Published in final edited form as:
Annu Rev Nutr. 2012 August 21; 32: 343–368. doi:10.1146/annurev-nutr-072610-145138.

Inflammation in Alcoholic Liver Disease

H. Joe Wang[1], **Bin Gao**[2], **Samir Zakhari**[1], and **Laura E. Nagy**[3]

H. Joe Wang: wangh4@mail.nih.gov; Bin Gao: bgao@mail.nih.gov; Laura E. Nagy: nagyL3@ccf.org

[1]Division of Metabolism and Health Effect, National Institute on Alcohol Abuse and Alcoholism, Bethesda, Maryland [2]Laboratory of Liver Diseases, National Institute on Alcohol Abuse and Alcoholism, Bethesda, Maryland [3]Center for Liver Disease Research, Departments of Pathobiology and Gastroenterology, Cleveland Clinic and Departments of Nutrition and Molecular Medicine, Case Western Reserve University, Cleveland, Ohio

Abstract

Frank Burr Mallory's landmark observation in 1911 on the histopathology of alcoholic liver disease (ALD) was the first identification of a link between an inflammation and ALD. In this review, we summarize recent advances regarding the origins and roles of various inflammatory components in ALD. Metabolism of ethanol generates a number of metabolites, including acetate, reactive oxygen species, acetaldehyde, and epigenetic changes, that can induce inflammatory responses. Alcohol and its metabolites can also initiate and aggravate inflammatory conditions by promoting gut leakiness of microbial products, by sensitizing immune cells to stimulation and by activating innate immune pathways, such as complement. Chronic alcohol consumption also sensitizes non-immune cells, e.g., hepatocytes, to inflammatory signals and impairs their ability to respond to protective signals. Based on these advances, a number of inflammatory targets have been identified with potential for therapeutic intervention in ALD, presenting new opportunities and challenges for translational research.

Keywords

Ethanol; liver injury; hepatic macrophage; cytokines; complement; inflammasome

INTRODUCTION

Acute inflammation is a critical homeostatic mechanism for protecting a host against noxious conditions, including infection and tissue injury. Such inflammation is characterized by innate immune cell infiltration into target tissues. These effector cells and their secreted mediators orchestrate the killing and removal of infected/damaged cells and promotes tissue repair and regeneration. Chronic inflammation occurs when an acute response becomes dysregulated or fails to resolve because of the continued presence of an inducer of inflammation. In addition to innate immune cells, adaptive immune cells are often involved in chronic inflammatory responses. The excessive (acute) or persistent (chronic) presence of cytotoxic immune cells and their mediators in tissues have been recognized to play important roles in the pathogenesis of human diseases.

Address Correspondence to H. Joe Wang, Division of Metabolism and Health Effect, National Institute on Alcohol Abuse and Alcoholism, Bethesda, Maryland; wangh4@mail.nih.gov OR Bin Gao Laboratory of Liver Diseases, National Institute on Alcohol Abuse and Alcoholism, Bethesda, Maryland; bgao@mail.nih.gov.

DISLOSURE TREATMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

Last year (2011) marks the centennial of Frank Burr Mallory's landmark report that described for the first time a large number of infiltrating neutrophils in cirrhotic livers from alcoholic patients (69). These liver-associated inflammatory activities were initially viewed as a host protective mechanism to remove damaged hepatocytes. This simplistic view was challenged and amended following a series of studies from the late Dr. Thurman's laboratory in the 1980–1990s which demonstrated that gut microflora-derived lipopolysaccharides (LPS) and the pro-inflammatory cytokine TNF-α play an important role in ALD development in alcohol-fed animal models, suggesting that alcohol-related inflammation contributes to the ALD development. Furthermore, elevated plasma LPS (endotoxemia) is commonly detected in patients with alcoholic steatohepatitis and cirrhosis, implicating a pathological role for LPS-induced inflammation in the pathogenesis of human ALD (28).

In the past decade, we have seen a significant expansion of research aimed to search for the key pro-inflammatory mediators that contribute to the ALD development (33). LPS was the first identified and best understood inflammatory inducer in the pathogenesis of ALD. In addition, many other alcohol-related mediators have also been identified to contribute to liver inflammation (Table 1). These mediators and LPS often co-exist and contribute, additively or synergistically, to ALD development. This review focuses on significant findings from recent studies, in particular: 1) the identification of the key role of microbial products and their host receptors (e.g., LPS/TLR4) in the development of ALD; 2) the identification of multiple inflammatory inducers in the pathogenesis of ALD, including alcohol, its metabolites, and alcohol-induced metabolic and cellular changes; and 3) functional study of inflammatory mediators, pro-inflammatory cytokines, anti-inflammatory cytokines, and hepatoprotective cytokines in the pathogenesis of ALD. These advances allow us to evaluate inflammation-based approaches for effective ALD intervention.

MICROBIAL PRODUCTS: THE LPS/TLR4 PARADIGM

The best known inflammation inducers include products of microbes and components of dead host cells. Their presence is detected by host-encoded receptors expressed predominantly on tissue specific and circulating innate immune cells, e.g., macrophages/monocytes and dendritic cells. Ligand binding to these receptors triggers a cascade of signaling events that induce the production of secreted mediators responsible for orchestrating an acute inflammatory response. The mammalian genome encodes a large number of pattern-recognition receptors that respond to diverse threats from pathogens and dead cells. The best characterized pattern-recognition receptor is Toll-like receptor (TLR) family, which has up to 13 members, each recognizing a unique set of microbial and host products. TLR4, for example, is the receptor for LPS.

As a cell wall component of Gram-negative bacteria, LPS is the best known for its role in bacteria-induced inflammation and multi-organ failure in sepsis. The ability of LPS to produce inflammation is mediated by the MD2 protein and the membrane receptor TLR4. In innate immune cells, such as tissue macrophages and their progenitor monocytes, the LPS-MD2/TLR4 complex triggers MyD88-dependent and MyD-independent (TRIF-dependent) signaling cascades, resulting in production of many inflammatory mediators, including TNF-α, IL-1β, and IL-6. The key findings linking LPS and ALD are summarized below.

Multiple mechanisms contribute to alcohol-related endotoxemia. In animal studies, continuous intragastric feeding of alcohol results in intestinal bacterial overgrowth and enteric dysbiosis, which is due, in part, to an alcohol-induced downregulation of expression of several intestinal antimicrobial molecules (139). In addition, accumulating evidence suggests that alcohol feeding increases acute LPS translocation due to an increase in gut

epithelial membrane permeability and impairment of tight junctions between epithelial cells (107). Moreover, chronic alcohol causes structural changes in the GI tract which might contribute to LPS translocation (107, 134). Following translocation across the intestinal epithelium, LPS enters the circulation by the lymphatic system and/or the portal vein-liver route; the relative contribution of these two dissemination routes in the presence of alcohol use is still not defined. Regardless of the route, the majority of circulating LPS is removed by a healthy liver, primarily through the abundant Kupffer cells and hepatocytes (134). Consequently, a healthy liver bears the brunt of inflammation induced by LPS. When the liver is damaged, the bioavailability of LPS in the circulation increases, which explains, in part, the prevalence of endotoxemia in cirrhotic patients (93).

