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Molecular mechanisms of alcoholic liver disease: Innate immunity and cytokines

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Abstract

Alcohol consumption is a predominant etiological factor in the pathogenesis of chronic liver diseases worldwide, causing fatty liver, alcoholic hepatitis, fibrosis/cirrhosis, and hepatocellular carcinoma. In the past few decades, significant progress has been made in our understanding of the molecular mechanisms underlying alcoholic liver injury. Activation of innate immunity components such as Kupffer cells, LPS/TLR4, and complements in response to alcohol exposure plays a key role in the development and progression of alcoholic liver disease (ALD). LPS activation of Kupffer cells also produces IL-6 and IL-10 that may play a protective role in ameliorating ALD. IL-6 activates signal transducer and activator of transcription 3 (STAT3) in hepatocytes and sinusoidal endothelial cells, while IL-10 activates STAT3 in Kupffer cells/macrophages, subsequently protecting against ALD. In addition, alcohol consumption also inhibits some components of innate immunity such as natural killer (NK) cells, a type of cells that play key roles in anti-viral, anti-tumor, and anti-fibrotic defenses in the liver. Ethanol inhibition of NK cells likely contributes significantly to the pathogenesis of ALD. Understanding the roles of innate immunity and cytokines in alcoholic liver injury may provide insight into novel therapeutic targets in the treatment of alcoholic liver disease.

Keywords

Alcohol liver injury; IL-6; STAT3; NK cells; IFN-γ

INTRODUCTION

In the last several decades, significant progress has been made in understanding the cellular and molecular mechanisms contributing to the pathogenesis of alcoholic liver disease (ALD), a predominant cause of cirrhosis and liver cancer in Western countries (O'Shea R, 2009). These mechanisms include direct hepatotoxicity and production of reactive oxygen species induced by ethanol and its metabolites, activation of innate immunity (such as Kupffer cells, LPS/TLR4 signaling, and complement system), and subsequent production of

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proinflammatory cytokines (such as TNF-α) (Arteel, 2003; Breitkopf et al., 2009; Hoek and Pastorino, 2002; Nagy, 2003; Rao, 2009; Tsukamoto and Lu, 2001). It is generally believed that chronic alcohol consumption increases the permeability of the gut, resulting in elevated levels of portal endotoxin (LPS). LPS then activates Kupffer cells to produce reactive oxygen species and TNF-α via MyD88-independent and TRIF-dependent pathways (Hritz et al., 2008; Zhao et al., 2008), leading to liver inflammation and injury. Recent studies, primarily from Dr. Nagy's lab, demonstrate that ethanol exposure also triggers the classical complement pathway activation via C1q binding to apoptotic cells in the liver, leading to an early increase in the expression of pro-inflammatory cytokines that contribute to the pathogenesis of ALD (Cohen JI, 2010; Roychowdhury et al., 2009). Kupffer cells activated by LPS also produce hepatoprotective (such as IL-6) and anti-inflammatory (such as IL-10 and adiponectin) cytokines that play compensatory roles in ameliorating ALD.

In addition, alcohol consumption also inhibits components of innate immunity, such as natural killer (NK) cells (Cook et al., 1997; Pan et al., 2006). As NK cells play key roles in anti-viral, anti-tumor, and anti-fibrotic defense in the liver (Gao et al., 2009), ethanol suppression of NK cells likely contributes to the pathogenesis of ALD.

Despite extensive research in last several decades, the mechanisms underlying ALD remain to be fully elucidated. In this review, we summarize two major findings recently from our laboratory showing that chronic alcohol exposure abrogates the hepatoprotective effect of interleukin-6/signal transducer and activator of transcription 3 (IL-6/STAT3) and abolishes the anti-fibrotic effect of natural killer (NK) cells/IFN-γ. These studies may add to our understanding of the complex mechanisms of ALD, and may help us find novel therapeutic targets to treat this disease.

HEPATOPROTECTION OF IL-6/STAT3 IN ALCOHOLIC LIVER INJURY: SUPPRESSION BY ALCOHOL

Emerging evidence suggests that elevated LPS in the liver after alcohol consumption stimulates Kupffer cells/macrophages to produce ROS and TNF-α, and also IL-6 and IL-10. The latter two cytokines seem to play a protective role in ameliorating alcoholic fatty liver, liver injury and inflammation via activation of STAT3 in various types of cells in the liver. The interplay of IL-6/IL-10/STAT3 in different types of cells in ameliorating alcoholic liver injury is described in a model in Fig. 1.

