Cellular Mechanisms of Tissue Fibrosis. 5. Novel insights into liver fibrosis

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Mallat A, Lotersztajn S. Cellular Mechanisms of Tissue Fibrosis. 5. Novel insights into liver fibrosis. Am J Physiol Cell Physiol 305: C789–C799, 2013. First published August 28, 2013; doi:10.1152/ajpcell.00230.2013.—Liver fibrosis is the common scarring reaction associated with chronic liver injury that results from prolonged parenchymal cell injury and/or inflammation. The fibrogenic response is characterized by progressive accumulation of extracellular matrix components enriched in fibrillar collagens and a failure of matrix turnover. This process is driven by a heterogeneous population of hepatic myofibroblasts, which mainly derive from hepatic stellate cells and portal fibroblasts. Regression of fibrosis can be achieved by the successful control of chronic liver injury, owing to termination of the fibrogenic reaction following clearance of hepatic myofibroblasts and restoration of fibrolytic pathways. Understanding of the complex network underlying liver fibrogenesis has allowed the identification of a large number of antifibrotic targets, but no antifibrotic drug has as yet been approved. This review will highlight recent advances regarding the mechanisms that regulate liver fibrogenesis and fibrosis regression, with special focus on novel signaling pathways and the role of inflammatory cells. Translation of these findings to therapies will require continued efforts to develop multitarget therapeutic approaches that will improve the grim prognosis of liver cirrhosis.

myofibroblasts; hepatic stellate cells; inflammation; chronic liver disease

HEPATIC FIBROSIS IS A COMMON pathological consequence of chronic liver diseases and results from the progressive accumulation of a qualitatively altered extracellular matrix that is highly enriched in type I and III fibrillar collagens (for reviews see refs. 23, 51, 77). Scar deposition results from an altered wound healing response to liver injury that is characterized by increased production of matrix proteins and decreased matrix remodeling. In a number of patients, iterative parenchymal insult ultimately leads to cirrhosis, a condition defined by an abnormal liver architecture, with fibrotic septa surrounding regenerating nodules and altered vascularization. Owing to decreased functional parenchymal reserve and altered hepatic blood flow, cirrhosis is associated with the life-threatening complications of liver failure, including hepatic encephalopathy, coagulation disorders and bacterial infections, and complications of portal hypertension such as ascites, variceal rupture, and hepatorenal syndrome. In addition, the cirrhotic liver is a precancerous state and thus requires the systematic screening for hepatocellular carcinoma. Recent reports from the Global Health Organization indicate that cirrhosis accounts for 170,000 deaths in Europe per year, which is 1.8% of all deaths. Predominant causes of liver fibrosis and cirrhosis include excessive alcohol intake, a leading factor in developed countries, and chronic hepatitis B and C in Asian and African countries (23, 51, 77). In addition, the rising epidemic of obesity and type 2 diabetes has dramatically increased the incidence of nonalcoholic fatty liver disease (58).

Detailed characterization of mechanisms that underlie liver fibrogenesis has disclosed striking similarities with fibrotic processes that occur with chronic injury in other tissues, including skin, lung, and kidney. Similar to what happens in other tissues, it is now well accepted that fibrosis is driven by a dynamic process composed of increased synthesis of matrix components and a failure of physiological mechanisms of matrix turnover. Moreover, the past 15 years have highlighted the capacity of the liver to undergo fibrosis regression, following cessation of the liver insult. These findings have constituted major breakthroughs in the understanding of the pathogenesis of chronic liver diseases and opened novel therapeutic approaches for the management of liver fibrosis.

This review will give an overview of new insights into the cellular and molecular events that regulate liver fibrogenesis, with special focus on pathways underlying the regression of fibrosis, the role of inflammatory cells, and emerging signaling pathways that may represent future therapeutic targets.

HETEROGENEITY OF LIVER FIBROGENIC CELLS

Extracellular matrix (ECM) accumulation during chronic liver injury is driven by a heterogeneous population of myofibroblasts that migrate and accumulate at the site of injury. Numerous studies have traced the origin of hepatic myofibroblasts and have led to the conclusion that extracellular ECM-producing cells mainly derive from resident mesenchymal cells, i.e., hepatic stellate cells (HSCs) and resident fibroblasts, whereas extrahepatic precursors are minor contributors to the fibrogenic population (Fig. 1).

Resident Mesenchymal Cells

Hepatic stellate cells. Hepatic stellate cells (HSCs) are considered as the main source of ECM during liver fibrosis and are the first identified fibrogenic cell population (20). HSCs are vitamin A-rich cells located in the perisinusoidal space, between endothelial cells and hepatocytes. In the normal liver, they display a quiescent phenotype, characterized by the expression of a large number of adipogenic genes and neural markers.

