Published in final edited form as:

Pharmacol Ther. 2017 December ; 180: 77–89. doi:10.1016/j.pharmthera.2017.06.007.

Targeting inflammation for the treatment of alcoholic liver disease^{☆,☆☆}

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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.

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 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.

[☆]Financial support: This work from Dr. Bin Gao's lab described in this review article was supported by the intramural program of NIAAA, NIH (B.G.).

^{☆☆}Conflict of interest: The authors declare that there are no conflicts of interest.

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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 GdCl₃, 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- κ B) 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- κ B kinase ϵ (IKK ϵ) 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 $\rightarrow$ ERK1/2 $\rightarrow$ early growth response protein 1 (Egr-1) $\rightarrow$ 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- κ B activation as PD98059, an ERK1/2 inhibitor reduces the activation of NF- κ B (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, protects mice from alcoholic liver injury by reducing the activation of NF- κ B 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, protects 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 augments 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*-acetylcysteine, 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- κ B 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 neutrophil infiltration and the severity of portal hypertension (Affo et al., 2013; Dominguez et al., 2009). A recent study also demonstrated that AH patients had a significant increase in albumin oxidation, which acts as a pro-oxidant that induces ROS and inflammation via the activation of neutrophils, suggesting a pathogenic role of neutrophils in AH (Das et al., 2017).

Intercellular Adhesion Molecule 1 (ICAM-1) is expressed constitutively on endothelial cells and reacts to the integrin CD11/CD18 on neutrophils to cause adhesion. During chronic alcohol intoxication, ICAM-1 expression is upregulated in hepatocytes, which facilitates the direct contact of neutrophils and hepatocytes (Bautista, 1997; Jaeschke, 2002). ICAM-1 deficient mice showed apparent resistance to alcohol induced liver injury (Kono et al., 2001). Another important molecule that mediates infiltration of neutrophils to liver is osteopontin (OPN), a matricellular protein that binds to integrins on neutrophils. In a model of alcoholic steatohepatitis induced by feeding Lieber-DeCarli diet plus LPS injection in rats, OPN expression is highly elevated. The elevation of OPN expression correlates with neutrophil infiltration and liver injury (Apte, Banerjee, McRee, Wellberg, & Ramaiah, 2005; Banerjee, Apte, Smith, & Ramaiah, 2006). However, a recent study using OPN deficient mice showed conflicting results regarding the role of OPN in a hybrid model with severe ALD. OPN deficient mice showed a tendency towards increased neutrophil infiltration and liver damage, although statistical significance was not reached (Lazaro et al., 2015). Thus, OPN may have a complicated function in alcoholic steatohepatitis. Finally, our recent study revealed that hepatic E-selectin is highly expressed in the chronic-plus-binge mouse model and human alcoholic fatty livers. Knockout of E-selectin diminished hepatic neutrophil infiltration and liver injury in the chronic-plus-binge ethanol feeding model (Bertola et al., 2013).

Neutrophils may also migrate in response to lipid signaling: Perez et al. provided evidence that hepatocytes generated a lipid material which was chemo-attractive to neutrophils (Perez, Roll, Bissell, Shak, & Goldstein, 1984), although the exact chemical nature of this lipid is not fully defined (Roll, Alexander, & Perez, 1989; Roll, Bissell, & Perez, 1986). Free fatty acids may be another source of signal for the production of CXCL1 (Chang et al., 2015; Roh et al., 2015). Hepatic CXCL1 is highly upregulated after high-fat diet (HFD)-plus-binge alcohol feeding, and blockage of CXCL1 markedly reduces HFD-plus-binge ethanol-induced hepatic neutrophil infiltration and liver injury (Chang et al., 2015).

In contrast to the evidence that neutrophils induce hepatocellular damage in animal models, histological studies in patients with severe AH have shown that greater neutrophil infiltration is associated with better outcome suggesting that presence of neutrophils is of benefit (Altamirano et al., 2014). The beneficial effect of neutrophils in severe AH may be because neutrophils play important roles in inhibiting bacterial infection and stimulating liver regeneration (Kwon, Won, Park, Feng, & Gao, 2014).

2.2.1. Therapeutic targeting of neutrophils for treatment of ALD—As neutrophils can play both detrimental (e.g. damaging hepatocytes) and beneficial (e.g. inhibiting bacterial infection and stimulating liver regeneration) roles in the pathogenesis of ALD, it may be more beneficial to restore neutrophil function in patients with ALD, rather than to seek to deplete or inhibit neutrophils in these patients. Indeed, there are some data suggesting that mobilization of neutrophils from the bone marrow with granulocyte colony stimulating factor (G-CSF) may be of benefit in AH (Singh et al., 2014).

2.3. T cells in ALD

It was reported that patients with AH had elevated circulating antibodies against lipid peroxidation adducts and significant infiltration of T cells in the liver (Albano & Vidali, 2010; Thiele, Freeman, & Klassen, 2004; Thiele et al., 2010). Other studies demonstrated hepatic T cells in ALD produced IL-17, and levels of IL-17 correlated with liver inflammation and injury (Lemmers et al., 2009). It is generally believed that excessive alcohol consumption causes hepatic oxidative stress, which then induces lipid peroxidation products (e.g. malondialdehyde and 4-hydroxynonenal) (Albano & Vidali, 2010; Thiele et al., 2004, 2010). These products then interact with many proteins to form protein adducts that can act as antigens to activate the adaptive immune response (Albano & Vidali, 2010; Liaskou et al., 2016; Thiele et al., 2004, 2010). However, the exact roles of adaptive immunity and T cells in the pathogenesis of ALD remain to be elusive. Future studies are urgently needed to unravel their functions to explore whether T cells and adaptive immunity are potential therapeutic targets for the treatment of ALD.

