FOCUS ON: ALCOHOL AND THE LIVER

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Thirty-five years ago Charles Lieber and colleagues (1975) published a seminal article in liver research, showing that alcohol itself is the primary cause for the higher prevalence of liver disease seen in alcoholic patients and not dietary deficiencies and malnutrition that often accompany alcoholism. Their groundbreaking research dispelled previously held theories that alcohol was not a major cause of liver damage and led to several decades of study of the deleterious effects of alcohol and its metabolism on the liver. Since that early study, clinical and experimental studies have continued to show a firm connection between high amounts of alcohol consumption and liver disease. This article tracks advances in alcohol-related liver disease research over the past 40 years and describes how these discoveries are helping scientists to gain insight into therapeutic targets that may help to combat this life-threatening disease. KEY WORDS: *Alcoholism; alcohol metabolism; liver; alcoholic liver disease; liver disease; alcohol-related liver injury; cirrhosis; hepatitis; steatosis*

Alcoholic liver disease (ALD)—and particularly cirrhosis—has long been one of the most prevalent and devastating conditions caused by alcohol consumption and is one of the leading causes of alcohol-related death. Liver cirrhosis is the 12th leading cause of death in the United States. The age-adjusted death rates attributed to ALD are higher in high-alcohol–consuming countries such as Spain and France and lower in countries with low alcohol consumption, with the United States ranking in between.

ALD encompasses a varied clinical and histological spectrum. On one end of the spectrum is fatty liver (steatosis), which is reversible with abstinence, and the more severe alcoholic hepatitis and fibrosis, which may or may not improve with abstinence. On the other end of the spectrum are cirrhosis (Laennec's cirrhosis) and end-stage liver disease, conditions that typically are irreversible and have a poor prognosis. In addition, continued consumption of alcohol combined with obesity can further increase a person's risk of cancer (hepatocellular carcinoma).

Steatosis may occur in 90 percent of individuals who consume more than 60 g/d of alcohol (or about five drinks per day) (Crabb 1999). In many cases, there are no clinical symptoms except for an enlarged liver (i.e., hepatomegaly), and steatosis can be reversed if alcohol consumption is stopped or significantly reduced (Mann et al. 2003). Histologically, steatosis is evidenced by an accumulation of fat molecules (i.e., lipids) in both small (i.e., microvesicular) and large (i.e., macrovesicular) droplets within liver cells.

Some biopsies from people with steatosis also show inflammatory changes, an early indicator of more serious liver disease. For example, alcoholic hepatitis is steatosis accompanied by inflammation, neutrophilic infiltration, hepatocyte necrosis, and Mallory bodies. Approximately 35 to 40 percent of alcoholic patients may progress from steatosis to fibrosis, characterized by increased deposition of extracellular matrix proteins, such as collagen, in the liver along with inflammation and, eventually, cirrhosis. Diagnosing the stage of disease is important for management and treatment of ALD. For more information, readers are referred to recently published guidelines using a data-supported approach, which address all three aspects of ALD—diagnosis, management, and therapy (O'Shea et al. 2010).

PATHOPHYSIOLOGY: CONCEPTS EMERGING FROM ALD RESEARCH

The pathogenesis of ALD is multifactorial (Lieber 2004; Tsukamoto 2001). Alcohol and its toxic metabolites can damage key liver cells (primarily hepatocytes and parenchymal cells) through the excessive generation of molecules called free radicals. Particularly important are the actions of oxygen-containing free radicals known as reactive oxygen species (ROS). ROS can modify the function of essential signaling pathways in the cells, including those regulating lipid or glucose metabolism; furthermore, ROS can directly modulate proteins and DNA. Excessive levels of ROS within a cell and/or the lack of molecules that can eliminate ROS (i.e., antioxidants) lead to a state of oxidative stress.