LPS clearly plays a pathological role in end-stage liver diseases and complications such as sepsis and the associated multi-organ failure. Reports in the last two decades have also built a strong case for a pathological role of LPS in the early phase of ALD. Pretreatment with non-absorbable antibiotics in alcohol-fed rats reduces alcohol-induced steatosis, liver injury, and inflammatory markers (2). Similarly, probiotic treatment mitigates the effect of alcohol on liver injury, reduces LPS in the circulation, and reduces inflammatory markers in alcohol-fed animals (88). Consistent with this, in a preliminary study of human alcoholic patients, probiotic use improved liver functions (52). In addition, administration of LPS to alcohol-fed rodents promotes pathogenesis from simple steatosis to steatohepatitis (105). Lastly, the disruption of the TLR4 gene or CD14 gene (a gene that encodes for the LPS-binding receptor) reduces liver pathology and inflammation in a murine model of alcoholic liver injury (41, 131, 140). The important role of LPS in inducing liver inflammation in ALD is likely mediated by binding to TLR4 on Kupffer cells/macrophages, which then produce a variety of pro-inflammatory cytokines and subsequently promote infiltration of inflammatory cells including neutrophils. Although neutrophil infiltration is always low to mild in rodents fed alcohol, when exogenous LPS is given to alcohol-fed animals, a significant number of neutrophils is observed in the liver (106). Such data support the idea that sufficient LPS (and consequently, the LPS-induced cytokine milieu) is required for neutrophil infiltration in alcoholic steatohepatitis. Comparative analysis of the circulating and liver cytokine milieu in alcohol and LPS-treated animals, and in alcoholic hepatitis patients might help identify the important mechanisms how LPS promotes liver inflammation in ALD.

The role of LPS in inducing inflammation can be augmented by alcohol and its metabolites. Prolonged alcohol exposure *in vitro* and *in vivo* potentiates LPS-induced cytokine production in monocytes and Kupffer cells, respectively (3, 29). Acetaldehyde, a metabolic product of alcohol, interacts with malondialdehyde, a product of lipid peroxidation, to form malondialdehyde-acetaldehyde adduct (MAA), another example of an LPS sensitizing agent (24). This suggests that alcohol consumption not only elevates LPS levels but also increases the sensitivity of Kupffer cells/macrophages to LPS stimulation, contributing to liver inflammation in ALD.

LPS is not the only gut microbial product that enters the circulation due to alcohol use. For example, a significant increase in circulating peptidoglycan, which is a bacterial wall product and TLR2 ligand, was observed in acute and chronic alcohol-fed rodents (18, 122). A few studies have examined the roles of these microbial products and their cellular sensors, other than LPS-TLR4, in the inflammatory state observed in ALD patients. Acute alcohol exposure augments TNF-α production in human monocytes when both TLR2 and TLR4 ligands are present (95). Increased expression of TLR2, 4, and 9 is associated with neutrophil dysfunction and endotoxemia; inhibition of these TLRs prevents the increase in oxidative burst and chemokine production, but has no effect on phagocytic activity in these neutrophils (118). In addition, deletion of TLR2 in mice has no significant impact on the

development of the early phase of ALD, such as the development of fatty liver and the elevation of liver enzymes (41, 118). Further studies are required to clarify the roles of TLR2 and TLR9 in the pathogenesis of ALD.

The mechanisms by which TLR4 induces early alcoholic liver injury have been extensively explored in last decade. LPS-MD2/TLR4 intracellular signaling is controlled by two adaptor molecules, MyD88 and TRIF(48), which induce activation of three key signaling transducer/regulators, NF-κB, MAPK, and IRF3, and subsequently control the expression a large array of inflammatory mediators. IRF3 is activated only by the TRIF dependent pathway whereas NF-κB and MAPK are activated by both the MyD88 and TRIF pathways. In addition, LPS/TLR4 receptor complex also activates NADPH oxidase to produce ROS, which then plays a supporting role in the activation of NF-κB and MAPK. Among these signaling molecules, NADPH oxidase and NADPH oxidase derived ROS, members of MAPK (p38, ERK1/2) and its downstream target (Egr1) have been found to play a causal role in developing ALD in animal models (53, 54, 56, 102, 125). Moreover, recent studies suggest that the role of LPS/TLR4 in the pathogenesis of ALD is mediated via activation of the MyD88-independent and TRIF/IRF3-dependent pathway, as IRF3-knockout mice, but not MyD88-knockout mice, are resistant to liver injury induced by an ethanol diet (41, 145). Further studies using bone marrow chimeric mice suggest that IRF3 in parenchymal cells is protective against ALD via the upregulation of type I IFNs and IL-10, whereas IRF3 in nonparenchymal cells is detrimental via the upregulation of TNF-α (98). Together, these results demonstrated the complex functions of the TLR4 downstream pathway in immune cells and hepatocytes that not only afford the liver to respond to an inflammatory challenge but also provide it with a certain level of protection.

Although an important role of LPS in the pathogenesis of ALD is well documented, it is worth noting that, at least in some animal models and a small percent of ALD patients, an increase in LPS level is not always apparent (31, 109). This suggests that LPS is not a sole factor to induce inflammation in ALD. Indeed, many other factors, in addition to LPS, have also found to play important roles in promoting liver inflammation in ALD (see below).

A DIRECT ROLE OF ALCOHOL AND ITS METABOLITES IN INDUCING INFLAMMATORY RESPONSES

Besides their ability to alter or enhance immune cell response to LPS, alcohol and its metabolites also play a direct role in activating an inflammatory program in immune cells. For example, recent studies from Guerri's group suggest that ethanol can directly induce activation of NF-κB and downstream Cox-1 and iNOs expression in cultured astrocytes and glial cells via the induction of the translocation of TLR4 into lipid rafts (4, 8, 9), contributing to ethanol-induced neuroinflamamtion. However, it is not clear whether ethanol can also directly activate TLR4 signaling in liver parenchymal and nonparenchymal cells, thereby promoting liver inflammation.

Alcohol metabolites, both acetaldehyde and acetate, have been shown not only to directly induce an inflammatory response but also to potentiate LPS-mediated induction of pro-inflammatory cytokines in Kupffer cells/macrophages. It was reported that acetaldehyde and acetate exposure of rodent macrophage cells caused activation of NF-κB signaling and production of TNF-α (114). This NF-κB activation is, in part, mediated via the down regulation of SIRT1, an NF-κB antagonist; such effect appears to be limited to alcohol metabolites, while alcohol itself has no effect (114). Additionally, acetate exposure also augments cytokine production in human macophages in response to LPS (49); this sensitizing effect of acetate appears to be dependent on the conversion of acetate to acetyl-CoA, which is the substrate for histone acetylation necessary for the enhanced transcription

of cytokine genes (49). Recent studies from Min You's group (You, M.: Adiponectin reverses impairment of Sirt1-TNF-α axis by LPS or acetate through stimulating AMPK signaling in cultured rat Kupffer cells. Alcoholism: Experimental and Clinical Research, volume 35, supplement S1, 302A) suggest alcohol and alcohol metabolites induce liver inflammation via the alteration of energy homeostasis. The end product of alcohol metabolism, acetate, is a highly usable substrate for energy production in the mitochondria via the TCA cycle. Metabolism of alcohol and acetaldehyde leads to an increase in the NADH/NAD+ ratio, which when reoxidized causes the mitochondrial electron transport chain to produce reactive oxygen species (ROS) as byproducts. Such observation is analogous to the finding that high glucose induces inflammatory response via ROS production (113). As discussed in the section below, ROS can activate inflammatory responses via activation of NF-κB and its downstream inflammatory events.