2.4. Natural killer (NK) and NKT cells in ALD

NK cells exert an anti-fibrotic effect in liver diseases (Jeong et al., 2011; Krizhanovsky et al., 2008; Peng, Jiang, et al., 2013; Radaeva et al., 2006). This effect primarily relies on NK cell killing of activated hepatic stellate cells (HSCs) (Jeong et al., 2011; Radaeva et al., 2006). Although NK cells effectively reduce liver fibrosis, alcohol drinking compromises this protective function through several mechanisms. First, chronic alcohol consumption hinders the development of conventional NK cells. Alcohol consumption directly prohibits the development of NK cells in the CD11b⁺CD27⁺ stage (Zhang, Little, & Zhang, 2017). Lack of IL-15/IL-15R signal availability is likely the reason of this effect of alcohol consumption, because restoring this signal recovers the NK cell number and activity (Zhang et al., 2017). Second, chronic alcohol feeding reduces conventional NK cell number in the liver and compromises the cytolytic functions of NK cells (Pan et al., 2006). NK cells from ethanol-fed mice have reduced expression of NKG2D, TRAIL and IFN- γ (Ben-Eliyahu, Page, Yirmiya, & Taylor, 1996; Jeong, Park, & Gao, 2008; Laso et al., 1997; Meadows, Blank, & Duncan, 1989). The shortage of these key molecules may explain the low cytolytic

activity of NK cells in ethanol-fed animals. Third, alcohol drinking increases the expression of suppressor of cytokine signaling (SOCS) 1 and TGF- β in HSCs (Jeong et al., 2008). SOCS1 is a natural antagonizing protein of the IFN- γ -STAT1 pathway. Increased SOCS1 expression diminishes the cell cycle arrest and apoptosis effect of IFN- γ on HSCs. TGF- β is a potent factor to inhibit the immune cell functions, including NK cell. By inducing the expression of TGF- β and SOCS1 in HSCs, alcohol consumption enhances the resistance of HSCs to the cytolytic activity of NK cells (Jeong et al., 2008). Thus, alcohol consumption enhances liver fibrosis by diminishing the endogenous anti-fibrotic mechanism provided by NK cells. This may underlie the effect of alcohol consumption in accelerating liver fibrosis from other fibrotic challenges.

NKT cells are another type of innate immune cells, which are enriched in the liver and play important roles in the pathogenesis of liver diseases (Bandyopadhyay, Marrero, & Kumar, 2016; Gao, Radaeva, & Park, 2009; Wang, Feng, Park, Yin, & Gao, 2013; Yin et al., 2014). NKT cells recognize lipid antigens presented by CD1d molecules. Interestingly, type I NKT cells and type II NKT cells are discovered to play opposite roles in the control of liver injury. Type I NKT cells are typically highly activated by α -GalCer and express IL-4 and IFN- γ . They manipulate other immune cells including neutrophils to induce liver injury (Maricic et al., 2015). In the chronic-plus-binge ethanol model, type I NKT cells are recruited and activated by IL-1 β produced by KCs (Cui et al., 2015; Mathews et al., 2016). Alcohol may also facilitate the assembly of α -GalCer to CD1d so that the type I NKT cells are easily activated in alcohol drinking (Buschard et al., 2011). In contrast, type II NKT cells are activated by sulfatide and inhibit the activity of type I NKT cells. Activation of type II NKT cells ameliorates liver damage in the chronic-plus-binge ethanol model (Maricic et al., 2015). As type I NKT cells are activated but type II NKT cells are not activated in alcohol drinking, manipulating the activation of these two cell subtypes may have therapeutic potential for the treatment of ALD (Maricic et al., 2015).

2.4.1. Therapeutic targeting of NK and NKT cells for treatment of ALD—NK cells have many beneficial effects in the liver, such as host defense against viral infection and tumor transformation, and anti-fibrotic functions. It is known that NK cell functions are suppressed in patients with ALD; thus restoration of NK cell functions may have therapeutic potential for the treatment of ALD (Gao et al., 2009).

NKT cells seem to play complex roles in the pathogenesis of ALD, and different types of NKT cells play opposite roles in the control of liver injury. More studies are needed to demonstrate whether NKT cells can be used as therapeutic targets for the treatment of ALD.

2.5. Potential role of innate lymphoid cells (ILCs) in ALD

ILCs are groups of lymphoid cells that belong to the innate immune system, and are usually divided into 3 groups according to the cytokine expression pattern in counterpart of helper T (Th) cells. Group 1 ILCs, which resemble the Th1 cells, express transcription factor T-bet and express Th1 cytokines IFN- γ and TNF- α to defend intracellular pathogens upon the activation of IL-12, IL-15 and IL-18 (Eberl, Colonna, Di Santo, & McKenzie, 2015; Sonnenberg & Artis, 2015). Group 2 ILCs are the counterpart of Th2 cells as they both

express transcription factor GATA3 and have similar function. They are built to protect from the extracellular parasites by receiving the signal from IL-25, IL-33, TSLP (thymic stroma lymphopoietin) and IL-4 to produce IL-4, IL-5, IL-9, IL-13 and amphiregulin. The counterpart of Th17 cells in the ILCs are the group 3 ILCs and they express transcription factor ROR γ t. Upon the activation with IL-33 and IL-1 β , they produce IL-17, IL-22, GM-CSF and lymphotoxins. The Group 3 ILCs are dedicated to defending the invading fungi and extracellular bacteria (Eberl et al., 2015; Sonnenberg & Artis, 2015). Modern classification of ILCs also casts NK cells alone as killer ILCs because of the direct cytotoxicity through expressing granzymes and perforin. The rest ILCs are called helper-like ILCs and further divided into groups mentioned above (Diefenbach, Colonna, & Koyasu, 2014; Eberl et al., 2015).

ILCs are important immune cells in the initiation and resolution of both acute and chronic inflammation in gastrointestinal and airway mucosa as well as in the pathogenesis of liver diseases (Eberl et al., 2015; Sonnenberg & Artis, 2015; Yang, Tian, Wu, van Velkinburgh, & Ni, 2015). To date, the roles of ILCs in the pathogenesis of ALD remain poorly understood. Given the substantial pathogenic effects of cytokines produced by ILCs in ALD, ILCs likely play an important role in the control of liver inflammation and injury in ALD. Future studies are required to identify the role of ILCs in the pathogenesis of ALD.