In addition to its direct effect on the liver, alcohol can increase the "leakiness" of the intestine cell wall, allowing a harmful component of Gram-negative bacteria called endotoxin to pass more readily into the blood. The body responds to this increase in endotoxin levels by launching a coordinated immune response, marked by activation of immune cells residing in the liver (i.e., Kupffer cells). Kupffer cells are macrophages whose main role—removing bacterial and foreign proteins from the blood—is essential to the liver's primary function of cleansing the blood of foreign materials and toxic substances. When activated, Kupffer cells secrete a variety of cytokines, including tumor necrosis factor (TNF)-α and several types of interleukins (ILs). All these molecules act as inflammatory cytokines, and, as such, aid the body in fighting against invading pathogens and tissue injury and assisting in the healing process. Cytokines also can have a damaging role, however, when produced in excessive amounts, pushing the immune system response into overdrive and, as a result, promoting the progression of liver disease.

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Research funded by the National Institute on Alcohol Abuse and Alcoholism (NIAAA)/National Institutes of Health (NIH) first identified the importance of Kupffer cells and their response to endotoxin in the progression of liver disease (Thurman et al. 1998). Researchers found that inhibiting Kupffer cells by sterilizing the gut significantly improved the signs of ALD in animal models (Adachi et al. 1994, 1995). Further research led to the discovery of pattern-recognition receptors (PRRs) expressed in the liver, not only on Kupffer cells but also on other immune cells and parenchymal cells. PRRs, including Toll-like receptors (TLRs), sense invading pathogen-derived molecular signals and activate inflammatory responses (Szabo et al. 2006). TLR4 can interact with endotoxin, also known as lipopolysaccharide (or LPS), though the coreceptor, CD14. TLR4 is now recognized as the major mediator of Kupffer cell activation in alcoholic liver injury (Hritz et al. 2008; Uesugi et al. 2001).

In addition to producing cytokines, activated Kupffer cells are the major source of ROS in the liver, which, in large amounts, can lead to oxidative stress. Research has found that oxidative stress not only affects fatty acid oxidation and key cellular function but also triggers activation of hepatic macrophages, thus adding to the inflammatory cascade (Mandrekar and Szabo 2009; Wheeler at al. 2001). These studies provide a strong basis for the current model of ALD (figures 1 and 2). These findings also provide possible mechanisms for developing therapeutics for ALD.

ALCOHOL, GUT PERMEABILITY, AND THE GUT–LIVER AXIS

Several mechanisms may underlie the significant increase in endotoxin levels in the bloodstream following chronic alcohol use (Mathurin et al. 2000). One hypothesis postulates that chronic drinking leads to higher endotoxin levels because Kupffer cells and/or hepatocytes are unable to clear these molecules from the circulation. As noted above, alcohol increases intestinal permeability—that is, it alters the degree to which the gut-lining inner mucosa allows the passage of various molecules, including endotoxin, into the blood, a condition called "leaky gut."

In healthy individuals, only trace amounts of bacteria, a source of endotoxin, are able to cross the intestinal wall and reach the liver through the portal circulation. There, the endotoxin is easily cleared by resident liver macrophages (Kupffer cells) and hepatocytes. However, patients with cirrhosis have reduced clearance of endotoxin and thus more unwanted bacteria are able to enter the bloodstream, presumably as a result of increased gut permeability. Studies confirm that alcohol can affect gut permeability by altering the integrity of the gut barrier and by disrupting the intestine's healthy microbial flora (Bode and Bode 2003; Bode et al. 1987; Keshavarzian et al. 2009; Rao et al. 2004).

Researchers hypothesize that the alcohol metabolite, acetaldehyde, increases intestinal permeability by disrupt-
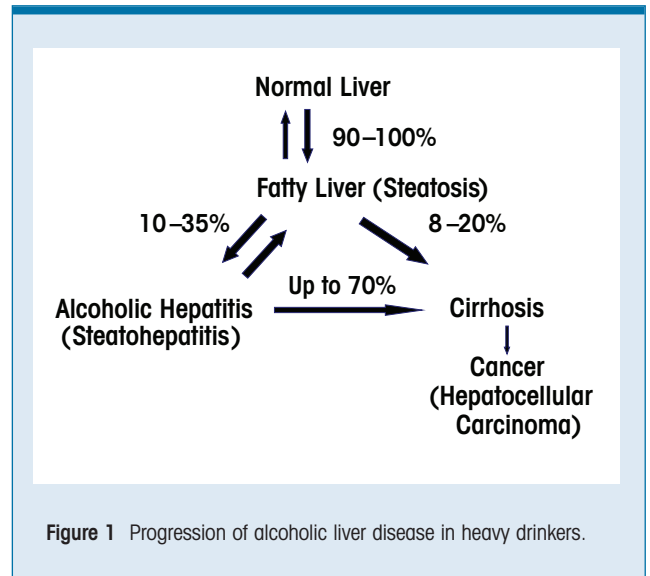

