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Review Article

Functions of hepatic non-parenchymal cells in alcoholic liver disease☆

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ABSTRACT

Alcoholic liver disease (ALD) represents a wide spectrum of disease from simple steatosis to cirrhosis. Although there have been multiple attempts to treat ALD, its treatment is still based on abstinence from alcohol and using corticosteroids in specified cases. However, nearly 40% of patients with ALD who are in need of treatment are unresponsive to the current treatments, which implies a new paradigm shift for the treatment of ALD. Traditionally, earlier studies have focused on the abnormal metabolism occurring in the hepatocytes as a protagonist in the pathogenesis of ALD. However, increasing evidence suggests that non-parenchymal cells, such as hepatic stellate cells (HSCs), Kupffer cells, liver sinusoidal endothelial cells, and immune cells around the hepatocytes have critical roles in multiple stages of ALD either by direct or indirect cell-to-cell interactions. For instance, in the early stage of ALD, Kupffer cells and HSCs located closely to hepatocytes contribute to the development of alcoholic steatosis and inflammation through the secretion of various inflammatory cytokines (immunologic pathways) and the activation of the endocannabinoid system (metabolic pathways). While the stage of ALD progresses to alcoholic hepatitis and fibrosis, various cell-to-cell interactions with infiltrating immune cells become highly significant at the multicellular level. This review explains the diverse roles of non-parenchymal cells in the progression of ALD, as well as potential therapeutic strategies to treat ALD.

1. Introduction

Alcohol consumption is a well-known risk factor for chronic liver disease worldwide, which accounts for as much as 5.9% of all death.[1] Especially, up to 25% of alcohol-mediated deaths are related with alcoholic cirrhosis and hepatocellular carcinoma.[1] Alcoholic liver disease (ALD) represents a broad spectrum of liver disease from simple steatosis to cirrhosis. Almost all individuals drinking more than 60 g/day of alcohol develop a fatty liver.[2] Moreover, about 50% of patients with alcoholic fatty liver will develop perivenular fibrosis if chronic alcohol consumption is continued for an average of 25 years; perivenular fibrosis has been identified as a major risk factor for the progression to end-stage liver disease including fibrosis and cirrhosis.[3,4] Along with the course of ALD, severe alcoholic hepatitis (AH), which has a fatal short-term prognosis, may occur in a subset of patients.[5]

The cessation of drinking is the cornerstone of therapy for ALD. However, histologic normalization of the liver occurs in only 27% of abstaining patients, whereas the remaining patients have persistent AH or progress to cirrhosis, which suggests that abstinence from alcohol does not ensure complete recovery of the liver.[6] For the targeted treatment of ALD, corticosteroids have been extensively studied for almost 40 years and strongly recommended for patients with severe AH (defined by a Maddrey's discriminant function ≥32, with or without hepatic encephalopathy) in the American Association for the Study of Liver Diseases (AASLD) and European Association for the Study of the Liver (EASL) guidelines.[7,8] Although corticosteroid treatment significantly increased short-term survival,[9] the 6-month mortality risk was still reported to be high (~30–40%).[7,10] Moreover, there is a subset of patients who do not respond to corticosteroids. Other therapies including treatments with anti-tumor necrosis factor (TNF) antibody, pentoxifylline, and antioxidant molecules have shown some promising results; however, further studies are needed to support using these

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drugs extensively in ALD.[8] Thus, these facts emphasize the need for developing novel therapeutic agents for ALD.

Previous studies have focused on the abnormal metabolism occurring in the hepatocytes, such as the production of acetaldehyde adducts, ethanol metabolism-induced oxidative stress, abnormal methionine metabolism, and glutathione depletion as important mechanisms in the pathogenesis of ALD.[11–13] However, increasing evidence has suggested that cell-to-cell interactions between parenchymal cells (hepatocytes) and non-parenchymal cells are deeply involved in the pathogenesis of ALD. For instance, neighboring non-parenchymal cells around hepatocytes, such as hepatic stellate cells (HSCs) and Kupffer cells directly interact with hepatocytes or are indirectly involved in the pathogenesis of ALD by interacting with various types of hepatic immune cells.[14,15] Hence, this review mainly focuses on recent advances in our understanding of the roles of non-parenchymal cells around hepatocytes in the pathogenesis of ALD and suggests potential molecules targeting these cell-to-cell interactions in relation to the stages of ALD.

2. Pathogenesis of alcoholic steatosis

Hepatic steatosis is the most common and earliest response to chronic alcohol consumption, which is characterized by the abnormal accumulation of neutral fat, mainly triglycerides, within hepatocytes. It might be closely associated with the interaction of hepatic non-parenchymal cells (Fig. 1).