ALCOHOL-INDUCED METABOLIC AND CELLULAR CHANGES CONTRIBUTING TO INFLAMMATION

Free fatty acids

Compared with a control cohort, liver biopsy samples from patients with ALD have a greater than 10 fold increase in free fatty acids (FFAs), consisted of saturated fatty acids (SFAs) and unsaturated fatty acids (UFAs)(77). Accumulating evidence suggests that these SFAs have the potential to directly or indirectly induce inflammatory responses in the liver (Table 1). First, SFAs promote inflammation by activating TLR4 on macrophages, contributing to liver inflammation. It was reported that free SFAs, but not UFAs, induce TLR4-mediated NF-κB activation and Cox-2 expression in macrophages *in vitro* (61). The role of this SFA-TLR4 link in liver inflammation was also confirmed *in vivo* by the fact that TLR4 knockout mice were partially resistant to a high fat infusion-induced liver TNF-α expression (116). Second, SFAs (e.g., palmitic acid) directly activate the inflammasome in hepatocytes or stimulate hepatocytes to produce danger signals that activate the inflammasome in immune cells (19). Inflammasome is an intracellular complex that controls the release of pro-inflammatory cytokines IL-1β and IL-18, and its activation promotes liver inflammation in nonalcoholic steatohepatitis and possibly in alcoholic steatohepatitis (19). Third, free SFAs can also directly promote hepatocytes to produce chemokines. Exposure of palmitic acid to rat or human hepatocytes promotes the NFκB-dependent production of pro-inflammatory chemokines CINC-2/MIP-2 and IL-8 (47). Finally, the liver might be particularly vulnerable to SFAs because, unlike those in circulation, liver FFAs are populated predominantly by SFAs (57, 77).

In light of these recent advances, it will be important in the near future to determine experimentally whether free SFAs also play a significant role in alcohol-related liver inflammatory conditions. Interestingly, early studies have shown that diets rich in esterified SFAs (beef tallow, cocoa butter, or medium-chain triglycerides) are protective against alcohol-induced liver pathology including inflammation (87, 110), an outcome believed to be attributable to an increased adiponectin production and/or increased resistance of membrane lipid to oxidation. However, whether or not consumption of such esterified SFAs modulates the levels of free SFAs in the liver is not known.

Free UFAs at a high level are also capable of inducing an inflammatory response. High fat diets consisting of free UFAs or esterified UFAs promote early ALD development in animal models through increasing lipid peroxidation and oxidative stress, which in turn induce inflammation by activating NFκB (86). Though free UFAs can inhibit SFA-induced inflammatory response from cultured macrophages (ref 62), a recent study showed that they

can act synergistically with alcohol in inducing oxidative stress and activating TNF-α production in Kupffer cells (20).

ROS

ROS are important signaling mediators for receptor-mediated activation of NFκB and the associated downstream cytokine production. Exogenous ROS can substitute for cytokine signaling to induce inflammatory responses. For example, H2O2 exposure of endothelial cells induces NF-κB-dependent ICAM-1 expression, which, as a ligand for integrin CD18/11A on leukocytes, is critical for neutrophil transmigration (59). ROS also mediate glucose-induced production of TNF-α by microglial cells and monocytes (103, 104).

It has been well documented that alcohol increases ROS production via multiple mechanisms, including the mitochondrial electron transport chain and CYP2E1-mediated alcohol metabolism (11, 143). Mechanistically, alcohol-induced ROS may contribute to inflammation by direct induction of the inflammatory response and/or amplification of inflammatory signaling. A close functional relationship between alcohol-induced ROS and liver inflammation is supported by the ability of N-acetylcysteine, an antioxidant, to reduce the level of inflammatory markers in alcohol-fed mice (146).

Additionally, ROS produced via respiratory burst during phagocytosis process by phagocytic cells is well established for its capacity of killing pathogens or pathogen-infected cells. Alcohol metabolism-induced ROS is also found to be sufficient in promoting cell injury, including necrosis (80). In turn, necrotic cells can activate the inflammatory response. Thus, alcohol-induced ROS may function as a catalyst to initiate a vicious cycle of cell killing, inflammatory responses, and further ROS production, thereby promoting liver inflammation.

Damaged cells

Necrosis and apoptosis, the two most common forms of cell death, are prominent features of human alcoholic steatohepatitis (69, 91, 129). Many factors have been shown to induce hepatocyte necrosis in ALD. These include activated neutrophils (6, 46), alcohol-related oxidative stress (80), pro-inflammatory cytokine TNF-α, and Fas ligand (17, 91). Necrotic cells, but not apoptotic cells, have the potential of inducing inflammatory responses; such induction requires the activation of the inflammasome, an intracellular sensor complex, in necrotic cells, which further leads to the release of IL-1α and, to some extent, IL-1β (45). These necrotic cell-derived IL-1α and IL-1β cytokines then activate macrophages to generate a full range of inflammatory responses including neutrophil infiltration (25, 55). Finally, RNA and DNA released from cells undergoing necrosis are capable of inducing inflammation via TLR7/9 (43). The important roles of these endogenous molecules are well documented in rodent models of nonalcoholic steatohepatitis (83) and drug-induced liver injury (43), but have not been explored in ALD-related liver inflammation.

Complement

When activated, key components of the complement system play several important functions in inflammation. For example, C3a and C5a are chemotactic factors and pathogen-associated molecular pattern sensors that can activate leukocytes to produce inflammatory cytokines. The level of complement is increased in alcoholics with withdrawal symptoms but is reduced in alcoholics with cirrhosis (67). Hepatic C1q and C3 deposition are markedly elevated in mice after 2-4 days of alcohol feeding. Further studies suggest that ethanol activates the classical complement pathway via C1q binding to apoptotic cells in the liver. Deletion of C1q abolishes ethanol-induced complement activation, prevents the upregulation of TNFα and IL-6 expression in the liver, and reduces ethanol-induced liver injury (16).

These findings indicate that the activation of complement components also contributes to inflammatory responses in ALD (Tables 1 and 2).

Adducts and immune complexes

Adducts generated by the reaction of lipid peroxide products or the alcohol metabolite acetaldehyde with proteins or lipids are commonly observed in alcohol-fed animals (130). Interestingly, adducts alone or in combination with LPS can stimulate the inflammatory response in rat sinusoidal endothelial cells and Kupffer cells (24). It was proposed that this inflammation-stimulating effect is mediated through the scavenger receptor (SR-A) on these cells. As neo-antigens, these adducts can also elicit adaptive immune responses, including antibody production and/or cytotoxic T cell activation. Adduct-specific antibodies (IgG and IgA) have been found in patients with ALD, and their titers correlate well with disease severity and TNFα levels (108, 132). Conceptually, antibody-adduct complexes formed on the cell surface can also activate the complement system, which in turn activates inflammatory responses.