Upon acute or chronic liver injury, a complex network of autocrine/paracrine fibrogenic signals promotes transdifferentiation of quiescent HSCs to a myofibroblastic phenotype characterized by the expression of α-smooth muscle actin and a parallel loss of retinoids and lipid droplets, a reduction in the expression of adipogenic/ligoprotein factors, and de novo expression of receptors for fibrogenic, chemotactic, and mitogenic factors. These phenotypic changes can be recapitulated...
in vitro, by the use of isolated quiescent HSCs plated on uncoated tissue culture plastic (for reviews see refs. 23, 51, 77).

**Portal fibroblasts.** Portal fibroblasts undergo myofibroblastic differentiation and play a prominent role in the fibrogenic process during biliary and cholestatic liver diseases (5, 91). They also probably contribute to fibrotic septa originating from portal tracts in other chronic liver diseases.

Activated HSCs and portal myofibroblasts express some common markers, but gene profiling has shown that one can differentiate the two cell populations by specific markers, including interleukin-6 (IL-6), Fibulin-2, Thy-1, elastin, and cofillin for portal myofibroblasts, and desmin, reelin, the protease P100, cytoglobin, α-2 macroglobin, and synaptophysin for activated HSCs (5). In terms of biological functions, both cell types show similar properties characteristic of fibrogenic cells, with the exception of a greater resistance to apoptosis and higher proliferative capacity for portal fibroblasts (43).

**Bone Marrow-Derived Myofibroblasts**

The bone marrow stem cell origin of a fraction of hepatic myofibroblasts has been suggested based on the detection of Y chromosome-positive myofibroblasts in the cirrhotic human or mice liver, following sex-mismatched bone marrow transplant (18). Discrepant findings were found in subsequent cell tracking studies in mice transplanted with bone marrow from green fluorescent protein (GFP)-positive animals (28). Bone marrow-derived circulating fibrocytes have also been evoked as precursors of hepatic myofibroblasts, but bone marrow transplant experiments into collagen I-GFP mice indicated that their contribution is probably minor (42).

**Epithelial to Mesenchymal Transition**

Cell culture studies have shown that hepatocytes and cholangiocytes exposed to transforming growth factor-β (TGF-β) may undergo epithelial-to-mesenchymal transition (EMT) and acquire mesenchymal features, including fibroblast-specific protein-1 (FSP-1) expression (98). However, cell fate mapping experiments did not support the in vivo relevance of these findings, by showing no colocalization of epithelial cell markers with mesenchymal markers during chronic liver injury; similar conclusions were drawn in studies of lung or kidney (95), therefore strongly arguing against an epithelial origin of ECM-producing cells (85).

**Fibrogenic Properties of Hepatic Stellate Cells and Portal Myofibroblasts**

Fibrogenic properties of hepatic myofibroblasts have been extensively discussed (for reviews see refs. 23, 51, 77) and are summarized in Fig. 2. Myofibroblasts are highly proliferative cells with enhanced survival that migrate and accumulate at sites of liver injury in response to paracrine/autocrine effects of a wide variety of growth factors, cytokines, lipid mediators, or adipokines produced by the injured liver (Fig. 2). Moreover, these cells synthesize and secrete an array of ECM proteins predominantly in fibrillar collagens. Finally, they also produce a wide range of ECM-degrading enzymes [matrix metalloproteinases (MMPs)], MMP activators that cleave pro-MMPs into their active form and specific tissue inhibitors of the metalloproteinase family (TIMPs). Production of MMPs and TIMPs is tightly regulated according to the activation state of HSCs and portal fibroblasts.
reflects ECM remodeling during chronic liver injury. At early stages, HSCs express MMPs and their activators, but not TIMPs. The resulting degradation of normal liver ECM allows substitution by fibrillar collagens. In contrast, fully activated HSCs shut down expression of MMPs and turn on expression of TIMPs, resulting in inhibition of matrix degradation (32, 51).

**HEPATIC MYOFIBROBLASTS: CELLS WITH MULTIPLE FUNCTIONS BEIDES FIBROGENESIS**

**Immunoregulation**

Hepatic stellate cells and portal myofibroblasts are now regarded as key regulators of the hepatic immune response (23, 51, 77), owing to their capacity to amplify the inflammatory response in the injured liver by promoting recruitment of inflammatory cells (Fig. 2). They produce chemokines [such as monocyte chemoattractant protein-1 (MCP-1) and RANTES] that regulate mononuclear cell and neutrophil recruitment (59, 78), express chemokine receptors with profibrogenic properties [e.g., CCR5 (78)], and behave as classical antigen presenting cells (93).