Figure 1 Progression of alcoholic liver disease in heavy drinkers.

ing the normal functions of proteins located within the cell walls. Those proteins are located in gut epithelial cell membranes (i.e., occludin and ZO-1 in the tight junctions and E-cadherin and β-catenin in the adherens junction) and play important roles in maintaining integrity and function of intestinal epithelial cells. Some of these effects have been linked to increased expression of inducible nitric oxide synthase (iNOS) and, recently, to alcohol-induced alterations in microRNA expression in the cell lining of the gut.

NIAAA-funded research also has revealed that microRNA targets ZO-1 protein translation, which causes a reduction in ZO-1 protein levels and, as a result, leads to increased intestinal permeability (Tang et al. 2008). The effects of alcohol on the gut–liver axis and the impact of various epidemiologic and genetic (i.e., epigenetic) influences on microRNAs are exciting new areas of alcohol research.

Chronic alcohol administration also affects the composition of intestinal bacteria, predominantly causing overgrowth of Gram-negative bacteria in alcohol-fed rats and in the gut of human alcoholics (Bode et al. 1984). Extensive studies show a strong association between endotoxin derived from the bacterial cell wall of Gram-negative bacteria and ALD. It is not yet determined if alcohol consumption affects Gram-positive bacteria, which are the source of peptidoglycan, a component of the cell wall that may be a marker of liver disease. In mice, prolonged administration of alcohol in the drinking water, a method that does not result in ALD, caused increased serum peptidoglycan levels, suggesting that intestinal translocation of bacterial components may not be restricted to Gram-negative organisms (Cook et al. 2007). The significance of the gut–liver crosstalk and its relation to some of the alcohol-induced other end-organ effects, such as brain functions, awaits further investigation.

THE ROLE OF INFLAMMATION

The development and progression of ALD is marked by onset of fatty liver, liver cell death, inflammation, regenerating nodules, scar tissue (i.e., fibrosis), and cirrhosis. Inflammation is the key to many of these detrimental effects, although the precise molecular mechanisms are only partially understood—primarily because of alcohol's (and its metabolite's) myriad immunologic and inflammatory cell effects. For example, endotoxin leaking from the gut as a result of alcohol interacts primarily with Kupffer cells, which, in turn, causes secretion of TNF-α, a cytokine that promotes a wide range of inflammatory responses, which, in turn, lead to the production of additional inflammatory cytokines such as IL-1, IL-6, and IL-8 (Diehl 2001; McClain et al. 1999; Tilg et al. 2003). This chain of events plays a key role in the development and progression of liver disease.

Inflammatory Cell Activation and Kupffer Cells in ALD

Persistent cytokine secretion results in chronic inflammation, which, in turn, leads to hepatitis, fibrosis, and cirrhosis. Cytokines such as TNF-α also regulate a process known as programmed cell death, or apoptosis, which is in part responsible for alcohol-induced damage of liver tissue. Apoptosis is important for eliminating hepatocytes that are no longer needed or whose DNA has been damaged.

Researchers have investigated the mechanisms through which endotoxin leads to TNF-α production by administering alcohol to normal mice and to mice that had been genetically engineered to lack the receptors with which endotoxin interacts on Kupffer cells—a molecule called CD14. Alcohol-fed CD14 knockout mice exhibited a reduction of liver injury (Yin et al. 2001). This finding confirmed that the binding of endotoxin to its receptors on Kupffer cells and the resulting Kupffer cell activation and production of TNF-α are key events in the development of alcoholic liver injury.