3. Pro-inflammatory cytokines and chemokines

Over the last four decades, studies using rodent models of ALD and human ALD samples have identified multiple inflammatory mediators that play important roles in the pathogenesis of ALD (Gao & Tsukamoto, 2016; Wang, Gao, et al., 2012). Although immune cells are the great sources of pro-inflammatory cytokines and chemokines, such as IL-8, TNF- α , CXCL1, IL-1, IL-17, CCL20, MCP-1 et al., hepatocytes and non-parenchymal liver cells also produce mediators including IL-1, TNF- α , CXCL1/GRO α , OPN, MCP-1, LCN-2 etc. The role of these pro-inflammatory cytokines and chemokines in the pathogenesis of ALD are summarized in Table 1.

3.1. TNF- α

AH patients demonstrate a high level of TNF- α , and TNF- α is also induced in alcohol-fed animals and is closely coupled with liver injury (Wang, Gao, et al., 2012). However, both chimeric monoclonal antibody to TNF- α (infliximab) and p75-soluble TNF receptor:FC fusion protein (etanercept) showed no benefit in clinical trials, instead both treatments increased mortality due to an increased incidence of severe infections (Boetticher et al., 2008; Naveau et al., 2004), leading to the early cessation of the studies. Pentoxifylline, an orally administered phosphodiesterase inhibitor was thought to have a beneficial effect on AH via the reduction of TNF- α but this has been shown to be ineffective for the treatment of severe AH (Thursz et al., 2015).

3.2. IL-8, CXCL1/GRO α

As discussed above, neutrophil infiltration is a key feature of AH (Gao & Tsukamoto, 2016). IL-8 and CXCL1/GRO α are the two most important chemokines for neutrophil recruitment.

Serum and hepatic levels of IL-8 and CXCL1/GRO α are elevated in AH patients, and correlate with hepatic neutrophils infiltration, disease severity and mortality (Dominguez et al., 2009; Maltby et al., 1996; Patel et al., 2015; Sheron et al., 1993). Patients with alcoholic cirrhosis have increased frequency of the rs4074 A allele and enhanced CXCL1 serum levels, indicating the CXCL1 rs4074 A allele as a genetic risk factor for alcoholic cirrhosis (Nischalke et al., 2013). In alcohol-fed animal models, blockade of IL-8 receptors (CXCR1/2) protects mice from liver injury, inflammation, steatosis, weight loss and mortality (Roh et al., 2015; Wieser, Adolph, et al., 2017). We recently also showed that CXCL1 was dramatically upregulated in HFD-plus-binge ethanol-exposed mice and *Cxcl1* knockout or anti-CXCL1 treatment protected mice against HFD-plus-binge ethanol-induced neutrophil-mediated liver injury (Chang et al., 2015). Thus, blockade of IL-8/CXCL1-induced neutrophil infiltration could be a potential therapeutic strategy for AH treatment. Interestingly, a recent study revealed that activation of peroxisome proliferator-activated receptor gamma markedly attenuated CXCL1 expression in hepatocytes and reduces hepatic neutrophil infiltration in high-fat diet-plus-binge ethanol fed mice (Wang et al., 2017), suggesting that manipulation of peroxisome proliferator-activated receptor gamma may also be a potential strategy to treat ALD via the inhibition of CXCL1 expression.

3.3. IL-1

The expression of IL-1 β , an important pro-inflammatory cytokine, is upregulated in AH patients and correlates with disease severity and short term mortality (McClain et al., 1986; Tilg, Moschen, & Szabo, 2016). The haplotype, IL-1b 2511 allele 2/13953 allele 1, is associated with the development of alcoholic cirrhosis (Takamatsu et al., 2000). IL1b –511CC genotype influences the total bilirubin, albumin and ALT levels among alcoholic “Bengalis” (Roy et al., 2012). IL-1 β is secreted by KCs via the activation of inflammasome and Caspase-1 (Mathews & Gao, 2013). An important role of the inflammasome-Caspase-1-IL-1 β signaling in the pathogenesis of AH was reported in mice, and antagonizing IL-1 β with IL-1Ra diminished the liver injury, suggesting a potential therapeutic strategy to treat ALD by blocking the inflammasome-Caspase-1-IL-1 β signaling (Petrasek et al., 2012; Tilg et al., 2016). The IL-1 receptor antagonist Anakinra and the IL-1 inhibitor Rilonacept are currently being evaluated in a clinical trial for severe AH (NCT01809132; NCT01903798).

3.4. IL-17

IL-17, a potent pro-inflammatory cytokine, participates in ALD by promoting both liver inflammation and fibrosis (Wang, Gao, et al., 2012). Several recent studies have shown that serum IL-17 levels and hepatic IL-17⁺ cell infiltration are increased in patients with AH and in mice with ALD (Chen et al., 2016; Shi et al., 2013; Stoy et al., 2013). HSCs produce IL-8 and GRO α upon IL-17 stimulation. Hepatic infiltration of IL-17 producing cells also correlates with liver fibrosis (Lemmers et al., 2009). Moreover, anti-IL-17 antibody treatment ameliorates liver steatosis in animal models (Shi et al., 2013). Thus, IL-17 is a potential target for the treatment of ALD.

3.5. CCL20

CCL20 is a chemokine highly expressed in peripheral blood lymphocytes, lymph nodes, the liver, the appendix, and the fetal lung upon the stimulation of many inflammatory mediators like LPS, TNF- α , and IL-1 β (Gao & Xu, 2014). The major target cells of CCL20 are lymphocytes. However, they can also attract neutrophils to a less extent through the binding of CCR6, which is found to regulate liver inflammation, fibrosis, and tumorigenesis (Aoki et al., 2011; Hammerich et al., 2014; Oo et al., 2012). Patients with AH have high serum levels of CCL20, which correlate with endotoxemia, liver fibrosis, portal hypertension, disease severity score, and short-term mortality (Affo et al., 2014). Macrophages and HSCs are major producers of CCL20 in mouse models of CCl₄ plus ethanol and CCl₄ plus LPS (Affo et al., 2014). Silencing CCL20 reduces LPS-induced liver inflammation and injury (Affo et al., 2014).

