Innate immunity in alcoholic liver disease

Bin Gao,1 Ekihiro Seki,3 David A. Brenner,3 Scott Friedman,4 Jessica I. Cohen,5 Laura Nagy,5 Gyongyi Szabo,6 and Samir Zakhari2

1Laboratory of Liver Diseases and 2Division of Metabolism and Health Effects, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, Maryland; 3University of California San Diego, School of Medicine, San Diego, California; 4Division of Liver Diseases, Mount Sinai School of Medicine, New York, New York; 5Cleveland Clinic Foundation, Lerner Research Institute, Cleveland, Ohio; and 6Department of Medicine, University of Massachusetts Medical School, Worcester, Massachusetts

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Excessive alcohol consumption is a leading cause of chronic liver disease in Western countries. It is well documented that chronic alcohol consumption leads to fatty liver; however, only up to 30% of heavy drinkers may develop more severe forms of chronic liver injury such as alcoholic hepatitis, cirrhosis, and hepatocellular carcinoma (83). Despite extensive research on alcoholic liver disease (ALD), how alcohol consumption damages the liver is still not completely understood. It is generally accepted that oxidative stress and ethanol metabolites (e.g., acetaldehyde, acetate) produced during alcohol metabolism are the major factors contributing to alcoholic liver injury. Emerging evidence suggests that activation or dysregulation of innate immunity also play important roles in the pathogenesis of ALD. Innate immunity can also specifically detect invading pathogens through pattern-recognition receptors (PRRs) expressed by host cells, which recognize common microbial patterns known as pathogen-associated molecular patterns (PAMPs) (54). Many PAMPs have been identified, including bacterial carbohydrates [e.g., lipopolysaccharide (LPS), mannosyl], bacterial peptides (flagellin), peptidoglycans and lipoteichoic acids (from gram-positive bacteria), N-formylmethionine, lipoproteins and fungal glucans, and nucleic acids (e.g., bacterial or viral DNA or RNA). These PAMPs can be recognized by secreted, membrane-bound, or phagocytic PRRs. Secreted PRRs include complements, pentraxins, and peptidoglycan-recognition proteins. Membrane-bound or intracellular PRRs include Toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD)-like receptors, and retinoic acid-induced gene I-like helicases. Phagocytic (or endocytic) PRRs include scavenger receptors, macrophage mannose receptors, and β-glucan receptors.

Evidence suggests that the liver plays a key role in the innate immune response (34). First, hepatocytes are responsible for biosynthesis of 80–90% of innate immune proteins including...
complement components and many secreted PRRs. Second, the liver contains a large number of Kupffer cells (KCs), which account for 80–90% of the total population of fixed tissue macrophages in the body. KCs, in combination with liver sinusoidal cells, are responsible for the elimination of molecular wastes from the body. Third, liver lymphocytes are rich in innate immune cells including NK, NKT, and T cell receptor γδ T cells. Fourth, liver nonparenchymal cells also express high levels of membrane-bound PRRs, such as TLRs (104, 112). Moreover, there is evidence that innate immunity in the liver not only plays a key role in host defense against microbial infection and tumor formation but also contributes to the pathogenesis of acute and chronic liver diseases, including ALD. Here we summarize the role of several components of innate immunity in the pathogenesis of ALD.

