38. Chua PJ, Yip GW, Bay BH: Cell cycle arrest induced by hydrogen peroxide is associated with modulation of oxidative stress related genes in breast cancer cells. Exp Biol Med (Maywood) 2009, 234:1086–1094
39. Shivapurkar N, Stastny V, Okumura N, Girard L, Xie Y, Prinsen C, Thunnissen FB, Wistuba II, Czerniak B, Frenkel E, Roth JA, Liloglou T, Xinarianos G, Field JK, Minna JD, Gazdar AF: Cytoglobin, the newest member of the globin family, functions as a tumor suppressor gene. Cancer Res 2008, 68:7448–7456
40. Whittaker S, Marais R, Zhu AX: The role of signaling pathways in the development and treatment of hepatocellular carcinoma. Oncogene 2010, 29:4989–5005
41. Bonner WM, Redon CE, Dickey JS, Nakamura AJ, Sedelnikova OA, Solier S, Pommier Y: γH2AX and cancer. Nat Rev Cancer 2008, 8: 957–967
42. Matsuda Y, Wakai T, Kubota M, Osawa M, Takamura M, Yamagiwa S, Aoyagi Y, Sanpei A, Fujimaki S: DNA damage sensor γ-H2AX is increased in preneoplastic lesions of hepatocellular carcinoma. In The Scientific World Journal. 2013, 2013:597095. doi:10.1155/2013/597095
43. Murata M, Thanan R, Ma N, Kawanishi S: Role of nitrative and oxidative DNA damage in inflammation-related carcinogenesis. J Biomed Biotechnol 2012, 2012:623019

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DOI: 10.3748/wjg.v20.i25.8048

*World J Gastroenterol* 2014 July 7; 20(25): 8048-8054
ISSN 1007-9327 (print) ISSN 2219-2840 (online)


TOPIC HIGHLIGHT

WJG 20th Anniversary Special Issues (10): Alcoholic liver disease

# Fibrogenesis in alcoholic liver disease

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Author contributions: Fujii H generated the figure; Fujii H and Kawada N wrote the manuscript.
Supported by A grant-in-Aid for Scientific Research (B) from the Japan Society for the Promotion of Science (JSPS) through grant No. 25293177 to Kawada N (2013-2016), and by a Grant-in-Aid for Scientific Research (C) from the JSPS through grant No. 25461007 to Fujii H (2013-2016)
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Received: November 24, 2013 Revised: January 28, 2014
Accepted: March 5, 2014
Published online: July 7, 2014

## Abstract

Alcoholic liver disease (ALD) is a major cause of morbidity and mortality worldwide. In developed countries, ALD is a major cause of end-stage liver disease that requires transplantation. The spectrum of ALD includes simple steatosis, alcoholic hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma. Alcohol abstinence is the most effective therapy for ALD. However, targeted therapies are urgently needed for patients with severe ALD (*i.e.*, alcoholic hepatitis) or those who do not abstain from alcohol. The lack of studies and the availability of animal models that do not reflect all the features of this disease in humans inhibit the development of new drugs for ALD. In ALD-associated fibrosis, hepatic stellate cells are the principal cell type responsible for extracellular matrix production. Although the mechanisms underlying fibrosis in ALD are largely similar to those observed in other chronic liver diseases, oxidative stress, methionine metabolism abnormalities, hepatocyte apoptosis, and endotoxin lipopolysaccharides that activate Kupffer cells may play unique roles in disease-related fibrogenesis. Lipogenesis during the early stages of ALD has recently been implicated as a risk factor for the progression of cirrhosis. Other topics include osteopontin, interleukin-1 signaling, and genetic polymorphism. In this review, we discuss the basic pathogenesis of ALD and focus on liver fibrogenesis.



**Key words:** Stellate cell; Kupffer cell; Steatohepatitis; Fibrosis; Cytokine; Oxidative stress

**Core tip:** Alcoholic liver disease (ALD) is a major cause of preventable morbidity and mortality worldwide. In ALD-associated fibrosis, hepatic stellate cells are the principal cell type responsible for extracellular matrix production. Although the mechanisms underlying ALD-associated fibrosis are largely similar to those observed in other chronic liver diseases, oxidative stress, abnormal methionine metabolism, hepatocyte apoptosis, and endotoxin lipopolysaccharides that activate Kupffer cells play unique roles in fibrogenesis in ALD. Recently, lipogenesis during the early stages of ALD has been implicated as a risk factor for progression of cirrhosis. Other critical factors include osteopontin, interleukin-1 signaling, and genetic polymorphisms.

Fujii H, Kawada N. Fibrogenesis in alcoholic liver disease. *World J Gastroenterol* 2014; 20(25): 8048-8054 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i25/8048.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i25.8048

## INTRODUCTION

Although the incidence of alcoholic liver disease (ALD) varies widely worldwide, the burden of ALD and ALD-induced death remains dominant in most countries[1]. ALD is the third highest risk factor for disease and disability worldwide. Almost 4% of all deaths in the world result from ALD, which is greater than deaths caused by

the human immunodeficiency virus/acquired immune deficiency syndrome, violence, or tuberculosis[1]. Furthermore, alcohol is associated with many serious social problems, including violence, child neglect, and abuse, and absenteeism in the workplace. A recent nationwide survey revealed that ALD was the third highest cause of liver cirrhosis in Japan (13.6%)[2], and the associated cost of medical care was estimated to be 6.9% of the total national medical expenditure[3]. Overall, ALD is recognized as a major but preventable public health problem.

The spectrum of ALD is broad: asymptomatic fatty liver, steatohepatitis, progressive fibrosis, end-stage cirrhosis, and hepatocellular carcinoma[4,5]. ALD may often resolve in those who become abstinent. However, for patients with severe ALD and those who do not completely abstain from alcohol, targeted therapies are urgently needed[4].

Patients with ALD can develop progressive liver fibrosis because of the accumulation of extracellular matrix (ECM) materials, including type I collagen, as generated by activated hepatic stellate cells (HSCs) and hepatic myofibroblasts. When liver injury occurs, HSCs are activated and differentiate into myofibroblast-like cells[6,7]. Activated Kupffer cells, infiltrating monocytes, activated and aggregated platelets, and damaged hepatocytes are the sources of platelet-derived growth factor and transforming growth factor-β1 (TGF-β1); these cells initiate intracellular signaling cascades leading to HSC activation. Although the key pathways of HSC activation are common to all forms of liver injury and fibrosis, disease-specific pathways also exist. Some specific signaling pathways regulating HSC activation in ALD are discussed below (Figure 1).

## CLASSICAL MECHANISMS UNDERLYING FIBROGENESIS IN ALD

### Alcohol metabolism

Approximately 90% of ingested alcohol is metabolized in the cytosol of hepatocytes. Cytosolic alcohol dehydrogenase[8] oxidizes alcohol to acetaldehyde that is then converted to acetate by acetaldehyde dehydrogenase. Acetaldehyde is considered the key toxin in alcohol-mediated liver injury that includes cellular damage, inflammation, ECM remodeling, and fibrogenesis[9]. Moreover, acetaldehyde triggers TGF-β1-dependent late-phase response in HSCs that maintains a pro-fibrogenic and pro-inflammatory cellular state[10]. Recently, Liu *et al*[10] indicated that, in vitro, leptin potentiates acetaldehyde-induced HSC activation and alpha-smooth muscle actin (SMA) expression by interleukin-6 (IL-6)-dependent signals such as p38 and phosphorylated-extracellular signal-regulated kinase 1/2. This report discusses the importance of a synergistic effect of leptin and acetaldehyde in the activation of HSCs in ALD.

### Oxidative stress

Alcohol consumed in chronic and heavy drinkers is also oxidized *via* the hepatocytic cytochrome P450 (CYP); previously termed inducible microsomal ethanol-oxidizing system[11]. CYP2E1 metabolizes various substances, including multiple drugs, polyunsaturated fatty acids, acetaminophen, and most organic solvents, and plays a critical role in the generation of reactive oxygen species (ROS), such as hydrogen peroxide and superoxide anions[11,12]. ROS are also generated from nitric oxide and reduced form of nicotinamide adenine dinucleotide phosphate oxidase by Kupffer cells[13]. ROS trigger inflammatory cascades and recruit neutrophils and other immune cells to the site of alcohol-induced hepatocyte damage, increasing levels of circulating pro-inflammatory cytokines, notably tumor necrosis factor (TNF)-α[14].

Accumulation of lipid peroxidation products, such as 4-hydroxynonenal (4-HNE), has been reported both in patients as well as animal models of ALD[15,16]. Several studies have shown that the lipid peroxidation reaction in the liver precedes the initial stages of fibrosis and is associated with the increased production of pro-fibrogenic TGF-β1 by Kupffer cells[14]. Nieto reported that ethanol-induced lipid peroxidation triggers the nuclear factor kappa B (NF-κB) transactivation of the collagen 2(I) gene promoter in HSCs by stimulating kinase cascades, including protein kinase C, phosphoinositide 3 kinase (PI3K), and protein kinase B/Akt[17]. These observations are agreement with the findings of previous reports, indicating that 4-HNE is pro-fibrogenic for collagen production in human HSCs[14] and that oxidative stress directly promotes collagen synthesis in HSCs over-expressing the CYP2E1 gene[14].

