LIVER FIBROSIS

Chronic alcohol liver injury leads to fibrosis, with the subsequent disruption of the liver architecture, and impairment of hepatic metabolism (15,60). The cross-talk between parenchymal and non-parenchymal cells and the excessive production of ECM components are main features in the development of hepatic injury and fibrosis (38,40). Soluble factors, such as cytokines, chemokines, and ROS are candidate mediators for the induction of the fibrogenic response (26,32,40,61). Reactive nitrogen species may also play a role. *Initiation* of HSC activation is mainly due to paracrine stimulation by injured hepatocytes/bile duct cells, inflammatory cells, activated macrophages and neutrophils, or to early changes in ECM composition (26,32,40). *Perpetuation* results from autocrine and paracrine stimulation, as well as from accelerated ECM remodeling (26,32,40).

—*Paracrine effect of hepatocytes*. Alcohol metabolism via CYP2E1 leads ROS production and lipid peroxidation-end products (62). Acetaldehyde, ROS, and long-chain polyunsaturated fatty acids can activate HSC in a paracrine mode (62-65). Acetaldehyde induces *COL1A1* and *COL1A2* through a TGF β -dependent mechanism

(63,64). TGF- β is considered to be the most potent pro-fibrogenic cytokine (35,66). It suppresses hepatocyte proliferation, stimulates HSC activation, promotes ECM production, and mediates hepatocyte apoptosis (3,35,53,67-69). ROS and lipid peroxidation products MDA and 4-HNE can increase collagen type I production in HSC (70-76). ROS also modulate the binding of transcription factors (e.g. c-Jun/AP1, NF κ B, Sp1, and Smads) which modulate *COL1A1* and *COL1A2* transactivation in HSC (27,40,77-79).

As already mentioned, alcohol modifies the cellular redox state by altering the ratio of NAD⁺/NADH and NADP⁺/NADPH, elevates lactic acid, and increases angiotensin II, a powerful pro-fibrogenic cytokine (2,80). Lastly, fatty acid ethyl esters activate quiescent HSC via a mitogen-activated protein kinase (MAPK)-dependent pathway (81).

—*Paracrine effect of Kupffer cells*. Ethanol impairs gut permeability leading to overgrowth of Gram negative bacteria (82-85). Endotoxin or lipopolysaccharide (LPS), a component of the Gram negative bacterial wall, translocates from the intestinal lumen into the portal circulation triggering Kupffer cell activation (82-85). Influx of Kupffer cells coincides with the appearance of HSC activation markers (e.g. PDGFR β and α -sma) (86). Kupffer cells may stimulate matrix synthesis, cell proliferation, and release of retinoids by HSC through the actions of cytokines and reactive species (57). They can also influence HSC through secretion of MMP-9 because it can activate latent TGF β 1, stimulating HSC collagen I synthesis (87). Lastly, Kupffer cells generate ROS either via NADPH oxidase (88), xanthine oxidase (89), mitochondria (89), or possibly CYP2E1 (90), which in turn, may enhance HSC activation and collagen I synthesis (13,91). These effects are enhanced under ethanol treatment, and a role for polyunsaturated fatty acids should be considered as critical components which promote injury in several *in vitro* and *in vivo* models of ethanol administration (85,92). Kupffer cells also produce nitric oxide (NO), which can counterbalance the stimulatory effects of ROS by reducing HSC proliferation, contractility, and collagen I production; however, NO may also react with O₂⁻ to generate peroxynitrite (ONOO⁻), whose potential effects on HSC collagen I production are unknown and worth exploring. TNF α acts as an antifibrogenic, down-regulating *COL1A1* and *COL1A2* promoters both in humans and in rodents (40,59,93).

—*Paracrine effect of sinusoidal endothelial cells*. Injury of sinusoidal endothelial cells by ROS and acetaldehyde stimulates the production of certain fibronectin isoforms, such as leptin, which may activate HSC (94). Moreover, damaged endothelial cells convert latent TGF- β 1 into its active form (22,95). Also, sinusoidal endothelial cells increase the expression of vascular endothelial growth factor (VEGF) in response to injury (96). VEGF binds to its receptors in HSC and promote their activation enhancing the expression of procollagen type I (96).

