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Concise Review

## Liver fibrosis and inflammation. A review

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### Abstract

**Hepatic fibrosis, is a wound healing process characterized by accumulation of extracellular matrix proteins (ECM) especially collagen types I and III, as well as an increase in other extracellular matrix constituents such as proteoglycans, fibronectin and laminin in response to liver injury. Recruitment of leukocytes takes place after the insult and requires several adhesion molecules. Monocytes and macrophages are involved in inflammatory actions by producing nitric oxide and inflammatory cytokines. As a consequence of chronic tissue damage stellate cells (SC) as well as extracellular matrix producing cells, undergo a process of activation characterized by proliferation, motility, contractility, and synthesis of extracellular matrix. Activation of SC is regulated by several soluble factors, including cytokines, chemokines, growth factors, and products of oxidative stress. TGF -  $\beta$  and IL- 6 are the two main fibrogenic cytokines. Potential regulatory factors of the activation of SC are important targets for future antifibrogenic treatments.**

**Key words: Fibrosis, inflammation, stellate cells, liver fibrogenesis.**

### Fibrosis and Inflammation

Knowledge on development and progression of liver fibrosis has grown exponentially. Complex cellular and molecular mechanisms resulting from chronic activation of tissue repair mechanism following tissue injury have been characterized. Recognition of fibrosis as a reversible process is a milestone.<sup>1</sup>

Hepatic fibrosis is a wound healing process characterized by accumulation of extracellular matrix (ECM) proteins, especially collagen types I and III, as well as an in-

crease in other extracellular matrix constituents such as proteoglycans, fibronectin and laminin in response to liver injury.<sup>1</sup> This ECM provides a scaffold for migrating cells, which ultimately contain the injury agent reaching a successful conclusion with dismantling of the repair apparatus. The ECM scaffold is taken down by matrix proteinases while activated stellate cells (SC) undergo apoptosis, restoring the normal tissue structure.<sup>2</sup> Extracellular degradation of matrix proteins is regulated by matrix metalloproteinases (MMPs) produced by hepatic SC.<sup>3</sup> When the injury is recurrent or chronic, collagen deposition occurs in excess of collagen resorption as a result of an imbalance between fibrogenesis and fibrolysis leading to scar formation. As scarring progresses from bridging fibrosis to the formation of complete nodules it results in architectural distortion and ultimately liver cirrhosis.

Liver fibrosis is a common sequel to diverse liver injuries such as chronic viral hepatitis, ethanol, biliary tract disease, iron, copper. Different types of disease produce different patterns of fibrosis as the disease progresses. These can be divided into those diseases in which the fibrosis is portal based and those that begin in acinar zone 3 and thus are central-based. The major portal based diseases that lead to cirrhosis include chronic viral hepatitis (viral and autoimmune), chronic cholestatic diseases (primary biliary cirrhosis, primary sclerosing cholangitis and chronic mechanical obstruction of any cause) and hemochromatosis. Central based diseases include steatohepatitis (alcoholic or non alcoholic) and chronic venous outflow obstruction of any cause.<sup>4</sup>

### Inflammation

Recruitment of leukocytes takes place following hepatic injury. Leukocytes become tethered to vessel wall through labile adhesions that permit their rolling in the direction of the blood flow. The recruitment of polymorphonuclear leukocytes to sites of inflammation requires several families of adhesion molecules. The initial contact of neutrophils with the endothelium is mediated by adhesion molecules of the selectin family and their ligands.<sup>5,6</sup> The rolling phenomenon is a prerequisite for the subsequent firm adhesion and transmigration, which is mediated by members of the integrin family, e.g. B2 integrins, and immunoglobulin gene superfamily, e.g. intercellular adhesion molecule-1 (ICAM-1).<sup>7</sup> In addition to being responsible for the rolling phenomenon, selectins have been

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implicated as important signaling molecules involved in leukocyte activation.<sup>8</sup> Low affinity, selectin-dependent interactions are converted into high affinity binding through action of chemoattractants, especially chemokines, produced at the tissue damage site. Chemokines establish a gradient surrounding the inflammatory trigger and may also be retained on ECM and the endothelial surface. Interaction between leukocytes and chemoattractants produces activation of integrin receptors leading to firm adherence and extravasation via binding to cell adhesion molecules of the immunoglobulin superfamily that function as integrin ligands.<sup>5</sup>