IL-6/STAT3 and IL-10/STAT3 in the liver

IL-6 was originally cloned as B-cell stimulatory factor-2 and is now known to involve in a variety of cellular functions in the hematopoietic, immune, neuronal and hepatic systems (Hibi et al., 1996). In the liver, IL-6 has been shown to play an important role in inducing acute phase response, liver regeneration, hepatoprotection, tumorigenesis etc (Gao, 2005b). The action of IL-6 in hepatocytes is mediated via binding IL-6 receptor and the signal-transducing receptor subunit gp130, followed by activation of the downstream signal Janus-Kinase (Jak)-STAT3. Activated STAT3 forms a dimer and subsequently translocates into nuclei as a transcription factor to induce expression of many genes encoding acute phase, anti-apoptotic, anti-oxidant, pro-oncongic proteins that attribute to a variety of liver functions.

IL-10 is one of the most important anti-inflammatory cytokines that selectively suppresses the expression of pro-inflammatory genes in myeloid cells, including macrophages and neutrophils, activated by pathogen recognition receptor ligands such as LPS (Mosser and Zhang, 2008). IL-10 exerts its anti-inflammatory function via binding IL-10R1 and IL-10R2, followed by activation of STAT3 and the subsequent induction of several

transcriptional repressors and co-repressors that attenuate the expression of inflammatory genes in myeloid cells (El Kasmi et al., 2007).

Protective role of IL-6 in ALD

Elevation of IL-6 has been reported in patients with ALD and correlates with the severity of disease (Hill et al., 1992), suggesting IL-6 may be involved in the pathogenesis of ALD. However, studies using animal models reveal that IL-6 protects against ethanol-induced liver injury. First, IL-6-deficient mice are more susceptible to ethanol-induced steatosis and liver injury (El-Assal et al., 2004; Hong et al., 2002); Second, *in vivo* treatment with IL-6 or ME3738, an inducer of IL-6, ameliorates ethanol-induced fatty liver disease in rodents (Fukumura et al., 2007; Hong et al., 2004); Third, *ex vivo* treatment with IL-6 reduces the mortality associated with ethanol-induced fatty liver transplant in rats (Sun et al., 2003); Finally, *in vitro* treatment with IL-6 prevents ethanol plus TNF-α-induced mouse hepatocyte apoptosis (Hong et al., 2002). These findings indicate that elevation of IL-6 associated with ALD may play a compensatory role in preventing hepatocellular damage in the early stage of ALD.

Hepatoprotective effect of STAT3 in ALD

STAT3 is a cell survival signal and has been shown to protect against hepatocellular damage in many rodent models of liver injury (Gao, 2005b; Horiguchi et al., 2010). Interestingly, conditional deletion of STAT3 in hepatocytes enhanced hepatic steatosis but not hepatocellular damage induced by feeding a diet containing 5% ethanol for 4 weeks (Horiguchi et al., 2008), suggesting that hepatocyte STAT3 plays a more important role in ameliorating steatossis but not hepatocellular damage in this model with mild liver injury. In contrast, conditional deletion of STAT3 in endothelial cells markedly enhanced sinusoidal endothelial cell and hepatocyte damage after ethanol feeding, as reflected by elevation of serum levels of hyaluronic acid and ALT, respectively (Miller et al., 2010). In addition, *in vitro* treatment with IL-6 induces STAT3 activation in isolated sinusoidal endothelial cells (Gao, 2004), and *ex vivo* treatment of isolated rat livers with IL-6 induces STAT3 activation and protects against sinusoidal endothelial cell apoptosis after liver transplantation (Sun et al., 2003).

Cell type-dependent pro- and anti-inflammatory role of STAT3 in ALD

In response to chronic ethanol feeding, hepatocyte-specific STAT3 knockout mice have reduced liver inflammation while myeloid cell-specific or endothelial cell-specific STAT3 knockout mice have markedly enhanced liver inflammation compared with wild-type mice (Horiguchi et al., 2008; Miller et al., 2010). These findings clearly indicate that hepatocyte STAT3 acts as a pro-inflammatory signal while myeloid and sinusoidal endothelial cell STAT3 play anti-inflammatory roles in ALD. The pro-inflammatory effect of STAT3 in hepatocytes is likely mediated via stimulating hepatocytes to produce chemokines and acute phase proteins such as complement 5 etc (Horiguchi et al., 2008). The anti-inflammatory role of STAT3 in macrophages and neutrophils, which is primarily stimulated by IL-10, is mediated via induction of several transcriptional inhibitors that block the expression of pro-inflammatory and chemokine genes (El Kasmi et al., 2007). On the contrary, the mechanisms underlying the anti-inflammatory effect of STAT3 in endothelial cells are less clear. It is plausible to speculate that STAT3 protects against sinusoidal endothelial cell apoptosis and subsequently prevents infiltration of inflammatory cells into liver parenchyma. The findings from Kano et al (Kano et al., 2003) suggest that activation of STAT3 in endothelial cells produces a yet-unidentified soluble factor that inhibits inflammation, which may also contribute to the anti-inflammatory role of endothelial cell STAT3 in the liver. What is the overall effect of the interplay of hepatic, myeloid, and endothelial STAT3 on hepatic pro-inflammatory response in ALD? Since myeloid cells