2.1. Imbalanced fat metabolism in hepatocytes

The principle mechanism of fat accumulation in hepatocytes can be explained by an imbalanced lipid metabolism. Up-regulation of sterol regulatory element-binding protein 1c (SREBP1c), a master regulator of *de novo* lipogenesis, and down-regulation of peroxisome proliferator-activated receptor α (PPARα), a nuclear hormone receptor, regulate various target genes involved in free fatty acid transport and synthesis.[8] In addition to genetic regulation, down-regulation of adenosine monophosphate-activated protein kinase (AMPK) is critical to alcoholic steatosis by stimulating SREBP1c and inhibiting PPARα activity.[13,16,17] Ethanol can directly increase the transcription of the SREBP1c gene and inhibit the PPARα activity in hepatocytes through its metabolite acetaldehyde,[18,19] or ethanol indirectly promote up-regulation of SREBP1c and down-regulation of the PPARα activity through the endoplasmic reticulum stress response,[20,21] cytochrome P450 2E1 (CYP2E1)-derived oxidative stress,[22] adenosine signaling,[23] sirtuin1-AMPK signaling,[24] adiponectin,[25,26] and endocannabinoid signaling pathways.[27]

2.2. Divergent role of Kupffer cells

Besides an imbalanced fat metabolism in hepatocytes, cell-to-cell interactions are deeply involved in the pathogenesis of alcoholic steatosis. Assume that alcoholic hepatic steatosis is a local rather than a systemic injury response, adjacent cells to the steatotic hepatocytes including Kupffer cells, liver sinusoidal endothelial cells (LSECs) and HSCs have more important roles than other immune cells in the development of alcoholic steatosis. Especially, Kupffer cells, resident macrophages lining the walls of hepatic sinusoids, are a major cell type involved in the development of alcoholic steatosis.[28,29] It is well-known that gut permeabilization is increased by alcohol intake, which leads to an increased portal entry of endotoxin/lipopolysaccharide (LPS).[28] In the liver, LPS in turn activates Kupffer cells through the toll-like receptor (TLR) 4 signaling pathways, leading to the production of various pro-inflammatory mediators including TNF-α, interleukin (IL)-1, IL-6, and reactive oxygen species (ROS).[30–32] Among these pro-inflammatory mediators, TNF-α is one of the important cytokines involved in the pathogenesis of alcoholic steatosis. Transgenic mice lacking the TNF receptor or treatment with anti-TNF-α showed attenuated alcoholic steatosis and alcohol-induced live injury.[33,34] TNF-α has been shown to increase the transcription of SREBP1c in the liver of mice and to enhance the maturation of SREBP1 in human hepatocytes,[35,36] leading to hepatic fatty acid synthesis by increasing the hepatic acetyl CoA carboxylase (ACC) and fatty acid synthase activities.[37] In contrast, IL-6 produced by Kupffer cells/macrophages activates signal transducer and activator of transcription (STAT) 3 in hepatocytes, which is protective against alcoholic steatosis.[38–40] In agreement with these findings, a recent study demonstrated that increased acetaldehyde byproducts, such as malondialdehyde-acetaldehyde in aldehyde dehydrogenase 2 *(ALDH2)* deficient mice stimulate Kupffer cells to produce IL-6, thereby ameliorating alcoholic steatosis, but aggravating inflammation and fibrosis.[41] The East Asian population, whose *ALDH2*2* allele frequency is up to 50%, should be monitored carefully because this population may resistant to alcoholic steatosis, but more susceptible to liver injury after alcohol consumption.[41] Moreover, our previous study demonstrated that TLR3 activation by polyinosinic-polycytidylic acid (poly I:C) treatment ameliorated alcoholic steatosis and injury through IL-10 production in Kupffer cells and HSCs and suggested that the TLR3 agonist as a novel therapeutic target for the treatment of alcoholic liver injury.[42] In addition, an interesting finding discovered that alternatively activated (M2) Kupffer cell polarization of female BALB/c mice prevented alcoholic steatosis by inducing apoptosis of classical (M1) Kupffer cells.[43] This study showed that IL-10 released from M2 Kupffer cells promote M1 Kupffer cell apoptosis through the activation of arginase in M1 Kupffer cells.[43]

2.3. HSCs and the endocannabinoid system

Under normal physiologic conditions, quiescent HSCs store retinol (vitamin A) lipid droplets and control retinoid homeostasis in our body.[44] When the liver is injured, HSCs are activated and become myofibroblast-like cells that mainly induce liver fibrosis by producing a huge amount of extracellular matrix (ECM).[14] However, recent studies have suggested that HSCs are involved in the pathogenesis of alcoholic hepatic steatosis as well. In our previous study, endogenous cannabinoids, lipid mediators that bind to cannabinoid receptors (CB_1 and CB_2) with similar effects as marijuana, were spotted as the culprit to provoke alcoholic fatty liver. Between the two main endocannabinoids, arachidonoyl ethanolamide (AEA) and 2-arachidonoylglycerol (2-AG), chronic ethanol consumption specifically increased the production of 2-AG by HSCs in mice, resulting in the paracrine activation of the CB_1 receptors in hepatocytes.[27] Moreover, alcohol-induced hepatic steatosis was significantly attenuated by the administration of rimonabant or by genetic ablation of the CB_1 receptors.[27] Interestingly, paracrine activation of CB_1 in hepatocytes induces CYP2E1 expression through estrogen-related receptor γ, promoting oxidative stress in response to ethanol exposure.[45] However, the use of CB_1 antagonists causes some serious side effects, such as depression, anxiety, and even suicide because of the fact that CB_1 receptors are highly expressed in the brain with many important psychoactive functions. Thus, peripherally restricted CB_1 antagonist could be a rational alternative to treat alcoholic steatosis.[46] In contrast to CB_1, CB_2 has protective effects against alcohol-induced inflammation and steatosis.[47,48] The activation of CB_2 in Kupffer cells induces heme oxygenase-1 (HO-1) expression, which inhibits LPS-induced nuclear factor-κB (NF-κB) activation and M1 polarization, thereby ameliorating hepatocyte steatosis via cell-to-cell interactions between the Kupffer cells and hepatocytes.[47]