LPS/TLR4 Signaling Pathway in Alcoholic Liver Disease

LPS/TLR4 signaling pathway. LPS is a component of Gram-negative bacteria and, biochemically, consists of an O-antigen, a core polysaccharide, and a lipid-A component (71). The lipid-A of LPS represents one of the PAMPs recognized by the innate immune system as a pathogen-derived danger signal to initiate immune activation. PRRs, including TLRs, are expressed on innate immune cells and play a central role in sensing pathogens (71, 87). TLR4 can specifically recognize LPS as a danger signal and induce activation of inflammation-associated genes (71, 87). Although TLR4 cannot directly bind LPS, the TLR4 adapter molecule, MD-2, and its coreceptor, CD14, do have the capacity to bind LPS and bring it to the receptor complex for recognition by TLR4. Since MD-2 and CD14 have no intracellular domains, it is the TLR4 that triggers downstream signaling via its intracellular TIR domain upon LPS-induced activation (71, 87). Activation of TLR4 induces two downstream signaling pathways: First, the MyD88-dependent pathway is initiated by recruitment of MyD88 to the TLR4 complex, resulting in downstream activation of IRAK1/4 and TRAF6, followed by activation of NF-κB and subsequent induction of the expression of NF-κB-controlled genes including proinflammatory cytokine and chemokine genes. Second, the MyD88-independent pathway is initiated after recruitment of the TRIF adapter to the TLR4 complex and results in activation of IKK/TAK1 kinase and IRF-3 phosphorylation as well as late activation of NF-κB (71, 87). Phosphorylated IRF-3 forms a complex and translocates to the nucleus to subsequently activate the transcription of IFN-α and β, as well as other interferon-induced genes (71, 87).

LPS/TLR4-MyD88-independent signaling pathway in alcoholic steatohepatitis. Among the various mechanisms contributing to the pathogenesis of alcoholic liver disease, gut-derived microbial products appear to play a significant role. This notion is supported by previous studies in which sterilization of the gut attenuated ALD in rodents (1). The bacterial endotoxin, LPS, has received particular attention since its levels are elevated in the plasma of both human alcoholics and in animal models of ALD (32, 81, 100).

The critical role of LPS in alcohol-induced steatohepatitis is believed to be mediated via targeting TLR4 on KCs (29, 118). In the liver, KCs are the primary cells that respond to LPS through TLR4 (104, 112). In the noninflamed liver, KCs secrete the anti-inflammatory cytokines IL-10 (63) and TGF-β (12); however, in response to LPS, they produce large amounts of proinflammatory cytokines including TNF-α, IL-1, IL-6, and IL-8, which contribute to liver inflammation (104, 112). Hepatocytes also express TLR4, but at low levels with a minimal response to LPS (51, 106). Excessive alcohol intake induces hepatocyte injury and lipid accumulation, which elevate TLR4 expression and potentially alters the sensitivity of TLR4 signaling on hepatocytes (41, 72). Recent studies by Machida et al. (72) have shown that elevated TLR4 expression on hepatocytes in hepatitis C virus (HCV) NSSA transgenic mice increases the sensitivity to alcohol and LPS, thereby exacerbating liver injury and tumor genesis. In addition, TLR4 also recognizes endogenous ligands such as hyaluronan and high-mobility group box 1 (HMGB1) (58, 117). In particular, HMGB1 has been shown to be released from damaged hepatocytes and contribute to liver injury (16). Thus HMGB1 may also serve as an endogenous ligand for TLR4 to promote the progression of ALD.

Although the critical role of TLR4 and its coreceptor CD14 in alcohol-induced liver injury has been well documented (118, 119, 124), their downstream signaling pathways that contribute to the pathogenesis of ALD has just been revealed recently. Disruption of the TLR4 downstream signaling molecule MyD88 in mice failed to prevent alcohol-induced steatohepatitis, reactive oxygen species (ROS) production, and inflammatory cytokines in the liver (48), whereas disruption of the MyD88-independent signaling molecule TRIF in mice abolished alcohol-induced steatohepatitis (128), suggesting that the MyD88-independent pathway contributes to TLR4-mediated alcoholic liver injury. Further studies suggest that TRIF/IRF-3 plays a critical role in alcohol-induced transactivation of the TNF-α gene in Kupffer cells/macrophages in vitro and in vivo, thereby initiating alcoholic liver injury (128). Finally, the critical role of TLR4 in the development of alcoholic and nonalcoholic fatty liver has been well documented in animal models (48, 102, 109, 118); however, how TLR4 activation affects lipid metabolism in hepatocytes is not fully understood. It is generally believed that the increased intestinal translocation of bacterial LPS during alcohol consumption leads to TLR4-dependent activation of Kupffer cells and subsequent induction of inducible nitric oxide synthase, formation of ROS, and induction of TNF-α and prostaglandin E2 that affect lipid metabolism in hepatocytes (30, 94). For example, TNF-α increases mRNA expression of hepatic SREBP-1c, a master transcription factor, to promote lipid synthesis in mice and stimulate the maturation of SREBP-1 protein in human hepatocytes (68). Hepatocytes also express TLR4, and LPS can directly regulate glucose metabolism in hepatocytes by targeting TLR4 (97); however, it is not clear whether activation of TLR4 by LPS can also directly affect lipid metabolism in hepatocytes.