### Methionine metabolism

Decreased intracellular levels of antioxidants such as vitamin C, vitamin E, and glutathione (GSH) in the blood and liver modify the process of alcohol-induced liver injury[18]. Excessive acute alcohol intake reduces GSH synthesis, and the acetaldehyde produced from alcohol metabolism inhibits GSH activity. Alcohol also disturbs the intracellular transport of GSH and preferentially depletes mitochondrial GSH, leading to apoptosis[18]. Levels of S-adenosylmethionine (SAMe), a universal methyl donor, are also markedly reduced in ALD due to the reduced activity of SAMe synthetase[18]. This fact is clinically important because therapy using SAMe increases survival of patients with alcohol-induced cirrhosis[18].

### Hepatocyte apoptosis

Hepatocyte apoptosis is pathophysiologically important in the progression of ALD[19]. There are two important apoptotic pathways: extrinsic (death receptor-mediated) and intrinsic (organelle-initiated)[20]. Most recently, Petrasek *et al*[21] revealed that interferon regulatory factor 3 (IRF-3) mediates ALD by linking endoplasmic reticulum (ER) stress with the mitochondrial pathway of hepatocyte apoptosis. Interestingly, ethanol induces ER stress and triggers the association of IRF-3 with the ER adaptor, stimulator of interferon genes, as well as the subsequent phosphoryla-

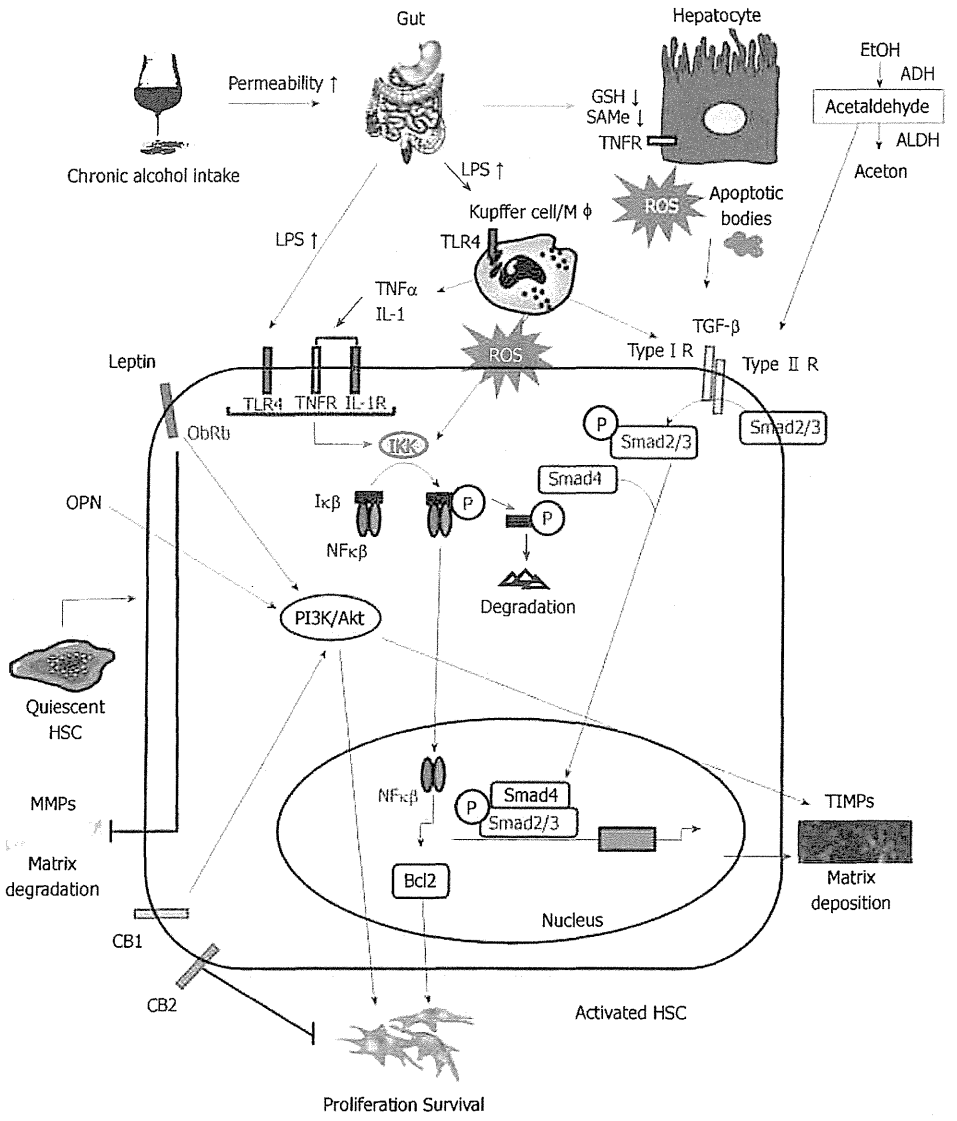



**Figure 1 Signaling pathways regulating hepatic stellate cell activation in alcoholic liver disease.** Alcohol consumption causes hepatocyte damage, which subsequently induces apoptosis. Alcohol dehydrogenase (ADH) oxidizes alcohol to acetaldehyde that is converted to acetate by acetaldehyde dehydrogenase (ALDH). Acetaldehyde directly targets hepatic stellate cells (HSCs). Alcohol reduces glutathione (GSH) synthesis and acetaldehyde inhibits GSH activity in hepatocytes. Levels of the S-adenosylmethionine (SAMe) are also markedly reduced. Alcohol consumption increases permeability of the intestine to bacterial endotoxin that in turn, elevates serum lipopolysaccharide (LPS) levels. LPS directly enhances HSCs activation by upregulating transforming growth factor (TGF)- signaling. TGF-β1 derived from activated Kupffer cells and damaged hepatocytes binds to TGF receptors. Phospho-Smad2/3 and Smad4 complexes translocate into the nucleus, display DNA-binding activity, and activate expression of genes related to fibrosis. Extracellular molecules, such as LPS, tumor necrosis factor (TNF)-α, interleukin (IL)-1, and reactive oxygen species (ROS), activate IκB kinase (IKK) that, in turn, phosphorylates IκB, resulting in ubiquitination, dissociation of IκBα from nuclear factor kappa B (NF-κB), and eventually, degradation of IκB by the proteasome. The activated NF-κB is then translocated into the nucleus and binds to specific DNA response elements. NF-B-dependent pathways are involved in the expression of the anti-apoptotic protein B-cell lymphoma 2 (Bcl2). Leptin binds ObRb, activating the phosphoinositide 3-kinase (PI3K)/Akt pathway and inducing matrix deposition by increasing expression of tissue inhibitor of metalloproteinases (TIMPs). Leptin also inhibits matrix degradation by decreasing expression of Matrix metalloproteinases (MMPs). Osteopontin (OPN) positively stimulates the PI3K/Akt pathway. The cannabinoid receptor CB2 mediates antifibrotic actions; in contrast, activation of CB1 receptors positively stimulates the PI3K/Akt pathway to promote the proliferation and apoptosis of HSCs. TLR: Toll-like receptor.

tion of IRF-3. Activated IRF-3 is associated with the proapoptotic molecule Bax (B-cell lymphoma 2-associated X protein) and contributes to hepatocyte apoptosis[21]. Apoptotic bodies induced by alcohol are phagocytosed by Kupffer cells and HSCs, which then produce TGF-β1 and subsequently activate HSCs[19,22]. Finally, increased serum levels of caspase-digested cytokeratin-18 fragments, a useful marker of hepatocyte apoptosis, are independent factors in predicting severe fibrosis in patients with ALD[23].

### *Lipopolysaccharide*

Increased serum lipopolysaccharide (LPS) levels are commonly found in patients with ALD[19]. Toll-like receptor (TLR) 4 is one of the multiple pattern recognition receptors that recognize both pathogen- and host-

derived factors that modulate inflammatory signals[24]. LPS interacts with TLR4 to activate the MyD88-independent toll-interleukin-1 receptor domain-containing adaptor-inducing interferon-β/IRF-3 signaling pathway that produces oxidative stress and proinflammatory cytokines (including TNF-α) causes hepatocellular damage and contributes to alcoholic steatohepatitis[15,22,25]. Recent studies have revealed that activation of TLR4 and complement factors also stimulates Kupffer cells to produce hepatoprotective cytokines, such as IL-6, and anti-inflammatory cytokines, such as IL-10[15,22,25,26]. These cytokines activate signal transduction and activator of transcription 3 in hepatocytes and macrophages/Kupffer cells, respectively, to prevent alcohol-induced liver injury and inflammation[15,22,26]. On the other hand, previous studies have reported that activation of TLR4 signaling in HSCs and liver sinusoidal endothelial cells (LSECs) promoted liver fibrogenesis[22,25], and that activation of TLR4 signaling in LSECs regulates angiogenesis through the MyD88-effector protein that regulates extracellular protease production, in turn, results in the development of liver fibrosis[27].

Experimental models of ALD have revealed that translocation of bacterial products across the intestinal barrier to the portal circulation triggers inflammatory responses in the liver and contributes to steatohepatitis[28,29]. Most recently, Hartmann *et al*[30] investigated the role of the intestinal mucus layer and found that mucin (Muc) 2 was involved in the development of alcohol-associated liver disease. The authors reported that *Muc2*$^{-/-}$ mice have significantly lower plasma levels of LPS than wild-type mice after alcohol administration. In addition, it was shown that *Muc2*$^{-/-}$ mice are effectively protected from intestinal bacterial overgrowth and the microbiome in response to alcohol administration[30]. This study clearly showed that the alcohol-associated alteration in the microbiome, and in particular, the overgrowth of intestinal bacteria contributes to the progression of ALD.