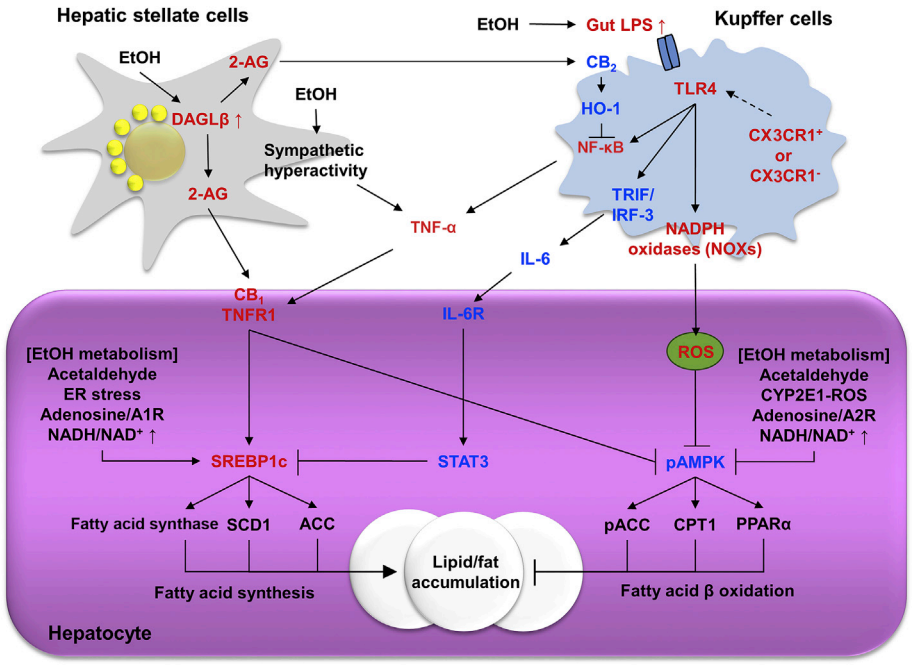

Fig. 1. Mechanisms of hepatic steatosis in ALD. Chronic alcohol consumption provokes hepatic steatosis through multiple pathways including cell-cell interactions. The results are the inhibition of peroxisome proliferator-activated receptor α (PPARα) and adenosine monophosphate-activated protein kinase (AMPK), which induce fatty acid oxidation, and the activation of sterol regulatory element-binding protein 1c (SREBP1c), which regulates *de novo* lipogenesis through fatty acid synthase, stearoyl-CoA desaturase 1 (SCD1), and acetyl CoA carboxylase (ACC) in hepatocytes. In detail, byproducts during the ethanol metabolism process, such as acetaldehyde, endoplasmic reticulum (ER) stress, cytochrome P450 2E1-reactive oxygen species (CYP2E1-ROS), adenosine pathway, and the increased ratio of reduced nicotinamide adenine dinucleotide (NADH) and oxidized nicotinamide adenine dinucleotide (NAD^+) can directly activate SREBP1c and inhibit PPARα. Chronic alcohol consumption also induces cell-cell interactions between hepatocytes and HSCs through the endocannabinoid systems or Kupffer cells through toll-like receptor (TLR)-mediated inflammatory cascade. All these mechanisms ultimately result in the disruption of hepatic lipid metabolism by upregulating lipogenesis and suppressing fatty acid oxidation. Abbreviations: A1R, adenosine receptor 1; A2R, adenosine receptor 2; CB_1, cannabinoid receptor 1; CB_2, cannabinoid receptor 2; CPT1, carnitine palmitoyltransferase 1; CX3CR1, CX3C chemokine receptor 1; DAGLβ, diacylglycerol lipase β; EtOH, ethanol; HO-1, heme oxygenase-1; IL, interleukin; IRF-3, interferon regulatory 3; LPS, lipopolysaccharide; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NF-κB, nuclear factor-κB; STAT3, signal transducer and activator of transcription 3; TNF-α, tumor necrosis factor α; TNFR1, tumor necrosis factor receptor 1; TRIF, TIR-domain containing adaptor-inducing interferon-β; 2-AG, 2-arachidonoylglycerol.