—*Paracrine and autocrine effect of HSC.* Activated HSC secrete inflammatory chemokines e.g. TNF α , TGF β , IL6, and PDGF which regulate collagen type I at the transcriptional and translational level (28,33,40,50,97). Activated HSC also secrete angiotensin II, which induces fibrogenic actions via NADPH oxidase (41,42). HSC are the master regulators for ECM remodeling. An expanding family of matrix-metalloproteinases (MMPs) contributes to either pathologic or restorative matrix degradation (41,42). These enzymes fall into five categories based on substrate specificity: interstitial collagenases (MMP-1, -8, -13), gelatinases (MMP-2, -9), stromelysins (MMP-3, -7, -10, -11), membrane-type metalloproteinases (MMP-14 or MT1-MMP, -15, -16, -17, -24, -25) and metalloelastase (MMP-12) (41,42). Metalloproteinases can be either activated through proteolytic cleavage, or inhibited by binding to specific inhibitors known as tissue inhibitors of metalloproteinases (TIMPs) (45). Liver 'pathologic' matrix degradation refers to early disruption of the normal subendothelial matrix which occurs through the actions of four enzymes: MMP-2 and MMP-9, which degrade type IV collagen; MT1-MMP, which activates latent MMP-2; and MMP-3, which degrades proteoglycans and glycoproteins, and also activates latent collagenases (43). Failure to degrade the accumulated scar is a major reason why fibrosis progresses to cirrhosis (7). In humans, MMP-1 (in rats, MMP-13) is the main protease which degrades type I collagen, the principal collagenous protein in liver fibrosis (98). Alternatively, it is possible that other enzymes such as MT1-MMP and MMP-2 may have interstitial collagenase activity (98). More importantly, progressive fibrosis is associated with marked increases in TIMP-1 and TIMP-2 (44,46), leading to a net decrease in protease activity, and therefore matrix accumulation. HSC are the major source of these inhibitors (44,46).

—*Role of innate immunity.* Alcohol consumption mediates suppression of the innate immunity as decreases NK cells activity and their numbers (99,100). The liver immune system is comprised of Kupffer cells, NK cells, NK-T cells and interferon alpha and gamma (IFN α and IFN γ). NK cells may kill activated HSC while IFN α and IFN γ block TGF- β 1 signaling and HSC activation (101,102).

REVERSION AND TREATMENT

Current questionable trends of thought suggest that reversion of fibrosis may happen by inducing apoptosis or necrosis of activated HSC, or less likely by transformation of activated HSC to a more quiescent phenotype.

—*Apoptosis of activated HSC.* Spontaneous resolution of experimental fibrosis is associated with the clearance of collagen type I-producing α -sma positive myofibroblasts (activated HSC and transdifferentiated portal fibroblasts) (103). HSC clearance has been attributed to the in-

duction of apoptosis (103). Apoptosis of myofibroblasts is associated with decreased expression of TIMP1, which protects activated HSC from apoptosis, and increased MMP-1 activity in the liver (103).

—*Reverse trans-differentiation of activated HSC to quiescent phenotype.* One theoretical approach to reverse fibrosis is the reverse trans-differentiation of activated HSC to a more quiescent phenotype. Quiescent HSC are full of vitamin A and triglycerides which are depleted in the activated HSC (26,28). The adipogenic/lipogenic transcriptional regulation conferred by PPAR, LXR, and SREBP-1c is required for the maintenance of the fat-storing quiescence phenotype of HSC (104). Expression of these transcription factors is lost in activated HSC (104). On the other hand, treatment of the activated HSC with an adipocyte differentiation cocktail or ectopic expression of PPAR γ or SREBP-1c causes their reversal to the quiescent phenotype (105-107).

