New Therapeutic Modalities in Liver Inflammation and Fibrosis.


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Received for Publication: July 21, 2010, Accepted for Publication: January 1, 2011.

Abstract:
Understanding the molecular and cellular mechanisms underlying liver inflammation and fibrosis would facilitate to design appropriate therapeutic interventions to halt or reverse the process of liver fibrosis. A review of literature was performed in 2010 to summarize scientific reports on inflammation pathways of hepatitis. Articles indexed in the Pub Med database during recent ten years searched for hepatitis, inflammation pathways, treatment modalities, and stem cell transplantation to investigate cellular and molecular pathways and biologic role of growth factors, cytokines, and mediators engaged in liver inflammation facilitate the development of pharmacological intervention. This manuscript reviews some recent scientific advances, and therapeutic interventions in liver inflammation, and fibrosis.

Keywords: Hepatitis, Inflammation pathways, Treatment modalities, Stem cell transplantation

Introduction
Hepatitis means inflammation of the liver. Inflammation of the liver is caused by a number of etiologic agents, including seven known types of viral hepatitis (A-G), bacteria, fungi, parasites, chemicals, and drugs. Hepatitis B virus (HBV) infection remains a public health problem with nearly 350 million carriers worldwide. The complexity of the natural history of hepatitis B depends on viral fea-
tures, hepatocyte behavior and patient immune response.\(^1\) Hepatitis C virus (HCV) is endemic in most parts of the world, with an estimated 170 million people infected worldwide and 3-4 million new cases each year. HCV-related end-stage liver disease is now the main indication for liver transplantation in the USA and Western Europe.\(^2\) Metabolic steatosis or non-alcoholic fatty liver (NAFLD) is the most common cause of chronic liver injury in Western countries.\(^3\) With obesity being an important risk factor universally, NAFLD is now receiving greater attention and is regarded as a public health issue.\(^4\) Liver cirrhosis is caused by iterative cycles of tissue injury, inflammation, and repair. Chronic inflammation and fibrosis are inextricably linked and the cellular interactions between immune effector cells, local fibroblasts, and tissue macrophages at sites of scar formation determine the outcome of liver injury and the development of scarring.\(^5\) A major advancement towards the understanding of the molecular mechanisms of fibrogenesis is derived from a consistent number of in vitro studies investigating the biological role of growth factors/cytokines and other soluble factors and their intracellular signalling pathways.\(^6\)

Stem cells are a promising source for liver repopulation after cell transplantation, but whether or not the adult mammalian liver contains hepatic stem cells is highly controversial. Part of the problem is that proliferation of mature adult hepatocytes is sufficient to regenerate the liver after two-thirds partial hepatectomy or acute toxic liver injury and participation of stem cells is not required. Mesenchymal stem cells and embryonic stem cells can be induced to differentiate along the hepatic lineage in culture, but at present these cells are inefficient in repopulating the liver.\(^7\) Mesenchymal stromal (MS) cells might be differentiated toward hepatocytes in vitro and thus are promising candidates for therapeutic applications in vivo. The efficacy of bone marrow-derived MS cells versus hepatocytes to contribute to liver regeneration was compared in a rat model of prolonged toxic hepatic injury.\(^8\)