(such as macrophages and neutrophils) play a dominant role in inducing inflammation in the liver, the anti-inflammatory effect of STAT3 in myeloid cells likely dominate the pro-inflammatory effect of STAT3 in hepatocytes. In addition, STAT3 in endothelial cells also acts as a strong anti-inflammatory effect. Thus, the net effect of STAT3 from different cell types is likely anti-inflammatory during alcoholic liver injury.

Effects of alcohol on STAT3 in the liver

The effects of ethanol on STAT3 signaling pathway have been intensely investigated in last several years. Acute ethanol exposure increases while chronic alcohol consumption inhibits liver STAT3 activation induced by partial hepatectomy (Chen et al., 1997) or T-cell hepatitis (Jaruga et al., 2004). Chronic ethanol exposure also abolishes endotoxin-induced STAT3 activation in the liver (Harrison-Findik D, 2009) and inhibits IL-6 activation of STAT3 in hepatocytes and sinusoidal endothelial cells (Fernandez D, 2009; Gao, 2004; Weng et al., 2008). Acute *in vitro* treatment with ethanol blocks IL-6 activation of STAT3 in freshly isolated hepatocytes (Chen et al., 2001). Clinical studies have shown that patients with ALD are often associated with elevated serum IL-6 levels (Hill et al., 1992). However, STAT3 activation in the liver is impaired in these patients compared to patients with other liver disorders such as chronic HCV infection (Horiguchi et al., 2007) or primary biliary cirrhosis (Starkel et al., 2005), suggesting that chronic alcohol consumption inhibits hepatic STAT3 activation in patients.

Acute alcohol consumption or exposure directly activates the STAT3 signaling pathway in human monocytes (Norkina et al., 2007) but attenuates cytokine-induced STAT3 activation in these cells via the induction of suppressor of cytokine signaling (SOCS) protein expression (Norkina et al., 2008). However, the effect of chronic alcohol consumption and exposure on the STAT3 signaling pathway in monocytes has not been examined. Since the anti-inflammatory effect of STAT3 in myeloid lineage cells has been well documented in several models of liver injury including alcoholic liver injury (Horiguchi et al., 2008), T cell hepatitis (Lafdil et al., 2009), and partial hepatectomy (Wang et al., 2010), modulation of the STAT3 signaling pathway by alcohol in monocytes and other myeloid lineage cells may contribute to the development of liver inflammation during alcoholic liver injury.

ANTI-FIBROTIC EFFECT OF NK/IFN-γ: SUPPRESSION BY ALCOHOL

Alcoholic liver fibrosis

It is well documented that excessive alcohol consumption leads to liver fibrosis in approximately 15-30% individuals and markedly accelerates the progression of liver fibrosis in patients with chronic hepatitis viral infection. Multiple mechanisms likely contribute to the development of alcoholic liver fibrosis and the alcohol-induced acceleration of liver fibrosis in viral hepatitis patients (Cubero et al., 2009; Gao, 2005a; Siegmund et al., 2005). It is generally believed that elevated hepatic LPS in response to alcohol consumption stimulates Kupffer cells to produce various factors that activate hepatic stellate cells (HSCs). In addition, LPS also directly targets HSCs and enhances pro-fibrogenic cytokine transforming growth factor β (TGF-β) signaling on these cells, subsequently leading to HSC activation and liver fibrosis (Cubero and Nieto, 2006; Cubero et al., 2009; Guo et al., 2009; Seki et al., 2007). Furthermore, ethanol and its metabolite acetaldehyde can directly induce HSC activation (Cubero et al., 2009). Recent findings from our own laboratory, as well as other laboratories, suggests that natural killer (NK) cells/IFN-γ play an important role in inhibiting liver fibrosis and that ethanol-inhibition of the anti-fibrotic effect of NK/IFN-γ is an important mechanism contributing to ethanol acceleration of liver fibrosis (Gao et al., 2009).