LPS/TLR4 in alcoholic liver fibrosis. Like other forms of hepatocellular damage, chronic alcoholic liver injury also leads to liver fibrosis, which is characterized by the accumulation of extracellular matrix proteins in the liver (10, 31). It is well known that chronic alcohol consumption induces elevation of portal endotoxin levels followed by activation of Kupffer cells via targeting TLR4 on these cells. Activated Kupffer cells produce ROS, lipid peroxidation products, inflammatory cytokines, and profibrogenic factors (such as TGF-β and PDGF), which subsequently induce hepatic stellate cell (HSC) activa-
tion and liver fibrosis (93, 108). In addition, the ethanol metabolite acetaldehyde can also act as a profibrogenic factor to cause HSC activation (17, 26, 39, 108).

Recent studies have shown that TLR4 signaling in HSCs and liver sinusoidal endothelial cells (LSECs) also plays an important role in the pathogenesis of liver fibrogenesis (53, 106). HSCs express TLR4 and respond to LPS stimulation by enhancing TGF-β signaling and producing cytokines/chemokines that contribute to liver fibrogenesis (86, 106). Quiescent HSCs are resistant to TGF-β-induced HSC activation due to expressing a high level of Bambi (bone morphogenetic protein and activin membrane-bound inhibitor) that inhibits TGF-β receptor signaling (106). Upon TLR4 activation, Bambi expression is promptly downregulated, leading to unrestricted activation of the signaling via the receptor for TGF-β that is secreted by Kupffer cells (106). In addition, activation of TLR4 signaling pathway in LSECs regulates angiogenesis through its MyD88 effector protein by regulating extracellular protease production and promoting liver fibrogenesis (53). Therefore, enhanced TLR4 activation on HSCs and LSECs by elevated portal LPS levels during alcohol consumption likely contributes to the pathogenesis of alcoholic liver fibrosis.

TLR4 signaling also strongly induces the production of CC chemokines including CCL2, CCL4 (MIP-1β), and CCL5 from HSCs (10, 106). These chemokines can interact with chemokine receptors (such as CCR1 and CCR5) on macrophages and HSCs and subsequently cause migration of these cells into the injured sites, playing an important role in inducing liver fibrosis (6, 105), whereas the interaction of CXCL9 and its receptor CXCR3 suppresses liver fibrogenesis by modulating the anti-fibrotic Th1-associated immune response (120). Although the recruitment of Kupffer cells and HSCs is considered to be crucial for the progression of liver fibrosis, the contribution of chemokine-chemokine receptor systems in ALD has not been evaluated. Microarray analyses show that expression of the CXC subfamily members IL-8, Gro-α, CXCL5, CXCL6, CXCL10, and the CC chemokine CCL2, but not CCL5, are significantly upregulated in the livers from patients with alcoholic hepatitis, and such upregulation correlates with worse prognosis, neutrophil infiltration, and the severity of portal hypertension (28), suggesting that the chemokine-chemokine receptor system plays an important role in the pathogenesis of ALD.