3.6. Osteopontin (OPN)

OPN is an extracellular matrix protein that has been implicated in the pathogenesis of liver injury, inflammation, and fibrogenesis. Serum and hepatic OPN levels are increased in patients with AH and correlate with disease severity and short term mortality (Affo et al., 2013; Morales-Ibanez et al., 2013; Patouraux et al., 2012; Seth et al., 2014). OPN gene knockout reduced chronic-plus-binge ethanol-induced liver steatosis, inflammation and injury (Morales-Ibanez et al., 2013). However, recent studies in OPN deficient mice revealed a slight but statistically non-significant improvement in liver injury in a chronic ethanol feeding model and a hybrid model with intragastric ethanol feeding plus multiple binges (Ge et al., 2014; Lazaro et al., 2015). It is possible that OPN only participates in the early stage of ALD, but not in the severe or late stages. Thus, OPN may not be a good therapeutic target for the treatment of ALD.

3.7. Lipocalin 2 (LCN2)

LCN2 was originally isolated from neutrophils and termed neutrophil gelatinase-associated lipocalin (NGAL). Recent studies show increased hepatic LCN2 levels in patients with ALD (Affo et al., 2013; Wieser, Tymoszek, et al., 2016). Similar increases of serum and hepatic LCN2 levels were also observed in ethanol-fed mice, and LCN2 deficiency ameliorated liver steatosis, inflammation and liver injury after chronic ethanol feeding (Bykov, Junnikkala, Pekna, Lindros, & Meri, 2007; Cai et al., 2016; Ki et al., 2010; Wieser, Tymoszek, et al., 2016). In addition, chronic infusion of recombinant LCN2 exacerbated diet-induced liver injury, inflammation and macrophage accumulation in a neutrophil-dependent manner in mice with non-alcoholic steatohepatitis (Ye et al., 2016). Because LCN2 plays an important role in inhibiting bacterial infection and promoting liver regeneration (Xu et al., 2015), inhibition of LCN2 is probably not a good strategy to treat ALD although LCN2 may promote inflammation in ALD.

4. Anti-inflammatory and hepatoprotective cytokines

To date, only a few anti-inflammatory and hepatoprotective cytokines are identified in ALD, i.e. IL-6, IL-22, and IL-10. All of these cytokines use signal transducer and activator of transcription 3 (STAT3) signal to regulate the expression of target genes, which are involved

in promoting cell proliferation, survival, and differentiation (Wang, Lafdil, Kong, & Gao, 2011). IL-6 treatment ameliorated ALD in mice and improved fatty liver transplantation in rats (Chen et al., 1997; Hong et al., 2004; Sun et al., 2003). However, it is not suitable for clinical use of IL-6 therapy because of many side effects. The IL-6 receptor (IL-6R) and the gp130 signal chain are widely expressed in many types of cells, which limits the specificity in clinical use (Wang, Gao, et al., 2012).

IL-10 is a strong anti-inflammatory cytokine and can block the production of TNF- α , IL-1 β , and IL-6 in macrophages. Byun et al. showed that treatment with poly I:C protected against alcoholic liver injury via the stimulation of IL-10 production in HSCs and KCs (Byun et al., 2013). However, IL-10 deficiency will elevate the expression of IL-6 and STAT3 signal as compensation. This effect reduces steatosis and liver injury after chronic ethanol feeding despite the increased liver inflammation in IL-10 knockout mice (Miller et al., 2011).

In contrast to the ubiquitous expression of IL-6R and IL-10R, IL-22R expression is restricted to epithelial cells, including hepatocytes and liver progenitor cells, but is not expressed by immune cells (Kong, Feng, Mathews, & Gao, 2013). The biological effect of IL-22 is therefore restricted to epithelial cells. This specificity should in theory minimize side effects of IL-22 in clinical usage. IL-22 treatment has been shown to ameliorate hepatocellular damage, promote liver regeneration, and alleviate liver fibrosis in several mouse models of liver injury (Kong et al., 2013). In addition, IL-22 treatment in mouse models also attenuated bacterial infection (Rubino, Geddes, & Girardin, 2012; Zheng et al., 2016) and kidney injury (Xu, Feng, et al., 2014), two deleterious and fatal conditions related to severe AH. These beneficial functions of IL-22 make it ideal for the clinical treatment of AH (Gao & Shah, 2015). The only concern for IL-22 therapy is its role in liver cancer. However, IL-22 transgenic mice with super high levels of serum IL-22 (>6000 pg/ml) do not spontaneously develop liver cancer, suggesting that IL-22 does not initiate the oncogenesis of liver cancer and may only impact on the existing liver cancer cell growth (Park et al., 2011). Short-term IL-22 therapy should be safe in AH patients without liver cancer. A phase Ib clinical trial examining IL-22 therapy for the treatment of patients with severe AH is currently ongoing in the United States (NCT02655510).

5. The role of microRNA in inflammation of ALD

MicroRNAs (miRNA) are a class of small non-coding RNAs involved in post-transcriptional regulation of gene expression, termed RNA interference (RNAi). In animals, pre-miRNAs are generated from the full-length primary transcript miRNAs, termed pri-miRNA through enzymatic cleavage by RNase III Drosha, and subsequently transport to the cytosol and are further processed by dicer to produce double-stranded mature miRNAs (~22 nucleotides). One strand is loaded into the silencing complex and imperfectly binds within the 3'-UTR of target transcripts to disrupt translation (Carthew & Sontheimer, 2009). In addition to intracellular localization, miRNAs are also detected in body fluids, such as urine, bile, saliva, serum and plasma, et al. (Mitchell et al., 2008; Shigehara et al., 2011; Weber et al., 2010). Recent studies have suggested that circulating miRNAs are present in either the protein fraction or extracellular vesicles (Bala et al., 2012; Eguchi et al., 2017; Momen-Heravi, Saha, et al., 2015). The high stability of circulating miRNAs makes them attractive

for biomarker discovery in liver diseases (Szabo & Bala, 2013). For example, miR-122 has been assessed as a biomarker for liver injury (Zhang, Jia, et al., 2010). Here we summarize the current data about miRNAs in ALD (Table 2).