Hypoxia

Hypoxia is a well established feature of alcoholic liver injury, resulting from increased consumption of oxygen by alcohol metabolism (5). HIF-1α is a transcription factor whose stability is negatively regulated by oxygen. Under hypoxic conditions, HIF1α is stabilized and cooperates with NF-κB to induce the production of pro-inflammatory and angiogenic chemokines and receptors, including CXCL12, CXCR4, and VEGF (92). Hepatocyte-specific deletion of HIF1α diminishes alcohol-induced liver fat accumulation, injury, and inflammation (90) (see table 1), suggesting that alcohol-induced hypoxia contributes to the inflammatory response in the liver.

In conclusion, the list of recognized alcohol-related inflammatory inducers has grown significantly in the last decade (Table 1 and depicted in Figure 1). Because many of these factors are closely linked to alcohol and its metabolism and are present at significant levels during chronic alcohol abuse, their roles in alcohol-related inflammation are of great interest. Nevertheless, many of the connections we made between these inducers and ALD pathogenesis are based on circumstantial evidence and still require vigorous examination.

PATHOLOGICAL *VERSUS* PROTECTIVE ROLE OF INFLAMMATORY MEDIATORS (CELLS, MEDIATORS, AND SIGNALING PATHWAYS) IN ALD

Infiltration of neutrophils and monocytes in the liver of alcoholics or alcohol-fed animals has received significant attention in the past. Neutrophil infiltration is the prominent feature of alcoholic steatohepatitis. Compared to neutrophils, monocyte infiltrates are often lower and are widely distributed in all stages of ALD, including fatty liver, the convalescent stage of steatohepatitis, and cirrhosis. In animal models, a pattern of neutrophil infiltration followed by monocytes infiltration is observed (7). There is also a significant lymphocyte infiltrate in ALD, yet the functions of these cells are poorly understood. The alterations in the activity of liver-specific resident immune cells, including Kupffer cells, NK cells, and NKT cells, have also been observed and reviewed recently (34).

Neutrophils

Intralobular neutrophil infiltration, secondary to hepatocyte degeneration, is a defining feature of alcoholic steatohepatitis. Accumulating evidence suggests that both IL-8 and IL-17 play an important role in inducing neutrophil infiltration in ALD. The elevation of these chemokines in the circulation and the liver has been consistently observed in alcoholic patients (62, 78). The grade of neutrophil infiltrate, e.g., density of these cells and frequency

of their presence, positively coincides with the peak of hepatocyte injury (ballooning and necrosis) and other diagnostic features of liver diseases such as serum bilirubin, Maddrey DF and Childs score (27, 99). With alcohol abstinence, the grade of neutrophil infiltrate in most patients declines, followed by an increase in monocyte infiltration and eventual resolution of inflammation (21, 27). Nevertheless, the inflammatory condition can last for days to months before resolution. With continuous alcohol use, such acute conditions can become prolonged and recurring events that are superimposed over the development of fibrosis and cirrhosis.

As suggested as early as 100 years ago by Dr. Frank Burr Mallory, liver neutrophil/monocyte infiltrates in alcoholic hepatitis patients may play a protective role for the host by killing and removing alcohol-damaged hepatocytes (69). However, the question remains whether this inflammatory response is excessive and contributes to liver injury, and thus pathological; conceptually, this hypothesis is highly plausible because the studies from other liver injury models in rodents suggest that activation of neutrophils contributes to the pathogenesis of hepatocellular damage by releasing reactive oxygen species and proteases (106).

Alterations in neutrophil function in alcoholics have been described in the literature. Neutrophils isolated from patients with alcoholic hepatitis, for example, have a lower baseline phagocytic capacity (117). Removal of LPS from serum restores neutrophil phagocytic function in a subset of patients whose neutrophils have a high frequency of resting oxidative burst, and probiotic treatment restores partially the phagocytic function of circulating neutrophils in patients with alcoholic cirrhosis, suggesting that the compromised neutrophil function in patients with alcoholic steatohepatitis is largely due to endotoxemia (84, 117). Interestingly, neutrophils from a small percentage of patients in this study had a low resting oxidative burst and a low phagocytic activity that was not restored by LPS removal, implicating that other mechanisms may also contribute to the compromised neutrophil functions in these patients (117). In addition, blockade of TLR2, TLR4 and TLR9 with neutralizing antibodies in these neutrophils prevented the increase in oxidative burst and chemokine production, but not phagocytic activity, suggesting that microbial ligands for TLR2, TLR4 and TLR9 play a role in neutrophil dysfunction (118). In patients with severe alcoholic hepatitis, one major cause of mortality is infection; thus, restoration of neutrophil function is expected to play a protective role against infectious agents. However, whether restoration of neutrophil functions is protective against or harmful to alcohol-induced liver injury is not known.

Monocytes and hepatic macrophages

A series of studies from Dr. Thurman's laboratory have provided strong evidence that liver-resident macrophages, Kupffer cells, play a pathological role in the early phase of ALD (127). Furthermore, they showed that chronic alcohol feeding in animals potentiates the ability of Kupffer cells to produce inflammatory cytokines in response to LPS.

Besides resident macrophages, infiltration of circulating monocytes, which become macrophages once in the tissue, is common at different stages of ALD, including fatty liver, steatohepatitis, and cirrhosis. Alcoholics with fatty liver often have macrophage aggregates (lipogranulomas) with fat droplets in the center that can be found in the majority of alcoholics without cirrhosis (15). In the intragastric infusion rat model, alcohol intoxication causes mild necrosis and infiltration of immune cells in the liver that are pericentrally localized and have a mostly mononuclear morphology (89, 128). A genetic study shows that mice deficient in monocyte chemoattrant protein-1 (MCP-1) attenuate alcohol-induced liver injury, supporting a pathogenic role for infiltrating monocytes (73). A separate study showed that feeding rats with alcohol plus a high-fat diet promotes the development of

infiltrated monocytes into macrophages with a pro-inflammatory phenotype (138), whereas another study has shown that voluntary feeding with an alcohol diet alone upregulates both hepatic classical (M1) and alternative (M2) markers (65). In a rat ALD model, chronic ethanol feeding itself is sufficient to cause a shift of Kupffer cells to an M1 macrophage polarization and upon LPS stimulation these cells had increased TNFα expression (71). Together, these pro-inflammatory M1 macrophages likely play an important role in inducing inflammation during ALD pathogenesis.

Like neutrophils, monocytes/macrophages also have a host-protective role because of their ability to remove damaged cells, combat infectious agents, produce hepatoprotective cytokines (such as IL-6, discussed below), and promote liver regeneration; such a host protective role has to be considered when these cells are targeted for ALD intervention. In addition to contributing to the development of liver fibrosis, macrophages/monocytes also play an important role in fibrosis resolution during the recovery phases of inflammatory fibrosis (23). These findings suggest that macrophages/monocytes play a complex role in the pathogenesis of ALD.

Lymphocytes and NK cells

Lymphocyte infiltrates in the liver are also commonly observed in patients with alcoholic steatohepatitis. Unlike neutrophils (which usually undergo rapid apoptosis after activation), the number of lymphocytes in the liver did not change significantly with alcohol abstinence and recovery (27). Very little is known about the functions of these lymphocytes except for a recent report on IL-17-secreting T cells in the circulation and the liver of alcoholics with ALD (see below). As discussed in the previous section, adducts between reactive MAA (malondialdehyde-acetaldehyde) and proteins, commonly observed in alcohol-fed animals and human alcoholics, have the potential to generate adaptive immune responses. A recent study has shown that immunization of mice with MAA-modified cytosolic proteins induces autoimmune-like hepatitis with elevated TNFα, IL-1β, and IFN-γ. Lymphocytes isolated from these mice, upon adoptive transfer, can also cause liver injury (126). These results provided proof of principle evidence that such adducts have liver disease-causing potential. However, whether endogenous adducts are sufficient to induce a disease-causing immune response in ALD remains to be demonstrated.