The identification of Toll-like receptors (TLRs) in HSCs has uncovered the major contribution of gut-derived bacterial products in the inflammatory reaction underlying progression of fibrosis (29). Chronic liver diseases are associated with enhanced portal inflow of gut-derived microbiota products [lipopolysaccharide (LPS), bacterial DNA, peptidoglycan, viral and fungal components host DNA], due to increased intestinal permeability (29). Several studies have reported the beneficial impact of intestinal decontamination by antibiotics on liver fibrogenesis, suggesting that gut microbiota enhances liver fibrogenesis (79). Consistent with this idea, mice with knockout (KO) of TLR4, TLR2, and TLR9 are protected from liver fibrosis (29). Moreover, TLR4 (the LPS receptor), TLR9, and TLR2 have been identified in HSCs, and their stimulation by LPS or bacterial products has been shown to induce a proinflammatory response (29).

**Angiogenesis**

Liver fibrosis is associated with alterations in hepatic vascular architecture that create an hypoxic milieu, which is a major stimulus to angiogenesis. In this environment, HSCs, which are located in close contact with endothelial cells, produce multiple angiogenic factors, including vascular endothelial growth factor (VEGF), angiopoietin 1 or 2, hedgehog ligands, and PDGF-BB, and express their receptors (63, 75, 84, 94). These angiogenic factors enhance the fibrogenic properties of HSCs and contribute to angiogenesis by eliciting paracrine signals for neighboring endothelial cells.

**Contribution of Hepatic Myofibroblasts to Liver Regeneration and Carcinogenesis**

HSCs and liver regeneration. The liver has a remarkable capacity to regenerate after resection or liver injury. The
regenerative response is promoted by mature hepatocytes or liver progenitor cells that differentiate into hepatocytes or cholangiocytes, depending on the nature and the extent of liver insult. Recent studies have demonstrated that cross-talk between fibrogenic cells and either hepatocytes or progenitor cells may also link fibrogenesis to liver regeneration. The underlying mechanism relies on the secretion by activated HSCs and portal fibroblasts of key mitogenic and antiapoptotic signals for hepatocytes or liver progenitor cells (i.e., hepatocyte growth factor, IGF-I, neurotrophins, interleukin-6, and Wnt ligands), which foster an environment favoring liver regeneration (6, 45, 68, 76, 86, 92). Activated HSCs can also orchestrate the specification of hepatic progenitor cells towards the biliary lineage, thereby contributing to biliary regeneration (6). Finally, activated HSCs also generate antiproliferative signals for hepatocytes such as TGF-β, which inhibit hepatocyte regeneration but which also have profibrogenic properties.

Another example is provided by the serotonin receptor 5-HT2B, which, in activated HSCs, combines profibrogenic properties with TGF-β-promoted antiproliferative properties towards hepatocytes (15).

HSCs and liver carcinogenesis. More than 80% of cases of hepatocellular carcinoma arise from a cirrhotic liver. Several studies have shown that activated HSCs may also contribute to liver carcinogenesis, owing to their capacity to generate a permissive environment that facilitates liver tumor cell growth. Coculture experiments have demonstrated that activated HSCs promote migration, growth, invasion, and survival of hepatocarcinoma and cholangiocarcinoma cells (11, 13). In addition, hepatic myofibroblasts secrete proinflammatory and proangiogenic factors essential for tumor growth, and in parallel inhibit immune surveillance by natural killer (NK) and natural killer T (NKT) cells (13, 53, 97).

HEPATIC MYOFIBROBLASTS: NOVEL MECHANISMS UNDERLYING ACQUISITION AND PERSISTENCE OF THE FIBROGENIC PHENOTYPE

Mechanisms governing acquisition of a myofibroblastic phenotype have been extensively characterized (23, 51, 77). Parenchymal injury and the resulting inflammatory reaction generate a large panel of signals that promote induction of specific sets of transcription factors and morphogens (Hedgehog ligands, Wnt) in quiescent HSCs, thereby triggering the activation program, and the acquisition of fibrogenic and proinflammatory properties (23, 51, 77). The activation process occurs in response to classical signals including lipid peroxides reactive oxygen species, proinflammatory and mitogenic cytokines and growth factors (Figs. 2 and 3). The matrix itself contributes to the activation process via integrin-mediated pathways activated by ECM molecules, matrix stiffness, and the degree of collagen cross-linking (32, 67). More recently, reprogramming of the HSC metabolic program and epigenetic events have been identified as additional mechanisms driving the HSC activation/deactivation program.