In another experiment, increased expression of mRNA of members of the TLR family (i.e., TLR1, TLR2, TLR4, TLR 6, TLR7, TLR8, and TLR9) was observed in mice chronically fed alcohol (Gustot et al. 2006). Further, when alcohol was fed to mice engineered to have an inactive TLR4 receptor (TLR4 mutant and knockout mice), those animals showed a reduction in liver injury (Hritz et al. 2008; Uesugi et al. 2001). Mediators derived from Kupffer cells also activate the other cell types in the liver, resulting in damage. Research shows that activation of the TLR4 pathway in Kupffer cells results in production of mediators, called cytokines and chemokines. Chemokines recruit additional immune responses, initiating the secretion of polymorphonuclear neutrophils and other inflammatory cells such as macrophages and T-cells, adding to the inflammatory cascade and further ramping up the inflammatory response.

CYTOKINES AND CHEMOKINES IN ALD

Cytokines and chemokines significantly contribute to the development and progression of alcoholic liver injury. Although increased levels of the cytokines TNF-α, IL-1, and IL-6 all have been observed in patients with alcoholic hepatitis, as well as animal models of liver disease, TNF-α is thought to be the key mediator of alcoholic liver injury (Diehl 2001; Hill et al. 2000; McClain et al. 1999).

Researchers observed a decrease in liver injury in alcohol-fed mice that lacked a receptor for TNF-α (TNF receptor I [TNFRI]) (Yin et al. 1999). A similar effect was found in mice administered TNF-α antibodies (Iimuro et al. 1997). Those antibodies interact with TNF-α to inhibit the cytokine's harmful actions. Although animal studies showed the efficacy of TNF-α inhibitors and antibodies, clinical trials using TNF-α antibodies had to be prematurely discontinued because of increased side effects with severe infections (Tilg et al. 2003).

In addition to TNF-α, the role of IL-6 in alcoholic liver injury has been recently investigated (El-Assal et al. 2004). Studies show that feeding alcohol to IL-6–deficient mice resulted in increased liver injury. This suggests a protective role for IL-6. Subsequent studies (Horiguchi et al. 2008) have shown that IL-6 mediates its effects by activating the transcription factor STAT3. STAT3 may help reduce or increase inflammation in ALD, depending on the specific cell type. For example, knockout mice that lack hepatocyte-specific STAT3 showed decreased inflammation but higher steatosis when fed alcohol. On the other hand, when alcohol was fed to macrophage/neutrophil-specific STAT3 knockout mice, they had both increased inflammation and greater injury. These studies indicate a crucial role for STAT3 in ALD.

IL-8 and monocyte chemoattractant protein (MCP)-1 are chemokines that recruit leukocytes to the sites of injury and inflammation. IL-8 induces neutrophil infiltration during alcoholic liver injury and perpetuates inflammation leading to alcoholic hepatitis. MCP-1 recruits monocytes/macrophages and is important in pathogenesis of alcoholic liver injury. Chemokines, such as MCP-1 and RANTES,[1] increase stellate cell activity in liver fibrosis and recruit inflammatory cells, amplifying a vicious cycle in which inflammatory and stellate cells stimulate one another (Karlmark et al. 2008). The observation that stellate cells, which are essential for the development of fibrosis (and also for the repair of damaged liver tissue), respond to the actions of IL-8 and MCP-1 may have important clinical applications. By blocking certain chemokines or chemokine receptors, scientists have been able to better understand the distinct effects these proteins have on the immune cells involved in ALD (Seki et al. 2009a, b).

[1] RANTES is defined as "regulated upon activation, normal T-cell expressed and secreted" and refers to a protein that is induced by TNF-α and IL1-α and which plays a role in recruiting immune cells.

OTHER MEDIATORS OF LIVER INJURY

Mediators of other than cytokines and chemokines also play an important role in alcohol-related liver injury. For example, the complement system bridges the gap between innate immunity, which responds to any pathogen it encounters, and acquired immunity, which amplifies the reaction of the innate immunity. Feeding alcohol increased one component of the complement system, C3, in rats (Roychowdhury et al. 2009); whereas C3-deficient mice were protected from injury (Pritchard et al. 2007). The precise mechanism of C3 in alcoholic liver injury is not clear; however, it is likely that C3 affects transcription of enzymes (lipogenic) that are important for the synthesis of fatty acids in the liver. Recent studies showed that C3 contributed primarily to the accumulation of triglyceride in the liver, whereas C5 was involved in inflammation and injury to hepatocytes.