Differential expression patterns of miRNAs in the liver of ALD patients and animal models have been studied (Dippold, Vadigepalli, Gonye, Patra, & Hoek, 2013; Liu, Chen, Jin, & Li, 2013). More recently, Blaya D et al. also identified 177 differentially expressed miRNAs in the liver of ALD patients (Blaya et al., 2016). In their studies, RNA samples from liver tissue of patients with severe AH, alcoholic cirrhosis, cirrhosis from hepatitis C, and healthy patients were examined and compared. The most interesting finding from these studies was that among the 177 differentially expressed miRNAs in the liver of AH patients, 19 miRNAs were found specifically dysregulated in AH with respect to other liver diseases, including miR-182, miR-503, miR-127-3p, miR-132, miR-3201, miR-3178, miR-432, miR-3128, miR-99b, miR-409-3p, miR-134, miR-4649-5p, miR-3613-3p, miR-3175, miR-4668-5p, miR-487b, miR-423-5p, miR-500a, and miR-371b-5p. A subsequent ingenuity pathway analysis revealed that the 19 miRNAs link to pathways related to the nuclear receptors pregnane X receptor (PXR), retinoid X receptor (RXR) and farnesoid X receptor (FXR), and to cholestasis. These findings may provide cues for exploring novel therapeutic options for AH. For example, the link to FXR raises the hypothesis that FXR agonists, like obeticholic acid that is currently under investigation for non-alcoholic fatty liver disease and primary biliary cholangitis (PBC), may deserve testing as a new therapeutic option for AH (Stickel & Dubuquoy, 2016).

Over the last 10 years, a large number of studies have examined the roles of miRNAs in the pathogenesis of liver diseases with a focus on hepatocyte-specific miR-122 (Wang, Heegaard, & Orum, 2012). MiR-122 is highly abundant in hepatocytes, and plays an important role in the control of lipid metabolism, differentiation, regeneration, and tumorigenesis in the liver (Hsu et al., 2016; Simerzin et al., 2016; Wang, Heegaard, et al., 2012). Here we mainly focus on the discussion of miRNAs in liver inflammation in ALD.

5.1. MiRNAs targeting hepatocytes and biliary epithelial cells

A recent study reported that miR-182 was the most highly expressed miRNA in AH and the levels of miR-182 correlated with degree of ductular reaction, disease severity and short-term mortality. MiR-182 mimic induced an upregulation of inflammatory mediators in biliary cells, suggest that miR-182 promotes liver inflammation by targeting biliary epithelial cells (Blaya et al., 2016).

5.2. MiRNAs targeting KCs/macrophages

Accumulating evidence suggests that many miRNAs also play an important role in the control of liver inflammation in ALD by targeting KCs/macrophages, which are discussed below and summarized in Table 2.

Inflammation-associated microRNAs (inflam miRs) are a group of microRNAs that has been implicated in inflammatory responses. These include miR-223, miRNA-155, miRNA-146a, miRNA-146b, miRNA-125, and miRNA-132, miR-150, miR-181, let-7 and miR-21 (Bala & Szabo, 2012; O'Neill, Sheedy, & McCoy, 2011). Among them, miR-27 was found to be able

to polarize macrophages into M2 phenotypes by targeting SPRY2 and subsequent phosphorylation of ERK (Saha et al., 2015, 2016). Chronic alcohol consumption also increases miR-155 in macrophages/KCs via an NF- κ B-dependent pathway and the increased miR-155 contributes to alcohol-induced elevation in TNF- α and IL-1 β production, indicating a central functional role of miR-155 for controlling pro-inflammatory (M1) polarization in macrophages (Bala et al., 2011, 2016). Similarly, miRNA-217 is found to be increased in ethanol-treated macrophages/KCs and leads to inflammatory activity by disrupting Siruin1-lipin-1 signaling (Yin et al., 2015). More recently, Dr. Nagy's group demonstrated that miR181b-3p in KCs plays an important role in the control of liver injury and inflammation in ethanol-fed mice via the regulation of importin α 5 and sensitivity of TLR4-mediated signaling (Saikia et al., 2017). A small specific-sized hyaluronic acid 35 (HA35) treatment can normalize the dysregulated miR181b-3p in KCs and ameliorate ALD in mice, suggesting that HA35, miR-181b-3p, or combination may have therapeutic potential for the treatment of ALD (He & Gao, 2017; Saikia et al., 2017).

5.3. MiRNAs targeting neutrophils

MiR-223 is a neutrophil-specific miRNA and is highly expressed in neutrophils, playing an important role in limiting neutrophil over-activation in a variety type of diseases, including drug-induced liver injury (He et al., 2017) and ALD (Li et al., 2017). In ALD, miRNA-223 is upregulated in neutrophils and serum. Mechanistically, miR-223 acts as an inhibitory factor of neutrophil activation in ALD by disrupting neutrophil IL-6 expression and subsequent p47^{phox}-mediated ROS production, suggesting miR-223 is an important regulator to block neutrophil infiltration in ALD and is a novel therapeutic target for the treatment of ALD (Li et al., 2017; Wang & Ding, 2017).

5.4. MiRNAs targeting intestinal epithelial cells

Gut-derived endotoxin is considered the key cause of endotoxemia in alcohol-fed animal models and human alcoholics. Therefore, targeting intestinal epithelial cells to protect against alcohol-induced gut leakiness is essential for the development of novel therapeutic interventions for prevention and/or treatment of ALD. The integrity of the intestinal barrier depends on both healthy epithelial cells and on an intact paracellular pathway. This pathway is a complex array of structures that include tight junctions between gut epithelial cells. Cytoplasmic plaque proteins such as Zonula occludens-1 (ZO-1) constitute a major component of tight junctions (Sawada et al., 2003). There are two miRNAs that have been identified involving in alcohol-induced gut leak, i.e. miR-155 and miR-212. Similar to the effect observed in the liver, chronic ethanol-induced upregulation of miR-155 stabilizes and increases the *Tnfa* mRNA and decreases its molecular target Reg3b, which cause intestinal inflammation and barrier dysfunction leading to endotoxemia (Lippai et al., 2014). Increased levels of miR-212 in the intestine were reported with alcohol feeding and this increased miR-212 targets tight junction proteins like ZO-1 causing gut leakiness by down-regulating ZO-1 translation (Tang et al., 2008, 2015; Zhao et al., 2015). Probiotic supplementation of *Lactobacillus rhamnosus* GG is shown to protect intestinal barrier dysfunction by decreasing the expression of miR-122 in chronic ethanol-fed mice (Zhao et al., 2015).