The strong association of the TLR4 signaling pathway and liver fibrosis has been recently confirmed in patients with chronic HCV infection by studying TLR4 single-nucleotide polymorphisms (SNPs). Huang et al. (50) have identified a series of SNPs that create a “Cirrhosis Risk Score” to predict the likelihood of fibrosis progression in patients with chronic HCV infection (50). Among the seven components of this score, a major CC allele of TLR4 (p.T399I) was the second most predictive SNP to indicate a protective role in the progression of liver fibrosis (50). The functional linkage of these TLR4 SNPs to HSC responses was further examined in a model of in vitro cultured cells, demonstrating that both the D299G and T399I TLR4 SNPs reduce TLR4-mediated inflammatory and fibrogenic signaling and lower the apoptotic threshold of activated HSCs (40). Thus the protective effect of the TLR4 SNP [c.1196C>T (rs4986791, p.T399I)] on liver fibrosis is explained at least in part by its ability to increase apoptosis and decrease fibrogenic signaling in HSCs (40).

These findings presage a new era in which genetic risks of disease conferred by specific SNPs will both regulate clinical management of patients with ALD and uncover novel pathways of fibrogenesis. Although the SNPs have been characterized in patients with HCV, future studies should address whether they also confer risk of progression in ALD, a likely outcome given the important role of LPS signaling in this disease as a result of increased gut permeability (100).

Complement and Alcoholic Liver Disease

In addition to LPS/TLR4 signaling pathway, a growing body of evidence in mouse models of ethanol exposure suggests that activation of the complement system also plays an important role in the pathogenesis of ALD. The complement system is an ancient part of the immune system that bridges innate and adaptive immunity and is comprised of more than 30 proteins, the majority of which are produced and secreted by the liver (36). Complement can be activated via three pathways: the classical, lectin, or alternative pathway. These three pathways converge on the third component of the complement system (C3), which is cleaved by convertases resulting in the formation of C3a and C3b (36). In addition to producing complement proteins, cells in the liver also express complement factor receptors, as well as intrinsic regulatory proteins. Under basal conditions, Kupffer cells and HSCs express the anaphylatoxin receptors C3a and C5a receptors (95). C5a receptor expression can be induced in proliferating hepatocytes or in response to inflam- matory cytokines (95). Recent evidence suggests that complement activation also contributes significantly to the pathogenesis of various forms of liver diseases, including ALD, through the production of proinflammatory cytokines (95). The role of complement in the pathogenesis of ALD is summarized in Fig. 1. Chronic ethanol feeding to mice for 4–6 wk increases activation of C3, as evidenced by increased C3a in the circulation (92), as well as increased accumulation of C3 or its proteolytic end product C3b/iC3b/C3c in liver (55, 103). Mice deficient in C5 and C5 are protected against ethanol-induced increases in hepatic triglycerides and circulating alanine aminotransferase, respectively (14, 92). Conversely, chronic ethanol-induced liver injury is exacerbated in mice lacking CD55/DAF, a complement regulatory protein, compared with wild-type controls (92). In rats, chronic ethanol exposure increases C3 activity and decreases expression of Crry, the rat homologue of CD55/DAF, and CD59 in the liver (55, 103). Additionally, rats deficient in complement component 6 (C6), a protein that makes up part of the terminal membrane attack complex, have increased hepatic steatosis and inflammation compared with wild-type controls (15).

Complement is activated early in the progression of ethanol-induced liver injury, prior to detectable increases in alanine aminotransferase/aspartate aminotransferase or accumulation of hepatic triglycerides (103). Early activation of complement contributes to increased inflammatory cytokine expression, mediated via the activation of the anaphylatoxin receptors, C3a and C5a, on Kupffer cells (103). The contribution of each pathway of complement activation in response to ethanol exposure is still unclear. It has also been suggested that ethanol-induced increases in LPS may contribute to activation of complement via the alternative pathway (55). It is also likely that activation of complement by any mechanism will initiate
Innate Immune Cells and Alcoholic Liver Disease

Liver lymphocytes are enriched in innate immune cells including Kupffer cells, NK cells, NKT cells, and dendritic cells (DCs) (34). Recent studies show that chronic alcohol consumption dysregulates functions of these cells. Such dys-regulation likely contributes to the pathogenesis of ALD and is discussed. In addition, alcoholic hepatitis is associated with infiltration of neutrophils, and alcohol consumption also alters the functions of neutrophils. The role of neutrophils in ALD is also briefly discussed.

