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Targeting inflammation for the treatment of alcoholic liver disease☆,☆☆,★★

Ming-Jiang Xu [a,1], Zhou Zhou [a,1], Richard Parker [a,b], Bin Gao [a,*]

[a] Laboratory of Liver Diseases, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD 20892, USA
[b] Centre for Liver Research, University of Birmingham, United Kingdom

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ABSTRACT

Alcoholic liver disease (ALD) is a leading cause of chronic liver disease with a wide spectrum of manifestations including simple steatosis to steatohepatitis, cirrhosis, and hepatocellular carcinoma. Liver injury in ALD is caused by chronic inflammation, which has been actively investigated as a therapeutic target for the treatment of ALD for over the last four decades. In this review, we summarize a wide variety of inflammatory mediators that have been shown to contribute to the pathogenesis of ALD, and discuss the therapeutic potential of these mediators for the treatment of ALD.

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1. Introduction

Alcoholic liver disease (ALD) is a major chronic liver disease which causes significant mortality worldwide. Chronic inflammation is a critical element in the development of ALD (Gao & Tsukamoto, 2016; Wang, Gao, Zakhari and Nagy, 2012). Inflammation is a series of responses to harmful stimuli with which the body maintains homeostasis, but which may cause collateral damage to normal tissue (Medzhitov, 2008). In ALD, inflammation is a consequence of excessive alcohol consumption causing direct and indirect damage to the liver, eventually

Abbreviations: AH, alcoholic hepatitis; ALD, alcoholic liver disease; ALT, alanine aminotransferase; CCL, chemokine (C-C motif) ligand; CXCL, chemokine (C-X-C motif) ligand; DAMP, danger-associated molecular pattern; DPI, diphenyleneiodonium; Egr-1, early growth response protein 1; ERK, extracellular signal–regulated kinase; GROα, growth-regulated alpha protein; HFD, high-fat diet; HSCs, hepatic stellate cells; ICAM-1, Intercellular Adhesion Molecule 1; IFN, interferon; IL, interleukin; IKKε, inhibitor-κB kinase ε; IRF, interferon-regulatory factor; KCs, Kupffer cells; LCN2, lipocalin 2; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinases; MCP1, monocyte chemoattractant protein-1; miRNA, microRNAs; NAPDH, nicotinamide adenine dinucleotide phosphate; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NK, natural killer; OPN, osteopontin; ROS, reactive oxygen species; SOCS, suppressor of cytokine signaling; TBK, TANK Binding Kinase; TGF, transforming growth factor; TNF, tumor necrosis factor; TLR, toll-like receptor; TRIF, TIR-domain-containing adapter-inducing interferon-β.

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* Corresponding author at: NIAAA/NIH, 5625 Fishers Lane, Bethesda, 20892, USA.
E-mail address: bgao@mail.nih.gov (B. Gao).
[1] Contributed equally to this work.

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leading to fibrosis and impaired liver function. Over the last four decades, clinical and experimental studies of ALD have identified many inflammatory mediators that play important roles in the pathogenesis of ALD (Gao & Tsukamoto, 2016; Wang, Gao, et al., 2012). In the present review article, we summarize the possible therapeutic targets on ALD to control inflammation that may induce liver damage, and these targets include inflammatory cytokines, immune cells, microRNAs, and gut microbiome.

2. Potential targets of immune cells for the treatment of ALD

2.1. Role of Kupffer cells (KCs) in alcoholic liver injury

KCs are resident macrophages in the liver (Ju & Tacke, 2016). They play important roles in the elimination of toxins and cell debris. KCs are activated by multiple factors in ALD, including gut-derived lipopolysaccharide (LPS), complement, and reactive oxygen species (ROS). Depleting KCs with $GdCl_3$, a macrophage-specific toxin, significantly reduced the injury in an ALD model (Adachi, Bradford, Gao, Bojes, & Thurman, 1994), suggesting that KCs are necessary for the development of ALD.

Alcohol ingestion compromises intestinal barrier function and increases the flux of lipopolysaccharide (LPS), derived from the bacterial cell wall, to the portal vein (Szabo & Bala, 2010). Excess LPS activates KCs by binding to toll-like receptor 4 (TLR4) (Uesugi, Froh, Arteel, Bradford, & Thurman, 2001), leading to nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation and secretion of inflammatory cytokines, including tumor necrosis factor alpha (TNF-α) (Gustot et al., 2006; Yamashina et al., 2005). A MyD88 independent signal pathway passes the signal from TLR4 to TIR-domain-containing adapter-inducing interferon-β (TRIF), TANK Binding Kinase (TBK) 1, inhibitor-κB kinase ε (IKKε) and finally the transcription factor interferon-regulatory factor (IRF) 3. IRF3 controls TNF-α gene expression by directly binding to its promoter. TRIF deficient mice, in which the TRIF pathway is absent, are resistant to alcoholic liver injury (Zhao et al., 2008). TLR4 also regulates KCs in alcoholic liver injury through extracellular signal–regulated kinase (ERKs) pathways. Among them, the TLR4 ➔ ERK1/2 ➔ early growth response protein 1 (Egr-1) ➔ TNF-α signal is the most recognized (Kishore, Hill, McMullen, Frenkel, & Nagy, 2002). Egr-1 deficient mice given chronic ethanol treatment have lower levels of hepatic TNF-α expression, alanine aminotransferase (ALT) elevation and hepatic steatosis than wild-type mice. The hypersensitivity of KCs to LPS stimulation is also abolished in Egr-1 deficient mice (McMullen et al., 2005). ERK1/2 signaling may also contribute to NF-κB activation as PD98059, an ERK1/2 inhibitor reduces the activation of NF-κB (Cao, Mak, & Lieber, 2002). Moreover, p38 phosphorylation is also upregulated after LPS stimulation, especially in ethanol-treated macrophages (Kishore, McMullen, & Nagy, 2001). SB203580, an inhibitor of p38, reduces the expression of TNF-α from KCs isolated from chronically ethanol-treated rats (Cao et al., 2002). P38 exerts this function by stabilizing TNF-α mRNA (Kishore et al., 2001). Conversely, Poly I:C, a TLR3 agonist, protectes mice from alcoholic liver injury by reducing the activation of NF-κB and the downstream inflammatory cytokines and chemokines (Byun, Suh, Yi, Lee, & Jeong, 2013).