Metabolic Control of the Myofibroblastic Phenotype

The parallel between adipocytes and hepatic stellate cells has been emphasized, with several studies reporting similar transcriptional programs between differentiated adipocytes and quiescent HSCs (8, 80). Reprogramming of the metabolic program. Quiescent HSCs display adipocyte-like features and are characterized by their abundance in lipid droplets, which contain retinyl esters, triglycerides, cholesterol, and fatty acids. They express lipogenic genes (acyl-CoA carboxylase, fatty acid synthase, genes controlling fatty acid oxidation and uptake, and fatty acid binding proteins) and lipogenic transcription factors (SREBP-1c, C/EBP, LXR-α, and PPAR-γ), all of which are downregulated along with HSC activation (8, 80). Interestingly, recent data indicate that more generally, transition to a myofibroblastic phenotype requires metabolic reprogramming of HSCs, characterized by inhibition of lipogenesis and gluconeogenesis, and parallel activation of aerobic glycolysis (9). The resulting accumulation of lactate enhances metabolic reprogramming of HSC gene expression, leading to the high proliferative fibrogenic myofibroblastic phenotype.

Autophagy. A number of cells maintain energy homeostasis through autophagic digestion of intracellular lipids (lipophagy). Because the loss of lipid droplets is a feature of HSC activation, autophagy was hypothesized to drive HSC activation by digesting lipid droplets, thereby providing the energy required for the activation process. Two groups independently reported that pharmacological or genetic inhibition of autophagy leads to growth inhibition and downregulation of the fibrogenic properties of HSC (24, 88). These results identified inhibition of HSC autophagy as a novel antifibrotic target. However, a more complex scheme is emerging, as recent data indicate that, in other hepatic cell types, autophagy reduces profibrogenic signals, by protecting hepatocytes from apoptosis (2) and eliciting anti-inflammatory effects in Kupffer cells (Lodder J, unpublished observations).

Epigenetic Regulation of Hepatic Stellate Cell Phenotype

Several recent studies have highlighted the role of molecular epigenomic events, including miRNA regulation, DNA methylation, and associated histone protein modification, in the activation process of HSCs (90).

miRNAs. MicroRNAs (mi-Rs) are small noncoding RNAs that regulate posttranscriptional gene repression, by decreasing target mRNA levels. A large panel of mi-Rs are expressed in HSCs and tightly control fibrosis progression. These mi-Rs include mi-R 29, mi-R 19b, and mi-R 221/222, among others. Mi-R 29 is a physiological inhibitor of various ECM proteins including collagens. Expression of mi-R 19b is strongly decreased in experimental models and in patients with advanced fibrosis (74), and mi-R 29 is downregulated by TGF-β and LPS in cultured HSCs (47, 74). Mi-R 19b, which is an inhibitor of TGF-β signaling, is downregulated in rodent and human fibrotic liver, and its overexpression in HSCs blocks the activation process (47). Finally, mi-R 221/222 is upregulated in human liver in parallel with progression of liver fibrosis. Its expression also increases during HSC activation, and its contribution to HSC proliferation has been proposed (66).

DNA methylation and histone modifications. DNA methylation of genes expressed in quiescent HSCs probably contributes to the maintenance of the quiescent phenotype. Upon activation, HSCs express DNA methyl-binding proteins (MeCP2) that promote silencing of antifibrogenic genes such as IkB-α or PPAR-γ and increase the expression of histone methyl transferase, leading in turn to enhanced transcription of...
collagen, TIMP-1, and TGF-β (90). Intriguingly, heritable epigenetic changes may also modulate fibrosis susceptibility, as demonstrated in a recent study showing that offspring from the progeny of male fibrotic rat ancestors are more resistant to liver fibrosis than counterparts with no previous history of fibrosis (99). Resistance to the wound healing process was ascribed to remodeling of DNA methylation and histone acetylation in the sperm of rats with fibrosis, with a subsequent hypomethylation of the PPAR-γ gene, resulting in elevated hepatic expression of this antifibrogenic transcription factor in adult offspring (99).