Kupffer cells. It is generally accepted that activation of Kupffer cells plays a key role in inducing alcoholic steatohepatitis. As mentioned above, an increased intestinal translocation of bacterial LPS during alcohol consumption is central to inducing Kupffer cell activation via targeting TLR4 on these cells (25, 116, 121). In addition, chronic alcohol intake also sensitizes Kupffer cell responses to LPS-mediated activation (115). Activated Kupffer cells produce inflammatory mediators (e.g., TNF-α and ROS) that contribute to hepatocyte necrosis and apoptosis and generation of extracellular matrix proteins leading to alcoholic liver injury and fibrosis (25, 82, 123). Many factors and their downstream signaling pathways attenuate Kupffer cell activation during alcohol consumption, such as adiponectin (74, 75, 90), IL-10 (75), STAT3 (47), Sirt1 (107), etc., and subsequently ameliorate alcoholic liver injury.

NK cells. Liver lymphocytes are rich in NK cells, which play an important role in antiviral and antitumor defenses in the liver (35). NK cells are activated during hepatitis B virus (HBV) or HCV infection especially in the early stage of infection (3, 5, 38, 84, 127). Such activation not only plays a critical role in spontaneous recovery from HCV infection but may also contribute to the hepatocellular damage by killing hepatocytes (3, 127). In several animal models as well as in human nonalcoholic fatty liver disease, recent evidence suggests that NK cells may also contribute to liver injury by killing hepatocytes expressing elevated NK cell-activating ligands (18, 20, 60, 61). However, NK cell function is suppressed rather than activated in ALD (7, 13, 22, 24, 27, 65, 88, 91, 126). Thus it is very unlikely that NK cells contribute to ethanol-induced hepatocellular damage. In contrast, ethanol inhibition of NK cells may play an important role in accelerating hepatitis viral infection, liver fibrosis, and liver tumors in alcoholic patients with hepatitis viral infection.

Fig. 1. Activation of the complement system contributes the pathogenesis of alcoholic liver disease (ALD). 1: Alcohol consumption leads to an early activation of complement (C3 and C5). Activated C3a and C5a interact with their receptors on Kupffer cells, leading to TNF-α production that induces hepatocyte damage. 2: Long-term alcohol consumption activates both TLR4- and complement-dependent pathways that contribute to the pathogenesis of ALD. Adapted from Roychowdhury et al. (103) with permission.

The alternative pathway-mediated feedback loop (36). Recent evidence shows that ethanol feeding activates the classical complement pathway via C1q binding to apoptotic cells in the liver, suggesting that the classical complement pathway also contributes to complement activation and the pathogenesis of ALD (21). Further studies are still needed to elucidate the specific roles of each pathway of complement activation in response to ethanol exposure. Although the critical role of complement in alcoholic liver injury has been well documented in rodent models, little is known about its role in human ALD. Recently, Rensen et al. (101) reported that the complement system is activated in human nonalcoholic fatty liver disease and its activation correlates with disease severity. Thus it will be very important to examine the role of complement in human ALD, which may provide novel therapeutic targets to treat this disease.