Liver-resident NK cells have a host protective role in blocking the activation of hepatic stellate cells (HSCs) and fibrogenesis; chronic alcohol intake impairs this protective function in these cells. These observations have been reviewed in detail elsewhere (34).

PRO-INFLAMMATORY CYTOKINES AND THEIR SIGNALING PATHWAYS

Elevated levels of circulating pro-inflammatory cytokines, specifically TNFα, IL-1β, and IL-8, have been consistently observed in ALD patients (78). These cytokines are, at least in part, produced by circulating monocytes in response to endotoxemia (increased circulating LPS). Later studies showed that an increased level of LPS, although not as high as in alcoholic hepatitis, is also a common feature in alcoholics without significant clinical liver disease (31). Interestingly, increased levels of IL-6 and IL-10, but not TNFα, are characteristics of this cohort (60). An elevated level of the newly identified proinflammatory cytokine IL-17 is also found in the circulation and liver of ALD patients (62).

TNFα

TNFα was one of first pro-inflammatory cytokines linked to ALD. A high level of circulating TNFα was found in patients with alcoholic hepatitis, but not in alcoholics without liver diseases. TNFα production is induced early in alcohol-fed animals and is closely coupled with liver injury (111, 146). A pathological role for TNFα in ALD was

demonstrated by studies showing that knockout of the TNFα receptor or administration of TNFα neutralizing antibody reduced liver injury induced by alcohol feeding (42, 141); Table 2). Other studies also showed that hepatocytes isolated from alcohol-fed animals are more susceptible to TNFα-induced cell death (97).

Based on these observations, TNFα antagonists have been used for treating patients with alcoholic steatohepatitis, but the results from these studies have been mixed (Table 3). On the one hand, pentoxifylline, a non-specific inhibitor of TNFα synthesis, improved the short-term survival of patients with alcoholic hepatitis (66). On the other hand, numerous trials with anti-TNFα antibody failed to demonstrate an improvement in patient survival, with one trial aborted because of increased mortality primarily owing to infections (10).

IL-8

IL-8 is a critical pro-inflammatory chemokine involved in many steps of neutrophil mobilization from bone marrow to tissue infiltration and activation. As discussed earlier, production of IL-8 is also induced by TNFα and by ligands for TLRs via the activation of NF-κB. Circulating IL-8 is highly elevated in patients with alcoholic hepatitis and is closely linked to neutrophil infiltration (30, 115). In comparison, IL-8 is only moderately elevated in alcoholic cirrhosis patients and alcoholics undergoing alcohol withdrawal.

IL-17

IL-17 is a pro-inflammatory cytokine with a relatively weak TNFα-like function. However, this cytokine can act synergistically with other cytokines, i.e., TNFα, to activate NF-κB and induce IL-8 production. A recent study found that elevated IL-17 is also a characteristic of alcoholic patients with liver disease (62). The cellular origins of IL-17 in this study were monocytes and T cells in the circulation and infiltrating neutrophils and T cells in the liver. Interestingly, expression of the IL-17 receptor was mainly localized to hepatic stellate cells in the liver biopsies of ALD patients. Exposure of hepatic stellate cells to IL-17 *in vitro* induced production of IL-8 and GROα, both of which are important for neutrophil recruitment. Finally, a correlation between liver IL-17-secreting cell infiltrates and fibrosis score was found (62). This suggests that IL-17 plays a role in promoting both liver inflammation and fibrogenesis of ALD.

ANTI-INFLAMMATORY AND HEPATOPROTECTIVE CYTOKINES

IL-6, IL-10, IL-22 and their downstream signaling molecule signal transducer and activator of transcription 3 (STAT3)

IL-6, IL-22, and IL-10 share a common downstream signal transducer, STAT3, which regulates expression of genes of diverse functions in immune defense and cell proliferation, survival, and differentiation. These cytokines, though all activating STAT3, sometimes have different and even opposing functions. For example, IL-6 has both pro- and anti-inflammatory roles, the latter of which is largely due to its hepatoprotective function, which ameliorates hepatocyte necrosis-associated inflammation (133). In contrast, the pro-inflammatory effect of IL-6 is mediated via the upregulation of pro-inflammatory cytokines in macrophages. IL-10 has a strong anti-inflammatory role by blocking the production of TNFα, IL-1β and IL-6 in macrophages.

Elevated IL-6 is found in chronically alcohol-fed animals and in alcoholics, even those without apparent liver disease (60). IL-6 knockout mice fed alcohol, surprisingly, had increased liver fat accumulation, lipid peroxidation, mitochondrial DNA damage, and sensitization of hepatocytes to TNFα-induced apoptosis, which was preventable by administration of recombinant IL-6 (26, 37, 144) (Table 2). The findings suggest that IL-6

has a host protective role at the early phase of ALD. Similarly, IL-22 administration to alcohol-fed mice also prevented liver steatosis and liver injury through activation of hepatic STAT3 (51, 136).

Similar to the IL-6 knockout mice, alcohol-fed mice with a hepatocyte STAT3 deletion had enhanced liver fat accumulation, but reduced inflammation, suggesting that hepatic STAT3 has a protective role against steatosis, but a stimulatory role in inflammation in hepatocytes (40). In contrast, alcohol-fed mice with a conditional STAT3 deletion in myeloid-derived cells or endothelial cells had an increased inflammatory response, consistent with a role for STAT3 in controlling inflammation in these immune cells, possibly via IL-10 (40). Indeed, alcohol-fed IL-10 knockout mice have increased hepatic and systemic inflammatory conditions compared to wild-type mice (81). Surprisingly, though, despite the increased inflammation, IL-10 knockout mice have a reduced fatty liver and lower serum ALT and AST levels after ethanol feeding compared with wild-type mice. This may be because IL-10 knockout mice have elevated IL-6 and STAT3 activation in the liver, which ameliorates steatosis and hepatocellular damage (81). Together, at the early stage of ALD, inflammation involving IL-6/STAT3 has a host protective role against alcoholic steatosis and liver injury.

Finally, hepatic STAT3 activation is impaired in alcoholics with ALD, suggesting the uncoupling of IL-6 from STAT3 activation (119); such an outcome can in part be explained by the fact that chronic alcohol exposure inhibits IL-6-induced STAT3 activation in hepatocytes and sinusoidal endothelial cells (12, 32). Thus, it is plausible that inflammation-associated activation of IL-6/STAT3 plays a compensatory role in ameliorating steatosis and liver injury at the early stage of ALD, whereas chronic alcohol consumption diminishes IL-6/STAT3 activation and its hepatoprotective function in the liver, leading to severe forms of ALD.