In liver, the inflammation process gives rise to different pathways of lymphocyte recruitment and migration, probably directly related to type of insult. These pathways involve portal tract, sinusoid and hepatic vein. Composition and distribution of the inflammatory infiltrate may include T lymphocytes (more peripheral) B lymphocytes (mainly central), plasma cells, histiocytes (granuloma), eosinophils, neutrophils, NK cells, and mast cells, which in turn give rise to secondary changes such as phenotypic differences among different vascular compartments.<sup>1</sup>

Leukocytes that are recruited to the liver during injury join with Kupffer cells in producing compounds that modulate stellate cell behavior. Monocytes and macrophages are involved in inflammatory actions by producing large amounts of nitric oxide (NO) and inflammatory cytokines such as tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) which have a direct stimulatory effect on stellate cell collagen synthesis. The influx of Kupffer cells coincides with the appearance of stellate cell activation markers. Kupffer cells can stimulate matrix synthesis, cell proliferation, and release of retinoids by stellate cells through the actions of cytokines especially TGF- $\beta$  1 and reactive oxygen intermediates/lipid peroxides. Kupffer Cells, the resident macrophages in the sinusoids of the liver, have been widely implicated in hepatic injury such as endotoxin-mediated liver injury. Kupffer cells are known to express the death ligands tumor necrosis factor  $\alpha$  (TNF  $\alpha$ ), TNF related apoptosis inducing ligand (TRAIL) and Fas ligand.<sup>9,10,11-13</sup> Expression of death ligands would be anticipated to further stimulate death receptor – mediated apoptosis in the liver. Emerging data suggest that death receptor – mediated apoptosis may contribute to liver inflammation and fibrosis.<sup>14-18</sup> Fas agonists induce chemokine expression in the liver, promote neutrophil infiltration into the liver, and stimulate hepatic fibrogenesis.<sup>18,19</sup>

The mechanism by which apoptosis promotes inflammation and fibrosis may relate to death receptor – initiated signaling cascades (e.g. gene expression of inflammatory mediators), secondary necrosis of apoptotic cells, or activation of phagocytes following engulfment of apoptotic bodies. Apoptotic body phagocytosis stimulates transforming growth factor (TGF)  $\beta$  production, a fibrogenic cytokine in the liver. Engulfment of neutrophil apo-

ptotic bodies by macrophages has also been reported to induce Fas ligand expression.<sup>20,21</sup> When expressed, Fas ligand has been shown to be proinflammatory.<sup>22</sup> Apoptotic body engulfment by SC has also recently been reported to trigger activation and collagen expression by this profibrogenic cell type.<sup>23</sup>

Hepatic neutrophil infiltration is a frequent occurrence in alcoholic hepatitis. Extravasation and transmigration of neutrophils into liver tissue are critical for neutrophil – induced injury and cytotoxicity.<sup>24</sup>

The CXC chemokine interleukin 8 (IL- 8) is a critical chemoattractant and activator for neutrophils, basophils, and T cells and is secreted by Kupffer cells, macrophages and hepatocytes. IL- 8 secretion is complex and is regulated primarily at the transcriptional level through cooperative interactions of the transcription factors nuclear factor  $\kappa\beta$  (NF- $\kappa\beta$ ), activator protein 1 (AP -1), and IL -6 in tissue.<sup>25,26</sup> IL- 8/CXC chemokines are associated with liver injury induced by lipopolysaccharide, tumor necrosis factor (TNF), Fas, and bacterial infections.<sup>27</sup> Increased plasma, hepatic and monocyte levels of IL- 8 are well documented in alcoholic liver disease (ALD) and is postulated to play a key role in the hepatic neutrophil infiltration in ALD.

## Stellate cells

As a consequence of chronic tissue damage, Stellate Cells (SC) as well as other extracellular matrix – producing cells such as fibroblasts and myofibroblasts, undergo a process of activation toward a phenotype characterized by increased proliferation, motility, contractility, and synthesis of extracellular matrix components. In the liver SC are located in the space of Disse in close contact with hepatocytes and sinusoidal endothelial cells. Hepatic SC express vimentin, desmin, a smooth muscle actin (a-SMA) glial fibrillary acidic protein, nestin, neural cell adhesion molecule and synaptophysin.<sup>28</sup> Quiescent SC are non – proliferative and at this stage collagen production is IV > III > I. During transition, collagen I > III > IV is produced. Transition of SC to myofibroblast – like phenotype is ultrastructurally characterized by appearance of dense bodies and patches of myofilaments through cytoplasm and by increased expression of a smooth muscle actin, which together with expression of integrin receptors specific for constitutive components of these ECM protein – particularly collagen type I and III – lead to structural configuration of activated SC, characterized by cytoskeletal tension or stress relevant to modulation of different cell functions in response to growth factors and vasoconstrictors.<sup>29</sup>

Activation of SC is regulated by several soluble factors, including cytokines, chemokines, growth factors, and products of oxidative stress as well as by extensive changes in composition and organization of extracellular matrix components.