By using intercross studies in animal models of liver fibrosis, Hillebrandt et al. (45) demonstrated that C5 plays an important role in promoting liver fibrogenesis via targeting C5aR on activated HSCs and Kupffer cells in mice. Thus C5 activation during alcohol consumption, as discussed above, likely also contributes to the development of alcoholic liver fibrosis. In addition, Hillebrandt et al. also reported that two C5 hSNPs (rs 2300929 and rs17611) are associated with the high risk for developing advanced fibrosis in patients with chronic HCV infection. However, Halangk et al. (42) recently found no evidence for the association of these two C5 SNPs with advanced liver fibrosis in a large number of patients with chronic viral hepatitis, ALD, autoimmune hepatitis, and other liver diseases. Thus further studies are required to clarify the role of C5 in liver fibrogenesis in patients with chronic liver diseases, including ALD.
The inhibitory effect of chronic alcohol consumption on NK cell functions has been observed for many years in alcoholic patients and rodents fed ethanol diets (7, 13, 22, 24, 27, 65, 88, 91, 126). Multiple mechanisms have been shown to contribute to alcohol inhibition of NK cells functions. First, alcohol consumption decreases expression of TRAIL, IFN-γ, and NK cell activating receptor NKG2D in liver NK cells (7, 56, 88). Second, alcohol consumption blocks NK cell release from the bone marrow and enhances splenic NK cell apoptosis (126). Third, alcohol consumption elevates serum levels of corticosterone, which inhibits NK cell functions (7). Lastly, alcohol consumption reduces central and peripheral levels of opioid peptide β-endorphin that can induce NK cell activation (13).

Clinical studies showed that individuals with a genetic predisposition to lower NK cell functions have greater propensity to develop chronic infection after acute HCV infection (62). Thus it is plausible to speculate that the high prevalence of chronic HCV infection in alcoholics could be partly attributed to ethanol’s inhibition of NK cell functions in these patients. In addition to their antiviral function, NK cells also have antifibrotic effect by directly killing early-activated and senescent-activated HSCs that express elevated levels of NK cell activating ligands (37, 46, 64, 76, 79, 80, 96) and via producing IFN-γ that induces HSCs cycle arrest and apoptosis (57). Recent studies reveal that chronic ethanol consumption not only inhibits NK cells function (7, 13, 22, 24, 27, 65, 88, 91, 126), thereby reducing their cytotoxicity to HSCs, but also renders activated HSCs resistant to NK cell killing and to the inhibitory effect of IFN-γ (56). It was shown that HSCs isolated from ethanol-fed mice were less sensitive to NK cell killing than those from pair-fed mice. This reduced sensitivity is due to higher levels of TGF-β, a potent inhibitor for NK cells, produced by HSCs from ethanol-fed mice than those from pair-fed mice (56). HSCs from ethanol-fed mice were also resistant to IFN-γ-induced cell cycle arrest and apoptosis by expressing higher levels of SOCS1, an inhibitor of IFN-γ signaling, and by producing higher levels of oxidative stress that inhibits IFN-γ activation of STAT1 (56). In summary, disruption of the antibifilic effects of NK/IFN-γ is an important mechanism contributing to ethanol-induced acceleration of liver fibrosis in alcoholics with viral hepatitis infection.