**Inflammation cascades and therapeutic interventions**

Liver fibrosis is defined as an excessive deposition of extracellular matrix. The accumulation of extracellular matrix proteins in liver fibrosis and cirrhosis is due to different cell types which acquire a myofibroblastic phenotype--the hepatic stellate cells, located in the space of Disse, portal fibroblasts as well as myofibroblasts of the portal and pericentral areas.\(^9\) It is now recognized that hepatic stellate cells (myofibroblast-like cells that encircle the sinusoids) are primarily responsible for hepatic fibrosis and subsequent progression to cirrhosis. In response to liver injury stellate cells undergo a phenotypic transformation that is termed activation, and characterized by chemotaxis, proliferation, contraction, fibrogenesis, and extracellular matrix degradation.\(^10\) Liver injury of any etiology will ultimately lead to activation of hepatic stellate cells (HSCs), which undergo transdifferentiation to fibrogenic myofibroblast-like cells. Different platelet-derived growth factor and transforming growth factor beta inhibitors have
been shown to effectively prevent liver fibrosis in animal models and represent promising therapeutic agents for humans.\(^{(11)}\) In liver fibrosis, alterations within the space of Disse microenvironment occur and facilitate further progression of chronic liver disease. The normal basement membrane-like matrix present within the space of Disse converts to a matrix rich in fibril-forming collagens during fibrosis.\(^{(12)}\) In liver fibrosis, alterations within the space of Disse microenvironment facilitate the migration of hepatic stellate cells (HSCs); the mechanism is associated with up-regulation of matrix metalloproteinases 2 (MMP-2) and with mediation of alpha1beta1 and alpha2 beta1 integrins. Extracellular matrix by itself shows feedback actions to migration of HSCs.\(^{(13)}\) Fatty liver has impaired regeneration that induces a secondary replicative pathway using bipotent, periportal, hepatic progenitor cells (HPCs). The findings of a study suggest that an altered replication pathway in active NASH promotes a periportal ductular reaction (DR), which in turn may provoke progressive periportal fibrogenesis.\(^{(14)}\) The HSCs produce collagen type I and secrete pro-fibrogenic cytokines and inhibitors of matrix-degrading enzymes (tissue inhibitor of matrix metalloproteinase), causing the production of extracellular matrix deposition over degradation. However, many clinical and experimental studies suggest that this process can be reversed, including the apoptosis of activated HSC. Thus, HSC represent an appealing target for antifibrotic therapy.\(^{(15)}\) Activated hepatic stellate cells, portal fibroblasts, and myofibroblasts of bone marrow origin have been identified as major collagen-producing cells in the injured liver. These cells are activated by fibrogenic cytokines such as TGF-beta1, angiotensin II, and leptin. Reversibility of advanced liver fibrosis in patients has been recently documented, which has stimulated researchers to develop antifibrotic drugs.\(^{(16)}\) Hepatic stellate cells have been considered the most important cell-type involved in hepatic fibrogenesis. Proliferation and differentiation of hepatic stellate cells into myofibroblast-like cells has been related to the development of liver fibrosis. The alpha-actin expressed by hepatic stellate cells was considered a marker of their activation to myofibroblast-like cell.\(^{(17)}\) The activation of hepatic stellate cells is the conversion of quiescent cells into proliferative, contractile, and fibrogenic myofibroblasts. The alpha-smooth muscle actin is a well known marker of hepatic stellate cells activation. Glial Fibrillary Acidic Protein expression in the liver is probably linked to the fine modulation of the cytoskeleton during the formation of cytoplasmic processes in both stellate cells and endothelial cells.\(^{(18)}\) In chronic liver injury, the stellate cell differentiates into a myofibroblast-like cell with marked expression of alpha-smooth muscle actin and occasional expression of desmin. Myofibroblast-like cells have a high fibrogenic capacity in the chronically diseased liver and are also involved in matrix degradation. The transition of stellate cells into myofibroblast-like cells is regulated by an intricate network of intercellular communication between stellate cells and activated Kupffer cells, damaged hepatocytes, platelets, endothelial and inflam-
matory cells, involving cytokines and nonpeptide mediators such as reactive oxygen species, eicosanoids and acetaldehyde.\(^{(19)}\)