Adiponectin

While increased expression of inflammatory mediators during chronic ethanol exposure, associated with both increased exposure and sensitivity of Kupffer cells to LPS (85), has been thought to be primarily a localized response within the liver, ethanol also has systemic effects that can impact the imbalance of pro-inflammatory cytokine production. In particular, recent studies have implicated ethanol-induced changes in the expression of adipokines in mediating the pathophysiological effects of ethanol. While some data suggest that ethanol can affect the release of the adipokines leptin and resistin (100), a considerable body of evidence now consistently demonstrates that chronic, heavy ethanol exposure decreases serum adiponectin concentrations in mice (137, 142) and rats (124). In rats, serum adiponectin concentrations decrease as early as 3 days on the Lieber-DeCarli ethanol diet, prior to the onset of liver injury (14). Adiponectin mediated responses can be regulated both by changes in adiponectin concentration and changes in the expression and functional activity of adiponectin receptors. Very little is known about the role of adiponectin and adiponectin receptors in mediating ALD in humans.

Adiponectin has broad effects on innate and adaptive immunity, with important anti-inflammatory activity. Kupffer cells isolated from ethanol-fed rats are very sensitive to the anti-inflammatory effects of adiponectin (124). Both globular and full-length adiponectin suppress LPS-stimulated cytokine expression, by decreasing TLR4-mediated signaling and shifting macrophages to an M2 phenotype (70-72).

TARGETING INFLAMMATION FOR ALD TREATMENT

ALD accounts for about 37,000 deaths per year in the USA. ALD in alcoholics begins with hepatic steatosis and progresses into more severe alcoholic hepatitis and/or cirrhosis. In the

clinical setting, only alcoholics with alcoholic hepatitis and/or cirrhosis have identifiable symptoms and receive treatment. Clinical manifestations, diagnosis, and treatment of alcoholic hepatitis has been thoroughly reviewed [for the most recent updates, see (33, 66, 94, 123)]. Severe alcoholic hepatitis patients, in particular, have a very high mortality rate of about 50%, and those who survive have a 70% probability of developing liver cirrhosis. Severe ALD is also the 2nd leading cause for liver transplant.

The cornerstone of the current treatment for alcoholic hepatitis is alcohol abstinence (33, 94, 123). Although alcohol abstinence is absolutely necessary, it is often insufficient or ineffective for some patients. Nutritional supplementation is also often recommended for alcoholic hepatitis patients because of the prevalence of malnutrition. Only two pharmacological agents, corticosteroids and pentoxifylline, are currently recommended for treating alcoholic hepatitis; significantly, both of these agents are aimed at reducing inflammatory conditions. Corticosteroids reduce cytokine production through transcriptional regulation whereas pentoxifylline has a similar effect through inhibition of phosphodiesterase.

The use of either corticosteroids or pentoxifylline is recommended only for individuals with severe alcoholic hepatitis because these pharmaceuticals do not provide a benefit for patients with milder conditions. In addition, the improvement in short-term survival of alcoholic hepatitis patients as a result of these drugs is modest. A high percentage of patients do not respond to either treatment. Furthermore, a significant side effect of corticosteroids is an increased susceptibility to infections, resulting from immune suppression.

Given that the current medication is limited to treating the severe stage of ALD and has modest efficacy, new and better pharmacological options are needed for treating and minimizing complications in patients with severe alcoholic hepatitis and cirrhosis.

TNFα antagonist

TNFα antagonists were thought to be promising for treating alcoholic hepatitis patients for a number of reasons. Elevated TNFα has long been described as a predictor of alcoholic hepatitis disease severity. Animal studies have shown that hepatocytes from alcohol-fed animals are more susceptible to TNFα-induced cell death. Administration of anti-TNFα antibodies in alcohol-fed animals also reduces liver injury. However, trials with TNFα antibody have had mixed results. Mechanistically, TNFα antagonist treatment causes systemic TNFα deficiency and subsequent infections (10). It is debatable whether a lower dose of a TNFα antagonist might be beneficial in treating severe or mild alcoholic hepatitis. Given the similarity in side effects of TNFα antagonists to corticosteroids, which induce a worse outcome, it will be difficult to justify additional human trials with TNFα antagonists for the treatment of ALD.

Antioxidants

Many antioxidants, such as N-acetylcysteine, are effective in treating alcohol-fed animals against liver fat accumulation, inflammation, and injury (146). Besides a study showing a limited improvement of AH patient survival by S-adenosylmethionine for restoring cellular anti-oxidant glutathione (76), however, studies using such agents have not been consistently successful in treating ALD patients (120). Because ALD in animal models is mostly mild in comparison to human ALD in the clinic, it is possible oxidative stress plays a minor role in developing severe forms of ALD. Laboratory studies with antioxidant post-treatment of severe forms of liver injury in animal models should be performed to confirm the effectiveness of these supplements in reducing liver oxidative stress.

Microflora products

Probiotics have been successfully used in treating mild liver diseases in both animal models (88) and alcoholic patients (52). In addition, the use of probiotics improves neutrophil function in patients with severe liver disease, although mortality is not improved (117). The advantage, and also a challenge, with probiotics is the availability of a wide variety of supplements. Analysis of the microflora in human alcoholics with or without different types of probiotics may provide useful information when developing treatments. The use of probiotics has a number of advantages, including likely affordability and low adverse side effects. However, whether probiotics are beneficial in treating mild to severe alcoholic hepatitis remains to be examined.

The factors that activate STAT3 in hepatocytes

As demonstrated in alcohol-fed animal models, STAT3 activation in hepatocytes induced by the administration of IL-6 or IL-22 reduces liver fat accumulation, ameliorates hepatocellular damage, and promotes liver regeneration (38, 51, 144). However, the clinical application of IL-6 therapy in ALD may be halted because of the many side effects of IL-6 treatment, owing to the ubiquitous expression of IL-6 receptors and their gp130 signaling chain in a wide variety of cell types. In contrast, IL-22 receptor expression is restricted to epithelial cells, including hepatocytes; thus, IL-22 treatment may have limited side effects and may be a promising option for treating ALD because of its antioxidant, antiapoptotic, antisteatotic, proliferative, and antimicrobial effects (51).

A separate application for IL-6 and IL-22 is in treating donor livers *ex vivo* for transplantation, as has been suggested by a earlier study (121). Liver transplantation is by far the most effective last resort for ALD patients who fail to respond to existing treatments. Steatosis in donor livers, which is prevalent in many countries as a result of obesity, alcohol and inactivity, is an important risk factor for transplant failure. Pretreatment of donor livers *in vitro* with IL-6 or IL-22 might significantly improve the success rate of transplantation.

The factors that activate STAT3 in myeloid cells

Conditional deletion of STAT3 in myeloid cells markedly increased liver inflammation in a variety of liver injury models, including Con A-induced T cell hepatitis (58), CCl4-induced liver injury (39), and ethanol-induced liver injury (40), suggesting that activation of STAT3 in myeloid cells inhibits liver inflammation. Any factors that activate STAT3 in myeloid cells, such as IL-10, may have beneficial effects in inhibiting liver inflammation in ALD.