2.4. Alcohol-induced sympathetic hyperactivity

Interestingly, recent studies have revealed that alcohol-induced sympathetic hyperactivity is also involved in the pathogenesis of alcoholic steatosis in the rat liver through the activation of HSCs leading to TNF-α overproduction.[49] Moreover, the development of alcoholic steatosis was significantly attenuated by carvedilol by the blockage of the sympathetic nervous system-activated HSC feedback loop.[49] Other evidence also supports the previous finding that sympathetic hyperactivity is associated with the development of alcoholic steatosis.[50,51] Chronic alcohol consumption is correlated with an increased level of 3-methoxy-4hydroxyphenylglycerol (noradrenaline metabolite) in the peripheral circulation and an increased level of tyrosine hydroxylase (the rate-limiting enzyme in the synthesis of catecholamine) in the liver.[49,50] Furthermore, previous study confirmed that the α2A-adrenoceptor of Kupffer cells was activated by chronic alcohol exposure, resulting in liver injury.[51]

2.5. Indistinct role of LSEC

During liver injury, LSEC-fenestrae undergo dynamic structural changes, whose diameter and number increase or decrease under various experimental and pathological conditions.[52] LSEC-fenestrae are also affected by acute and chronic alcohol consumption. Previous studies have demonstrated that acute alcohol injection increased the diameter of LSEC-fenestrae, but had no effect on the number of LSEC-fenestrae.[52] As alcohol consumption was prolonged up to 12 weeks, the number of LSEC-fenestrae was significantly decreased, and a basement membrane underneath the endothelium gradually developed.[52,53] Thus, alcohol consumption affects the function and structure of the LSEC, contributing to the development of alcoholic steatosis through the interaction with other non-parenchymal cells including Kupffer cells and HSCs. However, the effects of the altered function and structure of the LSEC on alcoholic steatosis are still not fully investigated.

3. Pathogenesis of alcoholic steatohepatitis (ASH)

A subset of patients with simple steatosis will progress to ASH; however, the exact prevalence of ASH and which patients are at high risk for progression have not been clarified. It is assumed to occur in 10–35% of hospitalized alcoholic patients based on histologic studies of patients with ALD.[54,55] ASH is characterized by infiltration of immune cells and hepatocellular injury. In response to chronic alcohol consumption and other unrevealed factors, various hepatic cells including immune cells are involved in the pathogenesis of ASH.

3.1. Bacterial overgrowth and dysbiosis

As described above, bacterial overgrowth and dysbiosis have been shown in animal models,[56] leading to intestinal oxidative stress and inflammation.[57,58] In agreement with animal models, some heavy drinkers have been revealed to have colonic dysbiosis (a lower percentage of *Bacteroidetes* and a higher percentage of

Proteobacteria) compared to nondrinkers, which causes higher levels of endotoxins.[59] Indeed, the administration of probiotics restored the gut microbiota and attenuated hepatic inflammation in animal models.[60] Based on this evidence, several clinical trials using antibiotics or probiotics for ALD are ongoing.

3.2. Involvement of innate immunity

Because Kupffer cells are located between the portal and systemic circulation, they have an important role in organizing the immune response against LPS through the TLR4 signaling pathways.[61,62] In Kupffer cells, TLR4 activates the MyD88-dependent or independent pathway involving the TIR-domain containing adaptor-inducing interferon-β (TRIF) and TRIF-related adaptor molecule activate interferon regulatory 3 (IRF3), leading to the production of pro-inflammatory cytokines, such as TNF-α, IL-6, monocyte chemotactic protein-1 (MCP-1), and type I interferons through the late activation of NF-κB.[61,63] Among the various pro-inflammatory cytokines, TNF-α has the most important role in ASH as well as in alcoholic steatosis,[33] providing a basis for the use of corticosteroid, anti-TNF antibodies, and pentoxifylline in patients with AH. Interestingly, our recent study showed that there are two subtypes of Kupffer cells, $CD11b^{+}F4/80^{high}CX3C$ chemokine receptor 1 $(CX3CR1)^{+}$ and $CD11b^{+}F4/80^{high}CX3CR1^{-}$ cells, in ALD.[64] $CX3CR1^{+}$ Kupffer cells showed a more pro-inflammatory phenotype by producing TNF-α and IL-1β than $CX3CR1^{-}$ Kupffer cells in mice after chronic ethanol consumption. Similarly, in vitro treatment of recombinant C-X3-C motif ligand 1 (CX3CL1) increased the gene expression of TNF-α and IL-1β in human $CD14^{high}CD16^{-}$ monocyte-derived Kupffer-like cells. In addition, ROS produced by reduced nicotinamide adenine dinucleotide phosphate oxidase (NOX) in Kupffer cells contributes to alcohol-mediated liver injury by enhancing pro-inflammatory signals.[65,66] However, the exact mechanism on how the activation of NOX in Kupffer cells regulates pro-inflammatory signals has not been fully elucidated. Interestingly, Kupffer cells can also produce hepatoprotective cytokines, such as IL-6 and anti-inflammatory cytokines, such as IL-10, resulting in the activation of signal transducer and activator of transcription 3 (STAT3) in hepatocytes and macrophages/Kupffer cells, respectively, to ameliorate alcohol-induced liver injury and inflammation.[40,67,68] However, targeting the hepatoprotective properties of IL-6 or IL-10 is halted by the fact that IL-6 and IL-10 receptors are expressed in many tissues, resulting in off-target effects. Unlike IL-6 or IL-10, IL-22 may be a promising target in two ways. First, IL-22 has hepatoprotective effects (antioxidant, anti-apoptotic, antisteatotic, proliferative, and antimicrobial effects) by activating STAT3 in hepatocytes.[69] Second, its receptor is mostly confined to epithelial cells, such as hepatocytes, which limits off-target effects in its use.[13] Other than Kupffer cells, chronic alcohol consumption induces the accumulation of hepatic infiltrating monocytes/macrophages in mice.[70] The infiltrated macrophages are composed of two distinct population with differential expression levels of Ly6C ($Ly6C^{+}$ and $Ly6C^{low}$). Whereas the $Ly6C^{+}$ macrophages exhibit a pro-inflammatory phenotype, the $Ly6C^{low}$ population retains an anti-inflammatory phenotype.[70] Compared with the Kupffer cells, these infiltrating macrophages have distinct roles in the development of ALD. The current knowledge on the roles of infiltrating macrophages in ALD has been reviewed elsewhere.[71,72]