REGRESSION OF FIBROSIS

Several clinical reports have documented that regression of liver fibrosis occurs in a substantial proportion of patients, provided that the factor responsible for liver insult is eradicated or controlled (7, 16, 57). Consistent with this observation, studies in rodents have also documented regression of fibrosis or early stage cirrhosis within weeks following eradication of the toxic insult. Reversal of fibrosis follows the restoration of fibrolytic activity in the liver, due to upregulation and activation of MMPs (31). Restoration of fibrolytic activity is initiated upon suppression of hepatic TIMPs, following elimination of hepatic myofibroblasts by apoptosis (31), senescence (46), or reversion to a quiescent phenotype (41, 89) (Fig. 3), suggesting that clearance of activated HSCs is a key step in the onset of regression of fibrosis. Myeloid cell subsets (“restorative” macrophages and dendritic cells), which constitute a major source of MMP critical for fibrosis resolution, and endothelial cells, which maintain HSCs in a quiescent phenotype, have also been identified as contributing to the resolution of fibrosis (see the section “control of the fibrogenic phenotype by neighboring liver cells” below).

Mechanisms of Resolution of Fibrosis

HSC apoptosis. Follow-up of rats exposed to CC\textsubscript{14} for 8 wk has shown that the recovery phase is associated with an early decrease in hepatic TIMP-1 and a parallel decrease in the density of activated HSCs due to apoptosis (31). Experiments in TIMP-1 transgenic mice and with TIMP-1 scavengers demonstrated the causal relationship between hepatic TIMP-1 expression, failure of fibrolysis, and increased HSC survival (32). Further studies identified NF-κB as an important transcription factor in the upregulation of antiapoptotic genes in activated

Fig. 3. Summary of the potential mechanisms governing acquisition of the myofibroblastic phenotype during fibrosis progression and elimination of activated fibrogenic cells during resolution. In response to acute or chronic parenchymal injury, fibrogenic cells acquire a myofibroblastic phenotype as a consequence of the action of cytokines, chemokines, growth factors, lipid mediators, and reactive oxygen species produced by epithelial cells (hepatocytes, cholangiocytes), endothelial cells, and cells of the innate and adaptive immune system (macrophages, dendritic cells, B and T lymphocytes). Activated myofibroblasts release chemokines and growth factors that further amplify the activation process and the inflammatory reaction. Clearance of fibrogenic cells is a key step in fibrosis resolution that precedes restoration of fibrolysis. Elimination of liver fibrogenic cells is controlled by neighboring cells and can occur via apoptosis, senescence, or reversal of the activated phenotype.
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HSCs and showed that inhibitors of NF-κB signaling induce apoptosis of activated HSCs and reversal of fibrosis (65).

**HSC senescence.** Senescent hepatic myofibroblasts may contribute to the regression of fibrosis because they stop proliferating, and upregulate the expression of matrix degrading enzymes and downregulate the expression of ECM proteins (46). Moreover, senescent hepatic myofibroblasts can be cleared by NK cells (46). Thus, senescence of hepatic myofibroblasts can prevent further proliferation of these ECM-producing cells, promote ECM degradation, and accelerate myofibroblast clearance from the site of injury.

**Reversion of HSC phenotype to an inactivated state.** Recent cell tracking studies have further documented earlier in vitro studies showing that activated HSCs can undergo deactivation to a quiescent phenotype following cessation of liver injury (41, 54, 89). However, reverted HSCs do not reacquire all of the characteristics of quiescent cells, but rather retain an activated intermediate state with enhanced susceptibility to a fibrogenic stimulus (41, 89). These data raise the intriguing possibility that reverted HSCs contribute to fibrosis reversal but may promote more rapid and severe fibrosis progression upon recurrence of liver injury.

**Irreversibility of Advanced Fibrosis**

Experiments in animals with advanced cirrhosis due to prolonged injury have shown that withdrawal of the liver insult is associated with partial remodeling of cirrhosis from a micronodular to a macronodular pattern, even after a prolonged period of observation, indicating that the potential for reversibility of fibrosis declines at advanced stages (32). Mechanisms underlying the limited remodeling of the scar matrix in models of advanced fibrosis remain only partially understood. Available data suggest that increased matrix stiffness and matrix cross-linking of collagen I in older septae induces resistance to the collagenolytic effects of MMPs (32, 67). Enzymes implicated in collagen cross-linking comprise tissue transglutaminase and lysyl-oxidase, which stabilize ECM (32, 70). Interestingly, the antifibrogenic efficacy of lysyl oxidase-like-2 (LOXL2) has been validated in preclinical models (4), and a clinical trial with a LOXL2 antibody is underway in patients with nonalcoholic steatohepatitis (NASH; NCT 01672866 and 01672879).

**CONTROL OF THE FIBROGENIC PHENOTYPE BY NEIGHBORING LIVER CELLS**

Acquisition of the fibrogenic phenotype is controlled by complex interactions with hepatocytes and nonparenchymal cells, including endothelial cells and immune cells.