Accumulating evidence shows an important contribution of KC-derived ROS in the pathogenesis of alcoholic liver injury. ROS sensitize the TLR4 pathway and independently stimulate the expression of TNF-α. Nicotinamide adenine dinucleotide phosphate (NAPDH) oxidase in KCs is believed to be the most important source of ROS (Thakur, Pritchard, McMullen, Wang, & Nagy, 2006). Blocking its function with the NAPDH oxidase inhibitor diphenyleneiodonium (DPI) or gene knock-out alleviates inflammation and liver injury (Thakur et al., 2006). The increase in production of TNF-α by alcohol treated KCs stimulated with LPS in vitro is abrogated by DPI (Thakur et al., 2006) and daily injection of DPI reduces the up-regulation of TLR4 mRNA in chronically alcohol treated rats (Gustot et al., 2006). Deficiency of p47, a subunit of NADPH oxidase, protectes mice from alcoholic liver injury by reducing ROS production and TNF-α production in KCs (Kono et al., 2000). In contrast, stimulation of the activity of NADPH oxidase with arachidonic acid augmentes ROS production and TNF-α expression in KCs (Cubero & Nieto, 2012).

The complement pathway is another important pathway of KC activation. KCs express a high amount of the complement receptors C3aR and C5aR (Qin & Gao, 2006). Alcohol administration rapidly causes deposition of C3b-iC3b/C3c complex in the liver. C3 deficient mice are resistant to alcohol induced liver steatosis (Bykov, Jauhiainen, et al., 2007) and do not show upregulation of inflammatory genes. Mice deficient of C3aR, C5aR or depletion of KCs all show diminished TNF-α induction during treatment with alcohol. These findings suggest that complement deposition and signal through C3aR/C5aR to KCs are responsible for early inflammatory gene induction after alcohol ingestion (Roychowdhury et al., 2009). The mechanisms of ethanol-induced TNF-α expression in KC are summarized in Fig. 1.

2.1.1. Therapeutic targeting of KCs for treatment of ALD

Inhibition of KCs via the reduction of LPS had beneficial effects for the treatment of ALD in animal models as described above. Indeed, liver injury is reduced by preventing LPS flux from the intestine through depleting normal intestinal microflora with antibiotics or improving intestinal barrier function (Adachi, Moore, Bradford, Gao, & Thurman, 1995; Gustot et al., 2006; Peng, Cui, et al., 2013). Current clinical studies targeting gut microbiota for the treatment of alcoholic hepatitis (AH) are described in the later paragraph in this paper. Inhibition of ROS production via the treatment with anti-oxidants (e.g. *N*-acetylecysteine, vitamin E) has shown mixed results in clinical trials (Nguyen-Khac et al., 2011; Phillips et al., 2006; Stewart et al., 2007). More clinical studies are required to clarify the therapeutic benefits of anti-oxidants for the treatment of AH.

In contrast to the devastating effect in alcoholic liver damage, KCs also promote liver regeneration, which may be a critical process of liver recovery after alcohol challenge. KC depletion significantly compromises liver regeneration after partial hepatectomy and partial liver transplantation (Luo, Ma, Qu, & Tian, 2015; Meijer et al., 2000), while activation of KCs improves liver regeneration in these animal models (Yoshiya et al., 2015). KCs promote liver regeneration through the production of TNF-α and IL-6, which activate NF-κB and STAT3 signals respectively to reduce hepatocyte apoptosis and increase their proliferation (Abshagen, Eipel, Kalff, Menger, & Vollmar, 2007; Luo et al., 2015; Yang et al., 2013; Yoshiya et al., 2015). Given these important functions of KCs in liver regeneration, long term inhibition of KCs may not be a good choice as a therapeutic target of ALD. Instead, keeping functional KCs in the recovery phase after eliminating the source of damage may be beneficial.

2.2. Role of neutrophils in ALD

Neutrophils are central to the development of ALD. Unlike other innate immune cells, neutrophils are not residential immune cells in the liver. The infiltration of neutrophils to the liver is a critical and necessary step for their biological functions. Neutrophils exert their damaging effects in ALD via the release of tissue dissolving enzymes and the production of ROS (Jaeschke, 2002). Depletion of neutrophils reduces liver damage in chronic-plus-binge mouse model (Bertola, Park, & Gao, 2013).

Neutrophils are typically attracted by CXC chemokines, such as CXCL8 (IL-8) in humans, CXCL1 in mice and cytokine-induced neutrophil chemoattractant in rats (Bautista, 2002). These cytokines are upregulated in clinical and experimental ALD (Chang et al., 2015; Roh, Zhang, Loomba, & Seki, 2015) (Shiratori et al., 1994). AH patients have elevated expression of many CXC and CC chemokines than healthy individuals, including IL-8, Gro-α, CXCL5, CXCL6, CXCL10, CCL20 and platelet factor 4. The expression of these chemokines correlates with the