Leukocyte infiltration is a prominent feature of ASH, amplifying the inflammatory response to LPS. A recent study revealed that a high-fat diet plus acute ethanol binge synergistically induced the expression of hepatic C-X-C motif chemokine ligand 1 (CXCL1; a.k.a. Gro-α), promoting hepatic neutrophil infiltration.[73] In AH patients, various pro-inflammatory mediators, such as IL-8, Gro-α, and IL-17 are up-regulated and contribute to the neutrophil infiltration and the severity of AH.[74–76] Besides their roles in neutrophil infiltration, they induce the secretion of pro-inflammatory chemokines and cytokines, such as C-C motif chemokine ligand 2 (CCL2), IL-8, TNF-α, and IL-1 by HSCs and Kupffer cells to enhance the neutrophil infiltration and severity of ASH.[77–79] Moreover, a recent important study has reported in both human alcoholics and chronic plus binge ethanol rodent model, which mimic some features of AH, that microRNA-223 is a critical regulator for neutrophil infiltration in ASH.[80] Interestingly, this study also suggested a novel finding that recent excessive drinking is mandatory in the increase of circulating neutrophils in patients.[80] Since the role of neutrophils in ASH is of importance, further studies are warranted to investigate the exact role of neutrophils in the pathogenesis of ASH.

3.3. Involvement of adaptive immunity

Although the activation of innate immunity is a key immunologic consequence in the pathogenesis of ASH, the activation of adaptive immunity also contributes to the progression of ASH as well. For instance, long-term alcohol consumption increases the levels of circulating antibodies against lipid peroxidation products and the numbers of T cells in the liver.[81–83] A recent study also suggested that inflammasome activation in Kupffer cell leads to the release of mature IL-1β, promoting alcoholic liver injury by recruiting and activating natural killer T (NKT) cells in the liver.[84] Regarding NKT cell-induced alcoholic liver injury, splenic T cells and NKT cells in the liver lead to hepatocyte apoptosis through the cytotoxic responses of these cells, such as Fas and TNF receptor 1 signaling.[85,86] Moreover, NKT cells play a critical role in the development of ASH by recruiting neutrophils to the liver.[87,88] Mucosal associated invariant T (MAIT) cells constitute a subset of T cells in the immune system, which shows innate, effector-like features. Interestingly, increased intestinal permeabilization, so-called a “leaky gut”, observed in ALD induces MAIT cell depletion and dysfunction by contact with microbial products in patients with severe AH or alcohol-related cirrhosis.[89] However, investigating the precise roles of MAIT cells in the development of ASH needs further studies.