**Hepatocytes**

Hepatocyte apoptosis is a common feature in chronic liver disease. Although apoptotic cells are usually eliminated by macrophages, recent data have shown that activated hepatic myofibroblasts may behave as nonprofessional phagocytes and phagocytize hepatocyte-derived apoptotic bodies (35). Interestingly, engulfment of hepatocyte-derived apoptotic bodies by HSCs is profibrogenic and promotes myofibroblast survival (35). Thus, phagocytosis links hepatocyte apoptosis to HSC activation, survival, and ECM production.

**Endothelial Cells**

Hepatic fibrogenesis is associated with an early activation of liver sinusoidal endothelial cells (LSEC), including loss of endothelial cell fenestration (i.e., capillarization of sinusoids), which precedes onset of fibrosis. Activated endothelial cells contribute to HSC activation, owing to their capacity to produce TGF-β and PDGF-BB, and to secrete ECM components, including collagen I. Conversely, recent findings indicate that restoration of LSEC differentiation may accelerate fibrosis regression by promoting HSC quiescence (96). The proposed paradigm is that differentiated LSECs maintain HSCs in a quiescent phenotype and that this gatekeeper function is lost with capillarization. Conversely, changes in LSEC differentiation during capillarization are key for progression of fibrosis.

**Immune Cells**

Sustained hepatic inflammation resulting from parenchymal injury is a driving force of fibrosis progression in the liver, as described in other organs. Selective depletion of individual inflammatory cells, either pharmacologically or genetically, has also revealed the impact of resident (Kupffer cells, dendritic cells) and infiltrating immune cells (macrophages, dendritic cells, and lymphocytes, including NK, NKT, T and B cells) on the resolution of fibrosis (Fig. 3).

**Innate Immune Cells**

**Monocyte/macrophages.** Activation of Kupffer cells and recruitment of monocyte/macrophages are key events governing initiation, perpetuation, and resolution of fibrosis. The influx of monocytes into the liver gives rise to inflammatory profibrogenic GR1hi (Ly6Chi) macrophages, expressing high levels of CD11b and F4/80 (38, 49). The important role of Kupffer cells and infiltrating macrophages in the initiation and progression of fibrosis has been extensively characterized, using pharmacological or conditional genetic ablation of monocytes/macrophages, in mice with ongoing liver injury (14, 30). These findings have been corroborated by in vitro studies showing that Kupffer cells promote activation and survival of HSCs (71).

In sharp contrast, experiments during the recovery phase have shown that macrophages harboring a distinct phenotype induce HSC apoptosis and produce active metalloproteinase, thereby inducing resolution of fibrosis (14, 17). Overall, these data indicated that in experimental models, distinct macrophage subsets regulate fibrosis progression and resolution. However, their characteristic profile remains incomplete, and classification according to an M1/M2 phenotype classification is probably oversimplified. “Restorative macrophages” show no expression of TGF-β, low levels of TNF-α mRNA and may promote HSC apoptosis through expression of HSC death ligands such as TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) and MMP-9 (17). However, their signature is characterized by a mixed expression of M1 and M2 markers that can be distinguished from their profibrogenic counterparts by a low versus high Gr1 (Ly6C) expression, a high phagocytic and matrix degrading activity, and a high expression of MMP-13 (71, 73). Additional studies are required to validate the relevance of these findings in patients with regression of liver fibrosis.
Kupffer cells and recruited monocytes express a number of chemokine receptors that control fibrosis progression and resolution. The proinflammatory/profibrogenic Gr1\(^{hi}\) (Ly6C\(^{hi}\)) monocytes express CCR1, CCR2, CCR6, CCR8, and CCR9 (10, 25, 61, 78). Activation of these receptors promotes differentiation of infiltrating monocytes into proinflammatory macrophages and helps HSC activation, as shown by genetic or pharmacological interventions or adoptive transfer experiments (10, 25, 61, 78). The Gr1\(^{lo}\) monocyte subset expresses chemokine receptors with anti-inflammatory and antifibrogenic functions, such as CXC1R and CX3CR1 (3, 39). For example, the CX3CR1 ligand CX3CL1 promotes macrophage survival and a shift towards an anti-inflammatory M2 phenotype, thereby reducing hepatic inflammation and fibrosis (3). Interestingly, a role for CCR2 in resolution of fibrosis has also been suggested (61).