## EMERGING MECHANISMS UNDERLYING FIBROGENESIS IN ALD

### *Lipogenesis in the early stages of ALD*

The development of steatosis due to chronic alcohol consumption is an important contributor to the progression of hepatic fibrogenesis[15]. Recent studies have found that direct or indirect alcohol exposure regulates transcription factors associated with lipid metabolism. Alcohol also stimulates lipogenesis and inhibits fatty acid oxidation[31]. There are two well-known pathways of lipogenesis: sterol regulatory element binding protein (SREBP)-1 activation and adenosine monophosphate kinase (AMPK) inhibition[15,31].

Alcohol consumption directly upregulates SREBP-1c gene expression through its metabolite acetaldehyde[19] or indirectly upregulates activating processes and factors such as ER stress[32], adenosine[33,34], endocannabinoids[35], LPS signaling *via* TLR4, and its downstream proteins, such as IRF-3, early growth response-1, and TNF-α.

AMPK is a key player in cellular and organism survival in metabolic stress through its ability to maintain metabolic homeostasis[36]. Chronic ethanol exposure inhibits AMPK activity, which increases activity of acetyl-CoA carboxylase and suppresses the rate of palmitic acid oxidation through the inhibition of liver kinase B1 phosphorylation[31,36].

The endocannabinoids, which are similar to the major active ingredient in marijuana, are endogenous lipid mediators that participate in the complex neural circuitry that controls energy intake[37]. There are at least two different cannabinoid receptors: CB1 and CB2. Recent studies indicate that while CB2 receptors mediate antifibrotic actions, the activation of CB1 receptors contribute to the development of fibrosis[37,38]. Both cannabinoid receptors are expressed in HSCs, and the inactivation of CB1 receptors decrease fibrogenesis by lowering TGF-β1 levels and reduce the accumulation of fibrogenic cells *via* downregulation of the PI3K/Akt signaling pathway[37]. Intriguingly, alcoholic liver steatosis is mediated mainly through HSC-derived endocannabinoids and their hepatocytic receptor[22,37]. Chronic alcohol consumption stimulates HSCs to produce 2-arachidonoylglycerol and its interaction with the CB1 receptor upregulates the expression of SREPB1c and fatty acid synthase, but downregulates the activities of AMPK and carnitine palmitoyltransferase 1[37,39].

### *Osteopontin*

Osteopontin (OPN) is a secreted, 44-66 kDa adhesive glycophosphoprotein that has involvement in both normal processes, such as bone development and immune system regulation, and pathologic processes, such as inflammation, cell transformation, tumor invasiveness, and metastasis[40]. OPN plays additional roles in ALD. In animal models, hepatic mRNA levels of OPN increased in ALD[15] and stimulated HSC activation in an autocrine and paracrine fashion[41]. Recently, Urtasun *et al*[42] investigated the mechanism of OPN in HSC activation. Recombinant OPN upregulated type I collagen production in primary HSCs in a TGF-β independent fashion, whereas it down-regulated matrix metalloprotease (MMP)-13. OPN induction of type I collagen occurred *via* integrin $\alpha_v\beta3$ engagement and activation of the PI3K/pAkt/NF-κB-signaling pathway[42]. On the other hand, recent studies indicate that OPN participates in the pathogenesis of hepatic steatosis, inflammation, and the fibrosis that results from non-alcoholic steatohepatitis[43]. OPN regulates steatohepatitis by stimulating the Hedgehog-signaling pathway[43]. In human ALD, hepatic mRNA levels of OPN correlate with hepatic neutrophil infiltration and the severity of fibrosis[44]. Finally, immunohistochemical detection of OPN is used as a prognostic biomarker to discriminate outcomes in some transplant patients with hepatocellular carcinoma derived from ALD[45].

### *IL-1 signaling*

Emerging data have provided evidence for the role of

IL-1 signaling in acute and chronic liver injury resulting from various causes, including acetaminophen-induced liver damage[46], nonalcoholic steatohepatitis[47], liver fibrosis[48], and immune-mediated liver injury[49]. However, the significance of IL-1 signaling in ALD has yet to be evaluated. A recent study from Petrasek *et al*[50] showed that activation of inflammasome-IL-1 signaling also plays a critical role in ethanol-induced liver injury in mice. Using IL-1 receptor antagonist-treated mice as well as 3 different mouse models deficient in regulators of IL-1β activation [caspase-1 (Casp-1) and ASC] or signaling (IL-1 receptor), they showed that IL-1β signaling is required for the development of alcohol-induced liver steatosis, inflammation, and injury. Interestingly, several fibrotic markers such as procollagen Ⅲ N-terminal propeptide (PⅢNP), tissue inhibitor of matrix metalloproteinase 1 (TIMP-1), and hyaluronic acid were downregulated in ethanol-fed Casp-1 knockout mice or in response to IL-1Ra treatment. Although the roles of inflammasome in HSC activation are not fully elucidated[51], it is suggested that targeting the inflammasome and/or IL-1 signaling pathways have therapeutic potential in ALD management. However, further studies are required to discover direct evidence of the relationship between IL-1 signaling and fibrogenesis in ALD.

### *Genetic variants associated with the fibrosis of ALD*

With the genotyping technique becoming more widely available, a great number of genetic case-control studies have evaluated candidate gene-variants that code proteins involved in the hepatic fibrosis[52]. Although two fibrosis-associated genes, including TGF-β and MMP 3, were evaluated in ALD[52], these genotypes are not associated with alcoholic liver cirrhosis[53,54]. Recent whole genome analyses of large numbers of genetic variants have identified novel yet unconsidered candidate genes[55]. Romeo *et al*[56] reported that the single-nucleotide polymorphism [rs738409(G), encoding I148M] in the patatin-like phospholipase domain-containing (PNPLA) 3 gene is a significant risk factor for increased hepatic fat accumulation and inflammation in nonalcoholic fatty liver disease. Subsequently, the strong association between the PNPLA3 I148 M allele and an increased risk of clinically evident alcoholic cirrhosis and liver cancer were confirmed in individual studies[57-60]. Most recently, Burza *et al*[61] reported that an increased age at onset of at-risk alcohol consumption and the PNPLA3 I148 M allele were independent risk factors for alcoholic liver cirrhosis (HR = 2.76; $P < 0.01$ *vs* 1.53; $P = 0.021$).

## CONCLUSION

In this review, several aspects potentially contributing to the mechanisms underlying fibrogenesis in ALD are discussed. Since there are no FDA-approved treatments for ALD at present, development of novel therapies for inhibiting inflammation and/or fibrogenesis associated with early stages of ALD will be beneficial for slowing disease progression and improving patient outcomes[51]. To achieve these objectives, animal models that accurately reflect the metabolic and histological characteristics of human ALD are needed.