6. Targeting intestinal microbiome for the treatment of ALD

The gut-liver interaction plays a critical role in the pathogenesis of ALD. The mucosal microbiota, integrity of the intestinal barrier and a healthy liver ensure the optimal working of the gut-liver axis. However, alcohol exposure damages both gut mucosa and liver health. Chronic alcohol consumption results in overgrowth of intestinal gram-negative bacteria, which consequently increases endotoxin production and release (Bode, Bode, Heidelberg, Durr, & Martini, 1984; Hauge, Persson, & Danielsson, 1997). In addition, alcoholic patients also have gut microbiota dysbiosis and elevated gut-derived serum endotoxins (Bode, Kolepke, Schafer, & Bode, 1993). Many factors have been found to contribute to alcohol consumption-induced intestinal dysbiosis including dietary habits, medications or xenobiotics, genetics, intestinal dysmotility, increased gastric pH, altered bile flow, and altered immune response (Hartmann, Seebauer, & Schnabl, 2015). Recently, Llopis M et al. showed a specific dysbiosis associated with ALD severity in patients (Llopis et al., 2016). Mice harboring the intestinal microbiota from a patient with severe AH developed greater degree of liver inflammation and necrosis, and greater intestinal permeability and higher translocation of bacteria than those harboring the intestinal microbiota from an alcoholic patient without AH (Llopis et al., 2016). More recently, a study suggests that excessive alcohol consumption is associated with an altered mycobiota and translocation of fungal products in patients and mice (Yang et al., 2017). Therefore, targeting intestinal dysbiosis and pathological bacterial and fungal translocation may help to prevent the progression of ALD. Treatment with probiotic VSL#3 improved gut permeability in chronically alcohol fed-rats (Chang et al., 2013). Administration of *Lactobacillus rhamnosus GG* or CCFM1107 to alcohol-fed mice resulted in a decrease in serum endotoxin level, and alcohol-induced liver damage by improving gut permeability, reducing oxidative stress, and restoring the intestinal flora (Bull-Otterson et al., 2013; Tian et al., 2015). Supplementation of diet with saturated fatty acid reverses alcohol-induced dysbiosis and liver damage (Chen et al., 2015). Fecal microbiota transplantation demonstrated beneficial effects in prevention of alcohol-induced dysbiosis, liver inflammation and injury in mice (Ferrere et al., 2017). Fecal microbiota transplantation has been tested as a therapeutic approach in patients with persistent positive HBeAg and demonstrated benefits in inducing HBeAg clearance after long-term antiviral therapy (Ren et al., 2017). Therefore, fecal microbiota transplantation may have beneficial effects for the treatment of ALD and intestinal microbiota could be considered as a new therapeutic target in ALD.

7. Ongoing clinical trials for the treatment of AH

Multiple clinical trials of anti-inflammatory treatments for AH are currently in progress (Table 3). All aspects of the inflammatory process are under investigation, including manipulation of the gut microbiome, promoting the development and release of neutrophils from bone marrow and agents targeted at pro-inflammatory mediators such as TNF- α or LPS. It has been notoriously difficult to show a survival benefit in ALD, the recent STOPAH trial in AH being a case in point (Thursz et al., 2015). In view of this, many trials currently underway use alternatives to mortality as a primary outcome measure as shown in Table 3.

8. Perspective

It is now clear that a large number of inflammatory mediators are elevated in severe forms of ALD, and these mediators likely interact with each other to synergistically promote the progression of ALD. Targeting a single inflammatory mediator will probably not be enough to control the severe inflammation in ALD. In addition, inflammation also plays an important role in promoting liver regeneration and attenuating bacterial infection. Inhibition of inflammation may further worsen the ongoing poor liver regeneration and bacterial infection in severe forms of ALD. Thus, combination therapy (ie. Immunosuppression, hepatoprotection, and anti-bacterial infection) is needed for the treatment of severe ALD (Gao & Shah, 2015).

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- κ B 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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Fig. 1.

Ethanol upregulates TNF- α in Kupffle cells via the activation of multiple pathways. Ethanol administration activates the TLR4 pathway, ROS production and complement activation to increase the expression of TNF- α . First, alcohol uptake increases intestine permeability and induces gut bacterial overgrowth. LPS from the intestinal bacteria will translocate into the liver via the portal vein and activates the TLR4 pathway. Upon activation, TLR4 will dimerize and recruit complicated adaptor proteins to initiate MyD88 dependent and independent pathways. A MyD88 dependent pathway mediates the activation of NF- κ B, which transactivates the *Tnf α* gene transcription. A MyD88 independent pathway activates another transcription factor IRF3 to increase the transcription of *Tnf α* gene transcription. Moreover, the TLR4 signal also activates ERK and p38 MAPK pathways through multiple signal cascades. Activation of ERK increases the activity of EGR-1, an important transcription factor to enhance the expression of *Tnf α* gene. The p38 signal pathway contributes to the Egr-1 mRNA stabilization in the presence of ethanol, which also increases TNF- α protein production. Second, ethanol treatment increases the ROS production from NADPH oxidase, which may enhance the ERK and p38 pathways. Finally, the C3 and C5 complements are also activated in ethanol-fed mice. The C3aR and C5aR mediated signals increase the *Tnf α* mRNA through yet unknown mechanisms.