NKT cells. NKT cells, which are abundant in the liver, are a heterogeneous group of T lymphocytes that recognize lipid antigens presented by the nonclassical MHC class I-like molecule CD1. Since they can rapidly produce a large amount of cytokines such as IFN-γ and IL-4 after stimulation with lipid antigens, NKT cells play an important role in regulating the innate and adaptive immunity. Although activation of NKT cells has been shown to induce hepatocellular damage in a variety of acute liver injury models, their role in chronic liver injury models seems more complex due to the existence of several subtypes of NKT cells that may have opposing functions. CD1d-dependent NKT cells can be divided into type I invariant NKT and type II NKT cells. Liver NKT cells consist of more than 90% type I NKT cells that promote liver injury induced by concanavalin A, α-Gal-cer, and ischemia-reperfusion but protect against liver injury induced by CCl4 and bile duct ligation (see review in Ref. 35). The detrimental effect of type I NKT cells on liver injury is mediated via killing of hepatocytes and production of various cytokines such as IFN-γ and IL-4 (see review in Ref. 35), whereas the hepatoprotective effect of type I NKT cells in CCl4 and bile duct ligation models is mediated via suppressing the neutrophil proinflammatory response (89, 122) and stimulating Kupffer cell-dependent IL-6 production that protects against liver injury (19). Type II NKT cells have been shown to protect against concanavalin A-induced liver injury via inhibiting the functions of type I NKT cells (43). Furthermore, it has been shown that, on one hand, NKT cells can kill activated HSCs and produce IFN-γ, which inhibits liver fibrosis (89), whereas on the other hand activation of NKT cells also promotes liver fibrosis via enhancing hepatocellular damage and promoting HSC activation (59, 111). Therefore, the final effect of NKT cells on liver fibrosis is determined by the balance between these inhibitory and stimulatory effects. However, the role of NKT cells in the pathogenesis of ALD remains unclear. In mice, NKT cell deficiency delayed alcohol-induced liver injury, and alcohol feeding enhanced NKT cell activator α-GalCer-induced liver injury (77), suggesting that activation of NKT cells may accelerate alcoholic liver injury.

Dendritic cells. DCs are the most efficient antigen-presenting cells (APCs) of the immune system, playing a crucial role in innate and adaptive immune responses. Liver DCs are comprised of several subsets including myeloid CD8α+ B220−, lymphoid CD8α+B220−, and plasmacytoid CD8α+B220− cells (49). Compared with peripheral DCs, liver DCs have a reduced ability to stimulate naive T cells but have enhanced ability to produce cytokines in response to TLR stimulation (49). In addition to acting as APCs, hepatic DCs also either aggravate or ameliorate hepatocellular damage via production of proinflammatory (23) or anti-inflammatory cytokines (9), respectively, in various liver injury models. Alcohol consumption can modulate the functions of DCs (66, 67) and subsequently impair the cellular response necessary for hepatitis viral clearance (4, 85, 113, 114), likely contributing to the synergistic effect of alcohol and viral hepatitis on liver injury. However, whether DCs directly contribute the pathogenesis of alcoholic liver injury via production of cytokines remains unknown.

Neutrophils. Infiltration of a large number of neutrophils is a very prominent feature of alcoholic hepatitis (52, 98); however, the pathogenic role of neutrophils in ALD has not been investigated because most animal models of alcoholic liver injury do not develop neutrophil accumulation. Evidence from other models of liver injury suggests that neutrophils play an important role in inducing liver dysfunction and injury (99). These models include ischemia-reperfusion (8, 44) and certain drug toxicities (70, 125), etc. Ziol et al. (129) analyzed 35 patients with alcoholic hepatitis and found that the hepatocyte apoptotic index, but not the ballooning hepatocyte index, was strongly correlated with the neutrophil infiltration index. Collectively, neutrophils likely contribute to hepatocellular damage in patients with alcoholic hepatitis by producing ROS and proteases (52).

As is well known, almost all heavy drinkers develop fatty liver but only up to 35% develop alcoholic hepatitis with infiltration of neutrophils. However, how neutrophils are recruited into the fatty liver in heavy drinkers who develop alcoholic hepatitis remains obscure. It is believed that activated Kupffer cells produce a variety of cytokines and chemokines, including IL-8, RANTES, MCP-1, IL-17, etc., that subsequently recruit neutrophils into the liver (2, 11, 28, 69, 73). Targeting these chemokines and their receptors could be a potential therapeutic option to treat alcoholic hepatitis.
Future Studies

In summary, the liver is an organ with predominant innate immunity. Dysregulation of many components of innate immunity in the liver due to chronic alcohol consumption likely contributes additively or synergistically to alcohol-induced liver injury, acceleration of viral infection and tumor formation (Fig. 2). Further research is needed to further clarify and identify the interrelationships between innate immunity involved in ALD. Examples of research on the role of innate immunity in ALD need to include, but are not limited to, the following.