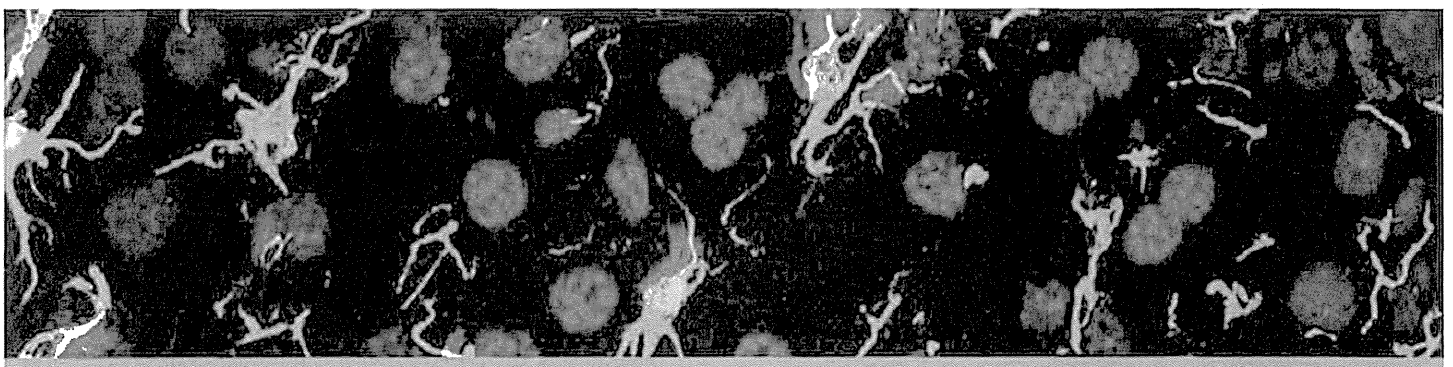
## REFERENCES


1 **Schwartz JM**, Reinus JF. Prevalence and natural history of alcoholic liver disease. *Clin Liver Dis* 2012; **16**: 659-666 [PMID: 23101975 DOI: 10.1016/j.cld.2012.08.001]
2 **Michitaka K**, Nishiguchi S, Aoyagi Y, Hiasa Y, Tokumoto Y, Onji M. Etiology of liver cirrhosis in Japan: a nationwide survey. *J Gastroenterol* 2010; **45**: 86-94 [PMID: 19789837 DOI: 10.1007/s00535-009-0128-5]
3 **Nakamura K**, Tanaka A, Takano T. The social cost of alcohol abuse in Japan. *J Stud Alcohol* 1993; **54**: 618-625 [PMID: 8412152]
4 **Altamirano J**, Bataller R. Alcoholic liver disease: pathogenesis and new targets for therapy. *Nat Rev Gastroenterol Hepatol* 2011; **8**: 491-501 [PMID: 21826088 DOI: 10.1038/nrgastro.2011.134]
5 **Crawford JM**. Histologic findings in alcoholic liver disease. *Clin Liver Dis* 2012; **16**: 699-716 [PMID: 23101978 DOI: 10.1016/j.cld.2012.08.004]
6 **Friedman SL**. Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. *Physiol Rev* 2008; **88**: 125-172 [PMID: 18195085 DOI: 10.1152/physrev.00013.2007]
7 **Kawada N**. Evolution of hepatic fibrosis research. *Hepatol Res* 2011; **41**: 199-208 [PMID: 21338451 DOI: 10.1111/j.1872-034X.2011.00776.x]
8 **Elamin EE**, Masclee AA, Dekker J, Jonkers DM. Ethanol metabolism and its effects on the intestinal epithelial barrier. *Nutr Rev* 2013; **71**: 483-499 [PMID: 23815146 DOI: 10.1111/nure.12027]
9 **Mello T**, Ceni E, Surrenti C, Galli A. Alcohol induced hepatic fibrosis: role of acetaldehyde. *Mol Aspects Med* 2008; **29**: 17-21 [PMID: 18164754 DOI: 10.1016/j.mam.2007.10.001]
10 **Liu Y**, Brymora J, Zhang H, Smith B, Ramezani-Moghadam M, George J, Wang J. Leptin and acetaldehyde synergistically promotes αSMA expression in hepatic stellate cells by an interleukin 6-dependent mechanism. *Alcohol Clin Exp Res* 2011; **35**: 921-928 [PMID: 21294755 DOI: 10.1111/j.1530-0277.2010.01422.x]
11 **Leung TM**, Nieto N. CYP2E1 and oxidant stress in alcoholic and non-alcoholic fatty liver disease. *J Hepatol* 2013; **58**: 395-398 [PMID: 22940046 DOI: 10.1016/j.jhep.2012.08.018]
12 **Zhu H**, Jia Z, Misra H, Li YR. Oxidative stress and redox signaling mechanisms of alcoholic liver disease: updated experimental and clinical evidence. *J Dig Dis* 2012; **13**: 133-142 [PMID: 22356308 DOI: 10.1111/j.1751-2980.2011.00569.x]
13 **Dey A**, Cederbaum AI. Alcohol and oxidative liver injury. *Hepatology* 2006; **43**: S63-S74 [PMID: 16447273 DOI: 10.1002/hep.20957]
14 **Albano E**. Oxidative mechanisms in the pathogenesis of alcoholic liver disease. *Mol Aspects Med* 2008; **29**: 9-16 [PMID: 18045675 DOI: 10.1016/j.mam.2007.09.004]
15 **Seth D**, Haber PS, Syn WK, Diehl AM, Day CP. Pathogenesis of alcohol-induced liver disease: classical concepts and recent advances. *J Gastroenterol Hepatol* 2011; **26**: 1089-1105 [PMID: 21545524 DOI: 10.1111/j.1440-1746.2011.06756.x]
16 **Smathers RL**, Galligan JJ, Stewart BJ, Petersen DR. Overview of lipid peroxidation products and hepatic protein modification in alcoholic liver disease. *Chem Biol Interact* 2011; **192**: 107-112 [PMID: 21354120 DOI: 10.1016/j.cbi.2011.02.021]
17 **Nieto N**. Ethanol and fish oil induce NFkappaB transactivation of the collagen alpha2(I) promoter through lipid peroxidation-driven activation of the PKC-PI3K-Akt pathway. *Hepatology* 2007; **45**: 1433-1445 [PMID: 17538965 DOI:

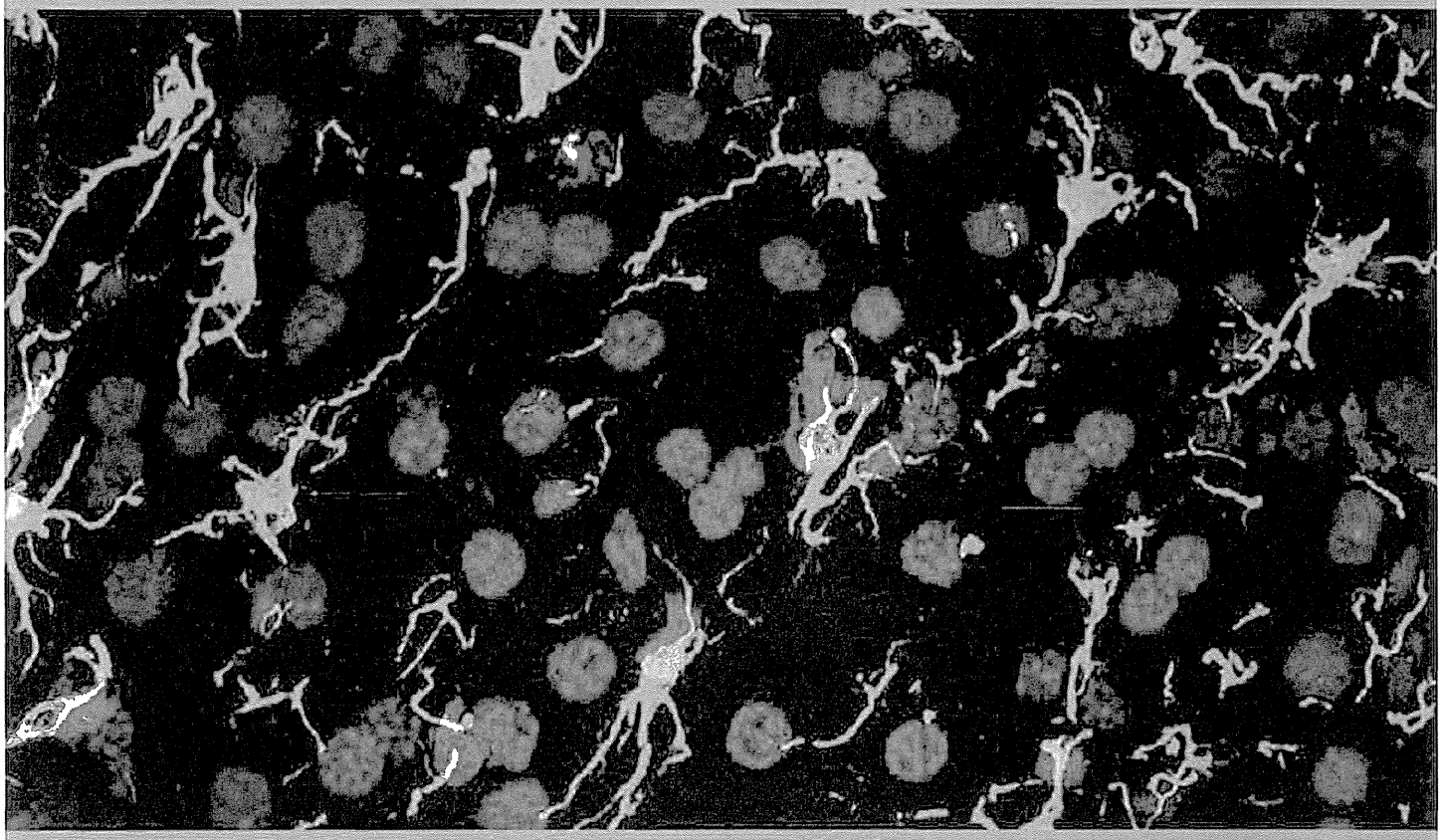
10.1002/hep.21659]
18 **Kharbanda KK**. Methionine metabolic pathway in alcoholic liver injury. *Curr Opin Clin Nutr Metab Care* 2013; 16: 89-95 [PMID: 23232418 DOI: 10.1097/MCO.0b013e32835a892a]
19 **Gao B**, Bataller R. Alcoholic liver disease: pathogenesis and new therapeutic targets. *Gastroenterology* 2011; **141**: 1572-1585 [PMID: 21920463 DOI: 10.1053/j.gastro.2011.09.002]
20 **Alkhouri N**, Carter-Kent C, Feldstein AE. Apoptosis in nonalcoholic fatty liver disease: diagnostic and therapeutic implications. *Expert Rev Gastroenterol Hepatol* 2011; 5: 201-212 [PMID: 21476915 DOI: 10.1586/egh.11.6]
21 **Petrasek J**, Iracheta-Vellve A, Csak T, Satishchandran A, Kodys K, Kurt-Jones EA, Fitzgerald KA, Szabo G. STING-IRF3 pathway links endoplasmic reticulum stress with hepatocyte apoptosis in early alcoholic liver disease. *Proc Natl Acad Sci USA* 2013; **110**: 16544-16549 [PMID: 24052526 DOI: 10.1073/pnas.1308331110]
22 **Suh YG**, Jeong WI. Hepatic stellate cells and innate immunity in alcoholic liver disease. *World J Gastroenterol* 2011; 17: 2543-2551 [PMID: 21633659 DOI: 10.3748/wjg.v17.i20.2543]
23 **Lavallard VJ**, Bonnafous S, Patouraux S, Saint-Paul MC, Rousseau D, Anty R, Le Marchand-Brustel Y, Tran A, Gual P. Serum markers of hepatocyte death and apoptosis are non invasive biomarkers of severe fibrosis in patients with alcoholic liver disease. *PLoS One* 2011; 6: e17599 [PMID: 21445263 DOI: 10.1371/journal.pone.0017599]
24 **Petrasek J**, Csak T, Szabo G. Toll-like receptors in liver disease. *Adv Clin Chem* 2013; **59**: 155-201 [PMID: 23461136]
25 **Gao B**, Seki E, Brenner DA, Friedman S, Cohen JI, Nagy L, Szabo G, Zakhari S. Innate immunity in alcoholic liver disease. *Am J Physiol Gastrointest Liver Physiol* 2011; **300**: G516-G525 [PMID: 21252049 DOI: 10.1152/ajpgi.00537.2010]
26 **Petrasek J**, Dolganiuc A, Csak T, Nath B, Hritz I, Kodys K, Catalano D, Kurt-Jones E, Mandrekar P, Szabo G. Interferon regulatory factor 3 and type I interferons are protective in alcoholic liver injury in mice by way of crosstalk of parenchymal and myeloid cells. *Hepatology* 2011; **53**: 649-660 [PMID: 21274885 DOI: 10.1002/hep.24059]
27 **Jagavelu K**, Routray C, Shergill U, O'Hara SP, Faubion W, Shah VH. Endothelial cell toll-like receptor 4 regulates fibrosis-associated angiogenesis in the liver. *Hepatology* 2010; 52: 590-601 [PMID: 20564354 DOI: 10.1002/hep.23739]
28 **Yan AW**, Fouts DE, Brandl J, Stärkel P, Torralba M, Schott E, Tsukamoto H, Nelson KE, Brenner DA, Schnabl B. Enteric dysbiosis associated with a mouse model of alcoholic liver disease. *Hepatology* 2011; **53**: 96-105 [PMID: 21254165 DOI: 10.1002/hep.24018]
29 **Rao R**. Endotoxemia and gut barrier dysfunction in alcoholic liver disease. *Hepatology* 2009; **50**: 638-644 [PMID: 19575462 DOI: 10.1002/hep.23009]
30 **Hartmann P**, Chen P, Wang HJ, Wang L, McCole DF, Brandl K, Stärkel P, Belzer C, Hellerbrand C, Tsukamoto H, Ho SB, Schnabl B. Deficiency of intestinal mucin-2 ameliorates experimental alcoholic liver disease in mice. *Hepatology* 2013; **58**: 108-119 [PMID: 23408358 DOI: 10.1002/hep.26321]
31 **Breitkopf K**, Nagy LE, Beier JI, Mueller S, Weng H, Dooley S. Current experimental perspectives on the clinical progression of alcoholic liver disease. *Alcohol Clin Exp Res* 2009; **33**: 1647-1655 [PMID: 19645734 DOI: 10.1111/j.1530-0277.2009.01015.x]
32 **Esfandiari F**, Medici V, Wong DH, Jose S, Dolatshahi M, Quinlivan E, Dayal S, Lentz SR, Tsukamoto H, Zhang YH, French SW, Halsted CH. Epigenetic regulation of hepatic endoplasmic reticulum stress pathways in the ethanol-fed cystathionine beta synthase-deficient mouse. *Hepatology* 2010; **51**: 932-941 [PMID: 19957376 DOI: 10.1002/hep.23382]
33 **Peng Z**, Borea PA, Varani K, Wilder T, Yee H, Chiriboga L, Blackburn MR, Azzena G, Resta G, Cronstein BN. Adenosine signaling contributes to ethanol-induced fatty liver in mice. *J Clin Invest* 2009; **119**: 582-594 [PMID: 19221436 DOI: 10.1172/JCI37409]
34 **Robson SC**, Schuppan D. Adenosine: tipping the balance towards hepatic steatosis and fibrosis. *J Hepatol* 2010; 52: 941-943 [PMID: 20395005 DOI: 10.1016/j.jhep.2010.02.009]
35 **Trebicka J**, Racz I, Siegmund SV, Cara E, Granzow M, Schierwagen R, Klein S, Wojtalla A, Hennenberg M, Huss S, Fischer HP, Heller J, Zimmer A, Sauerbruch T. Role of cannabinoid receptors in alcoholic hepatic injury: steatosis and fibrogenesis are increased in CB2 receptor-deficient mice and decreased in CB1 receptor knockouts. *Liver Int* 2011; 31: 860-870 [PMID: 21645218 DOI: 10.1111/j.1478-3231.2011.02496.x]
36 **Sid B**, Verrax J, Calderon PB. Role of AMPK activation in oxidative cell damage: Implications for alcohol-induced liver disease. *Biochem Pharmacol* 2013; 86: 200-209 [PMID: 23688501 DOI: 10.1016/j.bcp.2013.05.007]
37 **Tam J**, Liu J, Mukhopadhyay B, Cinar R, Godlewski G, Kunos G. Endocannabinoids in liver disease. *Hepatology* 2011; **53**: 346-355 [PMID: 21254182 DOI: 10.1002/hep.24077]
38 **Mallat A**, Teixeira-Clerc F, Deveaux V, Manin S, Lotersztajn S. The endocannabinoid system as a key mediator during liver diseases: new insights and therapeutic openings. *Br J Pharmacol* 2011; **163**: 1432-1440 [PMID: 21457226 DOI: 10.1111/j.1476-5381.2011.01397.x]
39 **Jeong WI**, Osei-Hyiaman D, Park O, Liu J, Bátkai S, Mukhopadhyay P, Horiguchi N, Harvey-White J, Marsicano G, Lutz B, Gao B, Kunos G. Paracrine activation of hepatic CB1 receptors by stellate cell-derived endocannabinoids mediates alcoholic fatty liver. *Cell Metab* 2008; **7**: 227-235 [PMID: 18316028 DOI: 10.1016/j.cmet.2007.12.007]
40 **Nagoshi S**. Osteopontin: Versatile modulator of liver diseases. *Hepatol Res* 2014; **44**: 22-30 [PMID: 23701387 DOI: 10.1111/hepr.12166]
41 **Seth D**, D'Souza El-Guindy NB, Apte M, Mari M, Dooley S, Neuman M, Haber PS, Kundu GC, Darwanto A, de Villiers WJ, Vonlaufen A, Xu Z, Phillips P, Yang S, Goldstein D, Pirola RM, Wilson JS, Moles A, Fernández A, Colell A, García-Ruiz C, Fernández-Checa JC, Meyer C, Meindl-Beinker NM. Alcohol, signaling, and ECM turnover. *Alcohol Clin Exp Res* 2010; **34**: 4-18 [PMID: 19860812 DOI: 10.1111/j.1530-0277.2009.01060.x]
42 **Urtasun R**, Lopategi A, George J, Leung TM, Lu Y, Wang X, Ge X, Fiel MI, Nieto N. Osteopontin, an oxidant stress sensitive cytokine, up-regulates collagen-I via integrin α(V)β(3) engagement and PI3K/pAkt/NFκB signaling. *Hepatology* 2012; **55**: 594-608 [PMID: 21953216 DOI: 10.1002/hep.24701]
43 **Syn WK**, Choi SS, Liaskou E, Karaca GF, Agboola KM, Oo YH, Mi Z, Pereira TA, Zdanowicz M, Malladi P, Chen Y, Moylan C, Jung Y, Bhattacharya SD, Teaberry V, Omenetti A, Abdelmalek MF, Guy CD, Adams DH, Kuo PC, Michelotti GA, Whitington PF, Diehl AM. Osteopontin is induced by hedgehog pathway activation and promotes fibrosis progression in nonalcoholic steatohepatitis. *Hepatology* 2011; **53**: 106-115 [PMID: 20967826 DOI: 10.1002/hep.23998]
44 **Patouraux S**, Bonnafous S, Voican CS, Anty R, Saint-Paul MC, Rosenthal-Allieri MA, Agostini H, Njike M, Barri-Ova N, Naveau S, Le Marchand-Brustel Y, Veillon P, Calès P, Perlemuter G, Tran A, Gual P. The osteopontin level in liver, adipose tissue and serum is correlated with fibrosis in patients with alcoholic liver disease. *PLoS One* 2012; **7**: e35612 [PMID: 22530059 DOI: 10.1371/journal.pone.0035612]
45 **Sieghart W**, Wang X, Schmid K, Pinter M, König F, Bodingbauer M, Wrba F, Rasoul-Rockenschaub S, Peck-Radosavljevic M. Osteopontin expression predicts overall survival after liver transplantation for hepatocellular carcinoma in patients beyond the Milan criteria. *J Hepatol* 2011; **54**: 89-97 [PMID: 20970216 DOI: 10.1016/j.jhep.2010.06.030]
46 **Imaeda AB**, Watanabe A, Sohail MA, Mahmood S, Mohamadnejad M, Sutterwala FS, Flavell RA, Mehal WZ. Acetaminophen-induced hepatotoxicity in mice is dependent on Tlr9

