STELLATE CELL BIOLOGY AND FIBROSIS

Innate immunity and alcoholic liver fibrosis

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Abstract

The hepatic innate immune system consists of predominant innate immunity, which plays an important role in innate defense against infection and tumor transformation. Emerging evidence suggests that innate immunity also contributes to liver injury, repair, and fibrosis. The present review summarizes the recent findings on the role of innate immunity in liver fibrosis. In general, Kupffer cells stimulate liver fibrosis via production of reactive oxygen species and pro-inflammatory cytokines, whereas natural killer (NK) cells inhibit liver fibrosis by directly killing activated hepatic stellate cells and production of $\gamma$-interferon (IFN-$\gamma$). Complement components, interferons, and Toll-like receptors have also been shown to regulate liver fibrosis. Recent evidence also suggests that modulation of innate immunity by alcohol plays an important role in the pathogenesis of alcoholic liver fibrosis. These include alcohol amplification of the profibrotic effects of Kupffer cells and suppression of the antifibrotic effects of NK/IFN-$\gamma$.

Key words
alcohol, hepatic stellate cell, innate immunity, liver fibrosis, natural killer cell.

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Alcoholic liver fibrosis

Chronic alcohol drinking is a leading cause of chronic liver disease worldwide. The typical spectrum of alcoholic liver disease includes fatty liver, alcoholic hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma. Interestingly, almost 100% of heavy drinkers have fatty liver, but only 10–20% of them develop alcoholic hepatitis, fibrosis, and cirrhosis. Additionally, chronic alcohol consumption has been shown to accelerate liver injury in patients with viral hepatitis infection. For example, it was reported that there was a 34% increased rate of fibrosis in heavy drinkers who are infected with hepatitis C virus (HCV) compared with those who drank less. However, the molecular and cellular mechanisms underlying alcoholic liver fibrosis and alcohol acceleration of liver fibrosis are not completely understood.

Liver fibrosis is a common scarring response to virtually all types of chronic liver injury, including chronic alcoholic liver disease. The mechanisms for liver fibrosis have been dramatically elucidated in the last several decades but are still not fully understood. It is generally believed that chronic liver injury leads to activation of hepatic stellate cells (HSC). Activated HSC produce a large amount of extracellular matrix (ECM) proteins, which accumulate within the subendothelial space of Disse and lead to liver fibrosis. Many other cell types may also be involved in the production of ECM proteins and collagens, but likely play a less important role compared to HSC. These include myofibroblasts, bone marrow-derived progenitor cells, and hepatocytes.

Emerging evidence has suggested that HSC activation and proliferation are controlled by a variety of pro-inflammatory cytokines and reactive oxygen intermediates. Among them, transforming growth factor (TGF)-$\beta$ and platelet-derived growth factor (PDGF) are considered the most potent stimulators for HSC transformation and proliferation, respectively. Recently, innate immunity has also been shown to play an important role in controlling the development and progression of liver fibrosis, which will be discussed below. In the present review, we will also discuss the role of innate immunity in alcoholic liver fibrosis.

Predominant innate immunity in the liver

The innate immunity system is responsible for the rapid, initial response of the organism to potentially dangerous stresses, including pathogens, tissue injury, and malignancy without specific
recognition of foreign antigens.\textsuperscript{14} It includes physical barriers (e.g. skin and mucous membranes), chemical barriers (e.g. enzyme and acid secretions), humoral factors (e.g. complement and interferons), phagocytic cells (e.g. neutrophils and macrophages), and lymphocytic cells (e.g. natural killer [NK] cells and NKT cells). Another important component of the innate immunity system is a group of recently identified pattern-recognition receptors that can specifically detect pathogens via recognizing specific structures called pathogen-associated molecular patterns expressed by invading pathogens.\textsuperscript{11} These include complements, pentraxins, peptidoglycan-recognition proteins, lipid transferases, Toll-like receptors (TLR), NOD-like receptors (NLR), RIG-like helicases etc.\textsuperscript{11}