Adiponectin

Previous studies have established that adipocyte-derived adiponectin has an inflammation-suppression effect on many cell types, including monocytes and endothelial cells (96). In addition, this chemokine stimulates fatty acid oxidation. These two properties of adiponectin have generated a broad interest of its application in combating atherogenesis and obesity-induced diabetes. Chronic alcohol intake inhibits adiponectin secretion by subcutaneous adipocytes (13). Administration of adiponectin in alcohol-fed mice significantly reduces liver fat, inflammation, and injury (137). A recent study showed that the effect of adiponectin is mediated by a heme oxygenase-1-dependent induction of the anti-inflammatory cytokine IL-10 (70). In summary, adiponectin treatment may have multiple advantages for treating ALD, including ameliorating steatosis and inflammation. Because very high levels of adiponectin are found in the serum of ALD patients, the administration of adiponectin may not be effective in these patients. Current challenges involve finding a pharmacologically suitable drug to mimic adiponectin and/or its down-stream effectors.

IL-17 and IL-8

The failure of TNFα antagonists raises the possibility that antagonists for pro-inflammatory cytokines/chemokines that are more specific and less pleiotropic in global immune defense may prove to be more effective for treating alcoholic hepatitis. IL-17 and IL-8 fit such a description because of their roles in inducing neutrophil infiltration and in synergizing TNFα function. These targets should be tested in animal models and in humans with alcoholic hepatitis in the near future.

In summary, existing therapies for alcoholic hepatitis are limited by their modest efficacy and undesirable side effects on immune suppression. Mitigating the pathology of ALD by targeting aspects of the inflammatory machinery has resulted in success in animal models, but not in patients. In fact, global blocking of inflammatory activity as a treatment option has a clear drawback in compromising immune defense. Interventions targeting narrower and more specific activity, such as IL-8/IL-17/neutrophil infiltration, remain to be explored. Recent findings on the hepatoprotective cytokine IL-22 and its downstream signaling STAT3 also provide an opportunity for interventions that target ALD.

CONCLUSIONS AND FUTURE PERSPECTIVES

Acute and chronic inflammatory conditions have been closely linked to the pathogenesis of ALD. In the past decade, significant advances have been made in identifying the inducers of inflammation and in determining the specific role of inflammatory components in ALD development. These advances have a number of important implications for future research directions and for ALD intervention development.

First, the causes of alcohol-related liver inflammation are complex. Besides the well known gut flora derived LPS, multiple factors have been identified with the capability of inducing liver inflammatory responses. These include, among others, alcohol metabolites, enriched FFAs, necrotic cell products, and complements. Evidence thus far supports that these agents are highly interactive via common downstream intermediates such as ROS. Significantly, many of these inflammatory inducing agents and their downstream intermediates, in addition to their important roles in inflammatory response, are also known to be common causes of cell/tissue injury. Examples include FFA- (68) and ROS-induced cell death. These close links between alcohol-related inflammation and tissue injury strongly support the effort of targeting inflammatory process for ALD intervention.

Second, inflammation and its key components often have dual roles, i.e., pathological and protective functions. The importance of this reflection is underscored by the many cases discussed in this review and the observed side effect of therapies based on corticosteroids and TNFα antagonists. Therefore, when developing effective anti-inflammatory interventions, the challenge is to identify the components and pathways that are primarily pathogenic and/or to develop a strategy that can mitigate the pathogenic effect without significantly compromising the protective functions of these components.

Lastly, we still need to gain a better understanding of the cellular and molecular changes that occur during the development of ALD and, in particular, alcoholic hepatitis. With the significant expansion of knowledge/technology on new types of immune cells and mediators, a renewed study of the cellular and molecular changes that occur during disease development is critically needed; an example is the recently identified cytokine IL-17, which is known for its key role in autoimmunity. Increased IL-17 and IL-17-secreting cells are now known to be unique characteristics of ALD, among liver diseases of different etiologies (62). The classification of patients based on an expanded cytokine profile, for example, could be instrumental in selecting patients with a clear need for treatment. For example, studies in the

1990s indicated that the rise and fall of circulating IL-6 and IL-8 over time in alcoholic hepatitis patients is closely associated with non-survival (death) and recovery, respectively (30, 50, 115). Discoveries made from such renewed efforts will stimulate mechanistic studies and translational research for more effective interventions for ALD.

Acknowledgments

The authors wish to thank Dr. Kathy Jung for critical reading of the manuscript. This work was supported in part by the intramural program of NIAAA, NIH (B. Gao) and grants P20 AA017837, R01 AA016399, R37 AA011876 and R01 AA011975 (L. Nagy).

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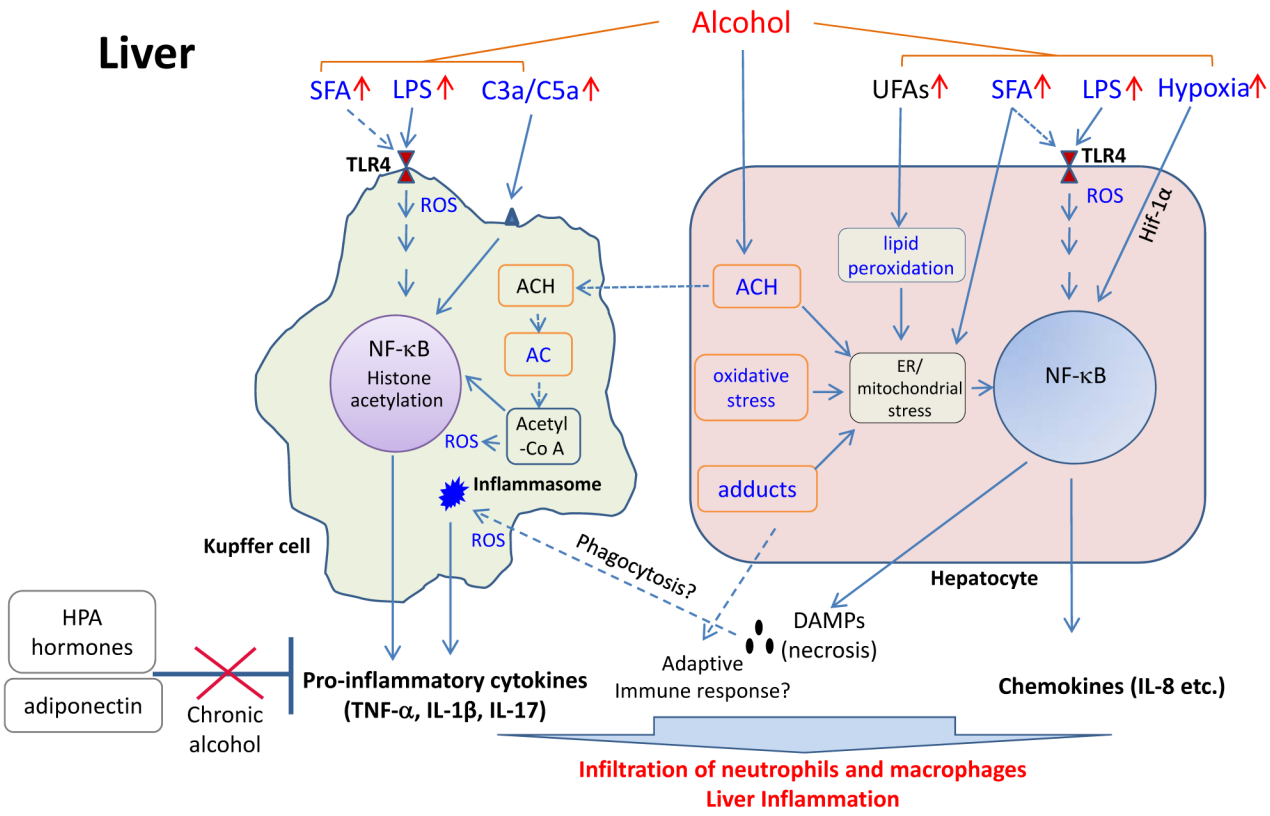