Hepatic SC have been suggested to play an important role in regulation of sinusoidal blood flow and tone in normal liver. Studies on kinetics and contraction of Hepatic SC suggest that Hepatic SC, as a consequence of their activated state, may affect sinusoidal resistance in presence of liver damage. Hepatic SC contribute significantly to alteration of sinusoidal structure in early stages of fibrogenesis. Capillarized sinusoids are characterized by accumulation of fibrillar extracellular matrix in the space of Disse that results in impairment in metabolic exchange between blood and hepatocytes. Capillarization of sinusoids is likely to be present as an initial cause of portal hypertension during development of hepatic fibrosis, contributing to early presinusoidal resistance resulting in obstruction to sinusoidal blood flow accompanied by parenchymal extinction, that is loss of contiguous tissue by collapse of sinusoidal stroma.<sup>30</sup> Parenchymal extinction appears secondary to vascular obliteration, portosystemic shunting, and replacement of parenchyma by mesenchyma. SC participate in up-regulating expression of several ECM components, metalloproteinases (MMPs), and tissue inhibitors of metalloproteinases (TIMPs). Controlled cell death (apoptosis) could also be a mechanism underlying the termination of Hepatic SC proliferation. Spontaneous apoptosis is detected in parallel with Hepatic SC activation. Both CD95 (APO-1/Fas) and CD95L (APO-1-/Fas-ligand) become increasingly expressed during the course of activation. Apoptosis can be fully blocked by CD95 blocking antibodies in normal cells and Hepatic SC already entering the apoptotic cycle.<sup>31</sup>

Potential regulatory factors of the activation of SC a central event in the pathogenesis of hepatic fibrosis have been proposed; this included a zinc finger molecule KLF6 which is upregulated in SC but it is also ubiquitously expressed and is found to upregulate p21.<sup>32</sup> Another is a novel family of RTKs known as DDR in particular DDR2 which regulates MMPs2 expression and cell growth.<sup>33</sup> As SC activate, the upregulation of DDR2 leads to accelerated degradation of basement of membrane by MMPs2, hastening its replacement by interstitial scar in the subendothelial space of Disse, which is rich in type I collagen, leads to further SC activation, creating a positive loop leading to the development of hepatic fibrosis.

Hepatic SC also display immune properties. SC express membrane proteins involved in antigen presentation, including membranes of the HLA family (HLA-I and HLA-II), lipid presentation molecules (CD1b and CD1c), and factors involved in T- cell activation (CD40 and CD80). Exposure of SC to proinflammatory cytokines markedly up-regulates these molecules. Cells freshly isolated from human cirrhotic livers highly express HLA- II and CD40, suggesting that SC can act as an antigen-presenting cells (APCs) in human fibrogenesis.<sup>34</sup>

SC can efficiently process exogenous antigens. Activated SC internalize low- and high-molecular-weight dextran and transferrin, indicating that they can per-

form fluid-phase and receptor-mediated endocytosis. Moreover, SC can perform phagocytosis of macromolecules because they internalize latex particles as well as bacteria. Both culture-activated and in vivo activated SC express high levels of CD68, a protein involved in antigen trafficking. Cytokine-stimulated SC stimulate the allogeneic T-lymphocyte response in an HLA-II-dependent manner. It has been recently demonstrated that human activated SC express molecules for antigen presentation, internalize macromolecules, and modulate T-lymphocyte proliferation.<sup>34</sup>