The most efficient treatment for alcohol liver diseases is usually the cessation of the contributing agent e.g. alcohol. Although liver fibrosis is reversible, cirrhosis, the end-stage consequence of fibrosis, is generally irreversible. Thus, efforts to understand fibrosis focus primarily on events that lead to the early accumulation of scar (e.g. collagen I) in hope of identifying therapeutic targets to slow its progression or help its resolution. Inhibition of HSC activation, proliferation, and the increased production of ECM are crucial steps for intervention in hepatic fibrogenesis.

REFERENCES

1. Friedman SL. Liver fibrosis-from bench to bedside. *J Hepatol* 2003; 38 (Supl. 1): S38-53.
2. Bataller R, Brenner DA. Liver fibrosis. *J Clin Invest* 2005; 115: 209-18.
3. Bissell DM. Chronic liver injury, TGF-beta, and cancer. *Exp Mol Med* 2001; 33: 179-90.
4. Williams EJ, Iredale JP. Liver cirrhosis. *Postgrad Med J* 1998; 74: 193-202.
5. Pinzani M. Liver fibrosis. *Springer Semin Immunopathol* 1999; 21: 475-90.
6. Stewart SF, Day CP. The management of alcoholic liver disease. *J Hepatol* 2003; 38 (Supl. 1): S2-13.
7. Siegmund SV, Dooley S, Brenner DA. Molecular mechanisms of alcohol-induced hepatic fibrosis. *Dig Dis* 2005; 23: 264-74.
8. Lieber CS. Metabolism of alcohol. *Clin Liver Dis* 2005; 9: 1-35.
9. Wu D, Cederbaum AI. Alcohol, oxidative stress, and free radical damage. *Alcohol Res Health* 2003; 27: 277-84.
10. Cahill A, Cunningham CC, Adachi M, Ishii H, Bailey SM, Fromenty B, et al. Effects of alcohol and oxidative stress on liver pathology: the role of the mitochondrion. *Alcohol Clin Exp Res* 2002; 26: 907-15.
11. Kessova I, Cederbaum AI. CYP2E1: biochemistry, toxicology, regulation and function in ethanol-induced liver injury. *Curr Mol Med* 2003; 3: 509-18.
12. Caro AA, Cederbaum AI. Oxidative stress, toxicology, and pharmacology of CYP2E1. *Annu Rev Pharmacol Toxicol* 2004; 44: 27-42.
13. Dey A, Cederbaum AI. Alcohol and oxidative liver injury. *Hepatology* 2006; 43: S63-74.
14. Lieber CS. Cytochrome P-4502E1: its physiological and pathological role. *Physiol Rev* 1997; 77: 517-44.
15. Lieber CS. The discovery of the microsomal ethanol oxidizing sys-