Adipokines are bioactive cytokines produced primarily in fat (adipose) tissue, two of which (i.e., adiponectin and leptin) are under investigation with regard to their link to liver disease. Adiponectin has been found to have an anti-inflammatory action that leads to decreases in liver injury. Rats fed alcohol over a long period of time had decreased adiponectin plasma levels. By administering adiponectin, scientists were able to reduce alcoholic liver injury in these animals (Rogers et al. 2008). It appears that adiponectin may exert its effect by increasing fatty acid oxidation (via carnitine palmitoyl transferase [CPT]-1) and decreasing two lipogenic enzymes—acyl CoA carboxylase (ACC) and fatty acid synthase (FAS)—all of which have important roles in hepatic steatosis. Activation of the mitochondrial enzymes, such as CPT-1, results in increased oxidation of fatty acids, whereas lipogenic enzymes increase fatty acid synthesis, and increased triglycerides in the liver result in steatosis (Reddy and Rao 2006). These studies clearly indicate a protective role for adiponectin in the pathogenesis of alcoholic steatosis and steatohepatitis.

SIGNALING MECHANISMS OF INFLAMMATION

Kupffer Cells

As endotoxin enters the liver, it binds to receptors on Kupffer cells, activating a variety of intracellular signaling molecules. For example, binding of LPS to TLR results in the activation of numerous intracellular adapters and kinases, such as MyD88, TRAF6, IKK, and MAP kinases (MAPKs), followed by DNA binding of transcription factors such as nuclear factor (NF) B and interferon (IFN) regulatory factor (IRF) 3 (see figure 3).

Long-term alcohol feeding to mice deficient in MyD88, which is involved in downstream activation of the transcription factor NF B and IRF3, exhibited increased liver injury (Hritz et al. 2008). Chronic alcohol feeding also increased NF B, which, in turn, controls the activities of numerous genes, including those that encode inflammatory cytokines such as TNF-α, IL-6, and IL-1β. Further, Zhao

and colleagues (2008) showed that feeding alcohol to IRF3-deficient mice protected them from liver injury. In their study, alcohol-induced binding of IRF3 to the promoter region of the TNF-α gene increased its expression. Studies have shown the role of TNF-α–mediated activation of apoptotic signaling pathways, resulting in increased cell death and liver damage (Hoek and Pastorino 2002).

Hepatocytes

Hepatocytes comprise about 70 to 80 percent of the total liver and play an important role in cellular homeostatic functions. Hepatocyte killing is considered the final step in alcohol-induced liver injury and is linked to oxidative stress resulting from ROS, fatty acid lipotoxicity, unfolded protein response resulting from endoplasmic reticulum (ER) stress, as well as cytokine effects and mitochondrial injury. Based on the two-hit hypothesis (Enomoto et al. 2000) for the pathogenesis of alcoholic liver injury, apoptosis of hepatocytes occurs as a result of both direct and indirect effects of alcohol. Indirectly, chronic alcohol exposure activates Kupffer cells, resulting in the production of TNF-α, which subsequently binds to receptors on hepatocytes and induces receptor-mediated cell death (Hoek and Pastorino 2002). Oxidative stress related to alcohol metabolism induces direct hepatocyte damage resulting from mitochondrial dysfunction and ER stress, leading to activation of intrinsic cell death pathways (Hoek et al. 2002).

Molecular Mechanisms of Steatosis

The hallmark of early alcohol-induced liver injury is steatosis or fat deposition as a result of increased intracytoplasmic triglyceride formation in hepatocytes. Several mechanisms have been linked to lipid accumulation in hepatocytes. Alcohol may cause steatosis via induction of TNF-α, decreased fatty acid oxidation in the liver, and increased lipogenesis in hepatocytes. Cytokines also influence impairment of transport and secretion of triglycerides. The two main lipogenic signaling pathways affected by alcohol in the liver are sterol regulatory element–binding protein (SREBP)-1 activation and adenosine monophosphate (AMP)-activated protein kinase (AMPK) inhibition. In mice fed alcohol, AMPK activity is inhibited but SREBP-1 is activated and influences upregulation of lipogenic genes and triglyceride accumulation in the liver (You and Crabb 2004). Supporting this data, SREBP-1 knockout mice were protected against alcohol-induced steatosis, thus establishing a strong link between fat deposition and SREBP-1 activation.