Interestingly, increasing evidence has suggested that the immune system in the liver consists predominantly of innate immunity.\textsuperscript{12} First, the liver is responsible for the biosynthesis of 80–90\% of innate proteins including complement, secreted pattern recognition receptors and acute phase proteins. Second, the liver contains a large number of sinusoidal endothelial cells and Kupffer cells, which are responsible for clearance of soluble macromolecules and insoluble waste in the body. Kupffer cells account for 80–90\% of the total population of fixed tissue macrophages in the body. Third, liver lymphocytes are enriched in innate immune cells including NK and NKT cells.\textsuperscript{13,14} For example, mouse liver lymphocytes contain 10\% NK cells, while rat and human liver lymphocytes contain approximately 30–50\% NK cells.\textsuperscript{13,14} NKT cells constitute up to 30\% of the intrahepatic lymphocyte population in mice.\textsuperscript{15} Fourth, liver cells also express a variety of pattern recognition receptors including TLR.\textsuperscript{16–18} Finally and interestingly, the adaptive immunity in the liver seems less active because the liver is a major site to induce T-cell apoptosis.\textsuperscript{19,20} Studies from the last several decades indicate that the hepatic innate immunity plays a key role not only in innate defenses against pathogens and transformed cells but also in regulating liver injury, repair, and fibrosis.\textsuperscript{21}

**Innate immunity and liver fibrosis**

Emerging evidence indicates that liver fibrosis is controlled by a variety of components of the innate immunity system including humoral factors (e.g. complement and IFN), phagocytic cells (e.g. neutrophils and macrophages), lymphocytic cells (e.g. NK and NKT cells), and pattern recognition receptors including TLR. (i) Among the humoral factors, complement 5 was shown to stimulate whereas both IFN-\(\alpha/\beta\) and IFN-\(\gamma\) inhibit liver fibrosis. (ii) Among the phagocytic cells, macrophages have been shown to play an important role in stimulating liver fibrosis whereas other phagocytic cells such as neutrophils and mast cells seem to have less effect. (iii) Among the lymphocytic cells, NK cells were shown to inhibit liver fibrosis via killing activated HSC whereas the role of NKT cells in liver fibrosis remain unclear. (iv) Finally, it was shown that several pattern recognition receptors including TLR are also involved in the pathogenesis of liver fibrosis.

**Complement**

The complement system consists of approximately 30 soluble and membrane-bound proteins that play a key role in the innate defense against microbe infection and in disposing immunoglobins and apoptotic cells. Recent studies indicate that the complement system is involved in the pathogenesis of a variety of liver disorders including liver fibrosis.\textsuperscript{22} Through genetic mapping, Hillebrandt\textit{et al.}\textsuperscript{23} identified the 44.7 Mb region on mouse chromosome 2 containing a gene that is responsible for the development of liver fibrosis, and further studies showed this gene is the complement 5 (C5) gene. Several inbred mouse strains with C5-deficiency were resistant to liver fibrosis whereas transgenic expression of the C5 gene increased the susceptibility of these mice to liver fibrosis.\textsuperscript{23} Clinical data also indicate that human C5 gene variants are associated with liver fibrosis in patients with chronic HCV infection.\textsuperscript{24} In addition, it was reported that other complement components such as C3, C4, and mannose binding lectin (MBL) correlate negatively with the Child–Pugh score in patients with liver cirrhosis.\textsuperscript{24,25} Collectively, these data suggest that C5 promotes whereas C3, C4 and MBL may inhibit liver fibrosis.

**Interferons**

Data from experimental studies in rodent models clearly show that both IFN-\(\alpha/\beta\) and IFN-\(\gamma\) negatively regulate liver fibrosis. It was reported that IFN-\(\gamma\)-deficient mice are more susceptible to liver fibrosis induced by administration of CCl\textsubscript{4} or feeding with a 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) diet,\textsuperscript{26,27} whereas treatment of animals with IFN-\(\alpha/\beta\) or IFN-\(\gamma\) ameliorates liver fibrosis in a variety of models.\textsuperscript{27–30} The antifibrotic effects of IFN are likely mediated via induction of HSC cycle arrest and apoptosis,\textsuperscript{31,32} activation of NK cell cytotoxicity against HSC,\textsuperscript{27} and inhibition of collagen synthesis.\textsuperscript{30}

IFN-\(\alpha\) in combination with ribavirin is currently the standard pharmacological treatment for HCV infection and is effective in approximately 60\% patients with HCV infection.\textsuperscript{33,34} Clinical data have shown that IFN-\(\alpha\) therapy improves serum levels of fibrotic markers and liver histology in both virological responder and non-responder patients with chronic HCV infection,\textsuperscript{35–39} but such therapy is less effective in patients with advanced fibrosis and cirrhosis.\textsuperscript{40} In contrast to IFN-\(\alpha\), clinical trial findings on antiviral and antifibrotic therapy of IFN-\(\gamma\) in HCV patients have been controversial, although IFN-\(\gamma\) has been shown to effectively inhibit liver fibrosis in animal studies (see above) and inhibit HCV replication \textit{in vitro}. Clinical trials with a small number of patients showed that IFN-\(\gamma\) treatment ameliorates liver fibrosis in patients with hepatitis B virus (HBV) infection\textsuperscript{41} and HCV infection,\textsuperscript{42} but a recent large clinical trial showed no beneficial effect of IFN-\(\gamma\) in patients with advanced liver disease.\textsuperscript{43} The difference between these studies may be due to the selection of different populations of patients. It is plausible to speculate that IFN-\(\gamma\) may be effective in treating early stages of liver fibrosis, but not the later stage of cirrhosis.