Direct stimulation of hepatic SC proliferation and collagen synthesis by products generated from hepatocytes is the leading hypothesis to account for iron and alcohol related fibrosis. In co-cultures of freshly isolated hepatocytes and a liver stellate cell line, ethanol induced  $\alpha 1$  (I) procollagen mRNA in a dose and time dependent manner via its metabolism by alcohol dehydrogenase.<sup>35</sup> There is considerable interest in the role of oxidative stress and the generation of ROS in the mechanisms by which alcohol is hepatotoxic. It has recently been reported that hepatocytes containing CYP2E1 release diffusible mediators including ROS which can activate hepatic SC. Reactive Aldehydes modulate stellate cell activation, lipid peroxidation increases production of ECM, including collagen,  $\alpha 1$  procollagen mRNA colocalizes with aldehyde adducted proteins, and liver collagen content correlates with the level of lipid peroxidation.<sup>36</sup>

## Cytokines

Of all the cytokines and growth factors produced TGF- $\beta$  and IL-6 are the two main fibrogenic cytokines. While Hepatic SC activation is taking place in liver and cytokines and signal transduction pathways are being stimulated, TGF- $\beta_1$  plays a central role in fibrosis, contributing to both influx and activation of inflammatory cells as well as activation of SC. TGF- $\beta$  is produced by Kupffer cells and SC, it up-regulates the transcription of the 1 (I) and 2 (I) collagen genes and induces the expression of TIMP-1, a tissue inhibitor of MMPs involved in collagen degradation. Moreover TGF- $\beta$  induces its own mRNA and establishes key autocrine and paracrine loops to sustain high levels of this cytokine in local sites of liver injury and/or inflammation. IL-6 which is produced by hepatic SC from normal or cirrhotic livers, it up-regulates the expression of TGF- $\beta$  in HSC from cirrhotic livers. Accordingly, it enhances the fibrogenic action of TGF- $\beta$ . The molecular mechanism by which TGF- $\beta$  up-regulates the expression of the type I collagen genes have not been completely defined. However, recent work indicates that reactive oxygen intermediates in general, and H<sub>2</sub>O<sub>2</sub> in particular, are important mediators of TGF- $\beta$  actions in HSC.<sup>37,38</sup>

Activated Hepatic SC in turn become an important source of TGF- $\beta_1$  and are major producers of ECM pro-

teins during liver injury. Cytokines of the transforming factor (TGF) family influence a wide spectrum of cellular processes including differentiation, proliferation, apoptosis and migration. Tissue and serum levels of active TGF- $\beta_1$  can induce organ fibrosis. Furthermore, experimental fibrosis, can be inhibited by anti-TGF- $\beta_1$  treatments, e.g. with neutralizing antibodies or soluble receptors. TGF- $\beta_1$  response is modulated during transdifferentiation process. Surface receptors for TGF- $\beta_1$  are serine – threonine kinases. Activation of type II TGF receptor lead to phosphorylation of two receptor-associated proteins, Smad 2 and Smad 3. Upon phosphorylation, Smad 2 and Smad 3 associated with Smad 4 in cytoplasm. Trimolecular complex are then translocated to nucleus, where it activates transcription of TGF- $\beta$ -responsive genes, e.g. those for plasminogen activator inhibitor (PAI-1), the 2 chain of collagen I, and Smad 7. Other members of Smad family, including Smad 6 and Smad 7, act as cytoplasmic inhibitors of TGF- $\beta$  signaling. Response of Hepatic SC to TGF- $\beta_1$  may vary. Whereas cells in earlier stages of activation have a huge response to TGF- $\beta_1$ , SC exhibit lack of this response in their myofibroblastic phenotype. This could be explained by loss of TGF- $\beta_1$ -dependent phosphorylation of Smad 2, a prerequisite for nuclear translocation of transcriptionally active Smad complexes.<sup>39</sup>

On the other hand, lines of evidence suggest that leptin modulates inflammatory and immune response. These observations suggest a possibility that leptin is involved in the progression of hepatic fibrosis. These findings support the hypothesis that leptin increases TGF- $\beta_1$  production from sinusoidal endothelial cells and kupffer cells, thereby up-regulating the production of extracellular matrix from HSCs.<sup>40</sup>

Glycosphingolipids, including gangliosides, are emerging as putative signaling lipid intermediates that play a role in stress response and cell death. They act at mitochondrial level, assembling cell death cascade initiated by stimulating a burst of reactive oxygen species (ROS). Identification of oxidative stress playing a significant role in fibrogenesis has been widely explored; in particular, ROS are necessary for mitochondrial permeability transition pore, cytochrome c release, and subsequent caspase activation. Their production has an impact on cell survival.<sup>41</sup>

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