**Dendritic cells.** Dendritic cells are CD11c\(^{+}\) cells that play a key role in the regulation of inflammation, owing to their capacity to modulate innate and adaptive immune responses, via recruitment of immune effectors cells such as NK cells, NKT cells, and neutrophils. Similarly to monocyte/macrophages with which they may share a common Gr1\(^{hi}\) monocyte precursor, dendritic cells orchestrate the inflammatory response during both progression and resolution of liver fibrosis (12, 22, 36). However, their direct contribution to the fibrogenic process has recently been challenged (22, 71), and conditional ablation of CD11c\(^{+}\) cells in fibrotic mice has suggested that dendritic cells promote fibrosis resolution via the release of MMP-9, a gelatinase active on collagens I, III, IV, and elastin (36). Detailed comparison of the respective contribution of dendritic cells and macrophages to fibrolysis will require better phenotypic discrimination between the two cell types.

**Natural killer and natural killer T cells.** Liver-specific NK and NKT cells are lymphocytes with antiviral and antitumorigenic properties. In the context of liver fibrosis, compelling evidence suggests that NK cells reduce fibrogenesis by inducing apoptosis of early activated and senescent HSCs via TRAIL-mediated pathways (46, 72). Susceptibility of HSCs to NK cell-mediated killing results from increased expression of retinoic acid early inducible 1 (RAE1), a ligand for the NK cell-activating receptor NKG2D, and from decreased expression of the NK cell-inhibitory ligand major histocompatibility complex class I (MHC-I) by HSCs (46, 72). In addition, NK cells also limit HSC accumulation by releasing IFN-\(\gamma\), an antiproliferative and apoptotic mediator of HSCs. Interestingly, fully activated HSCs lose their capacity to produce RAE1, which may contribute to their survival in the chronically injured liver (19, 72).

The functions of NKT cells have been investigated through the use of CD1d-deficient mice (type I iNKT and type II NKT-deficient) and Jo18-KO mice (iNKT-deficient). Both antifibrogenic and profibrogenic effects have been described, depending on the model and the extent of liver injury (19). Profibrogenic effects rely on, T helper (Th) 2 (Th2) and Th17 mediators produced by NKT cells, including IL-4, IL-13, or IL-17, whereas NKT cell-mediated apoptosis of HSCs has been ascribed to the release of IFN-\(\gamma\) (19). Further studies are required to understand whether the heterogeneous nature of NKT cells may account for the apparently opposite functions of NKT cells in the control of liver fibrosis.

**Adaptive Immune Cells**

**T lymphocytes.** CD4\(^{+}\) T lymphocytes [Th1, Th2, Th17, and regulatory T cells (Tregs)] are major regulators of the hepatic immune response that control the fibrogenic process with positive or negative outcome depending on their phenotype (95). A number of studies have shown that the release of IFN-\(\gamma\) by Th1 effector T cells reduces liver fibrogenesis via inhibition of the transduction cascade elicited by TGF-\(\beta\) and antiproliferative and apoptotic effects for hepatic myofibroblasts (55, 96). In contrast, Th2 polarization promotes liver fibrosis via production of IL-13 (50). More recently, Th17 lymphocytes, characterized by the production of their signature cytokine IL-17, as well as other interleukins such as IL-21 and IL-22, have also emerged as regulators of organ fibrosis (95). In the liver, the number of IL-17-positive cells is enhanced in patients with chronic hepatitis B or alcoholic liver disease and correlates with severity of fibrosis (48). Hepatic IL-17 levels are elevated in various experimental models of liver fibrosis, and mice deficient for IL-17 show resistance to liver fibrosis (21, 60, 83). IL-17 targets hepatic myofibroblasts by enhancing their proinflammatory and profibrogenic potential (21, 48) and Kupffer cells by switching their phenotype towards M1 polarization (21). However, Th17 cells also produce the hepatoprotective and antifibrogenic cytokine IL-22 (44, 60).

Although the role of regulatory T cells (Tregs) in the control of liver fibrosis has not been investigated in detail as yet, antifibrogenic properties of Tregs might be anticipated from results obtained in cardiac and pulmonary fibrosis (95). Preliminary studies indicate that the number of Tregs is increased in mice following bile duct ligation and that depletion of Tregs enhances cholestasis, inflammation, and fibrosis (40). Although these data suggest a protective role of Tregs during liver fibrosis, further studies are needed inasmuch as Tregs are also known to release proinflammatory mediators such as TGF-\(\beta\) (95).

**B lymphocytes.** A profibrogenic role for B lymphocytes has been suggested by the resistance of B cell-deficient mice to liver fibrosis (64). Mechanisms underlying profibrogenic effects of B cells have been partially delineated and may involve direct interactions with HSCs or indirect effects on CD4\(^{+}\) T lymphocytes or NK cells (19).