Table 1

ALD interventions by targeting cytokines

Targets	Outcome in animal models	References	Outcome in human patients	References
Proinflammatory cytokines				
TNF- α	Anti-TNF- α treatment reduced hepatic necrosis and inflammation in rodents.	Imuro, Gallucci, Luster, Kono, and Thurman (1997)	Elevated in circulation and liver in patients with ALD; TNF- α -238G > A polymorphism was associated with ALD risk; Inhibition of TNF- α increases bacterial infections in ALD patients.	McClain, Barve, Deaciuc, Kugelmas, and Hill (1999) and references therein, Zhao, Xiao, Zhang, Xie, and Zeng (2016), Boetticher et al. (2008), Naveau et al. (2004)
IL-8	Blockade of IL-8 receptors (CXCR1/2) reduces liver injury, inflammation, steatosis, weight loss and mortality in mice ALD.	Wieser, Adolph, et al. (2017)	Elevated in circulation and liver in AH, correlated with hepatic neutrophils infiltration, disease severity and mortality.	Afford et al. (1998), Dominguez et al. (2009), Gonzalez-Reimers et al. (2012), Hill, Marsano, and McClain (1993), Homann, Benfield, Graudal, and Garred (2000), Huang et al. (1996), Patel, Noor, Kumar, and Thakur (2015), Sheron et al. (1993), Swiatkowska-Stodulska, Bakowska, and Drobinska-Jurowiecka (2006)
CXCL1 (GRO- α)	Upregulation of CXCL1 in the serum and liver in ALD; Blockage of CXCL1 or its receptor CXCR2 reduces ALD.	Chang et al. (2015), Lazaro et al. (2015), Roh et al. (2015)	CXCL1 rs4074A allele is a genetic risk factor for alcoholic cirrhosis; Liver CXCL1 correlates with hepatic neutrophils infiltration in AH patients.	Nischalke et al. (2013), Affo et al. (2013), Dominguez et al. (2009), Maltby, Wright, Bird, and Sheron (1996)
IL-17	Serum IL-17 is upregulated in mice with ALD; Anti-IL-17 ameliorates liver steatosis.	Chen et al. (2016), Shi et al. (2013)	Serum IL-17 levels and hepatic IL-17 ⁺ cell infiltration are elevated in patients with AH.	Stoy et al. (2013)
IL-1	Serum and hepatic IL-1 β are elevated in mice with ALD; IL-1R1 deficiency or IL-1R antagonist ameliorates alcohol-induced liver injury	Petrasek et al. (2012)	Serum and hepatic IL-1 levels correlate with disease severity in AH patients; IL-1b 2511, 2/13953 allele 1 is a risk factor for alcoholic cirrhosis; IL1b -511CC genotype affects the total bilirubin and ALT levels in alcoholic "Bengalis"; Anakinra, IL-1R antagonist, and IL-1 inhibitor, Rilonacept, are being evaluated in a clinical trial for severe 32; NCT01903798).	McClain et al. (1986), Takamatsu et al. (2000), Roy et al. (2012)
OPN	Hepatic OPN is elevated in ethanol-fed rats and mice; The role of OPN in animal ALD is still contradictory.	Apte et al. (2005), Banerjee et al. (2006), Morales-Ibanez et al. (2013), Seth, Duly, Kuo, McCaughan, and Haber (2014), Ge et al. (2014), Lazaro et al. (2015)	Serum and hepatic OPN levels are elevated in AH patients and correlate with disease severity and short-term mortality.	Affo et al. (2013), Morales-Ibanez et al. (2013), Patouraux et al. (2012), Seth et al. (2014)
CCL20	CCl ₄ plus ethanol or CCl ₄ plus LPS induces CCL20 production in macrophages and HSCs; silencing CCL20 ameliorates LPS-induced liver injury and inflammation.	Affo et al. (2014)	Serum and hepatic CCL20 levels are elevated in AH patients and correlate with disease severity and short-term mortality.	Affo et al. (2014)
CCL2/MCP-1	Hepatic CCL2 levels are elevated in ethanol-fed mice.	Mandrekar, Ambade, Lim, Szabo, and	Serum and hepatic CCL2 levels are increased in patients with ALD.	Afford et al. (1998), Colmenero et al. (2007), Devalaraja, McClain,

Targets	Outcome in animal models	References	Outcome in human patients	References
	CCL2 deficiency ameliorates ALD.	Catalano (2011), Na et al. (2015)		Barve, Vaddi, and Hill (1999), Dominguez et al. (2009), Fisher, Neil, Williams, and Adams (1999)
LCN2	Serum and hepatic LCN2 are elevated in ethanol-fed mice; LCN2 deficiency ameliorates liver steatosis, inflammation and liver injury after chronic ethanol feeding.	Bykov, Junnikkala, et al. (2007), Cai et al. (2016), Ki et al. (2010), Wieser, Tymoszyk, et al. (2016)	Hepatic LCN2 level are increased in patients with ALD.	Affo et al. (2013), Wieser, Tymoszyk, et al. (2016)
Anti-inflammatory and hepatoprotective cytokines				
IL-6	Hepatic IL-6 is upregulated in ethanol-fed mice; IL-6 KO exacerbates ALD; IL-6 treatment ameliorates ethanol-induced liver injury.	Horiguchi et al. (2008), El-Assal, Hong, Kim, Radaeva, and Gao (2004), Hong et al. (2002), Zhang, Tachibana, et al. (2010), Chen, Bao, Sawyer, Kunos, and Gao (1997), Hong et al. (2004)	Serum IL-6 level is elevated in ALD patients and correlates with the severity of liver disease.	Hill et al. (1992)
IL-10	IL-10 KO exacerbates liver inflammation, decreases liver steatosis and injury in ethanol-fed mice.	Miller et al. (2011)	The results from clinical studies on the association of IL-10 polymorphisms and ASH are inconsistent.	Grove, Daly, Bassendine, Gilvarry, and Day (2000), Richardet, Scherman, Costa, Campillo, and Bories (2006), Yang et al. (2014), Zhao et al. (2016)
IL-22	Administration of IL-22 reduces acute and chronic alcohol-fed induced liver steatosis and injury in mice.	Ki et al. (2010), Xing et al. (2011)	A phase Ib clinical trial examining IL-22 treatment in AH patients is currently ongoing (NCT01918462).	