- tem and its physiologic and pathologic role. *Drug Metab Rev* 2004; 36: 511-29.
16. Fernandez-Checa JC, Hirano T, Tsukamoto H, Kaplowitz N. Mitochondrial glutathione depletion in alcoholic liver disease. *Alcohol* 1993; 10: 469-75.
  17. Bailey SM, Patel VB, Young TA, Asayama K, Cunningham CC. Chronic ethanol consumption alters the glutathione/glutathione peroxidase-I system and protein oxidation status in rat liver. *Alcohol Clin Exp Res* 2001; 25: 726-33.
  18. Carrasco MP, Jiménez-López JM, Martínez-Dueñas L, Ubina S, Segovia JL, Marco C. Ethanol specifically alters the synthesis, acylation and transbilayer movement of aminophospholipids in rat-liver microsomes. *Life Sci* 2006; 78: 2781-6.
  19. Seenaiah B, Bichenkov E, Ellingson JS. The effects of chronic ethanol consumption on the formation of phosphatidylethanolamine molecular species and their appearance at the plasma membrane. *Alcohol Clin Exp Res* 1998; 22: 1245-54.
  20. Sarin SK, Dhingra N, Bansal A, Malhotra S, Guptan RC. Dietary and nutritional abnormalities in alcoholic liver disease: a comparison with chronic alcoholics without liver disease. *Am J Gastroenterol* 1997; 92: 777-83.
  21. Daniluk J, Szuster-Ciesielska A, Drabko J, Kandefer-Szerszen M. Serum cytokine levels in alcohol-related liver cirrhosis. *Alcohol* 2001; 23: 29-34.
  22. Friedman SL. Cytokines and fibrogenesis. *Semin Liver Dis* 1999; 19: 129-40.
  23. Enomoto N, Ikejima K, Yamashina S, Hirose M, Shimizu H, Kitamura T, et al. Kupffer cell sensitization by alcohol involves increased permeability to gut-derived endotoxin. *Alcohol Clin Exp Res* 2001; 25: 51S-4S.
  24. Thakur V, Pritchard MT, McMullen MR, Wang Q, Nagy LE. Chronic ethanol feeding increases activation of NADPH oxidase by lipopolysaccharide in rat Kupffer cells: role of increased reactive oxygen in LPS-stimulated ERK1/2 activation and TNF-alpha production. *J Leukoc Biol* 2006; 79: 1348-56.
  25. Lieber CS, Packer L. S-Adenosylmethionine: molecular, biological, and clinical aspects--an introduction. *Am J Clin Nutr* 2002; 76: 1148S-50S.
  26. Friedman SL. Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. *J Biol Chem* 2000; 275: 2247-50.
  27. Nieto N, Friedman SL, Cederbaum AI. Stimulation and proliferation of primary rat hepatic stellate cells by cytochrome P450 2E1-derived reactive oxygen species. *Hepatology* 2002; 35: 62-73.
  28. Friedman SL. Stellate cells: a moving target in hepatic fibrogenesis. *Hepatology* 2004; 40: 1041-3.
  29. Geerts A. History, heterogeneity, developmental biology, and functions of quiescent hepatic stellate cells. *Semin Liver Dis* 2001; 21: 311-35.
  30. Li D, Friedman SL. Liver fibrogenesis and the role of hepatic stellate cells: new insights and prospects for therapy. *J Gastroenterol Hepatol* 1999; 14: 618-33.
  31. Lieber CS. CYP2E1: from ASH to NASH. *Hepatol Res* 2004; 28: 1-11.
  32. Pinzani M, Marra F, Carloni V. Signal transduction in hepatic stellate cells. *Liver* 1998; 18: 2-13.
  33. Pinzani M, Marra F. Cytokine receptors and signaling in hepatic stellate cells. *Semin Liver Dis* 2001; 21: 397-416.
  34. Pinzani M. PDGF and signal transduction in hepatic stellate cells. *Front Biosci* 2002; 7: d1720-6.
  35. Gressner AM, Weiskirchen R, Breitkopf K, Dooley S. Roles of TGF-beta in hepatic fibrosis. *Front Biosci* 2002; 7: d793-807.
  36. Rockey DC, Fouassier L, Chung JJ, Carayon A, Vallee P, Rey C, et al. Cellular localization of endothelin-1 and increased production in liver injury in the rat: potential for autocrine and paracrine effects on stellate cells. *Hepatology* 1998; 27: 472-80.
  37. Rojkind M. Role of metalloproteinases in liver fibrosis. *Alcohol Clin Exp Res* 1999; 23: 934-9.
  38. Nieto N, Domínguez-Rosales JA, Fontana L, Salazar A, Armendariz-Borunda J, Greenwel P, et al. Rat hepatic stellate cells contribute to the acute-phase response with increased expression of alpha1(I) and alpha1(IV) collagens, tissue inhibitor of metalloproteinase-1, and matrix-metalloproteinase-2 messenger RNAs. *Hepatology* 2001; 33: 597-607.
  39. Lindquist JN, Parsons CJ, Stefanovic B, Brenner DA. Regulation of alpha1(I) collagen messenger RNA decay by interactions with alpha-haCP at the 3'-untranslated region. *J Biol Chem* 2004; 279: 23822-9.