Recent data suggest that hepatocytes and biliary epithelial cells undergo an epithelial to mesenchymal transition, similarly assuming a fibrogenic phenotype.\(^{(20)}\) In hepatic fibrosis process, activation of hepatic stellate cells is characteristic of cell proliferation and migration, production of collagen and other extracellular matrix (ECM) molecules, and contraction after transforming into myofibroblasts. It has been shown that the fibrogenic process is prominently regulated by transforming growth factor-beta1 (TGF-beta1) and that the specific blockade of TGF-beta1/Smad3 signaling may therapeutically intervene the fibrosis of various tissues.\(^{(21)}\) The identification of activated hepatic stellate cells (HSCs) as the major fibrogenic cell type in the injured liver, as well as the recognition of key cytokines involved in this process, have facilitated the design of promising new antifibrotic therapies. These therapies are aimed at inhibiting the accumulation of activated HSCs at the sites of liver injury and preventing the deposition of extracellular matrix.\(^{(22)}\) Recent work in human and animal models has shown that liver fibrosis is potentially reversible and, in specific circumstances, demonstrates resolution with a restoration of near normal architecture.\(^{(23)}\) Portal hypertension is primarily caused by an increase in resistance to portal outflow and secondly by an increase in splanchnic blood flow. Among vasoactive substances activated in portal hypertension, nitric oxide (NO) is considered as the most important vasodilator. Endothelin-1 and cyclooxygenase-derived prostaglandins are the foremost vasoconstrictor factors. In addition to an imbalance in vasoactive substances, a major role has been attributed to activated hypercontractile hepatic stellate cells which cause vascular remodelling as an adaptive response to the changed balance in vasoactive substances.\(^{(24)}\) Connective tissue growth factor (CTGF=CCN2), one of six members of cysteine-rich, secreted, heparin-binding proteins with a modular structure, is recognized as an important player in fibrogenic pathways as deduced from findings in non-hepatic tissues and emerging results from liver fibrosis.\(^{(25)}\) The renin-angiotensin system (RAS) is frequently activated in the patients with chronic liver diseases, and plays a pivotal role in the progression of chronic liver diseases, i.e., liver fibrosis and hepatocellular carcinoma (HCC). Angiotensin-II (AT-II) reportedly stimulates contractility and proliferation of the activated hepatic stellate cells, and increases the transforming growth factor-beta (TGF-beta) expression through angiotensin type-I receptors (AT1-R).\(^{(26)}\) Toll-like receptors (TLRs) recognize pathogen-associated molecular patterns (PAMPs) to detect the presence of pathogens. In addition to their role in innate immunity, TLRs also play a major role in the regulation of inflammation, even under sterile conditions such as injury and wound healing. The liver not only represents a major target of bacterial PAMPs in many disease states but also upregulates several DAMPs following injury. TLR-mediated signals have been implicated in a number of chronic liver diseases.\(^{(27)}\) TLR signaling also plays
an important role in the activation of the adaptive immune system by inducing proinflammatory cytokines and upregulating costimulatory molecules of antigen presenting cells. The dysregulation of TLR signaling may cause autoimmunity. The involvement of TLR signaling in the pathogenesis of autoimmune diseases may provide novel targets for the development of therapeutics. Activation of TLR’s leads to production of pro-inflammatory cytokines such as tumour necrosis factor (TNF)-alpha. Increased monocyte expression of TLR2, but not of TLR4, correlates significantly with both increased circulating TNF-alpha levels and hepatic necroinflammatory activity in patients with chronic hepatitis C. Innate immune cells, particularly dendritic cells, have a pivotal role in sensing pathogens and initiating adaptive immune responses by activation and regulation of T-lymphocyte responses. Although the liver provides a "tolerogenic" immune environment for antigen-specific T-cells, activation of Kupffer cells, recruited macrophages, and inflammatory cells results in production of cytokines and chemokines that can lead to prolonged inflammation, hepatocyte damage, and/or cholestasis. Nuclear factor-kappaB (NF-kappaB) is a transcriptional regulator of genes involved in immunity, inflammatory response, cell fate, and function. In vivo studies using rodent models of liver disease and cell-targeted perturbation of NF-kappaB activity have revealed complex and multicellular functions in hepatic inflammation, fibrosis, and the development of hepatocellular carcinoma - a process we have termed the "inflammation-fibrosis-cancer axis". Observations that hepatic inflammation and cirrhosis are associated with the presence of thrombi within the hepatic microvasculature and fibrin-fibrinogen deposition have led to epidemiologic studies showing that carriage of the factor V Leiden mutation, protein C deficiency, and increased expression of factor VIII are associated with rapid progression to cirrhosis in a chronic hepatitis C virus. Interference with either the generation of thrombin or its downstream activity may reduce hepatic fibrosis. In preclinical studies it has been shown that activation of ribosomal S-6 kinase (RSK) and phosphorylation of the CCAAT/enhancer-binding protein (C/EBP) beta in activated HSC is critical for the progression of liver fibrosis, RSK has been considered as a therapeutic target for the liver fibrosis. Thus, appropriate RSK inhibitors may be beneficial in the prevention and treatment of liver injury and liver fibrosis.

Bone marrow–derived mesenchymal stem cells can effectively rescue experimental liver failure and contribute to liver regeneration and offer a potentially alternative therapy to organ transplantation for treatment of liver diseases. Bone marrow stem cells have been shown to contribute to parenchymal liver cell populations, and although this may not be functionally significant, it has sparked interest in the field of autologous stem cell infusion as a possible treatment for cirrhosis. The mechanisms by which cells are trafficked from the bone marrow to the liver are complex; the stromal derived factor-1/CXC receptor 4 axis is central to this process. Autoimmune hepatitis recurs after transplantation in at
least 17% of patients, and it typically improves after adjustments in the immunosuppressive regimen. Future therapies are likely to include mesenchymal stem cell transplantation, adoptive transfer of T regulatory cells, and cytokine manipulation.\(^\text{36}\)

**Conclusions**

Understanding of inflammation signaling pathways and the molecular alterations associated with hepatitis would provide new therapeutic modalities and better management the cost of disease burden, and to halt or reverse the process of liver fibrosis. Drugs targeted based on these processes, would have more specific effect. Stem cell therapy may have the advantage of replacing cells with inadequate function or may target for certain immunologic responses. All these modalities need further investigation for possible adverse complications and ethical consequences.

**References:**