**Kupffer cells**

Kupffer cells are liver-resident macrophages, which play a key role in the clearance of insoluble wastes in the body. In addition, Kupffer cells also contribute significantly to the pathogenesis of liver injury, fibrosis, and regeneration. In general, it is believed...
that, after injury, Kupffer cells become activated and release a variety of pro-inflammatory cytokines and reactive oxygen intermediates, which then activate HSC, leading to liver fibrosis. \(^{35-46}\) However, Kupffer cells or macrophages also play an important role in the resolution of liver fibrosis via production of metalloproteinase-13.\(^ {45,47}\)

**Natural killer cells**

Over last several decades, many studies have shown that hepatic NK cells play an important role in the innate immune response against tumors and microbial pathogens including viruses, bacteria and parasites. NK cells can kill virus-infected cells and tumors by releasing granules containing granzyme and perforin, death ligands such as tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) and a variety of pro-inflammatory cytokines such as IFN-\(\gamma\) and TNF-\(\alpha\). Increasing evidence suggests that NK cells may also be involved in the pathogenesis of liver injury, fibrosis, and regeneration.\(^ {27,32,46-51}\) For example, we and others have recently shown that activation of NK cells inhibits liver fibrosis in vivo.\(^ {32,51}\) Further studies suggest that the inhibitory effects of NK cells on liver fibrosis are mediated via multiple mechanisms (Fig. 1). First, NK cells can directly kill early activated HSC in NKG2D+, TRAIL+, major histocompatibility complex (MHC) class I molecules- and granzyme-dependent manners. Interestingly, NK cells do not kill quiescent or fully activated HSC. This is because only early activated HSC express increased levels of NKG2D activating ligand retinoic acid early inducible gene 1 (RAE-1) but decreased levels of NK cell inhibiting ligand (MHC-1). Second, NK cells can also ameliorate liver fibrosis by releasing IFN-\(\gamma\), which subsequently induces HSC cell cycle arrest and apoptosis via a signal transducer and activator of transcription 1 (STAT1)-dependent mechanism.\(^ {32}\) Moreover, we examined the molecular mechanism by which RAE-1 expression was upregulated in early activated HSC. Our findings suggest that during the early stages of activation, HSC loss retinol, which can be metabolized into retinal through alcohol dehydrogenases (ADH) 1 and ADH3, and subsequently metabolized into retinoic acid (RA) through retinal dehydrogenases (Rdhs). RA induces expression of RAE-1. Finally, clinical data show that NK cells from HCV patients were able to kill human HSC and their activities correlated negatively with the grade of liver fibrosis in these patients.\(^ {51,55}\) Taken together, NK cells seem to have antifibrotic functions and activation of NK cells could be a novel strategy to treat liver fibrosis.\(^ {53}\)

**Natural killer T cells**

Natural killer T cells are a subset of lymphocytes expressing both NK and T cell markers and consist of many subtypes. NKT cells are activated very early in the course of an immune response and play a key role in the innate defense against infection and tumor transformation. Recent studies suggest that NKT cells also contribute to liver injury and regeneration.\(^ {56-60}\) However, the role of NKT cells in liver fibrosis remains obscure, although the antifibrogenic effects of NKT cells have been documented in other organs.\(^ {61,62}\) As NKT cells contain several subtypes and produce an array of profibrogenic cytokines (such as interleukin [IL]-4 and IL-13) and antifibrogenic cytokines (such as IFN-\(\gamma\)), they may have a dual role in regulating liver fibrosis. Further studies are required to identify the role of NKT cells in liver fibrosis.