  40. Purohit V, Brenner DA. Mechanisms of alcohol-induced hepatic fibrosis: a summary of the Ron Thurman Symposium. *Hepatology* 2006; 43: 872-8.
  41. Arthur MJ. Collagenases and liver fibrosis. *J Hepatol* 1995; 22: 43-8.
  42. Arthur MJ. Fibrogenesis II. Metalloproteinases and their inhibitors in liver fibrosis. *Am J Physiol Gastrointest Liver Physiol* 2000; 279: G245-9.
  43. Iredale JP. Tissue inhibitors of metalloproteinases in liver fibrosis. *Int J Biochem Cell Biol* 1997; 29: 43-54.
  44. Xu GF, Li PT, Wang XY, Jia X, Tian DL, Jiang LD, et al. Dynamic changes in the expression of matrix metalloproteinases and their inhibitors, TIMPs, during hepatic fibrosis induced by alcohol in rats. *World J Gastroenterol* 2004; 10: 3621-7.
  45. Senties-Gómez MD, Gálvez-Gastelum FJ, Meza-García E, Armendariz-Borunda J. Hepatic fibrosis: role of matrix metalloproteinases and TGFbeta. *Gac Med Mex* 2005; 141: 315-22.
  46. Benyon RC, Arthur MJ. Extracellular matrix degradation and the role of hepatic stellate cells. *Semin Liver Dis* 2001; 21: 373-84.
  47. Nie QH, Zhang YF, Xie YM, Luo XD, Shao B, Li J, et al. Correlation between TIMP-1 expression and liver fibrosis in two rat liver fibrosis models. *World J Gastroenterol* 2006; 12: 3044-9.
  48. Yoshiji H, Kuriyama S, Miyamoto Y, Thorgeirsson UP, Gómez DE, Kawata M, et al. Tissue inhibitor of metalloproteinases-1 promotes liver fibrosis development in a transgenic mouse model. *Hepatology* 2000; 32: 1248-54.
  49. Yoshiji H, Kuriyama S, Yoshiji I, Ikenaka Y, Noguchi R, Nakatani T, et al. Tissue inhibitor of metalloproteinases-1 attenuates spontaneous liver fibrosis resolution in the transgenic mouse. *Hepatology* 2002; 36: 850-60.
  50. Marra F. Chemokines in liver inflammation and fibrosis. *Front Biosci* 2002; 7: d1899-914.
  51. Marra F, Romanelli RG, Giannini C, Failli P, Pastacaldi S, Arrighi MC, et al. Monocyte chemoattractant protein-1 as a chemoattractant for human hepatic stellate cells. *Hepatology* 1999; 29: 140-8.
  52. Hellemans K, Verbuyst P, Quartier E, Schuit F, Rombouts K, Chandraratna RA, et al. Differential modulation of rat hepatic stellate phenotype by natural and synthetic retinoids. *Hepatology* 2004; 39: 97-108.
  53. Blomhoff R. Retinoids may increase fibrotic potential of TGF-beta: crosstalk between two multi-functional effectors. *Hepatology* 1997; 26: 1067-8.
  54. Schwabe RF, Bataller R, Brenner DA. Human hepatic stellate cells express CCR5 and RANTES to induce proliferation and migration. *Am J Physiol Gastrointest Liver Physiol* 2003; 285: G949-58.
  55. Pinzani M, Abboud HE, Gesualdo L, Abboud SL. Regulation of macrophage colony-stimulating factor in liver fat-storing cells by peptide growth factors. *Am J Physiol* 1992; 262: C876-81.
  56. Marra F, DeFranco R, Grappone C, Milani S, Pastacaldi S, Pinzani M, et al. Increased expression of monocyte chemoattractant protein-1 during active hepatic fibrogenesis: correlation with monocyte infiltration. *Am J Pathol* 1998; 152: 423-30.
  57. Knittel T, Mehde M, Kobold D, Saile B, Dinter C, Ramadori G. Expression patterns of matrix metalloproteinases and their inhibitors in parenchymal and non-parenchymal cells of rat liver: regulation by TNF-alpha and TGF-beta1. *J Hepatol* 1999; 30: 48-60.
  58. Tsukamoto H. Is interleukin-10 antifibrogenic in chronic liver injury? *Hepatology* 1998; 28: 1707-9.
  59. Iraburu MJ, Domínguez-Rosales JA, Fontana L, Auster A, García-Trevijano ER, Covarrubias-Pinedo A, et al. Tumor necrosis factor alpha down-regulates expression of the alpha1(I) collagen gene in rat hepatic stellate cells through a p20C/EBPbeta- and C/EBPdelta-dependent mechanism. *Hepatology* 2000; 31: 1086-93.
  60. Pritchard MT, Nagy LE. Ethanol-induced liver injury: potential roles for egr-1. *Alcohol Clin Exp Res* 2005; 29: 146S-50S.
  61. Friedman SL. Mechanisms of disease: mechanisms of hepatic fibrosis and therapeutic implications. *Nature Clinical Practice* 2004; 1: 98-105.
  62. Wu D, Cederbaum AI. Oxidative stress mediated toxicity exerted by ethanol-inducible CYP2E1. *Toxicol Appl Pharmacol* 2005; 207: 70-6.