4. Pathogenesis of alcoholic liver fibrosis

Liver fibrosis is characterized by the excessive accumulation of ECM and a wound-healing response to all forms of chronic liver injury. Chronic alcohol consumption is one of the major causes of liver fibrosis. Generally, if chronic alcohol consumption more than 40–80 g/day is continued for an average of 25 years, perivenular fibrosis, an early stage of liver fibrosis, occurs in 40–60% of patients.[3] The increased production of ECM by activated HSCs is the major pathway in the fibrogenesis of virtually all causes of chronic liver injury. Recent studies have revealed some unique mechanisms restricted to alcohol-mediated fibrogenesis, in particular cell-to-cell interactions between activated HSCs and other liver cells including Kupffer cells and liver lymphocytes. First, acetaldehyde produced mainly by hepatocytes during alcohol metabolism can act on HSCs in a paracrine manner, leading to the production of collagen and transforming growth factor (TGF)-β1 in HSCs.[90] Second, ROS generated by TLR4-mediated NOX activation in Kupffer cells and CYP2E1 expression in hepatocytes can activate HSCs in a paracrine manner.[78,91,92] Stimulation of HSCs with LPS alone is not enough for the activation of HSCs, but the MyD88-dependent pathway by TLR4 activation sensitizes HSCs to TGF-β through the down-regulation of the TGF-β pseudoreceptor, Bambi, resulting in the aggravation of liver fibrosis.[93] Besides these mechanisms described above, recent studies performed by our group and other

groups have demonstrated the important role of cell-to-cell interactions between HSCs and immune cells, especially natural killer (NK) cells, in the pathogenesis of alcoholic fibrosis (Fig. 2).

4.1. Interactions between activated HSCs and NK cells

Among the various immune cells, NK cells have an important role in fibrosis prevention by killing activated HSCs in a natural killer group 2 member D (NKG2D)- and TNF-related apoptosis and inducing ligand (TRAIL)-dependent manner.[94,95] However, quiescent HSCs can evade this mechanism because the expression of an NK cell-activating ligand, retinoic acid early inducible 1 (RAE1), is down-regulated, and the expression of an NK cell-inhibitor ligand, major histocompatibility complex-1 (MHC-1), is up-regulated only when HSCs are in their quiescent forms.[95,96] Moreover, NK cells can also suppress liver fibrosis by the production of interferon (IFN)-γ, resulting in HSC cell cycle arrest and apoptosis in a STAT1-dependent manner.[97,98] However, chronic alcohol consumption impairs the immune surveillance and cytotoxicity of NK cells against HSCs.[99,100] This is because of the alcohol-mediated down-regulation of NKG2D- and TRAIL and IFN-γ expression on NK cells.[99] Indeed, in a murine model, direct IFN-γ treatment failed to ameliorate alcoholic liver fibrosis; however, it recovered the potency by the addition of TGF-β1 neutralizing antibody.[99] Although it was not derived from an alcohol model, our previous study demonstrated that the antifibrogenic effects of NK cell/IFN-γ are reduced during advanced liver injury due to enhanced TGF-β and suppressor of cytokine signaling 1 in HSCs, whereas those effects are valid during an early stage of liver fibrosis.[101] From these findings, IFN-γ treatment still can be a potential candidate for anti-fibrotic agents if it is used selectively in the early stage of alcoholic fibrosis or with the combination of the TGF-β neutralizing antibody. We also reported an interesting finding that alcohol dehydrogenase 3 (ADH3) has an important role in aggravating liver fibrosis by the production of retinoic acid and collagen in HSCs and inhibition of NK cells through retinol metabolism-mediated TGF-β production.[102] Moreover, inhibition of ADH3 by genetic ablation or 4-methylpyrazole ameliorates liver fibrosis by the activation of NK cells and suppression of HSCs.[102,103] Therefore, ADH3 might be a potential therapeutic target in the treatment of liver fibrosis.

4.2. Interactions between activated HSCs and other immune cells

Besides NK cells, other immune cells also participate in the dynamic interactions with activated HSCs. For instance, NKT cells can also inhibit HSC activation by direct killing and IFN-γ production similar to NK cells but only in the early stage of liver fibrosis.[104] However, another group has suggested an opposite role of NKT cells which aggravate alcoholic liver injury by Fas and TNF receptor 1 signaling on alcohol-stressed hepatocytes.[85] Thus, the role of NKT cells on alcoholic liver injury is still controversial and further studies are needed. Recently, accumulating evidence has revealed that IL-17 production by Th17 lymphocytes is involved in the development of fibrosis. For instance, IL-17 promotes liver fibrosis by stimulating its cognate receptors on human HSCs, resulting in increased migration of neutrophils by secreting IL-8 and Gro-α.[76] Indeed, fibrosis scores in patients with ALD closely correlate with IL-17 levels.[76] In addition, γδ T cells, a minor population of liver lymphocytes, ameliorated liver fibrosis by Fas ligand-mediated apoptosis of HSCs in chemical and methionine-choline-deficient diet-induced liver fibrosis.[105] In contrast, hepatic γδ T cells are identified as a major type of IL-17A production in chemical-induced