**THERAPEUTIC PERSPECTIVES**

Regression of fibrosis can be achieved in a proportion of patients following treatment of the underlying cause in a variety of chronic liver diseases. Although recent years have seen tremendous advances in the development of effective treatment for chronic liver diseases such as chronic viral hepatitis B and C, a large number of patients remain with active ongoing chronic liver insult, therefore warranting the development of antifibrotic drugs. Nevertheless, despite experimental characterization of an array of antifibrotic pharmacological targets (Table 1), no clinical translation has as yet been achieved. This lack of success may be attributable to a number of obstacles. A primary concern arises from the slow progression of liver fibrosis over decades in humans compared with a much faster time course in rodents, therefore requiring therapeutic trials of prolonged duration. In addition, there is a crucial need for noninvasive specific and sensitive surrogate markers of fibrosis progression/regression, so as to allow...
precise monitoring of the evolution of fibrosis. Also, avoiding type II errors requires the design of large trials including homogeneous cohorts of patients with a high risk of progression of fibrosis (77). Altogether, therapeutic trials primarily focused on antifibrotic endpoints remain scarce and have thus far failed to demonstrate any benefit. Lastly, fibrogenic and fibrolytic pathways are complex and probably variable, depending on the cause of the liver disease, host, and environmental factors. Therefore, targeting a single pathway may be of limited efficacy, and strategies that combine multiple antifibrotic targets may be preferred. Nevertheless, such approaches may be limited by an increased risk of adverse event due to off-target effects. This concern has stimulated efforts to develop cell-specific drug carriers (69).

The following section summarizes selected examples of potential multitargeted antifibrotic approaches that might be future therapeutic options.

COMBINED CB1 ANTAGONISM AND CB2 AGONISM

CB1 and CB2 receptors are G protein-coupled receptors and components of the endocannabinoid system that regulates several steps of the fibrogenic cascade. Expression of both receptors is increased in experimental models of liver fibrosis and in liver samples obtained from patients with chronic liver disease or cirrhosis (37, 56, 87). Studies with cultured cells and in animals have shown that an increased CB1 tone contributes to the pathogenesis of alcohol-induced liver disease and nonalcoholic fatty liver disease by enhancing hepatocyte injury (34, 56, 62, 82). In addition, CB1 receptors expressed by hepatic myofibroblasts promote liver fibrogenesis (87). Studies in patients with nonalcoholic fatty liver disease and hepatitis C support the relevance of these experimental data to human liver diseases (26, 27). However, although promising, the first-generation CB1 antagonist, Rimonabant, was withdrawn from clinical use because of a high rate of mood disorders (55). Current research efforts are directed to the development of non-brain penetrant CB1 antagonists; such compounds have shown promising results in experimental models (ref. 82 and our unpublished observations). In contrast to CB1-dependent effects, an increase in CB2 signaling is associated with hepatoprotective effects, reduced liver inflammation, and improved liver fibrogenesis (21, 37, 52, 86). Overall, the combined use of CB1 antagonists with CB2 agonists is considered to be a promising multitarget antifibrotic approach.

PPAR-α/δ AGONISTS

Peroxisome proliferator-activated receptors (PPARs) belong to the nuclear receptor family and play key roles in the regulation of metabolic balance, inflammation, and differentiation. PPAR-α agonists reduce liver inflammation and steatosis in rodent models of nonalcoholic fatty liver disease (1). PPAR-δ ligands also improve hepatic glucose homeostasis, display hepatoprotective properties, and reduce liver fibrosis in CCL4-treated or bile duct-ligated rodents (33). These observations have prompted the development of GFT505, a mixed PPAR-α/δ agonist that predominantly accumulates in the liver. In experimental models of NASH, administration of GFT505 improved steatosis, liver inflammation, and fibrosis (81). The molecule has also shown promise for the management of dyslipidemia and diabetes in phase II trials; phase IIb studies in NASH patients are underway.

CONCLUSION

There have been tremendous advances in the understanding of mechanisms that underlie the fibrogenic response to chronic liver insult. A complex network has been identified that integrates multiple interactions between liver fibrogenic cells, parenchymal cells, endothelial cells, and immune cells as well as signals originating from extrahepatic sites. Translation of these data to the therapeutic field remains a crucial challenge that might be achieved in the coming years, owing to the availability of multitargeted therapies and to the validation of novel biomarkers that allow precise monitoring of their efficacy.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

A.M. and S.L., conception and design of research; A.M. and S.L., prepared the figures; A.M. and S.L., drafted the manuscript; A.M. and S.L., edited and revised the manuscript; A.M. and S.L., approved the final version of the manuscript.
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MECHANISMS OF LIVER FIBROSIS