63. Greenwel P, Domínguez-Rosales JA, Mavi G, Rivas-Estilla AM, Rojkind M. Hydrogen peroxide: a link between acetaldehyde-elicited alpha1(I) collagen gene up-regulation and oxidative stress in mouse hepatic stellate cells. *Hepatology* 2000; 31: 109-16.
64. Svegliati-Baroni G, Inagaki Y, Rincón-Sánchez AR, Else C, Saccomanno S, Benedetti A, et al. Early response of alpha2(I) collagen to acetaldehyde in human hepatic stellate cells is TGF-beta independent. *Hepatology* 2005; 42: 343-52.
65. Galli A, Svegliati-Baroni G, Ceni E, Milani S, Ridolfi F, Salzano R, et al. Oxidative stress stimulates proliferation and invasiveness of hepatic stellate cells via a MMP2-mediated mechanism. *Hepatology* 2005; 41: 1074-84.
66. García-Trevijano ER, Iraburu MJ, Fontana L, Domínguez-Rosales JA, Auster A, Covarrubias-Pinedo A, et al. Transforming growth factor beta1 induces the expression of alpha1(I) procollagen mRNA by a hydrogen peroxide-C/EBPbeta-dependent mechanism in rat hepatic stellate cells. *Hepatology* 1999; 29: 960-70.
67. Border WA, Noble NA. Transforming growth factor beta in tissue fibrosis. *N Engl J Med* 1994; 331: 1286-92.
68. Bissell DM, Roulot D, George J. Transforming growth factor beta and the liver. *Hepatology* 2001; 34: 859-67.
69. Leask A, Abraham DJ. TGF-beta signaling and the fibrotic response. *Faseb J* 2004; 18: 816-27.
70. George J, Pera N, Phung N, Leclercq I, Yun Hou J, Farrell G. Lipid peroxidation, stellate cell activation and hepatic fibrogenesis in a rat model of chronic steatohepatitis. *J Hepatol* 2003; 39: 756-64.
71. Niemela O. Aldehyde-protein adducts in the liver as a result of ethanol-induced oxidative stress. *Front Biosci* 1999; 4: D506-13.
72. Niemela O, Parkkila S, Pasanen M, Iimuro Y, Bradford B, Thurman RG. Early alcoholic liver injury: formation of protein adducts with acetaldehyde and lipid peroxidation products, and expression of CYP2E1 and CYP3A. *Alcohol Clin Exp Res* 1998; 22: 2118-24.
73. Niemela O, Parkkila S, Pasanen M, Viitala K, Villanueva JA, Halsted CH. Induction of cytochrome P450 enzymes and generation of protein-aldehyde adducts are associated with sex-dependent sensitivity to alcohol-induced liver disease in micropigs. *Hepatology* 1999; 30: 1011-7.
74. Nieto N, Friedman SL, Cederbaum AI. Cytochrome P450 2E1-derived reactive oxygen species mediate paracrine stimulation of collagen I protein synthesis by hepatic stellate cells. *J Biol Chem* 2002; 277: 9853-64.
75. Nieto N, Friedman SL, Greenwel P, Cederbaum AI. CYP2E1-mediated oxidative stress induces collagen type I expression in rat hepatic stellate cells. *Hepatology* 1999; 30: 987-96.
76. Nieto N, Greenwel P, Friedman SL, Zhang F, Dannenberg AJ, Cederbaum AI. Ethanol and arachidonic acid increase alpha 2(I) collagen expression in rat hepatic stellate cells overexpressing cytochrome P450 2E1. Role of H₂O₂ and cyclooxygenase-2. *J Biol Chem* 2000; 275: 20136-45.