Liver NK cells

Liver lymphocytes are enriched in NK cells (Gao et al., 2008; Gao et al., 2009; Nemeth et al., 2009), which are activated during viral hepatitis infection and contribute to anti-viral immunity and hepatocellular damage via killing of virus-infected hepatocytes (Ahlenstiel et al., 2010; Oliviero et al., 2009). NK cells distinguish tumor cells or "stressed" cells from normal healthy autologous cells via the interactions of specific NK cell receptors (stimulatory and inhibitory receptors) on NK cells with specific ligands (stimulatory and inhibitory ligands) expressed on target cells. When cells are under stress, transforming into tumors, or infected, various molecules, including NK cell stimulatory ligands, are upregulated on the their surfaces. These upregulated ligands can induce NK cell killing of these stressed cells, tumor cells, or infected cells. Upregulation of NK cell stimulatory ligands has been reported in hepatocytes and biliary epithelial cells in a variety of liver injury models as well as in the patients with nonalcoholic steatohepatitis (Cheng et al., 2009; Hou et al., 2009; Kahraman et al., 2008; Kahraman A, 2009; Shivakumar et al., 2009). Such upregulation induces NK cell activation and attributes to NK cell-induced hepatocyte and biliary epithelial cell damage and HSC apoptosis (Gao B, 2010).

Anti-fibrotic function of NK cells/IFN-γ

Several studies have shown that during liver injury, activated hepatic stellate cells (HSCs) have elevated expression of NK cell stimulating ligands. These ligands then stimulate NK cells to kill activated HSCs and inhibit liver fibrosis (Jeong et al., 2006; Krizhanovsky et al., 2008; Melhem et al., 2006; Radaeva et al., 2006). Several mechanisms have been implicated in the increased sensitivity of activated HSCs to NK cell killing. First, HSCs contain a large amount of vitamin A (retinol), which can be metabolized into retinoic acid during stellate cell activation. Retinoic acid then induces expression of retinoic acid-induced early gene 1 (RAE1) that in turn activates NK cell killing of activated HSCs (Radaeva et al., 2007). Second, DNA damage associated with senescence-activated HSCs also upregulates NK cell activating ligands on these cells, followed by stimulating NK cell cytotoxicity against these cells (Krizhanovsky et al., 2008). Third, expression of NK cell inhibitory ligands are downregulated on activated HSCs, resulting in loss of inhibition of NK cell function and the subsequent activation of NK cells to kill activated HSCs (Melhem et al., 2006). Fourth, activated HSCs have increased expression of TRAIL receptor (Fischer et al., 2002; Taimr et al., 2003) and become sensitive to NK cell killing via the interaction of TRAIL-TRAIL receptor (Radaeva et al., 2006). Activated NK cells also produce a large amount of IFN-γ, which induces HSC apoptosis and cell cycle via activation of STAT1 signaling pathway, thereby inhibiting liver fibrosis (Jeong et al., 2006).

Although the anti-fibrotic effect of NK cells in mice is well documented, the role of NK cells in controlling liver fibrosis in humans remains unclear. It is known that NK cells are enriched in human liver lymphocytes (Gao et al., 2008; Nemeth et al., 2009) and are activated in the liver and peripheral blood of patients with chronic hepatitis virus infection (Ahlenstiel et al., 2010; Oliviero et al., 2009). Clinical data also show that NK cell activity negatively correlates with the stage of liver fibrosis in patients with chronic hepatitis C infection (Morishima et al., 2006). These findings suggest that NK cells play an important role in inhibiting the progression of liver fibrosis in patients with viral hepatitis infection.

Alcohol suppression of anti-fibrotic function of NK cells/IFN-γ

The inhibitory effect of alcohol consumption on NK cells is well documented (Cook et al., 1997). Thus, it is probable that the anti-fibrotic effect of NK cells is also attenuated in viral hepatitis patients with chronic alcohol consumption, potentially contributing to the alcohol-induced acceleration of liver fibrosis in these patients (Gao, 2005a; Wiley et al., 1998). Indeed, by using animal models, we have recently demonstrated that chronic ethanol feeding

abolishes NK cell killing of activated HSCs and diminishes the anti-fibrotic effect of NK cells and IFN-γ (Jeong et al., 2008). These observed effects are likely mediated by several mechanisms (Fig. 1). First, chronic ethanol feeding directly attenuates NK cell cytotoxicity against activated HSCs via downregulation of NK cell-associated molecules (such as NKG2D, TRAIL, FAS ligand, perforin, and IFN-γ). Second, chronic ethanol feeding stimulates HSCs to produce TGF-β, a potent inhibitor of NK cells, thereby attenuating NK cell killing activity. Third, chronic ethanol exposure induces expression of SOCS1 protein, followed by inhibiting IFN-γ signaling in HSCs. Lastly, chronic ethanol consumption stimulates hepatocytes to produce oxidative stress, which subsequently inhibits IFN-γ signaling in HSCs (Jeong et al., 2008).

