Alcoholic Liver Disease

22

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Key Points

- Alcoholic liver disease is the liver manifestation of the end-organ effects of chronic excessive alcohol intake.
- The effects of alcohol on gut integrity and the adipose tissue contribute to the development of ALD.
- Alcohol and its metabolites have some direct effects on the liver and reactive oxygen radicals generated during alcohol metabolism modulate functions of hepatocytes and other cell types in the liver.
- Activation of the innate immune system is a major component in the development and progression of alcoholic liver disease.
- Gut-derived and endogenous danger signals contribute to innate immune activation in ALD.
- Acute alcoholic hepatitis is mediated by pro-inflammatory cytokines.
- Understanding specific molecular mechanisms involved in ALD may guide development of new therapeutic interventions.

Introduction

This chapter focuses on the immune-mediated aspects of the pathogenesis of alcoholic liver disease (ALD). Within the frame of the effects of alcohol on the liver and organ interactions, we discuss the cellular effects of alcohol and its metabolites, innate and adaptive immune responses, intracellular signaling pathways, and nuclear receptors. Current and emerging therapeutic approaches are discussed as potential translation of the basic findings in ALD to clinical applications.

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Clinical Characteristics of Alcoholic Liver Disease

Epidemiology and Natural History of ALD

It is estimated that there are 17.6 million alcoholic individuals in the USA and 140 million worldwide; while not all alcoholics develop symptomatic liver disease, about 12,109 deaths/year are attributed to ALD in the USA [1, 2]. The clinical spectrum of ALD includes liver steatosis, steatohepatitis, steatohepatitis with fibrosis, and cirrhosis that increases the risk for the development of hepatocellular cancer (HCC) [3]. Heavy alcohol consumption, including binge drinking, leads to liver steatosis in over 90 % of individuals, and fat deposition resolves after cessation of alcohol use in the absence of advanced liver disease (Fig. 22.1). Persistent heavy alcohol use leads to liver steatosis with inflammation and sets the stage for progressive liver disease. Inflammation triggers fibrosis, a deposition of extracellular matrix and collagen that over time leads to irreversible cirrhosis [1, 3, 4]. Continued alcohol intake is the most important risk factor for progression of ALD [2, 4, 5]. Cirrhosis, decompensated liver disease, and HCC can be life threatening, and liver transplantation is not typically offered to individuals with ongoing active alcohol use in most transport centers (in the USA) [6].

Clinical Findings and Diagnosis of ALD

Clinically, most patients with persistent alcohol use have nonspecific symptoms that may include nausea, vomiting, diarrhea, or hepatomegaly [2–4]. Typical laboratory findings in ALD often show increased transaminases (transaminases rarely increase above 300 mg/dL) with an aspartate aminotransferase/alanine aminotransferase (AST/ALT) ratio >1. Serum bilirubin and alkaline phosphatase are often elevated and indicate more severe forms of ALD. In patients with severe forms of ALD, impaired liver synthetic function is indicated by abnormal prothrombin time (PT/INR),

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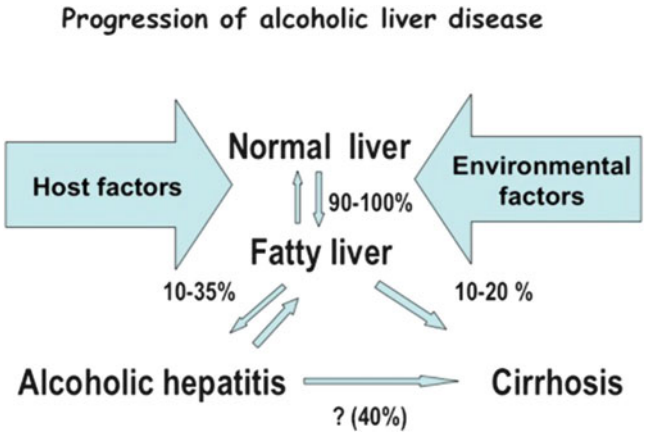

Fig. 22.1 Progression of alcoholic liver disease (ALD). Percentages represent the proportion of alcoholic individuals who will develop liver disease

decreased serum albumin, and thrombocytopenia [2–4]. Patients often have increased circulating white cell count (CBC). This does not necessarily indicate infection as it could be simply a manifestation of recruitment of immune cells from the bone marrow to the liver in response to the massive pro-inflammatory cytokine activation.

Different scoring systems are in use to establish the severity of ALD. The Maddrey discriminant factor >32 is the usual cutoff for defining severe alcoholic hepatitis. More recently, Model of end-stage liver disease (MELD) score >21 has been introduced as a cutoff for severe alcoholic hepatitis. The advantage of the MELD score is that it eliminates the variability of prothrombin time (PT) measurements that could vary between different diagnostic laboratories.

Acute Alcoholic Hepatitis

Acute alcoholic hepatitis (AAH) is the most severe form of ALD. It is a state of hepatic and systemic pro-inflammatory cascade activation with hepatocyte/liver dysfunction. Molecular mechanisms and biomarkers that trigger the development of AAH from stable ALD are yet to be delineated. Previous studies identified tumor necrosis factor (TNF) as a central mediator of ALD [7–11]. TNF-α was increased both in the serum and liver in human alcoholic hepatitis [7, 8, 12–14].