and the Nalp3 inflammasome. *J Clin Invest* 2009; 119: 305-314 [PMID: 19164858 DOI: 10.1172/JCI35958]
47 **Miura K**, Kodama Y, Inokuchi S, Schnabl B, Aoyama T, Ohnishi H, Olefsky JM, Brenner DA, Seki E. Toll-like receptor 9 promotes steatohepatitis by induction of interleukin-1beta in mice. *Gastroenterology* 2010; **139**: 323-324.e7 [PMID: 20347818 DOI: 10.1053/j.gastro.2010.03.052]
48 **Gieling RG**, Wallace K, Han YP. Interleukin-1 participates in the progression from liver injury to fibrosis. *Am J Physiol Gastrointest Liver Physiol* 2009; **296**: G1324-G1331 [PMID: 19342509 DOI: 10.1152/ajpgi.90564.2008]
49 **Petrasek J**, Dolganiuc A, Csak T, Kurt-Jones EA, Szabo G. Type I interferons protect from Toll-like receptor 9-associated liver injury and regulate IL-1 receptor antagonist in mice. *Gastroenterology* 2011; **140**: 697-708.e4 [PMID: 20727895 DOI: 10.1053/j.gastro.2010.08.020]
50 **Petrasek J**, Bala S, Csak T, Lippai D, Kodys K, Menashy V, Barrieau M, Min SY, Kurt-Jones EA, Szabo G. IL-1 receptor antagonist ameliorates inflammasome-dependent alcoholic steatohepatitis in mice. *J Clin Invest* 2012; **122**: 3476-3489 [PMID: 22945633 DOI: 10.1172/JCI60777]
51 **Mathews S**, Gao B. Therapeutic potential of interleukin 1 inhibitors in the treatment of alcoholic liver disease. *Hepatology* 2013; **57**: 2078-2080 [PMID: 23609413 DOI: 10.1002/hep.26336]
52 **Stickel F**, Hampe J. Genetic determinants of alcoholic liver disease. *Gut* 2012; **61**: 150-159 [PMID: 22110053 DOI: 10.1136/gutjnl-2011-301239]
53 **Osterreicher CH**, Halangk J, Berg T, Patsenker E, Homann N, Hellerbrand C, Seitz HK, Eurich D, Stickel F. Evaluation of the transforming growth factor beta1 codon 25 (Arg--& gt; Pro) polymorphism in alcoholic liver disease. *Cytokine* 2008; **42**: 18-23 [PMID: 18337117 DOI: 10.1016/j.cyto.2008.02.003]
54 **Stickel F**, Osterreicher CH, Halangk J, Berg T, Homann N, Hellerbrand C, Patsenker E, Schneider V, Kolb A, Friess H, Schuppan D, Puhl G, Seitz HK, Leathart JL, Day CP. No role of matrixmetalloproteinase-3 genetic promoter polymorphism 1171 as a risk factor for cirrhosis in alcoholic liver disease. *Alcohol Clin Exp Res* 2008; **32**: 959-965 [PMID: 18445105 DOI: 10.1111/j.1530-0277.2008.00654.x]
55 **Krawczyk M**, Müllenbach R, Weber SN, Zimmer V, Lammert F. Genome-wide association studies and genetic risk assessment of liver diseases. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 669-681 [PMID: 21045792 DOI: 10.1038/nrgastro.2010.170]
56 **Romeo S**, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, Boerwinkle E, Cohen JC, Hobbs HH. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008; **40**: 1461-1465 [PMID: 18820647 DOI: 10.1038/ng.257]
57 **Tian C**, Stokowski RP, Kershenobich D, Ballinger DG, Hinds DA. Variant in PNPLA3 is associated with alcoholic liver disease. *Nat Genet* 2010; **42**: 21-23 [PMID: 19946271 DOI: 10.1038/ng.488]
58 **Trepo E**, Guyot E, Ganne-Carrie N, Degre D, Gustot T, Franchimont D, Sutton A, Nahon P, Moreno C. PNPLA3 (rs738409 C& gt; G) is a common risk variant associated with hepatocellular carcinoma in alcoholic cirrhosis. *Hepatology* 2012; **55**: 1307-1308 [PMID: 22162034 DOI: 10.1002/hep.25518]
59 **Trépo E**, Gustot T, Degré D, Lemmers A, Verset L, Demetter P, Ouziel R, Quertinmont E, Vercruysse V, Amininejad L, Deltenre P, Le Moine O, Devière J, Franchimont D, Moreno C. Common polymorphism in the PNPLA3/adiponutrin gene confers higher risk of cirrhosis and liver damage in alcoholic liver disease. *J Hepatol* 2011; **55**: 906-912 [PMID: 21334404 DOI: 10.1016/j.jhep.2011.01.028]
60 **Seth D**, Daly AK, Haber PS, Day CP. Patatin-like phospholipase domain containing 3: a case in point linking genetic susceptibility for alcoholic and nonalcoholic liver disease. *Hepatology* 2010; **51**: 1463-1465 [PMID: 20373372 DOI: 10.1002/hep.23606]
61 **Burza MA**, Molinaro A, Attilia ML, Rotondo C, Attilia F, Ceccanti M, Ferri F, Maldarelli F, Maffongelli A, De Santis A, Attili AF, Romeo S, Ginanni Corradini S. PNPLA3 I148M (rs738409) genetic variant and age at onset of at-risk alcohol consumption are independent risk factors for alcoholic cirrhosis. *Liver Int* 2014; **34**: 514-520 [PMID: 24102786 DOI: 10.1111/liv.12310]

**P- Reviewers**: Dragoteanu M, He ST, Streba CT, Zhang SJ
**S- Editor**: Qi Y **L- Editor**: A **E- Editor**: Wang CH



# STELLATE CELLS
# IN HEALTH AND DISEASE



Edited by Chandrashekhar R. Gandhi
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# Stellate Cells in HEALTH AND DISEASE

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# CHAPTER 12

# Interactions of Stellate Cells with Other Non-Parenchymal Cells

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## 12.1 HEPATIC STELLATE CELLS: INTRODUCTORY REMARKS

Hepatic stellate cells (HSCs), formerly known as Ito cells, fat-storing cells, or lipocytes, reside in the space of Disse in the hepatic sinusoid, encapsulate sinusoidal endothelial cells with their well-branching dendritic processes on one side, and face hepatocytes on the other side [1]. In physiological conditions, the principal function of quiescent HSCs is storing vitamin A in their cytoplasm; 50–80% of vitamin A in the body is accumulated in the liver and 90% is stored in HSCs. Quiescent HSCs express neural crest markers, such as glial fibrillary acidic protein (GFAP) and neurotrophins and their receptor (p75), and an intermediate filament, desmin. Quiescent HSCs secrete extracellular matrix materials (ECMs) including laminin, proteoglycan, and type IV collagen, which form basement membrane-like structure [2–4]. Because HSCs function also as liver-specific pericytes, their contractility in response to endothelin-1 (ET-1), angiotensin-II, and relaxation by nitric oxide (NO) controls the diameter of the sinusoidal lumen and regulates the local microcirculation [5].

Following liver injury caused by hepatitis virus B or C (HBV or HCV) infection, alcohol abuse, drug toxicity, autoimmunity, or steatosis, HSCs undergo activation and transdifferentiate to myofibroblast (MF)-like cells [8]. These MF-like cells are characterized by a loss of vitamin A droplets, increased expression of α-smooth muscle actin (α-sma) and growth factor receptors, augmented contractile activity, and increased generation of multiple ECMs, largely types I and III collagens [2–4]. Activation of HSCs is triggered by paracrine stimulation by hepatic constituent cells, including sinusoidal endothelial cells, Kupffer cells (liver macrophages), hepatocytes, and cholangiocytes as well as platelets and following interactions with cells of the immune system. Activated HSCs secrete profibrogenic transforming growth factor β (TGF-β) as their autocrine stimulant and liver sinusoidal endothelial cells (LSECs) participate in conversion of TGF-β from its latency associated peptide-binding form to active form [6]. Cholangiocytes and platelets are also a source of platelet-derived growth factor

*Stellate Cells in Health and Disease*
DOI: http://dx.doi.org/10.1016/B978-0-12-800134-9.00012-9

(PDGF), TGF-β, and epidermal growth factor (EGF). Hepatocytes constitute another source of fibrogenic lipid peroxides and their apoptosis initiates the activation of HSCs via a process mediated by Fas and tumor-necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL). Apoptotic fragments derived from hepatocytes stimulate HSC activation in culture, and phagocytosis of apoptotic hepatocytes by MFs stimulates their fibrogenic activity via reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 2 and the janus kinase/signal transducer and activator of transcription (STAT) and phosphoinositide 3-kinase/Akt pathways [7,8].

Activation of HSC is controlled by transcription factors, such as activated protein-1, Jun D, Sp1, Kruppel-like factor 6, and nuclear factor kappa B (NF-κB), leading to transcriptional upregulation of latent TGF-β [9]. In this process, intracellular signaling molecules, such as Smad, Ras, Raf-1, and mitogen-activated protein (MAP) kinase, play important roles [10]. In addition, augmented production of the tissue inhibitor of matrix metalloproteinases (TIMPs) hampers the degradation of ECMs and conversely stimulates their accumulation in the inflamed liver [11]. Involvement of leptin and other adipocytokines in the HSC activation process is also notable [12]. Activated HSCs are also characterized by an increased expression of receptors for several polypeptides, including PDGF, TGF-β, vascular endothelial growth factor (VEGF), angiotensin-II, and ET-1 [13].

Along these lines, chronic activation of wound-healing reaction and of hepatic MFs is then essentially sustained by several growth factors, cytokines, and chemokines, as well as several additional mediators and environmental conditions (i.e., hypoxia). Thus, the development of progressive CLD may be envisaged to rely on a long-standing postulate of crosstalk/interactions between quiescent or activated HSCs and other populations of liver cells, with non-parenchymal cells playing a fundamental role.

## 12.2 THE CROSSTALK OF HSCs WITH MACROPHAGES

### 12.2.1 Liver macrophages (Kupffer cells)

Kupffer cells are resident liver macrophages adherent to sinusoidal endothelial cells inside the sinusoid and are the largest population of innate immune cells in the liver [14]. The principal function of Kupffer cells is to perform scavenger and phagocytic functions to remove protein complexes, small particles, senescent red blood cells, and cell debris from portal blood flow through pattern recognition receptors (PRRs). Kupffer cells also perform primary immune surveillance against gut-derived toxic materials, including endotoxin lipopolysaccharide (LPS) and pathogens from the intestinal flora. Thus, Kupffer cells, the most abundant pool of macrophages in the body, participate in the homeostasis by protecting the host and are able to trigger both immunogenic and tolerogenic immune responses [15,16].