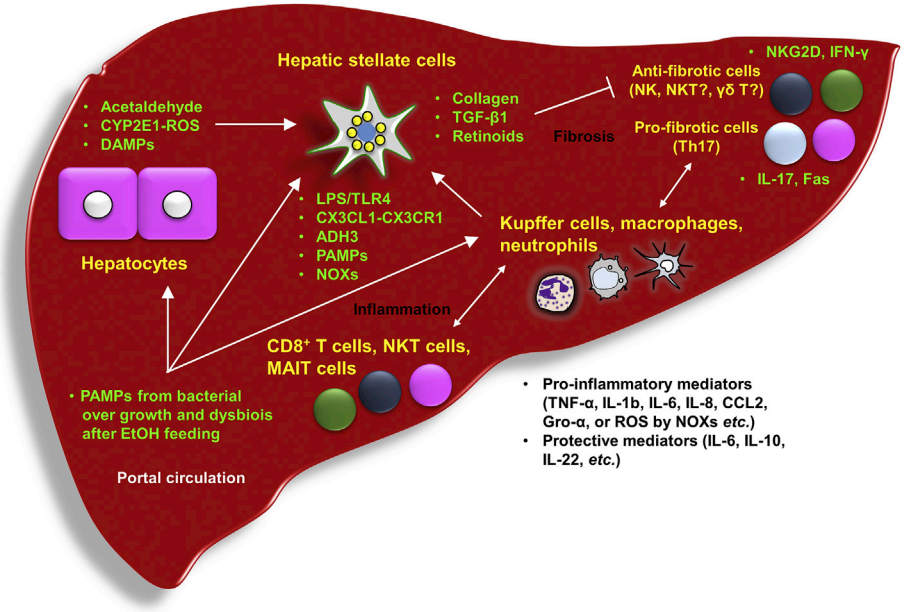

Fig. 2. Mechanisms of inflammation and fibrosis in ALD. Chronic alcohol consumption causes hepatic inflammation by recruiting various innate and adaptive immune cells including Kupffer cells, infiltrating macrophages, neutrophils, $CD8^+$ T cells, NKT cells, and MAIT cells. Intercellular interactions between innate and adaptive immune cells contribute to the exacerbation of hepatic injury via various pro-inflammatory mediators. Repetitive hepatic inflammation leads to the activation of HSCs through ROS generated by hepatocytes and Kupffer cells, TLR4 activated by LPS, and IL-17 secreted by Th17 cells, which further aggravates neutrophil infiltration and inflammation. On the other hand, NK cells and γδ T cells induce the apoptosis of activated HSCs by natural killer group 2 member D (NKG2D)-retinoic acid early inducible 1 (RAE1) interaction and Fas-FasL activation. However, in the advanced stage of liver fibrosis, chronic alcohol consumption impairs the immune surveillance and the cytotoxicity of NK cells against HSCs by enhancing TGF-β and SOSC1 in HSCs and downregulating NKG2D and interferon (IFN) γ expression in NK cells. Abbreviations: ADH3, alcohol dehydrogenase 3; CCL2, C-C motif chemokine ligand 2; CX3CL1, C-X3-C motif ligand 1; CX3CR1, CX3C chemokine receptor 1; CYP2E1, cytochrome P450 2E1; DAMPs, damage-associated molecular patterns; EtOH, ethanol; FasL, Fas ligand; Gro-α, C-X-C motif chemokine ligand 1; HSC, hepatic stellate cell; IL, interleukin; LPS, lipopolysaccharide; MAIT, mucosal associated invariant T; NK, natural killer; NKT, natural killer T; NOXs, nicotinamide adenine dinucleotide phosphate oxidases; PAMPs, pathogen-associated molecular patterns; ROS, reactive oxygen species; TNF, tumor necrosis factor; TGF, transforming growth factor; TLR4, toll-like receptor 4.

acute liver injury and in the early stage of liver fibrosis, in which the interaction between activated HSCs by hepatic exosomes and γδ T cells has a critical role.[106] Therefore, the exact roles of hepatic γδ T cells should be elucidated especially in alcoholic liver fibrosis.

5. Novel therapeutic targets for ALD

As described above, a distinct pathway in each stage of ALD as well as common pathways penetrating all stages of ALD contributes to the pathogenesis of ALD. Thus, each treatment strategy should be individualized according to its contribution to the pathogenesis of ALD. For instance, a broad antioxidant cocktail treatment is inferior to corticosteroids in patients with severe AH.[107] Moreover, the treatment outcome of antioxidant therapy in combination with corticosteroids is also disappointing.[108,109] This is probably because of the fact that oxidative stress is primarily involved in the early stage of ALD, in particular alcoholic steatosis. Antioxidant molecules could be considered in the early stage of ALD to ameliorate alcoholic steatosis and to reduce the risk of disease progression; prospective studies are needed for the confirmation of their clinical efficacy.