Patients with severe AAH have a high mortality and often develop jaundice, portal hypertension, and other signs of hepatic decompensation. While many cases of AAH manifest as acute on chronic liver injury, portal hypertension develops even in the absence of cirrhosis as a result of sinusoidal congestion in the inflamed liver [3]. The clinical course of AAH is often complicated with upper GI bleeding, ascites, peripheral edema, and renal insufficiency. Systemic infections or SBP are other complicating factors often emerging in patients with ALD. Renal failure and hepatorenal syndrome in AAH carry high mortality [3]. Alcohol withdrawal and its physical and behavioral symptoms provide additional challenges in the clinical management of these patients.

Pathogenesis of ALD

Multiple key elements have been identified in the pathogenesis of ALD that include but are not limited to direct effects of alcohol and its metabolites on liver cells, alcohol-induced mitochondrial damage, production of reactive oxygen species (ROS), and induction of pro-inflammatory cytokines.

Organ Interactions in ALD

Alcohol affects virtually all organs in the body and it is increasingly evident that alcohol-induced changes in one organ can affect the function of other organs. Experimental evidence suggests a cross talk between the liver and intestine as well as the liver and adipose tissue in ALD [15, 16].

Gut–Liver Axis in ALD

Increasing evidence suggests that interactions between the liver and gut contribute to the development of ALD. In normal homeostasis, a balance is maintained between the gut microbiome, gut permeability, and translocation of gut-derived substances that reach the liver via the portal circulation summarized in [15, 17, 18]. The liver, as an immune organ, contains sensitive receptor systems on all of its cell types that trigger responses to pathogen-derived signals from the gut. Lipopolysaccharide (LPS), a component of Gram-negative bacteria, is present at increased levels in the portal and systemic circulation in humans and in animals after excessive alcohol intake [17, 19, 20]. The central role of LPS has been demonstrated by several studies in animal models of ALD [19, 21–23]. Increased serum levels of peptidoglycan were found in mice after chronic alcohol administration suggesting that components of Gram-positive microbes may also increase in the serum after prolonged alcohol use [24]. These effects of alcohol have been attributed to changes in intestinal permeability. Indeed, chronic alcohol exposure increases gut permeability by reducing epithelial cell barrier functions [20, 25]. Specifically, in vitro alcohol treatment of colonic epithelial cells decreases the expression of tight junction proteins such as zona occludin-1 (ZO-1) and the expression of the antimicrobial peptide, Reg3b [25]. Mechanistically, alcohol-induced ROS contributes to increased expression of microRNA-221 that in turn downregulates ZO-1 protein levels in intestinal epithelial cells [25].

In addition to the direct effects of alcohol on gut epithelium, alcohol consumption results in changes in the gut microbiome. Animal studies have revealed that there are quantitative and qualitative changes in the gut microbiome after prolonged alcohol feeding [26]. Specifically, there was a significant increase in the amount of bacteria in the cecum of alcohol-fed mice compared to controls [26]. Furthermore, the composition of the bacterial species has changed after alcohol treatment where the relative proportions of Firmicutes have increased at the expense of Bifidobacteria in alcohol-fed mice [26]. The specific role of these changes in the pathogenesis of ALD remains unclear; however, previous studies elegantly demonstrated that "sterilization" of the gut with nonabsorbable antibiotics has a significant protective effect on alcohol-induced steatosis and inflammation in animal models of ALD [21].

Liver and Adipose Interactions

The role of adipose tissue-derived adipokines, including adiponectin, has been highlighted in ALD [16]. Adiponectin contributes to the development of fatty liver and it also has pro-inflammatory effects. In animal models alcohol decreases gene expression and secretion of adiponectin in adipose tissues [27]. In vitro experiments revealed that alcohol decreases the activity of the mouse adiponectin promoter and decreases adiponectin secretion in differentiated adipocytes. Adiponectin exerts its biological effects through the adiponectin receptors 1 and 2. In mice AdipoR2 is downregulated in the human liver and decreased AdipoR1 was found in micropigs after chronic alcohol feeding [16].

Fat metabolism is also regulated by osteopontin, which is increased in the adipose tissue, liver, and serum of patients with fibrosis induced by chronic alcohol use [28]. Osteopontin has been suggested as a marker of liver disease progression [29–31].

The Effects of Alcohol, Metabolites, Reactive Oxygen Species, and Oxidative Stress

Alcohol Metabolism

Alcohol is metabolized by alcohol dehydrogenase (ADH) into acetaldehyde which is further metabolized into acetate by aldehyde dehydrogenase (ALDH) [32]. Acetaldehyde and acetate are short-lived and have high tissue toxicity; thus, many of the direct tissue effects of alcohol have been attributed to these metabolites (Fig. 22.2). Both of ADH and ALDH enzymes have limited capacity due to their low Michaelis constant. Thus, higher tissue concentration of alcohol is broken down by alternate enzyme systems including cytochrome P450 2E1 (CYP2E1) and microsomal enzymes that are upregulated in chronic alcohol use. Their by-products are ROS that contribute to direct cellular oxidative stress in hepatocytes and immune cells [33–35]. Alcohol metabolism results in increase in NADH/NAD+ ratio in the cytoplasm and mitochondria of hepatocytes [33, 36]. The increased NADP inhibits mitochondrial β oxidation and accumulation of lipids in hepatocytes [33].