77. Carmona-Cuenca I, Herrera B, Ventura JJ, Roncero C, Fernández M, Fabregat I. EGF blocks NADPH oxidase activation by TGF-beta in fetal rat hepatocytes, impairing oxidative stress, and cell death. *J Cell Physiol* 2006; 207: 322-30.
78. Czaja MJ, Liu H, Wang Y. Oxidant-induced hepatocyte injury from menadione is regulated by ERK and AP-1 signaling. *Hepatology* 2003; 37: 1405-13.
79. Wu J, Danielsson A, Zern MA. Toxicity of hepatotoxins: new insights into mechanisms and therapy. *Expert Opin Investig Drugs* 1999; 8: 585-607.
80. Bataller R, Gabele E, Schoonhoven R, Morris T, Lehnert M, Yang L, et al. Prolonged infusion of angiotensin II into normal rats induces stellate cell activation and proinflammatory events in liver. *Am J Physiol Gastrointest Liver Physiol* 2003; 285: G642-51.
81. Li J, Hu W, Baldassare JJ, Bora PS, Chen S, Poulos JE, et al. The ethanol metabolite, linolenic acid ethyl ester, stimulates mitogen-activated protein kinase and cyclin signaling in hepatic stellate cells. *Life Sci* 2003; 73: 1083-96.
82. Brock RW, Lawlor DK, Harris KA, Potter RF. Initiation of remote hepatic injury in the rat: interactions between Kupffer cells, tumor necrosis factor-alpha, and microvascular perfusion. *Hepatology* 1999; 30: 137-42.
83. Thurman RG, Bradford BU, Iimuro Y, Knecht KT, Arteel GE, Yin M, et al. The role of gut-derived bacterial toxins and free radicals in alcohol-induced liver injury. *J Gastroenterol Hepatol* 1998; 13 (Supl.): S39-50.
84. Thurman RG. II. Alcoholic liver injury involves activation of Kupffer cells by endotoxin. *Am J Physiol* 1998; 275: G605-11.
85. Tsukamoto H. Redox regulation of cytokine expression in Kupffer cells. *Antioxid Redox Signal* 2002; 4: 741-8.
86. Friedman SL, Arthur MJ. Activation of cultured rat hepatic lipocytes by Kupffer cell conditioned medium. Direct enhancement of matrix synthesis and stimulation of cell proliferation via induction of platelet-derived growth factor receptors. *J Clin Invest* 1989; 84: 1780-5.
87. Winwood PJ, Schuppan D, Iredale JP, Kawser CA, Docherty AJ, Arthur MJ. Kupffer cell-derived 95-kd type IV collagenase/gelatinase B: characterization and expression in cultured cells. *Hepatology* 1995; 22: 304-15.
88. Kono H, Rusyn I, Yin M, Gabele E, Yamashina S, Dikalova A, et al. NADPH oxidase-derived free radicals are key oxidants in alcohol-induced liver disease. *J Clin Invest* 2000; 106: 867-72.
89. Maemura K, Zheng Q, Wada T, Ozaki M, Takao S, Aikou T, et al. Reactive oxygen species are essential mediators in antigen presentation by Kupffer cells. *Immunol Cell Biol* 2005; 83: 336-43.
90. Lieber CS. New concepts of the pathogenesis of alcoholic liver disease lead to novel treatments. *Curr Gastroenterol Rep* 2004; 6: 60-5.
91. Wheeler MD, Kono H, Yin M, Nakagami M, Uesugi T, Arteel GE, et al. The role of Kupffer cell oxidant production in early ethanol-induced liver disease. *Free Radic Biol Med* 2001; 31: 1544-9.