ROS, Cyp2E1, GSH

Generation of ROS is important in alcohol-induced liver damage: Alcohol increases generation of ROS in Kupffer cells and hepatocytes. TNF-α–induced hepatocyte death is attributed to increased ROS-mediated oxidative stress.

In the liver, Kupffer cells produce ROS, and recent studies show a role for reduced nicotinamide adenosine

dinucleotide phosphate (NADPH) oxidase, an enzyme that catalyzes the production of superoxide. Activation of NADPH during alcohol metabolism contributes to increases in TLR2, -4, -6, and -9 mRNA and NFκB activation—all key players in alcohol-related liver injury. When alcohol-fed rats were pretreated with a compound that inhibits NADPH oxidase, diphenyliodonium (DPI), ROS production, TLR mRNA, and NFκB activation were reduced, resulting in decreased TNF-α production (Gustot et al. 2006). Studies (De Minicis and Brenner 2008) in mice that lacked a subunit p47 phox of this enzyme (i.e., NADPH oxidase subunit p47 phox–deficient mice) found that these animals had increased protection against alcohol-induced liver injury, possibly because of a decreased accumulation of lipid peroxidation products. This further supports a role for NADPH oxidase in ALD (Kono et al. 2000).

Additionally, researchers have examined the effects of an antioxidant called dilinoleoylphosphotidylcholine or DLPC in rats fed alcohol and found that it prevented LPS-induced NFκB and extracellular signal–regulated kinase 1/2 activation and TNF-α production in Kupffer cells (Cao et al. 2002). These findings indicate that the effects of ROS also may modulate inflammatory responses in Kupffer cells, adding to the endotoxin-induced injury

in ALD. Thus, ROS not only plays a critical role in oxidative injury but also contributes to increased inflammatory responses resulting in hepatocyte injury.

The role of cytochrome P450 2E1 (CYP2E1), an enzyme that is induced by alcohol and contributes to alcohol metabolism, has been extensively studied. CYP2E1 generates ROS, resulting in the oxidative stress that can lead to liver cell damage. The effects of ROS are exacerbated if the body's normal defense systems against this damage—antioxidants, such as glutathione (GSH) and vitamin E α-tocopherol—are impaired. Alcohol and its metabolism have been shown to reduce the levels of both GSH and vitamin E. Feeding alcohol to mice that lack CYP2E1 can prevent alcoholic liver injury (Lu et al. 2008), probably as a result of a reduction in oxidative stress.