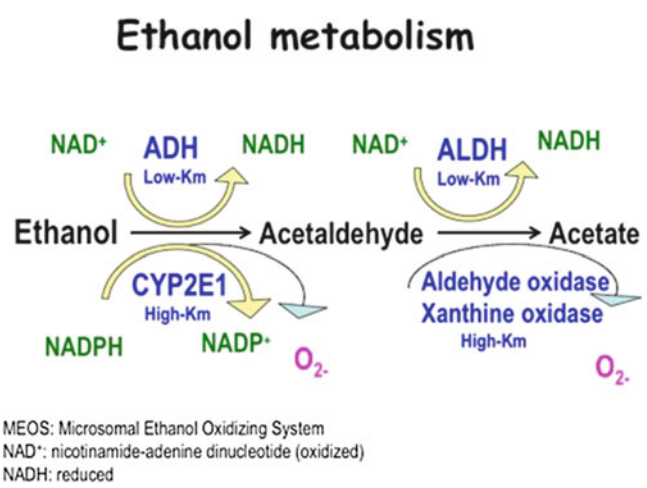

Fig. 22.2 Ethanol metabolism. The enzymes and intermediates of alcohol metabolism

CYP2E1 is an effective generator of ROS such as the superoxide anion radical and hydrogen peroxide and, in the presence of iron catalysts, produces powerful oxidants such as the hydroxyl radical. The role of CYP2E1 in hepatocyte damage in ALD has been established using elegant in vitro cell models and animal models [33, 37].

Reactive Oxygen Species and Mitochondrial Stress in ALD

In addition to ROS associated with direct alcohol metabolism, alcohol also increases mitochondrial oxidative stress [10]. Alcohol leads to alteration in mitochondrial membrane permeability and transition potential and contributes to apoptosis, release of cytochrome c, and caspase-3 activation [33, 38]. ROS also damages mitochondrial DNA and ribosomes.

The NADPH oxidase complex, involving various Nox proteins p47phox and p40, plays a role in ROS generation both in immune and parenchymal cells in the liver [39]. NADPH oxidases are activated in ALD in immune as well as in liver parenchymal cells [40, 41]. NADPH p47phox was shown to contribute to Kupffer cell activation in ALD [40, 42, 43].

Endoplasmic Reticulum (ER) Stress

The unfolded protein response also referred to as ER stress is a protective cellular mechanism that is disturbed by alcohol [44, 45]. Alcohol consumption results in increased expression of key components of the unfolded protein response including glucose regulatory proteins (GRP78, GRP 94, CHOP, and caspase-12) [46]. Intracellular glutathione levels

are depleted by chronic alcohol use and ER stress contributes to increased homocysteine levels [46, 47]. Upregulation of transcription factors SREBP-1c and SREBP-2 is associated with lipid accumulation.

Decreased Antioxidants

While alcohol increases ROS, it also reduces the availability of most antioxidant systems, thereby promoting oxidative stress and ROS-induced liver damage. Alcohol-fed mice had decreased expression of the antioxidant, superoxide dismutase (SOD) [44]. Glutathione sulfhydryl (GSH) and glutathione-S-transferase (GST) activity are also decreased in ALD [46].

Innate and Adaptive Immune Responses

The liver is a major immune organ that contains all cell types of the immune system. In ALD, there is evidence for recruitment of immune cells to the liver including cell populations of neutrophil leukocytes, monocytes, macrophages, T cells, and B cells [48, 49]. Other key aspects in the evaluation of immune responses in the liver are the interactions between the different immune cell types, including cross talk between liver parenchymal cells and immune cells. It is important to consider that the normal liver has an immunotolerant tissue environment that is profoundly changed in ALD where a state of pro-inflammatory cell and cytokine activation prevails and disturbs parenchymal cell functions in the liver [50]. The pathomechanism of ALD involves complex interactions between the effects of alcohol and its toxic metabolites on various cell types in the liver and gut, induction of ROS, and upregulation of the inflammatory cascade [8, 10, 35, 51–53]. Studies using antibiotics to "sterilize" the gut and experiments with elimination of Kupffer cells (KC) identified both gut-derived factors, such as LPS, and Kupffer cell activation as central components in ALD (Fig. 22.3) [15, 17, 20, 21, 25, 42, 54–56]. Chronic alcohol sensitizes macrophages to LPS-induced inflammatory cytokine production [57, 58].

Role of Innate Immunity

The innate immune system is the first line of defense in recognition and response to danger signals in the liver [52]. Innate immune cells and signaling pathways recognize exogenous danger signals such as pathogen-derived molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs) that are released from stressed, injured of dying cells [59–63]. The slow blood flow in the liver sinusoids and the proximity of liver parenchymal cells and immune cell in the liver sinusoids allow ample interactions between danger signals, immune cells, and parenchymal cells during the different states of ALD. Both soluble mediators and different cell types of the innate system contribute to the liver and systemic inflammation that characterizes ALD and particularly AAH. Overexpression of pro-inflammatory cytokines and chemokines (TNF-α, interleukin (IL)-1α, IL-1β, MCP-1, IL-8) and decreased levels of anti-inflammatory mediators (IL-10) in AH represent dysregulation of innate immunity [23, 48, 51, 64, 65].

Soluble Mediators

Complement

Complement and complement activation are involved in the development of ALD. Specifically, C1q, the recognition subunit of the first complement component, binds to apoptotic cells.