**Toll-like receptors**

There are 13 different TLR identified so far. Several of them have been implicated in regulating the development and progression of liver fibrosis. Activated human HSC express TLR2 and TLR4, activation of which results in nuclear factor (NF)-kB and JNK activation, followed by upregulation of chemokines and adhesion, suggesting that activation of TLR2 and TLR4 on HSC may be involved in the inflammatory response.\(^ {63,64}\) Activation of TLR9 on HSC by apoptotic hepatocyte DNA has also been implicated in induction of liver fibrosis.\(^ {65}\) In contrast, activation of TLR3 by polyinosinic : polycytidylic acid (poly I:C) inhibits liver fibrosis.\(^ {27,32}\) The antifibrotic effect of poly I:C is likely mediated via activation of NK cells and production of IFN-\(\gamma\) because such an effect was diminished in NK cell-depleted mice and IFN-\(\gamma\) knock-out mice.\(^ {27,32}\) Finally, several other TLR have been implicated in the pathogenesis of human primary biliary cirrhosis.\(^ {56-58}\)
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Alcohol amplification of the profibrogenic effect of Kupffer cells

There is increasing evidence that chronic alcohol consumption leads to liver fibrosis via upregulation of the profibrogenic effects of Kupffer cells (Fig. 2). It is well established that chronic alcohol consumption increases gut endotoxin leakage in humans and rodents, leading to endotoxemia, which likely contributes to the development of alcoholic hepatitis, fibrosis, and cirrhosis. Lipopolysaccharide (LPS) concentration was found at elevated levels in alcoholic patients and rodent models exposed to ethanol. In addition, chronic alcohol consumption upregulates expression of CD14 on Kupffer cells, further increasing the sensitivity of Kupffer cells to endotoxin via upregulation of CD14 expression.

Alcohol abrogation of the antifibrogenic effect of NK/IFN-γ

Ethanol has been recognized for a long time as an immunosuppressant, including suppression of NK cell activity. Recent studies from our laboratory suggest that alcohol inhibition of NK/IFN-γ could be an important mechanism contributing to alcohol-induced liver fibrosis and alcohol acceleration of liver fibrosis. We have integrated these findings in Figure 3. First, chronic ethanol consumption decreases the number of NK cells in the liver and inhibits NK cell accumulation in the liver induced by poly I:C or IFN-β. We have demonstrated that chronic ethanol consumption directly inhibits NK cell killing of activated HSC via induction of suppressor of cytokine signaling 1 (SOCS1) protein on HSC, followed by inhibiting IFN-γ signaling in HSC.

Modulation of other innate immunity components contributes to alcoholic liver fibrosis

In addition to amplification of the profibrogenic effect of Kupffer cells and inhibition of the antifibrogenic effect of NK/IFN-γ in
the liver, alcohol modulation of other innate immunity components may also contribute to alcoholic liver fibrosis. For example, it was shown that chronic ethanol consumption induced accumulation of hepatic NKT cells, and NKT cells contributed to alcoholic liver injury. It is plausible that modulation of NKT cells by alcohol may also contribute to alcoholic liver fibrosis. Several components of the complement system are involved in liver fibrosis and alcoholic liver injury. However, it is not clear whether modulation of the complement system by alcohol also contributes to alcoholic liver fibrosis or alcohol acceleration of liver fibrosis. Feeding mice with an alcohol-containing diet for 2 weeks upregulated multiple TLR in the liver and increased the sensitivity to liver inflammation induced by multiple bacterial products recognized by TLR, suggesting that modulation of TLR likely contributes to alcoholic liver fibrosis.

**Conclusion**

The hepatic immune system consists of predominant innate immunity, which plays an important role in regulating liver fibrosis. The role of Kupffer cells in liver fibrogenesis has been extensively investigated and the profibrogenic effects of Kupffer cells are well documented. In addition, Kupffer cells also contribute to the resolution of liver fibrosis. NK cells were recently identified to have antifibrogenic functions by direct killing of activated HSC and production of IFN-γ. Complement 5 was shown to contribute to liver fibrosis, whereas the roles of other complement components in liver fibrosis remain obscure. TLR4 and TLR9 have been implicated in contributing to the pathogenesis of liver fibrosis but activation of TLR3 seems to inhibit liver fibrosis. Modulation of innate immunity by alcohol is an important mechanism contributing to alcoholic liver fibrosis and alcohol acceleration of liver fibrosis in patients with HCV infection. These include alcohol amplification of the profibrogenic effect of Kupffer cells and inhibition of the antifibrogenic effect of NK/IFN-γ in the liver. Further understanding the effects of innate immunity in alcoholic liver fibrosis and injury may help us to identify novel therapeutic targets to treat alcoholic liver disease.

**Conflict of interest**

No conflict of interest has been declared by the authors.

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