Although endotoxin and CYP2E1 are considered independent risk factors, the synergistic effect of these two factors on hepatotoxicity involving oxidative stress, activation of MAPKs, and mitochondrial dysfunction recently was described (Lu and Cederbaum 2009). Understanding the precise role of alcohol-induced oxidative stress in the mechanisms of liver injury is needed and will aid researchers in better defining strategies to alleviate ALD.
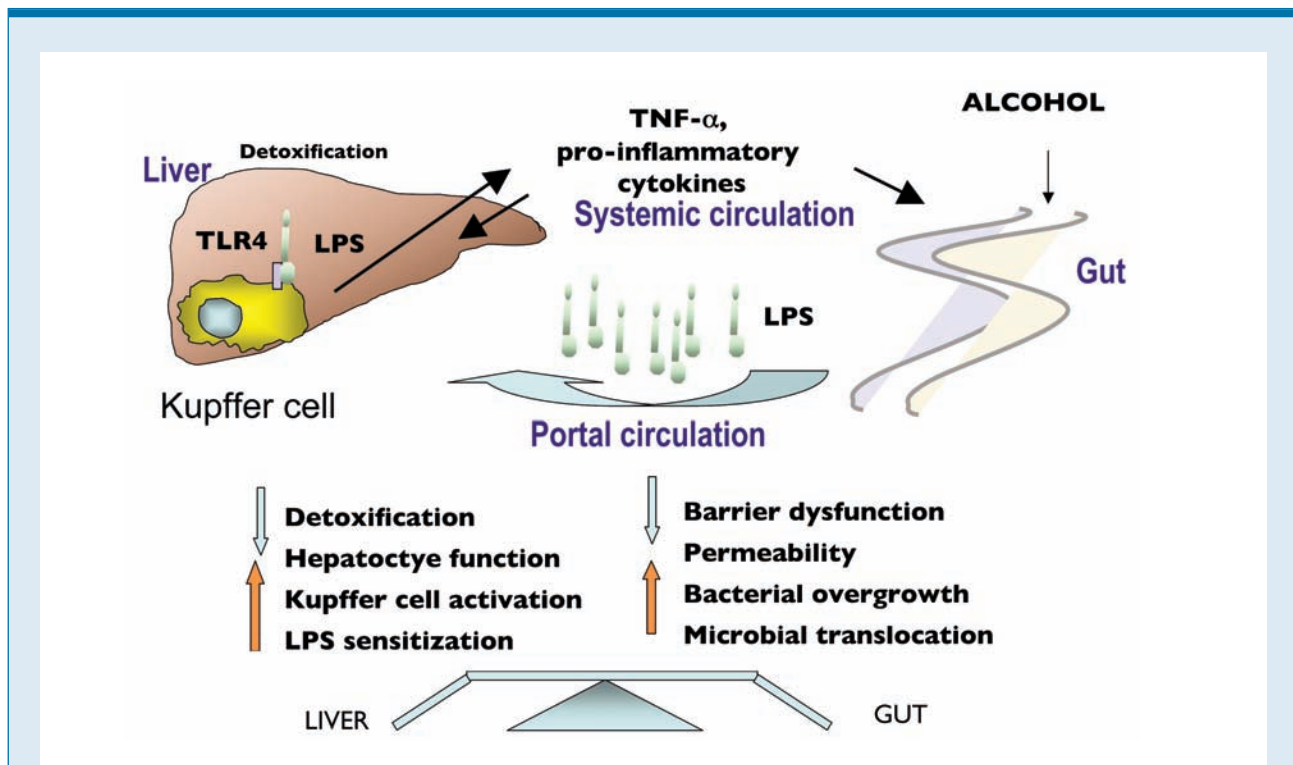

Figure 2 Proposed mechanism for alcoholic liver disease. Chronic alcohol abuse increases gut permeability resulting in high circulating endotoxin that reaches the liver via portal circulation. Endotoxin (lipopolysaccharide or LPS) is recognized by the Toll-like receptor (TLR)-4 complex on resident macrophages or Kupffer cells in the liver, leading to production of proinflammatory cytokines, particularly tumor necrosis factor (TNF)-α, and resulting in injury to liver cells (hepatocytes).

GSH, which limits toxicity of alcohol and other drugs in the liver, also has been extensively studied. Chronic alcohol use has been shown to deplete GSH levels, particularly in the mitochondria,[2] and this decline is seen to precede liver injury (Lee et al. 2004). Studies (Garcia-Ruiz and Fernández-Checa 2006) have shown that mitochondrial GSH depletion occurs primarily in the pericentral hepatocytes, where most of liver injury originates. Mitochondrial GSH depletion is attributed to a defective transport of GSH from the cellular fluid (cytosol) into the mitochondria, and this can be prevented by administering a nutritional supplement called *S*-adenosylmethionine (SAMe). Although reports on the effects of alcohol on mitochondrial GSH alterations are conclusive, how alcohol affects total hepatic GSH levels remains unclear.

Determinants of Disease Progression in ALD and Liver Fibrosis

As mentioned above, ALD often proceeds along a continuum, from fatty liver to simple liver scarring or fibrosis to full-blown cirrhosis and even cancer. Hepatic fibrosis is a healing process gone awry in response to ongoing liver injury in ALD (Siegmund et al. 2005). To repair the damage, excessive collagen is deposited into the extracellular matrix and the resulting loss of parenchymal structure leads to liver fibrosis and, over time, cirrhosis (Friedman 2008). It has been shown that endotoxin can directly induce hepatic stellate cell activation through TL4. Hepatic stellate cells are mostly responsible for deposition of extracellular matrix. As the liver becomes increasingly fibrotic, the number of functional hepatocytes decreases, and the liver loses its capacity to remove toxic substances from the blood.