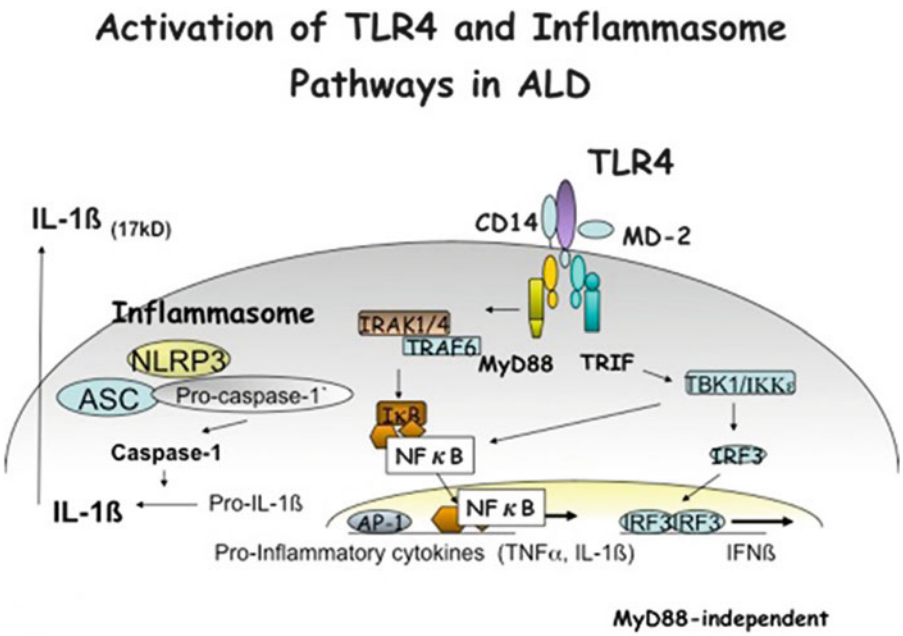

Fig. 22.3 Activation of TLR4 and inflammasomes in ALD. Pattern recognition receptors (PRRs) are activated by danger signals, resulting in the production of inflammatory cytokines

A recent study indicated that ethanol activates the classical complement pathway via C1q binding to apoptotic cells in the liver and thereby plays a role in the early stages of ALD [48, 53, 66].

Chemokines

Monocyte chemoattractant protein (MCP)-1, a CXC chemokine, contributes to recruitment of monocytes and macrophages to the liver in ALD [53, 67, 68]. Monocyte production of MCP-1 is increased in AAH [69]. MCP-1 also has direct effects on hepatocytes as it induces lipid accumulation [49]. It has been proposed that MCP-1 exerts its lipogenic effect via induction of the hypoxia-inducible factor-1 (HIF-1) in hepatocytes [70]. In a recent study, total body deficiency in MCP-1 in mice resulted in attenuation of alcohol-induced liver steatosis and inflammation [68]. It has been proposed that MCP-1 modulated PAPR-γ activity in hepatocytes as a mechanism for lipid accumulation in hepatocytes [68].

IL-8 is involved in many steps of neutrophil recruitment and activation. Increased levels of IL-8 were found in patients with alcoholic hepatitis while IL-8 was only moderately increased in patients with alcoholic cirrhosis [71].

Cytokines

The critical role of pro-inflammatory cytokines has been validated by several studies in ALD [51, 53, 72]. Pro-inflammatory cytokines not only mediate the pathogenesis of ALD but also account for many of the clinical symptoms in these patients. TNF-α has been identified as a central mediator of ALD [8, 9, 73, 74]. There is evidence for increased circulating and liver levels of TNF-α, IL-6, IL-8, and IL-1 [7, 9, 12–14]. Isolated monocytes from patients with alcoholic hepatitis produce increased levels of these pro-inflammatory cytokines [8, 9, 75]. In animal models, increased gene expression and liver and circulating protein levels of TNF-α, IL-1β, MCP-1, and IL-6 were found in several studies [49, 58, 67, 68]. In the liver, Kupffer cells have been identified as the major source of the pro-inflammatory cytokine production [23, 48, 54]. The mechanistic role of pro-inflammatory cytokines is suggested by experiments that featured cytokine knockout mice and found that deficiency either in TNF receptor 1 (TNFR1), MCP-1, or IL-1 receptor (IL-1R) ameliorated ALD [49, 68]. Furthermore, administration of recombinant IL-1R antagonist, that prevents the biological effects of IL-1β and IL-1α on the IL-1R, attenuated the development of ALD in a mouse model [49]. These observations indicate that pro-inflammatory cytokine production is upregulated at multiple levels in ALD and that there is a positive amplification loop between these cytokines to perpetuate inflammation.

In addition to fueling inflammation, TNF-α, IL-1, and IL-6 have important effects on hepatocytes that contribute to the pathogenesis of ALD [58, 67]. By engaging its receptors on normal hepatocytes, TNF-α does not induce apoptosis. In injured hepatocytes that are present in the alcohol-exposed liver, TNF-α can trigger the death pathway [76]. The role of TNF-α is more complex, however, as it is also involved in liver regeneration that is a major element in compensation in liver homeostasis in the alcohol-exposed organ [58].

IL-1β is an endogenous pyrogen, an inducer of other pro-inflammatory mediators [77]. It also has direct effects on hepatocytes by inducing steatosis [49]. Furthermore, IL-1β sensitizes hepatocytes to the killing effect of TNF-α, thereby fueling a synergistic effect between pro-inflammatory cytokines on hepatocyte injury [49].

IL-6 also promotes fat accumulation in hepatocytes and, most importantly, has protective effects on the liver in steatohepatitis including ALD [59].

IL-22, a member of the IL-10 family, was shown to have hepatoprotective effects in ALD. IL-22 is produced by Th17 T and natural killer (NK) cells, and its levels were diminished in the liver after chronic alcohol feeding [78]. Furthermore, administration of recombinant IL-22 resulted in hepatoprotection in an acute alcohol binge drinking model, and the protective effects of IL-22 were attributed to STAT3 activation in the hepatocytes [65, 79].