1) Further understanding is needed of the role of Kupffer cells/TLRs/NOD in ALD; for example:
   - Identification of the correlation between TLR4 SNPs and the progression of ALD.
   - Alcohol’s effects on Kupffer cells to release cytokines, chemokines, eicosanoids, ROS, proteolytic enzymes, and NO.
   - The effect, if any, of alcohol on Kupffer cell recruitment of NKT lymphocytes, neutrophils, and monocyte-derived macrophages via influencing adhesion molecules and processing and presenting antigens. Is alcohol-induced liver damage due to Kupffer cell inability to recognize and eliminate danger molecules, or excessive mobilization of cytotoxic molecules and failure to halt inflammation?
   - Role of damage-associated molecular patterns (heat shock proteins, hyaluronan, ureate) and TIRAP/Myd88-dependent pathways in ALD progression.
   - Further understanding of the role of TLR4 in ALD by neutralizing LPS or blocking LPS-signaling through the use of TLR4 antagonists (e.g., CyP, CRX-526, eritoran), or LPS signaling interfering molecules (e.g., TAK-242, besifloxacin, compound K, etc.).

2) Recent evidence shows that IFN activation of NK cells plays an important role in inhibition of HCV replication in patients with chronic HCV infection (38, 110). It would be very important to determine the effect of alcohol consumption on IFN activation of NK cells, NK cell-mediated inhibition of HCV replication, and the expression and signaling of NK cell stimulatory and inhibitory receptors.

3) NKT cells seem to play a complex role in the pathogenesis of liver disease owing to existence of various subtypes of NKT cells and NKT cell tolerance induction. The role of NKT cells in the pathogenesis of ALD and the effects of ethanol on NKT cells remain largely unknown.

4) Alcoholic hepatitis is associated with accumulation of neutrophils (69); however, the role of neutrophils in the pathogenesis of ALD remains obscure (52) and needs further study.

5) Hepatic DCs seem to determine the balance between liver tolerance and immunity. How alcohol disturbs this balance leading to ALD needs further investigation.

6) Since the liver is the major site for synthesis of complement components, alcohol may directly or indirectly affect the level of serum complements. Further work is needed to identify how alcohol derails the complement system, resulting in ALD.

7) Further investigation is needed into the role of epigenetic changes induced by alcohol on the innate immune system in ALD; e.g., both IFN-α and β have been shown to

Fig. 2. Ethanol (EtOH) dysregulation of innate immunity contributes to the pathogenesis of ALD. Chronic alcohol consumption results in activation of innate immunity components such as Kupffer cells/LPS/TLR4 and complements or inhibition of innate immunity components such as natural killer (NK) cells, contributing to the pathogenesis of ALD. First, alcohol consumption increases gut permeability and subsequently hepatic LPS levels via binding to TLR4; LPS then stimulates Kupffer cells to produce TNF-α in a TRIF/IRF-3-dependent manner. TNF-α induces hepatocellular damage. LPS can also directly target hepatic stellate cells (HSCs) and subsequently enhance TGF-β signaling and expression of chemokines, contributing to liver fibrogenesis. Second, alcohol consumption results in activation of complement components C3a and C5a, which then stimulate Kupffer cells to produce TNF-α, contributing to hepatocellular damage. Third, alcohol consumption inhibits the antifibrotic effect of NK cells and IFN-γ via multiple steps. Alcohol inhibits NK cell functions via blocking IFN-γ and TRAIL production, inhibits IFN-γ signaling in HSCs via induction of SOCS1, and renders HSC resistance to NK cell killing via production of TGF-β that inhibits NK cell function.
modulate expression of several microRNAs (miRNAs), and miRNA-146a/b is upregulated in response to TLR4 ligands in an NF-kB-dependent manner. Further insights into alcohol’s effects on regulation of cytokine signaling by miRNAs, DNA methylation, and histone acetylation/deacetylation should help designing new approaches to modulate inflammation in ALD.

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