SUMMARY

Studies from the last several decades demonstrate that alcohol exposure activates innate immunity such as complement system, LPS/TLR4, and Kupffer cells, followed by the induction of several pro-inflammatory cytokines, including TNF-α, subsequently inducing hepatocellular damage. The data demonstrates activation of innate immunity also results in elevation of hepatoprotective (such as IL-6) and anti-inflammatory cytokines (such as IL-10, adiponectin), which play an important role in ameliorating alcoholic liver injury and inflammation (Fig. 1). However, chronic alcohol exposure attenuates the signaling pathways activated by these cytokines, thereby limiting their hepatoprotective and anti-inflammatory effects, and contributing to the pathogenesis of ALD.

Alcohol consumption also suppresses components of innate immunity, such as NK cells (Cook et al., 1997; Pan et al., 2006) (Fig. 1). Since NK cells play key roles in anti-viral, anti-tumor, and anti-fibrotic defenses in the liver (Gao et al., 2009), ethanol inhibition of NK cells likely contributes significantly to the pathogenesis of ALD.

FUTURE STUDIES

Role of IL-10 in ALD

In contrast to IL-6 and adiponectin, whose roles in ALD are well documented (see above) (Huang et al., 2008; Mandal et al., 2010; Rogers et al., 2008), the effect of IL-10 in ALD has not been extensively investigated. It was reported that chronic ethanol feeding sensitizes mice to LPS or D-galactosamine-induced liver injury (Hill et al., 2002; Sermon et al., 2003). However, it is difficult to rule out whether the enhanced liver injury in IL-10 deficient mice observed in these studies is due to the ethanol or the toxins such as LPS or D-galactosamine. Thus, the role of IL-10 in ALD should be further investigated.

Therapeutic potential of cytokines in ALD

The therapeutic application of IL-6 and adiponectin in treating ALD is impeded as ALD patients are associated with high serum levels of IL-6 and adiopnection. In addition, treatment with IL-6 is accompanied with numerous side effects. Future studies to seek the therapeutic agents or cytokines that target similar signaling pathways to IL-6 or adiponectin while limiting the side effects and maintaining low levels in ALD patients are warranted.

Regulation of NKT cells by ethanol

NKT cells are a distinct T lymphocyte lineage with expression of both T and NK cell markers. After recognition of antigen or inflammatory stimulations, NKT cells are rapidly activated and produce a variety of cytokines that influence many types of cells, allowing them to functionally link the innate and adaptive immune responses. Previous studies reported that NKT cells contribute to ethanol-induced liver injury in mice (Minagawa et al.,

2004); however, how alcohol consumption affects NKT cells remains unknown. Future studies on the study of acute and chronic alcohol exposure on NKT cells in the liver are needed.

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Abbreviations

ALD	alcoholic liver disease
HSC	hepatic stellate cells
IFN-γ	interferon-gamma
NK	natural killer
RAE-1	retinoic acid-induced early gene 1
SREBP1	sterol regulatory element binding protein-1
STAT3	signal transducer and activator of transcription 3
SOCS	suppressor of cytokine signaling.

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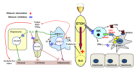

Fig. 1. Molecular mechanisms of alcoholic liver disease (ALD): roles of innate immunity and cytokines

Chronic alcohol exposure leads to innate immunity activation such as Kupffer cells and LPS/TLR4. Activated Kupffer cells produce TNF-α and toxins that contribute to ALD. LPS also stimulates Kupffer cells to produce hepatoprotective cytokines (such as IL-6) and anti-inflammatory cytokines (such as IL-10). Both IL-6 and IL-10 play protective roles in ameliorating ALD via activation of STAT3 in different types of liver cells. Alcohol consumption inhibits IL-6 activation of STAT3 in hepatocytes and sinusoidal endothelial cells, and inhibits IL-10 activation of STAT3 in monocytes, contributing to the pathogenesis of ALD. Alcohol consumption also suppresses some other components of innate immunity such as NK cells. NK cells play important roles in anti-viral, anti-tumor, and anti-fibrotic defenses in the liver. Alcohol inhibits NK cell functions via multiple mechanisms as described in the text, contributing to the pathogenesis of ALD.