Immune Cells

Neutrophil Leukocytes

In human ALD, the histopathological pattern of alcoholic hepatitis includes infiltration of neutrophil leukocytes, hepatocyte degeneration ballooning, and oncotic necrosis [31, 80]. Induction of chemokines (IL-8, cytokine-induced neutrophil chemoattractant (CINC)) and cytokines in addition to apoptosis of hepatocytes has been suggested as a mechanism for neutrophil infiltration [81].

A recent study demonstrated a correlation between neutrophil recruitment and the presence of IL-17 producing T-helper cells within the inflammatory liver infiltrates in patients after alcohol-induced liver intoxication [82]. They found that ALD patients showed a significant increase in both IL-17 plasma titers and frequency of IL-17$^+$ T cells and displayed a correlation between liver infiltration of neutrophils and Th17 cells. Furthermore, they found that Th17 cells produced IL-8 as well as GRO-α and that these factors were both necessary and sufficient to induce recruitment of neutrophils [82].

Kupffer Cells, Macrophages, and Monocytes

A central role has been suggested for Kupffer cells (KC) in ALD. KCs are liver resident macrophages that express surface markers of F4/80 and are enriched in livers of chronic alcoholics and alcohol-fed mice [49, 83]. There is an increase in the number of F4/80 cells that most likely represent KCs and/or newly recruited macrophages. Blood monocytes are activated in ALD and produce cytokines [75].

The tremendous plasticity in the phenotype of macrophages has recently been recognized. Depending on the tissue environment, danger signals, and cytokine milieu, blood monocytes differentiate into M1 or M2 macrophages or similar phenotypes. M1 macrophages are "classically" activated by LPS, IFN-γ, or pro-inflammatory cytokines and have high phagocytic activity while M2 macrophage differentiation is triggered by IL-4, IL-10, TGF-β, or adiponectin [84, 85]. M2 macrophages are "alternately activated" macrophages and express CD206, CD163, as well as arginase-1 [86, 87]. The role of the M1 and M2 macrophages in ALD is yet to be explored.

Most investigations focused on KCs have found that KCs isolated from ALD are in vivo "sensitized" to stimulation with LPS to produce increased amounts of TNF-α [88]. This has been linked to increased expression of NF-κB, ERK, and MAPK pathways [48, 89–91]. In vivo studies elegantly demonstrated that elimination of KC by gadolinium chloride in rats or clodronate in mice attenuated alcohol-induced liver injury [54, 92]. Recent studies using bone marrow transplantation corroborated the early findings to demonstrate the critical role of bone marrow-derived inflammatory and Kupffer cells in ALD. For example, while mice deficient in caspase-1 or IRF3, molecules that mediate IL-1β and TNF-α, respectively, are protected from ALD [19, 49], alcohol feeding after transplantation of these mice with wild-type bone marrow resulted in steatosis, liver damage, and inflammation [19].

Human studies from patients with ALD demonstrated increased production of monocyte IL-1β, TNF-α, and IL-6 [8, 9]. Furthermore, NF-κB activation was also observed in circulating monocytes from patients with ALD [8, 9, 75].

Dendritic Cells

Dysfunction of dendritic cells (DCs) including their antigen presentation capacity in inducing antigen-specific T cell activation, immunomodulatory cytokines (IL-12) production, and expression of co-stimulatory molecules is altered by acute and chronic alcohol use [50, 52, 93]. The composition of the dendritic cell population was changed in the liver in mice after alcohol administration, and DC functions were also altered in favor of an immature DC phenotype that is characterized by reduced antigen presentation capacity [50].

Adaptive Immunity

It has been shown that T cell, NK cell, and B cell functions are altered by chronic alcohol use [48, 53, 67]. In the liver, there is enrichment of T lymphocytes although their specific role to the local tissue pathology is less clear. In ALD, the formation of protein adducts was shown as a result of ROS-induced modification. Reactive acetaldehyde, malondialdehyde (MDA), and 4-hydroxy-2-nonenal (HNE) can bind to proteins to form adducts [94]. These adducts are recognized by KCs, endothelial cells, and stellate cells in the liver via the scavenger receptor and induce cytokines [94]. In addition, protein adducts elicit antibody responses, in response to protein adducts [94, 95].

Table 22.1 Potential danger signals activating innate immune responses in alcoholic liver disease

Danger signal	Sensor/receptor	Mediators
Exogenous danger signals		
LPS	TLR4	Inflammatory cytokine
	TLR2	Inflammatory cytokine
Endogenous danger signals		
Saturated fatty acids	TLR4, inflammasome	IL-1, inflammatory cytokine
Unsaturated fatty acids		
ROS		NF-κB, SIRT1
Apoptotic cells	Inflammasome	CIg
Necrotic cells (ATP?)	Inflammasome	
Hypoxia		HIF1α

Signaling Pathways

Pattern Recognition Receptors

Innate immune responses are triggered by danger signals from pathogens or injured self through recognition by pattern recognition receptors (PRRs) (Table 22.1). The major families of PRRs in the liver are Toll-like receptors (TLRs), RIG-I-like RNA helicase receptors (RLHs), and NOD-like receptors (NLRs) [59, 60, 63, 96, 97]. Ample evidence demonstrates that activation of TLRs and NLRs is a pivotal element in the pathogenesis of ALD (Fig. 22.4). While most studies focus on the role of LPS as a trigger of innate immune activation, the role of other pathogen-derived or endogenous danger signals remains to be evaluated.