Although it has been long considered that circulating monocytes contribute to the Kupffer cell pool, Naito et al. already showed in 1997 that Kupffer cells originate from fetal yolk sac precursor and undergo self-renewal throughout adult life [17]. More recent fate-mapping studies by Yona et al., using a mouse expressing constitutive and conditional CX3CR1 promoter-driven Cre recombinase, demonstrated that Kupffer cells are established prior to birth, maintained in adult steady state via self-renewal, and are independent of the input of bone marrow-derived monocytes [18]. Thus, Kupffer cells are able to renew themselves under stimulation with granulocyte/macrophage-colony stimulating factor (GM-CSF) and M-CSF. Although a specific marker to identify hepatic Kupffer cells is absent, human Kupffer cells are identified by their expression of CD14, CD16, and CD68, rat Kupffer cells by CD68 or CD163 (ED1 and ED2, respectively), and mouse Kupffer cells by F4/80.

### 12.2.2 Kupffer cells in hepatic inflammation and fibrogenesis

Upon inflammatory stimuli, Kupffer cells become activated and initiate their biological response. Endotoxin LPS derived from intestinal flora is a well-known stimulus of Kupffer cell activation. LPS binds to the toll-like receptor 4 (TLR4) with co-receptor CD14 and MD-2. TLR4 activates MyD88-dependent pathway to induce NF-κB and p38/c-jun N-terminal kinase (JNK) activation. When activated, Kupffer cells produce multiple bioactive substances, including cytokines such as TNF-α, interleukin 1 (IL-1), IL-6, IL-10, and IL-18, chemokines such as macrophage inflammatory protein 2 (MIP-2, CXCL2), macrophage chemotactic protein-1 (MCP-1, CCL2), RANTES (CCL5), MIP-1α (CCL3), MIP-1β (CCL4) and osteopontin, and reactive oxygen species (ROS), which lead to the infiltration of inflammatory cells into the liver [19]. In addition, activated Kupffer cells generate PDGF and TGF-β1, which in turn induce HSC activation (Figure 12.1).

*In vivo* macrophage depletion or blockade strategies revealed a major role for macrophages in liver fibrosis. For instance, Ide et al. showed that, in an animal model, depletion of Kupffer cells using the administration of gadolinium chloride ($GdCl_3$) suppresses α-sma–positive MF activation and ameliorates hepatic fibrosis in response to thioacetamide administration [20]. Rivera et al. also demonstrated that destruction of Kupffer cells with $GdCl_3$ or their inactivation with glycine ameliorates liver fibrosis induced by carbon tetrachloride ($CCl_4$) accompanied with downregulation of α-sma, α1(I) collagen mRNA and TGF-β1 protein expressions in the liver [21]. Aoyama et al. showed that CX3CR1 expression in Kupffer cells regulates HSC activation via the binding of HSC-derived CX3CL1 in $CCl_4$-induced mouse liver fibrosis [22]. Pradere et al. also demonstrated that hepatic macrophages enhance the survival of activated HSCs in a NF-κB-dependent manner and thereby promote liver fibrosis induced by bile duct ligation (BDL) in mice [23]. Taken together, liver macrophages

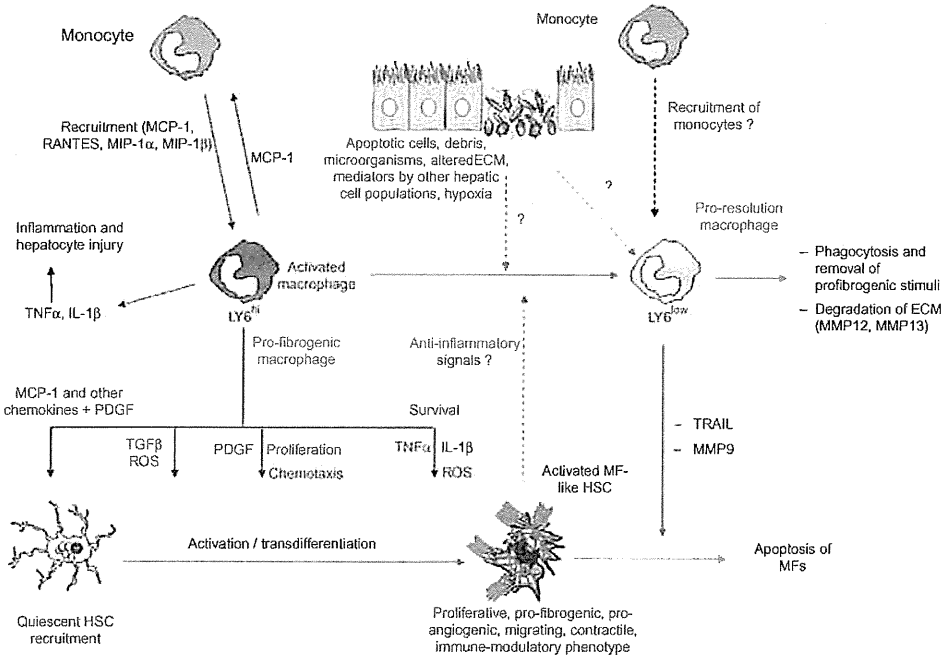


**Figure 12.1** ***Simplified scheme of interactions between macrophages and HSCs in CLD.*** During the course of fibrogenic progression of a CLD, inflammatory monocytes are recruited to the site of injury/inflammation by different chemoattractants, with a major role described for MCP-1 (CCL2), leading to a population of proinflammatory and profibrogenic macrophages ($LY6^{hi}$). These macrophages, in addition to sustained inflammation and further hepatocyte injury by releasing TNF and IL-1β, can release mediators able to sustain fibrogenesis by favoring the process of activation/transdifferentiation of HSC to MF-like, to regulate the related classic phenotypic responses of activated cells and enhance their survival. Pro-resolution macrophages ($LY6^{low}$) originate as a consequence of signals/mediators coming either from activated MFs (possibly CX3CL1) or from other hepatic cell populations in the scenario of chronic live injury. Pro-resolution macrophages remove cell debris and potential profibrogenic signals and express TRAIL and MMP9 that can promote apoptosis of MFs. In addition, these macrophages can release matrix metalloproteases such as MMP12 and MMP13 that can degrade/remodel extracellular matrix. More details in the text.

generally participate in the augmentation of liver fibrosis through the activation of HSCs primarily via TGF-β1 cascade.

### 12.2.3 Macrophage heterogeneity

Macrophage heterogeneity has been described by a diversity in cytokine production, cell surface markers, and transcriptional profiles. Macrophages have been classified either into M1 or M2 macrophages; M1 macrophages are linked to Th1 primed CD4 T cells and induced by IL-12, interferon (IFN)-γ, and LPS, whereas M2 macrophages associate with Th2 CD4 T cells and are controlled by IL-4, IL-13, and GM-CSF. It is

generally accepted that M1 macrophages are activated immediately as a defense against bacteria and viruses and release pro-inflammatory cytokines, such as TNF, IL-1β, IL-12, and ROS. M2 macrophages are generally thought to promote tissue-remodeling and secrete immune-modulatory mediators such as IL-4 and IL-13 via IL-4 receptor and IL-13 receptor α1, and characterized by unique signal transducers such as STAT6, arginase, or scavenger receptor (CD206) [24,25]. However, liver macrophages are shown to express markers of M1 and M2 differentiation simultaneously, indicating their ability to change phenotype markedly in response to injury.

As mentioned earlier, resident Kupffer cells play a role in early response of liver injury and secrete CCL2 and CCL5 chemokines and multiple cytokines. HSCs, upon activation by TLR4 ligands, are also a source of CCL2 and initiate monocyte recruitment. While the number of Kupffer cells decreases during inflammation and fibrogenesis, the number of monocyte-derived macrophages increases in inflamed liver. By using a model of conditionally depleted macrophages ($CD11b^+F4/80^+$) in CD-11b-diphtheria toxin receptor transgenic mice, Duffield et al. showed that macrophage depletion resulted in marked reduction of ECM deposition and of the number of α-sma–positive MFs in $CCl_4$ intoxication for 12 weeks, indicating that infiltrating monocytes exert a pro-fibrogenic role [26]. Karmark et al. tracked LY-6C, a cell surface glycoprotein that is widely used to identify functionally discrete mouse circulating monocyte population, and found that $Gr1^+$ ($Ly6C^{hi}$) inflammatory monocytes (their human counterpart is $CD14^+CD16^-$ monocytes expressing CCR2, CD64, and CD62L) are recruited into the liver in a CCR2-dependent manner during $CCl_4$-induced chronic liver injury in mice [27]. This interaction caused increase in the inducible NO synthase (iNOS)-positive $CD11b^+F4/80^+$ intrahepatic macrophages, which promote profibrogenic actions through direct activation of HSCs [28]. Thus, in the context of liver fibrogenesis in mice, infiltrating $Ly6C^{hi}CD11b^+F4/80^+$ macrophages play a central role in wound-healing response compared to $Ly6C^{low}CD11b^{low}F4/80^{hi}$ matured monocyte-derived and resident Kupffer cells.