Table 2

The role of miRNAs in ALD

MicroRNA	Source	Target genes	Outcomes	References
miR-21	Liver	<i>Faslg, Dr5</i>	Upregulated in the liver, promote cell survival, reduce liver injury and fibrosis	Francis et al. (2014)
miR-26a	Liver	<i>Dusp4, Dusp5</i>	Enhances autophagy in liver cells and ameliorates ethanol-induced liver injury	Han et al. (2015)
miR-27a	Serum EV, monocytes derived EV, monocytes	<i>SPRY2</i>	Elevated in serum EV; Induces M2 macrophage phenotypes	Saha, Bruneau, Kodys, and Szabo (2015), Saha, Momen-Heravi, Kodys, and Szabo (2016)
miR-34a	Liver, hepatocyte-derived EV	<i>Sirt1, Casp2</i>	Increases cell survival and transformation in vitro	Francis et al. (2014), Liu, French, Li, Tillman, and French (2015), Meng et al. (2012)
miR-122	Liver, serum/plasma, exosomes	<i>Hif1a, Ccng1 and Bcl-W</i>	Elevated in plasma/serum and correlated with liver injury; loss of miR-122 in the liver increases stemness, progenitor cell markers and enhanced EMT	Ambade, Satishchandran, and Szabo (2016), Bala et al. (2012), Francis et al. (2014), Meng et al. (2012), Momen-Heravi, Bala, Kodys, and Szabo (2015), Zhang, Jia, et al. (2010)
miR-155	Liver, intestine, macrophages, serum, exosomes	NA	Upregulated in the liver, macrophages and serum; promotes liver steatosis, injury, inflammation and fibrosis; causes gut leakiness	Bala et al. (2016, 2011, 2012), Bala and Szabo (2012), Lippai, Bala, Catalano, Kodys, and Szabo (2014), Momen-Heravi, Bala, et al. (2015)
miR-181-3p	KCs	<i>Kpna1</i>	A miR181b-3p → importin α 5 axis contributes to the ethanol-induced sensitization of inflammatory signaling pathways in hepatic macrophages	Saikia et al. (2017)
miR-182	Liver	NA	Upregulated in liver tissue, correlates with degree of ductular reaction, disease severity and short-term mortality. Blockage of miR-182 reduces liver injury, inflammation and bile acid accumulation	Blaya et al. (2016)
miR-212	Intestinal Epithelial Cells	<i>Zo-1</i>	Increased in intestinal epithelial cells; causes gut leakiness by down-regulating ZO-1 translation	Tang et al. (2008, 2015), Zhao et al. (2015)
miR-214	Liver	<i>Gsr, Por</i>	Upregulated in liver; induce oxidative stress	Dong, Liu, Chen, Li, and Zhao (2014)
miR-217	Liver, macrophage/KCs	<i>Sirt1</i>	Upregulated in the liver; promotes ethanol-induced liver steatosis and hepatic inflammation	Yin et al. (2012), Yin, Liang, Jogasuria, Davidson, and You (2015)
miR-223	Serum, neutrophils	<i>Il-6</i>	Increased in serum and neutrophils leading to hepatic neutrophil infiltration and liver injury	Li et al. (2017), Wang and Ding (2017)

Table 3

Ongoing clinical trials for the treatment of AH. Details are taken from clinicaltrials.gov (accessed 8th June 2017) (modified from the reference (Xu, Chang, Mathews, & Gao, 2014))

	Experimental treatment	Design	Primary outcome	Title/trial number
Gut microbiota				
Probiotics	Dietary Supplement: Lactobacillus Rhamnosus GG	Randomised, placebo controlled	Change in MELD score after 30 days	Novel therapies in moderately severe acute alcoholic hepatitis (NTAH-Moderate) NCT01922895
Antibiotics	Vancomycin, Gentamycin and Meropenem	Single group, compared to historic control group	M ϕ activation, Differences in serum levels of M ϕ activation markers	The effect of gut sterilization on M ϕ activation in patients with AH (NCT03157388)
Fecal microbiota transplantation (FMT)	FMT	Randomised, open label trial with pentoxifylline as control	Survival at 3 months	Pentoxifylline versus fecal microbiota therapy in severe AH (NCT02458079)
LPS	Hyperimmune bovine colostrum enriched with anti-LPS antibodies, versus placebo	Phase 2: randomised, placebo control	Circulating LPS at 7 months	Safety and efficacy of IMM 124-E for patients with severe AH (NCT01968382)
Immune cell mobilization				
G-CSF	G-CSF	Randomised, placebo controlled trial	Four and twelve week survival	Efficacy of G-CSF in the management of steroid non-responsive severe AH (NCT01820208)
G-CSF and N-acetylcysteine (NAC)	G-CSF, or G-CSF with NAC	Phase 4: Randomised, placebo controlled, open label trial	90-day survival	G-CSF plus NAC in severe AH (NCT02971306)
LPS	Hyperimmune bovine colostrum enriched with anti-LPS antibodies, versus placebo	Phase 2: randomised, placebo controlled double blinded trial	Circulating LPS at 7 months	Safety and efficacy of IMM 124-E for patients with severe AH (NCT01968382)
Cytokine-targeted therapy				
IL-1 antagonism	Rilonacept or Mycophenolate	Phase 2: randomised, open label trial	Day 29 survival, change in bilirubin	A safety and efficacy study of Mycophenolate Mofetil and Rilonacept in patients with AH (NCT01903798)
IL-1 antagonism, TNF inhibition, gut barrier function	Anakinra, Pentoxifylline, and Zinc Sulfate versus methylprednisolone	Phase 2/3: randomised, blinded trial	6-month survival	Efficacy study of Anakinra, Pentoxifylline, and Zinc compared to Methylprednisolone in severe acute AH (NCT01809132)
Recombinant IL-22-Fc protein	IL-22 Fc protein	Phase 1b:	Safety in AH patients	Safety and efficacy of IL-22 protein for patients with severe AH (NCT02655510)

	Experimental treatment	Design	Primary outcome	Title/trial number
Antibiotics				
Rifaximin	Prednisolone with or without Rifaximin	Phase 2: single group, open label	Bacterial infections at 90 days	Effects of Rifaximin in patients with acute AH (NCT02116556)
Ciprofloxacin	Ciprofloxacin versus placebo	Phase 1: randomised, open label, placebo controlled study	28-day, 3-month and 6-month survival	NCT02326103
Amoxicillin	Amoxicillin versus placebo	Phase 3: randomised, double blinded, placebo controlled trial	2 month survival	Efficacy of antibiotic therapy in severe AH treated with Prednisolone (NCT02281929)