TLRs

Recent advances in the understanding of ALD show the contribution of the different members of these receptors. Of the 13 TLRs, TLRs 1–6 are expressed on the cell surface recognize extracellular PAMPs, while intracellularly localized TLRs (TLR3, 7, 8, 9) sense nucleic acid sequences [59, 62, 63, 98]. The cytoplasmic TIR domain of TLRs interacts with the TIR domain of adapter molecules such as the My88, the common adapter utilized by all TLRs except for TLR3, or TRIF that is involved in TLR3 and TLR4 signaling. MyD88 recruitment triggers downstream signaling via IRAK1/4 kinases and leads to NF-κB activation and induction of pro-inflammatory cytokine genes reviewed in [63, 99, 100]. The TRIF adapter activates IKKε/TBK leading to IRF3 or IRF7 phosphorylation and Type I Interferon (IFN) induction. TLR4 recognizes endotoxin derived from Gram-negative bacteria, TLR2 senses microbial lipopeptides, while TLR1 and TLR6 combined with

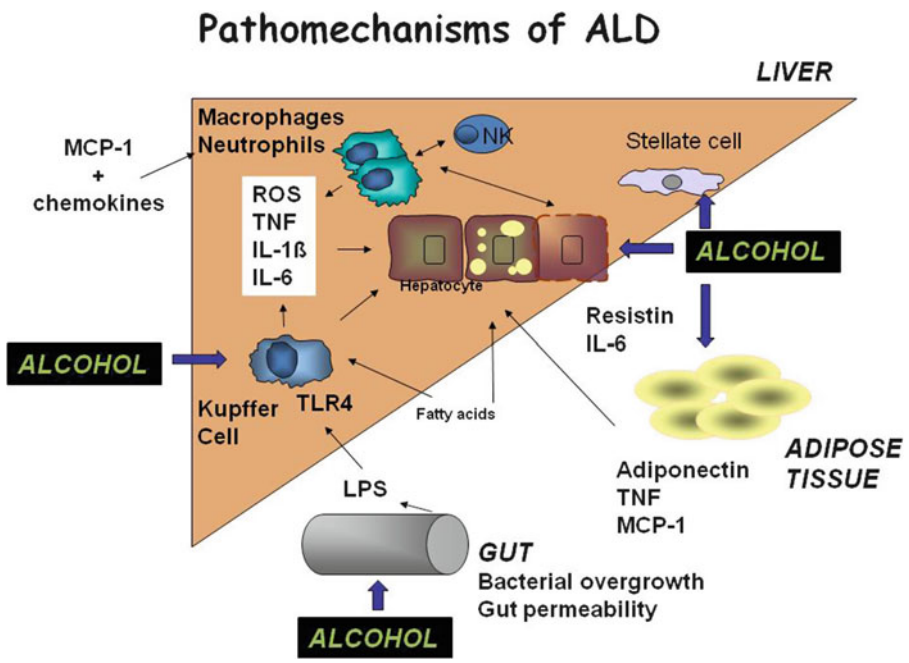

Fig. 22.4 Pathomechanisms of ALD. Both hepatic and immune-derived cells are involved in the pathogenesis of ALD. Mediators include cytokines, chemokines, and reactive oxygen species. Cell types are shown in blue, whereas extracellular mediators are shown in black

TLR2 distinguish between triacyl- and diacyl-lipopeptides. TLR3 recognizes viral double-stranded RNA, and the bacterial flagellin stimulates TLR5. TLR7 and TLR8 are sensors of single-stranded RNA (Nan, Campoy, and Bird 1997, 471–481) and TLR9 recognizes CpG-rich DNA reviewed in [63, 99, 100]. All TLRs are broadly expressed in the liver in different cell populations across immune and parenchymal cells [63].

TLR4, the receptor that senses LPS, plays a central role in ALD. TLR4 recognition of LPS is facilitated by the co-receptors CD14 and MD-2. CD14, a GPI-anchored protein, facilitates the transfer of LPS to the TLR4/MD-2 receptor complex that modulates LPS recognition [96]. MD-2 associates with TLR4 and binds LPS directly to form a complex with LPS in the absence of TLRs. The association between LPS and CD14 can be further facilitated by LPS-binding protein (LBP) [96].

Studies in animal models demonstrated that mutation in TLR4 or deficiency (knockout) of TLR4 attenuated alcohol-induced liver steatosis, inflammation, and injury [22, 49]. The TLR4 receptor complex includes the TLR4 co-receptors CD14 and MD2 that contribute to alcohol-related liver damage [101]. Ligand engagement of TLR4 triggers rapid downstream signaling by recruitment of the adaptor molecules, MyD88 or TRIF. MyD88 recruitment leads to IRAK-1/4 activation and phosphorylation that triggers downstream activation of the inhibitory kinase (IKK) complex and NF-κB activation [98]. NF-κB activation has been shown in ALD. NF-κB has a complex role in ALD, including protecting hepatocytes from apoptosis and pro-inflammatory cytokine activation in Kupffer and immune cells [51, 53]. Nuclear translocation of the NF-κB p65/p50 dimer in immune cells correlates with pro-inflammatory cytokine induction in ALD [51]. Recruitment of the TRIF adapter to TLR4 triggers downstream activation of the TBK/IKKε complex that phosphorylates IRF3 leading to IRF3 nuclear translocation and induction of Type I IFNs. Recent studies evaluated the involvement of TLR4, MyD88, and IRF3 in a mouse model of ALD and found that TLR4 and IRF3 were critical in the development of liver steatosis, inflammation, and liver damage after chronic alcohol feeding in mice [19, 22, 102]. Bone marrow chimera experiments revealed a cell-specific role for IRF3. Specifically, the absence of IRF3 in bone marrow-derived cells resulted in protection from alcohol-induced steatosis, inflammation, and liver damage. Conversely, IRF3 deficiency in the liver parenchymal cells promoted alcohol-induced liver injury [19].