### 12.2.4 Role of macrophages in fibrosis regression

It is generally accepted that regression of liver fibrosis occurs clinically in patients who achieve a sustained viral response (SVR) after the eradication of HCV by antiviral therapy or whose HBV viral level is well controlled by using nucleot(s)ide analogs, such as entecavir or tenofovir. In addition, substantial clinical data indicate that these treatments can not only regress cirrhosis but also promote recovery of hepatic functions of the liver and improve the prognosis of patients. Thus, the development of common anti-fibrotic therapy is anticipated regardless of the etiology of liver disease [6,29]. Regression of liver fibrosis is mechanistically explained by the following four aspects: (i) regeneration of hepatocytes, (ii) reversal of activated and MF-like HSCs to

vitamin A-storing quiescent phenotype, (iii) removal of MFs by apoptosis, and (iv) lysis of ECMs. Advanced liver fibrosis is far less reversible in humans while rodent models exhibit induction and spontaneous resolution of fibrosis.

Macrophages are crucial in the resolution process of liver fibrosis (Figure 12.1). They are a source of fibrolytic MMPs, including MMP-12 and MMP-13, and also express TRAIL that promotes apoptosis of activated MFs [30]. Recent understanding of macrophage heterogeneity, as shown by Ramachandran et al., has illustrated that $Ly6C^{low}CD11b^{hi}F4/80^{int}$ subset of macrophages is most abundant in the liver in a resolution stage and represents the principle MMP-expressing subset. It is noteworthy that $Ly6C^{low}$ macrophages are derived from a phenotypic transition of the profibrogenic $Ly6C^{hi}$ macrophages and characterized by evidence of prior phagocytosis of dying cells [31].

Regression of liver fibrosis is also characterized by the removal of activated HSCs and MFs from the scar. Two potential pathways, either reversal to a quiescent phenotype or clearance through apoptosis, are considered. While the former can be completely accomplished in culture model [32], recent studies by Troeger et al. using genetic tracking techniques have revealed that reversal of activated HSCs toward a more quiescent phenotype occurs in a mouse model of liver injury; approximately 50% of HSC-derived MFs adopted an intermediate phenotype and reacquired features of quiescence [33]. On the other hand, activated HSCs express CD95, equivalent to Fas, TNF receptor 1, p75 and TRAIL receptors, resulting in the apoptosis after the binding of respective ligands. In fact, gliotoxin, an inducer of apoptosis of activated HSCs *in vivo*, enhances the resolution of liver fibrosis [34,35].

## 12.3 HSC/MFs AND INTERACTIONS WITH OTHER CELLS OF INNATE AND ADAPTIVE IMMUNITY

### 12.3.1 HSCs in the scenario of an immunological organ like the liver

As mentioned in the previous section, activated HSCs closely interact with liver macrophages and this interaction is believed to be critical for fibrogenic progression of CLD, regardless of the etiology. In this scenario, several laboratories have provided compelling evidence that HSCs, in addition to the widely accepted conventional "profibrogenic" role, can serve as immune-regulatory cells because of their ability to secrete a number of mediators and polypeptides, including critical chemokines like MCP-1 or CCL2, regulated and normal T cell expressed and secreted (RANTES or CCL5) as well as several isoforms of MIPs. Moreover, it should be considered that HSCs express several TLRs and chemokine receptors including CCR5, CCR7, CXCR3, and CXCR7, thus also becoming a "target cell" for several pro-inflammatory mediators. Finally, HSCs have also been reported to function as putative antigen presenting cells (APCs) [2,36]. For these reasons, HSCs may be considered innate immune cells [3].

The interactions between HSCs and cells of either innate or adaptive immunity (Figure 12.2) are relevant for two main reasons: (i) they occur in the scenario of an organ like the liver, which is considered to be an "immunological" or "lymphoid" organ and (ii) these interactions as well as the activation of specific immune signaling pathways within HSC function together to promote or modulate liver fibrogenesis [3,37–39].

The definition of the liver as an "immunological" organ relies on its unique structural and anatomical organization, with the organ receiving blood flow mostly from the gastrointestinal (GI) tract through the portal vein. This antigen-rich blood from the GI tract is then forced to pass through liver sinusoids where it is continuously monitored by resident APCs (including Kupffer cells, LSECs, and dendritic cells but also even HSCs) [3,36–39] as well as by a heterogeneous lymphocyte population that is enriched particularly in natural killer (NK) and natural killer T (NKT) cells, and B lymphocytes as well as "conventional" T lymphocytes ($CD8^+$ and $CD4^+$ T cells). The definition of "conventional" T lymphocytes is useful in order to distinguish them from

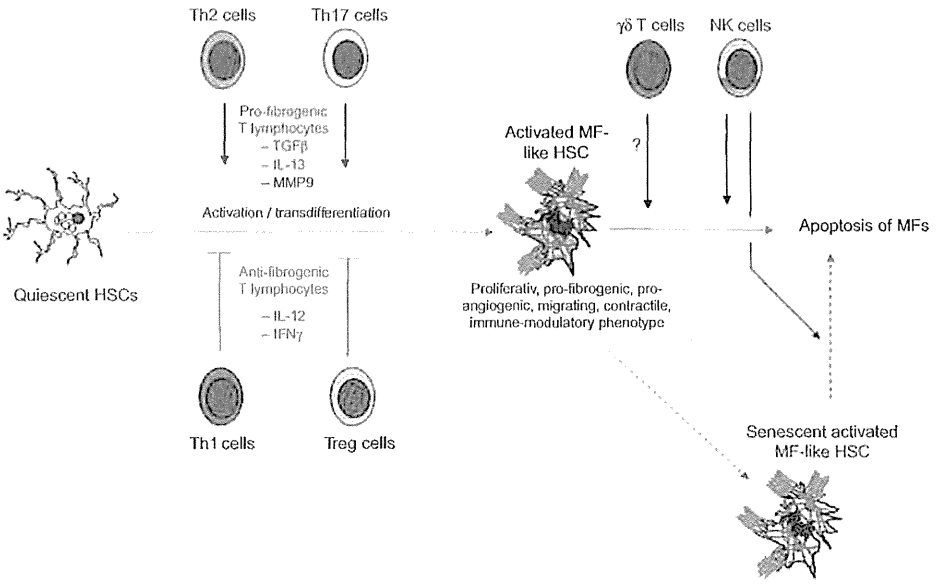


**Figure 12.2** ***A simplified scheme of interactions between immune cells and HSC in a CLD.*** Immune cells can differently affect fibrogenesis by modulating the process of activation/transdifferentiation of HSCs to activated and MF-like cells. In synthesis, Th2 and Th17 lymphocytes, directly by releasing mediators like IL-13 and MMP9 or indirectly by stimulating the expression of TGFβ, can sustain the process of activation. By contrast, Th1 and Treg cells are believed to slow down or inhibit the same process through the release of IFNγ and IL-12 and then by inhibiting/limiting Th2 response. The atypical γδ T cells, together with ROS and other cytokines and death ligands, can induce apoptosis of activated and MF-like HSC. NK cells can kill early activated HSC and senescent activated HSC but not fully activated and MF-like HSC.

those defined as "unconventional" T cells. The latter comprise various cell types that can be categorized into two major populations: (i) cells that express NK cell markers, also defined as classical NKT cells (either CD4-positive or CD4/CD8 double negative cells), which are abundant in the liver, and their migration or expansion in the liver being controlled by NK cells and (ii) cells that do not express NK cell markers or TCR γδ T cells (i.e., nonclassical NKT) a population representing 15–25% of all intrahepatic T cells; the liver is one of the richest sources of TCR γδ T cells, which are able to recognize a limited range of antigens such as stress proteins and non-protein antigens [37,40].

In general terms, NK and NKT cells play critical roles as first-line cellular immune defense against invading pathogens and they also play an important role in the modulation of liver injury and in the recruitment of circulating lymphocytes [3,37–39]. Indeed, circulating lymphocytes make contact with liver resident APC displaying antigens and can also contact hepatocytes directly, due to fenestrated sinusoidal endothelium that lacks a basement membrane. This overall "hepatic" scenario is believed to facilitate direct or indirect priming of lymphocytes as well as to modulate the immune response to hepatotrophic pathogens. Such interactions contribute to some of the unique immunological properties of the liver, including its capacity to induce antigen-specific tolerance [3,37–39].

### 12.3.2 NK and NKT cells: Their role in modulating HSC activity and fibrogenesis

Interest in the role of NK and NKT cells in modulating liver fibrogenesis has recently emerged on the basis of the fact that these cells are enriched among liver lymphocytes and are markedly altered in CLDs of different etiology, with NK cells proposed to prevent fibrogenic progression of CLDs and NKT cells to act by either inhibiting or favoring liver fibrosis [41].

NK cells, which belong to the innate immune system, are able to recognize and kill target cells by employing several cell surface receptors. NK cells detect changes in the expression of host cell surface molecules that usually appear on either viral-infected, transformed, or injured cells [42]. This results in NK cell activation followed by the killing of target cell mediated by either exocytosis of perforin and granzyme granules or through FAS ligand, TNF-α, and TRAIL [41,42]. NK cells are somewhat strategically located in the hepatic sinusoids close to liver non-parenchymal cells and represent a rather unique organ-associated NK cell population. They are characterized by a rapid turnover, with continuous substitution by bone marrow-derived cells. NK cells, which normally account for approximately 30–50% of human liver lymphocytes, increase dramatically in number in pathological conditions such as those related to viral infection as well as acute and chronic inflammation [41]. The most important message from studies in this field (reviewed in Ref. [41]) is that liver NK cells