92. Lieber CS, DeCarli LM. Quantitative relationship between amount of dietary fat and severity of alcoholic fatty liver. *Am J Clin Nutr* 1970; 23: 474-8.
93. Ferraz JG, Tigley AW, Appleyard CB, Wallace JL. TNF-alpha contributes to the pathogenesis of ethanol-induced gastric damage in cirrhotic rats. *Am J Physiol* 1997; 272: G809-14.
94. Ikejima K, Takei Y, Honda H, Hirose M, Yoshikawa M, Zhang YJ, et al. Leptin receptor-mediated signaling regulates hepatic fibrogenesis and remodeling of extracellular matrix in the rat. *Gastroenterology* 2002; 122: 1399-410.
95. Breitkopf K, Sawitza I, Westhoff JH, Wickert L, Dooley S, Gressner AM. Thrombospondin 1 acts as a strong promoter of transforming growth factor beta effects via two distinct mechanisms in hepatic stellate cells. *Gut* 2005; 54: 673-81.
96. Yoshiji H, Kuriyama S, Yoshii Y, Ikenaka Y, Noguchi R, Hicklin DJ, Wu Y, et al. Vascular endothelial growth factor and receptor interaction is a prerequisite for murine hepatic fibrogenesis. *Gut* 2003; 52: 1347-54.
97. Kershenobich Stalnikowitz D, Weissbrod AB. Liver fibrosis and inflammation. A review. *Ann Hepatol* 2003; 2: 159-63.
98. Nagase H, Woessner JF, Jr. Matrix metalloproteinases. *J Biol Chem* 1999; 274: 21491-4.
99. Cook RT. Alcohol abuse, alcoholism, and damage to the immune system--a review. *Alcohol Clin Exp Res* 1998; 22: 1927-42.
100. Collier SD, Pruett SB. Mechanisms of suppression of poly I: C-induced activation of NK cells by ethanol. *Alcohol* 2000; 21: 87-95.
101. Friedman SL. Mac the knife? Macrophages- the double-edged sword of hepatic fibrosis. *J Clin Invest* 2005; 115: 29-32.
102. Inagaki Y, Nemoto T, Kushida M, Sheng Y, Higashi K, Ikeda K, et al. Interferon alpha down-regulates collagen gene transcription and suppresses experimental hepatic fibrosis in mice. *Hepatology* 2003; 38: 890-9.
103. Iredale JP, Benyon RC, Pickering J, McCullen M, Northrop M, Pawley S, et al. Mechanisms of spontaneous resolution of rat liver fibrosis. Hepatic stellate cell apoptosis and reduced hepatic expression of metalloproteinase inhibitors. *J Clin Invest* 1998; 102: 538-49.
104. Miyahara T, Schrum L, Rippe R, Xiong S, Yee HF, Jr., Motomura K, et al. Peroxisome proliferator-activated receptors and hepatic stellate cell activation. *J Biol Chem* 2000; 275: 35715-22.
105. Hazra S, Miyahara T, Rippe RA, Tsukamoto H. PPAR gamma and hepatic stellate cells. *Comp Hepatol* 2004; 3 (Supl. 1): S7.
106. Hazra S, Xiong S, Wang J, Rippe RA, Krishna V, Chatterjee K, et al. Peroxisome proliferator-activated receptor gamma induces a phenotypic switch from activated to quiescent hepatic stellate cells. *J Biol Chem* 2004; 279: 11392-401.
107. She H, Xiong S, Hazra S, Tsukamoto H. Adipogenic transcriptional regulation of hepatic stellate cells. *J Biol Chem* 2005; 280: 4959-67.