Fig. 1. Alcohol–induced inflammation in the liver

Emphasis is given to the contribution from hepatocytes and macrophages (Kupffer cells and likely infiltrating monocytes) in inducing proinflammatory mediators required for attracting immune cells (neutrophils, monocytes and lymphocytes) to the liver. Solid lines indicate data with strong evidences and dotted lines indicate data still needs confirmation, including adaptive immune cells. Contributions from stellate cells and endothelial cells to inflammation are not discussed. Terms and items in bright blue indicate immediate inducers for inflammatory response. Abbreviations: ACH, acetaldehyde; AC, acetate; DAMPs, damage associated molecular pattern molecules; HPA, Hypothalamic–pituitary–adrenal; Hif–1α, hypoxia–inducible factor 1 alpha; ROS, reactive oxygen species; C3/C5, complement factors 3a and 5a; SFA, free saturated fatty acid; UFA, unsaturated free fatty acids.

Table 1

Inflammation inducers associated with ALD.

	Sensors/early mediators	Reference
LPS	TLR4	(131, 140)
Alcohol & metabolite	TLR4; (alcohol metabolites): SIRT; HDAC and Acetyl-coA synthetase	(8, 9, 49, 114)
Saturated fatty acids	TLR4 , Inflammasome?	(19, 61, 135)
Unsaturated fatty acids	ROS	(112)
ROS	NF-κB; Sensitization of existing response	(146)
Apoptotic cells	C1q	(16) and reference within
Necrotic cells	Inflammasome NLD3 via ATP	(45, 63)
Hypoxia	HIF-1α via VHL (von Hippel-Lindau tumor suppressor gene product)	(90, 92)

Table 2

Genetic and functional studies of inflammatory components associated with ALD

	ALD Association[*1]	Knockout/antagonist phenotype	References
Pathogen/Danger Sensors			
TLR4	Increased expression on the neutrophils and in the liver in patients with alcoholic cirrhosis	TLR4KO (whole body or bone-marrow derived cells): reduced inflammation and injury in mice;	(44, 131, 140)
TLR2	Increased liver expression in alcohol-fed mice and in neutrophils of alcoholic cirrhotic patients	TLR2KO: No appreciable effect on inflammation and liver injury in alcohol-fed mice	(35, 41)
Cytokines/chemokines			
TNFα	Elevated in circulation and liver in alcoholics with ALD; elevated in alcohol-fed rodents	TNF-αKO and antagonists: reduced liver fat and injury in rodents; TNF-α antagonists: increased infections in AH patients	(10, 42)
TNFR1	Receptor for TNFα	TNFR1KO: reduced liver fat, inflammation, and hepatocyte necrosis	(141)
IL-6	Elevated in circulation and liver in alcoholics with or without liver disease;	IL-6KO: increased liver fat and injury; increased mitochondrial DNA damage;	(26, 144)
IL10	Reduced in chronic alcohol fed mice; Moderately to highly elevated in alcoholics with liver diseases	IL-10KO: [alcohol only] reduced liver fat but increased inflammation w/o increased injury; [alcohol+LPS]: increased liver	(36, 81, 117)
IL-8, IL-17	Elevated in alcoholics with liver disease	N/D	(62, 115)
HIF-1α	Induced in alcohol-fed animal model in response to hypoxia	Hepatocyte-specific HIF-1αKO: reduced liver fat, inflammation, and hepatocyte necrosis	(90)
Inflammatory Signaling pathways			
IRF3	(One of two major signal transducers for TLR4)	Global IRF3KO: reduced liver fat, inflammation and injury	(98, 145)
		IRF3 deficiency in parenchymal cells: aggravated liver fat, inflammation and injury	(98, 145)
		IRF3 deficiency in myeloid cells: reduced inflammation, but no change in liver injury	(98, 145)
STAT3	(downstream target for IL-6, 10, and 22) Activation at early phase in animal; low activation in AH patients	STAT3 deficiency in hepatocyte: increased steatosis & lipogenic gene expression	(40)
		STAT3 deficiency in macrophage/neutrophil or endothelial cells: increased inflammation and liver injury	(40, 82)
Immune cells			
Kupffer cells (macrophage)	(acute alcohol) Increased tolerance to LPS; (Chronic alcohol) Increased sensitization to LPS.	Depletion: reduced liver injury	(1)
Neutrophils	Increased liver infiltration in AH with high resting respiratory burst	Anti-PMN antibody attenuated alcohol- induced liver injury in rat	(6, 84)
monocytes	Increased spontaneous and LPS induced cytokine production	Knockout of monocyte chemoattractant protein-1 (MCP-1) attenuates alcohol- induced liver injury, steatosis and oxidative stress in mice	(73, 79)
Plasma components			
complement	Increases C1q, C3b liver deposition in alcohol fed mice; Low in alcoholics with cirrhosis	C1q KO: blocked an early inflammation and attenuated liver injury	(16)
		C5KO: reduced liver inflammation and injury with no effect on liver fat accumulation	(101)

	ALD Association[*1]	Knockout/antagonist phenotype	References
		C3, C3R, or C5RKO: reduced an early phase of inflammation and injury	(111)

Table 3

ALD interventions by targeting inflammation

Target	Outcome in animal models	Outcome in human patients	
General inflammation	N/A	Corticosteriods (inhibitor of cytokine production) improved the short term survival of severe AH patients; but not recommended for less severe AH patients due to increased infection;	(74, 75)
		Pentoxifylline (inhibitor of TNFα synthesis and has vasoactivity) improved the short term survival of AH patients;	(66) and references within
TNFα	Anti-TNFα antibody reduced liver pathology in mice	Anti-TNFα antibody increased AH patients' short term mortality by infections	(10)
ROS	Antioxidant N-acetal cysteine (NAC) reduced liver cell injury and inflammatory conditions	Antioxidant cocktail (alone or with corticosteroid) showed no benefit;	(22, 146)
	S-adenosyl-L-methionine (restoring alcohol depleted anti-oxidant gluthione) in alcohol-fed baboons attenuate liver injury	S-adenosyl-L-methionine supplement improved mortality in AH patients (after excluding patients with the most severe class Child C)	(64, 76)
Microflora product	Probiotic (Lactobacillus) significantly reduced liver pathology and circulating LPS; Lactobacillus GG ameliorates alcohol-induced gut leakiness and liver injury; Non-absorbable antibiotics reduced liver injury	Probiotics: 1) reduced liver injury in alcoholics with pycholoysis; 2) improved neutrophil phagocytic capacity and ex vivo LPS stimulated cytokine production from whole blood from patients with compensated alcoholic cirrhosis. Non-absorbable antibiotics moderately improve ALD patients with encephalopathy	(2, 52, 88, 117)
STAT3	Exogenous IL22 reduced liver fat accumulation and injury	N/D	(51)
Adiponectin	Exogenous adiponection reduced liver fat, inflammation, and injury	N/D	(137)