Increasing evidence suggests that non-parenchymal cells around hepatocytes are not just bystanders but deeply involved in the pathogenesis of all stages of ALD. Hence, it is important to target simultaneously not only hepatocytes but also non-parenchymal cells around hepatocytes to improve the treatment efficacy. The following could be good candidates for novel therapeutic targets that satisfy the above criteria. For targeting alcoholic steatosis and preventing disease progression, endocannabinoids can be an attractive target because CB_1-deficient mice are resistant whereas CB_2-deficient mice are more susceptible to the development of alcoholic steatosis.[27,47] From these findings, peripherally restricted CB_1 antagonists (due to the neuropsychiatric side effects of the blood-brain barrier penetrating CB_1 antagonists) and CB_2 agonists could be tried to ameliorate alcoholic steatosis. Although more studies should be conducted to consolidate the finding, beta-blockers that can block alcohol-induced sympathetic hyperactivity might be tried to attenuate alcoholic steatosis through the inhibition of TNF-α production by HSCs.[49]

Increasing evidence suggests that bacterial overgrowth and dysbiosis are one of the key mechanisms that cause alcoholic steatosis and hepatitis. Thus, modulation of the gut microbiota and LPS pathway by probiotics, antibiotics and TLR4 antagonists could be promising targets to treat patients with ALD. Several clinical trials using probiotics or antibiotics are ongoing, awaiting their results. As described previously, targeting NOX in Kupffer cells might be one of the interesting targets through the reduction of ROS.[62,66,110] Neutrophil infiltration is one of the salient features in the pathogenesis of ASH. IL-8 and Gro-α are responsible for the recruitment of neutrophils to the liver,[74–76] which might be a promising target to reduce inflammation. Moreover, IL-22 might be an attractive target due to its antioxidant, antiapoptotic, antisteatotic, and antimicrobial effects as well as the absence of off-target effects because its expression is only limited to epithelial cells, such as hepatocytes.[69]

The activation and transdifferentiation of HSCs into myofibroblast-like cells are the key mechanisms in virtually all causes of liver fibrosis. However, there is no effective treatment for the reversal of liver fibrosis. Augmentation of cell-mediated killing of activated HSCs by NK cells might be a future direction of targeted treatment for alcoholic fibrosis. Based on the previous findings, the treatment of IFN-γ in an early stage of liver fibrosis or the combinatorial treatment of IFN-γ and TGF-β neutralizing antibody might be a possible approach for the treatment of alcoholic fibrosis.[99,101] According to the different role of ADH3 in activated HSCs and NK cells, targeting ADH3 might be a potential therapeutic target in the treatment of liver fibrosis.[102] Indeed, treatment of 4-methylpyrazole that blocks ADH3 activity attenuates liver fibrosis in a murine model.[103] Further studies investigating this finding are needed. Small pilot studies using small molecules that specifically target ADH3 might be tried in human based on those future studies.

6. Limitations of the current approaches in the study of ALD

The discovery of novel therapeutic targets for ALD has been limited by the fact that there are no reliable animal models that mimic the entire spectrum of ALD in humans. Most animal models induce varying degrees of hepatic steatosis but little or no liver inflammation and fibrosis. Recently, a model of chronic ethanol feeding (8–12 weeks)-plus-binge ethanol feeding in mice (single or multiple) has been recommended, which shows severe steatosis and inflammation with mild fibrosis.[111] From its transcriptome analysis, it is encouraging that this model mimics severe ASH in human patients, but further studies are needed to confirm this model. Moreover, there is no appropriate model that mimics advanced to end-stage ALD with moderate to severe alcoholic fibrosis and cirrhosis. Due to the limitations of rodent models, human samples are probably the most suitable way to understand the pathogenesis of ALD and to identify therapeutic targets. However, we should be cautious to interpret the results from human samples due to confounding factors as exemplified by anti-TNF-α therapies for patients with AH. Based on data from animal models and human studies that TNF-α contributes to the pathogenesis of ALD as described previously, Etanercept, one of the agents that block TNF-α signals, was tried in patients with AH, which showed a disappointing outcome.[112] Two valuable lessons can be inferred from this study. First, rodent models that mostly mimic early-stage ALD cannot be extrapolated to humans with AH where inflammation is more severe. Second, confirming data from animal models in human samples are usually carried out by cross-sectional studies, for which it is difficult to determine a causal relationship. For instance, increased levels of TNF-α in AH patients might be due to impaired liver clearance or bacterial infections. Because of the above limitations based on current approaches, it is necessary to develop animal models that reflect the full spectrum of ALD in humans. In addition, the serial collection of human samples could help substantially in the understanding of the pathogenesis of ALD.

7. Conclusions

Recently, major progress in understanding the pathogenesis of ALD has been made at the experimental level. However, the development of novel agents for the treatment of ALD has been hampered by critical hurdles, including the lack of reliable animal models and discrepancies between animal studies and human clinical trials. The present review summarizes the recent advances in understanding the pathogenesis of ALD based on the stages of the disease and explains the leading roles of hepatic non-parenchymal cells in the development of ALD. Moreover, novel therapeutic targets are suggested based on those studies regarding cell-to-cell interactions between hepatic parenchymal and non-parenchymal cells. The insight obtained from these complex cell-to-cell interactions may provide potential leads for developing novel therapeutic agents to expand the currently available therapies for ALD.

Authors' contributions

W.M.Choi and W.I.Jeong wrote the manuscript. M.H.Kim and W.I.Jeong gave a critical revision of the manuscript and draw

figures. All authors were involved in deciding the subject and content of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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