NOD-Like Receptors and the Inflammasome

Inflammasomes are multiprotein complexes that include NLR sensors, adapter molecules, and pro-caspase-1 that cleave pro-caspase-1 into active caspase-1 upon ligand engagement [97]. Caspase-1 activation results in cleavage of pro-IL-1β, pro-IL-18, or IL-33 into a biologically active IL-1β (17 kD), IL-18, or cleaved IL-33 [103]. The family of

NLR is characterized by the presence of a central nucleotide-binding and oligomerization (NACHT) domain, which is flanked by C-terminal leucine-rich repeats (LRRs) and N-terminal caspase recruitment domain (CARD) or Pyrin (PYR) domains [97, 103]. NLRs function as receptors with ligand sensing in the LRRs region, whereas the CARD and PYR domains provide protein–protein interactions for downstream signaling. Based on their domain structures, the NLR family consists of subfamilies including NODs (NOD1-9), NLRPs (NLRP1-14, also called NALPs), IPAF (IPAF or NLRC4 and NAIP), and AIM2. The AIM2 inflammasome is not a formal member of the NLRs but like NLRs is composed of ASC and caspase-1 leading to IL-1β activation [104]. These NLRs all lead to caspase-1 activation and IL-1β cleavage while their ligand activation is unique.

Previous reports document increased serum IL-1β as a feature of human ALD [77]. Indeed, Il-1β levels are also increased in a mouse model of ALD while IL-1α, which is mostly cell-associated, is not elevated. Recent investigations revealed that IL-1β increase in ALD is due to inflammasome activation as caspase-1-deficient mice had significantly attenuated alcoholic liver steatosis, inflammation, and liver damage [49]. Interestingly, interruption of inflammasome activation prevented alcohol-induced increase in MCP-1 and TNF-α, suggesting amplification between these pro-inflammatory cytokines [49].

Nuclear Receptors

Most nuclear receptors that have received attention in ALD are involved in regulation of both lipid metabolism and inflammation [105]. Hypoxia has been shown to play a role in the pathogenesis of ALD [64]. Hypoxia-inducible factor-1 (HIF-1α) messenger RNA was increased in livers of chronic alcoholics and in mice after chronic alcohol administration [70]. Alcohol-induced steatosis was mediated by HIF-1α, and involvement of HIF-1α activation was found in both hepatocytes and liver immune cells [70].

Retinoid X receptor (RXR) was found to modulate alcohol metabolism by affecting ADH expression. Blood ethanol levels in hepatocyte-specific RXRα-KO mice were significantly lower than in wild-type controls, and the same mice had significantly increased liver damage and more pronounced liver steatosis [106–109].

PPAR-α is responsible for regulation of lipid metabolism. Decrease in PPAR-α was linked to liver steatosis after alcohol feeding and PPAR-α agonist treatment ameliorated ALD in mice [61, 110]. Likewise, PPAR-γ is also regulated in chronic alcohol exposure in KCs and hepatocytes. Treatment with the PPAR-γ agonist pioglitazone prevented the development of alcohol-induced steatosis and inflammation [111]. SREBP contributes to lipophilic pathway in ALD [112].

MicroRNAs in ALD

MicroRNAs (miRNAs) are a class of evolutionarily conserved, single-stranded, noncoding RNAs of 19–24 nucleotides that control gene expression at the posttranscriptional levels [113]. MicroRNAs contribute to the regulation of liver parenchymal and immune cells [114]. The expression and potentially the function of many miRNAs are changed in ALD in mice [114, 115]. MicroRNAs also regulate stem cell differentiation, regeneration, and cell death [116]. Innate immune responses are fine-tuned by miR-155, miR-125b, and miR-146a as these miRNAs positively or negatively regulate target genes/proteins in the family of TLR signaling, NF-κB, ERK, and MAPK inflammatory intracellular signaling pathways [117]. MiR-155 positively regulates TNF-α through enhancing its translation [114, 118]. One of the important effects of alcohol is sensitization of KCs to LPS-induced TNF-α production [8]. It has recently been shown that miR-155 levels are increased in the liver after chronic alcohol feeding and that alcohol-induced upregulation of miR-155 is a major molecular mechanism for LPS sensitization in mice [119]. Increased miR-155 expression was particularly prominent in Kupffer cells after chronic alcohol administration and it had a causative role in increased TNF-α production by KCs [119].

Alcohol-induced liver steatosis has also been linked to alterations in miRNA expression. For example, miR-122, which regulates many targets in lipid metabolism, is decreased in the liver in ALD while miRNA-217 was shown to promote ethanol-induced fat accumulation in hepatocytes [120]. Epigenetic regulation of miR-34 has recently been linked to miR-34 expression and fibrosis in ALD [121].

MicroRNAs are present in the circulation and are stable in the serum and plasma, making them attractive targets in biomarker discovery [114, 122]. For example, mir-122 represents 80 % of the total liver miRNAs and is abundantly expressed in hepatocytes where it regulates fat metabolism [123]. Recent reports demonstrated that circulating miR-122 is increased in different forms of liver injury, and in a mouse model of ALD, increased circulating miRNA-122 correlated with reduced levels of miR-122 in the liver [11]. The utility of circulating miRNAs as biomarkers in AAH and ALD is an area of active research [124–126].

Treatment for Alcoholic Liver Disease

Abstinence

Cessation of alcohol intake is the first-line intervention in patients with alcoholic hepatitis [127]. This fully depends on the patient's motivation and often requires participation in detox programs and a supportive domestic environment.

Steatosis and early steatohepatitis are reversible, while cirrhosis may not regress after discontinuation of alcohol use.

Current Medical Treatment

Alcoholic hepatitis (AH), the most severe form of ALD, has high morbidity and limited treatment options [128]. While corticosteroid treatment improves short-term survival, it increases the risk of infections [129]. The standard of care is prednisolone 40 mg daily for 28 days. A recent study demonstrated that using the Lille score at day 7 of steroid treatment, patients can be stratified to those who respond to therapy where continued treatment has benefits in contrast to those who show no decrease in serum bilirubin after 7 days of prednisone treatment [130]. In the latter group steroids should be discontinued.

Pentoxifylline, a weak phosphodiesterase inhibitor, has been evaluated as an alternate to steroid treatment in AH; however, most studies found it inferior compared to steroids [131, 132]. A recent study investigated the combination of steroids and pentoxifylline and found no benefits over single therapy except for a small population of patients with hepatorenal syndrome as well as in animal models of ALD [133, 134].

Liver Transplantation in ALD

In the USA, patients with AAH that is linked to recent alcohol abuse are not considered candidates for liver transplantation. Most transplant centers in the USA require at least 6 months of abstinence and participation in support groups for eligibility for listing for liver transplantation. These rules obviously eliminate many patients because of the high 6-month mortality associated with AAH. In a recent multicenter study in the European Union, liver transplantation was effective as a treatment in patients with AAH [135]. While in pre-transplant all of the recipients heavily used alcohol, <10 % had relapse in alcohol use after liver transplantation for AAH [135].

Liver transplantation for alcohol-induced liver cirrhosis is highly successful and part of standard of care in the USA and other parts of the world. Transplanted organ survival is excellent both in 1 and 5 years, and recipient survival is also high compared to transplantations for many other etiologies, particularly viral hepatitis [136].

Potential Therapeutic Targets and Considerations in Future Therapies

Advances in the understanding of the cellular and molecular mechanism of ALD in the last decades provide multiple attractive therapeutic targets in ALD. Table 22.2 lists the most actively studied potential targets in the pathogenesis of ALD that may provide the basis for new therapeutic interventions. For example, considering that AAH is a state of hepatic and systemic pro-inflammatory cascade activation with hepatocyte/liver dysfunction, approaches to interrupt these vicious cycles are highly attractive. In addition, molecular mechanisms and biomarkers that distinguish the development of AAH from stable ALD are yet to be delineated.

Table 22.2 Current therapies and emerging therapeutic targets in alcoholic liver disease

Target	Functional effect
Current therapies	
Steroid	Anti-inflammatory
Pentoxifylline	Phosphodiesterase inhibitor
Liver transplantation	Healthy liver
Zinc	Intestinal barrier
Emerging therapeutic targets	
TNFA	Inflammation, hepatocyte death
IL-1β	Inflammation, steatosis
IL-1 receptor antagonist	Inflammation, steatosis
IL-17	Inflammation, hepatocyte death
IL-22	
IL-6	Inflammation, regeneration
Chemokines	
MCP-1	Inflammatory cell recruitment steatosis
IL-8	Neutrophil recruitment
GRO-α	Neutrophil recruitment
Osteopontin	Inflammation, regeneration
Signaling molecules	
TLR4	Inflammation, fibrosis
IRF3	TLR signaling
NF-κB	Inflammation, cell survival
Caspase-1	IL-1β production
Heat shock protein 90	Steatosis, inflammation
Hypoxia-inducible factor-1	Steatosis
Heme-oxygenase1	Inflammation
SIRT1	ROS steatosis, inflammation
PPAR-α	Steatosis
Cell death	
Fas	Apoptosis
Bcl-2	Apoptosis
Microbiome	
LPS	Inflammation
Pro-/prebiotics	Inflammation of gut phase

Previous studies identified TNF-α as a central mediator of ALD and TNF-α was increased both in the serum and liver in human alcoholic hepatitis [8, 9, 58]. While TNF-α blockade showed protection in animal models, human clinical trials using anti-TNF antibodies with steroids were discontinued due to infectious complications [137–140]. These studies had several limitations including high doses of anti-TNF-α and co-administration with steroids that increased immunosuppression. Pro-inflammatory

cytokines, other than TNF-α, are also increased in AH including IL-6, IL-8, and IL-1.

Recent preclinical data demonstrated upregulation of IL-1β in the liver after chronic alcohol administration and showed amelioration of liver steatosis and inflammation after therapeutic blockade of IL-1-mediated signaling. This may provide basis for translation to clinical application by evaluation of the therapeutic utility of IL-1R blockade or anti-IL-1 antibodies in ALD. There are several reasons for this. First, IL-1 inhibition can prevent the autoregulatory amplification loop of IL-1α and IL-1β upregulation. Second, inhibition of IL-1 should attenuate TNF-α induction and break the vicious cycle of pro-inflammatory cytokine cascade activation in AH. Third, because IL-1 induces steatosis and sensitizes hepatocytes to the cytotoxic effects of TNF-α, IL-1 inhibition should attenuate hepatocyte damage in AH [141].

Inhibition of MCP-1 could be another attractive approach considering that MCP-1 is an early mediator in ALD that contributes to steatosis and inflammatory cell recruitment. Additional potential targets are listed in Table 22.2; all of these potential therapeutic targets were identified based on experimental evidence and their role in the pathomechanisms of ALD and further preclinical and potential clinical investigations.

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