Certain immune cells, i.e., natural killer (NK) T-cells, are known to induce hepatic stellate cell apoptosis during liver fibrosis. Preliminary findings show that NK T-cells play a diverse role in acute liver injury but are depleted in chronic liver injury, suggesting that NK T-cells may inhibit the early stage of liver fibrosis but not the late stage of disease (Gao et al. 2009). Likewise, the compound c-Jun N-terminal kinase (JNK) regulates several important cellular functions, including hepatic stellate cell activation. Scientists have found that inhibiting the production of this enzyme is a potential target for antifibrotic treatment approaches (Kluwe et al. 2010).

Fibrosis also may be caused by cells other than the traditionally investigated stellate cells, including hepatocytes themselves. For example, in addition to mediating hepatocyte apoptosis, transforming growth factor (TGF)-β induces early fibrosis. Inhibition of the TGF-β pathway, in turn, has been found to prevent liver injury (Meyer et al. 2010).

COFACTORS OF LIVER DISEASE

Additional health and medical issues also may put some people at greater risk for liver disease. For example, patients infected with hepatitis C virus (HCV) who drink heavily are likely to suffer more severe liver injury. Although researchers do not fully understand how alcohol consumption accelerates liver injury in patients with HCV infection, the following mechanisms have been proposed:

- Increased replication of HCV in the liver. Higher HCV RNA blood concentrations (Pessione et al. 1998).

- Mutations of the HCV (known as quasi-species), increasing the viral complexity and making it difficult for the immune system to control mutated viruses, leading to progressive injury (Takahashi et al. 2001).

- Increased apoptosis is induced by alcohol consumption in people with HCV (Szabo 2003).

- Higher levels of inflammation and immunoregulatory proteins (specifically ILs, TNF, and IFN). Geissler and colleagues (1997) reported that chronic alcohol feeding in mice inhibited their immune responses, specifically by T-helper cells and cytotoxic T-lymphocytes, which are important in removing HCV from the body.

- Fatty liver. Serfaty and colleagues (2002) found that HCV-related fibrosis progressed about twice as fast among drinkers with steatosis compared with those without steatosis or nondrinkers with or without steatosis.

- Accumulation of excess iron. Piperno and colleagues (1998) found that alcohol increases iron stores in the liver, and iron overload seems to contribute to HCV disease progression by inducing fibrosis.

- Oxidative stress (Szabo et al. 2005).

- Depression of the immune system by alcohol (Szabo 2003; Dolganiuc et al. 2003).

- The innate immune cells (NK/IFN-γ) may be attenuated by alcohol use, thus contributing to the acceleration of liver fibrosis in patients with chronic HCV (Jeong and Gao 2008).

Gender

Another factor associated with increased rates of alcohol-related liver injury is gender. Cirrhosis mortality rates are about two times higher in men than women. These rates reflect the fact that men typically drink more than women and that the proportion of heavy drinkers and alcoholics is much higher among men. However, it also appears that at any given level of alcohol consumption, women have a

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[2] Structures within the cell that generate most of the cell's energy through the production of adenosine triphosphate (ATP).

higher likelihood of developing cirrhosis than men (Tuyns and Pequignot 1984).

Several possible explanations exist. One is that levels of alcohol dehydrogenase (ADH), the enzyme involved in breaking down alcohol, may be lower in the women than in men, which would result in higher blood alcohol content for women than for men who consume similar amounts of alcohol (Frezza et al. 1990). Higher blood alcohol content and longer exposure to alcohol's toxic byproducts then could lead to higher rates of cirrhosis. Another possible explanation is that estrogen may increase the susceptibility of the liver to alcohol-related damage (Colantoni et al. 2003; Ikejima et al. 1998). Genetic factors, including those that influence metabolism and risk for alcohol also may be involved (Reed et al. 1996).

Corrao and colleagues (1998) found that 98 percent of cirrhosis cases in men but only 67 percent of cases in women could be attributed to alcohol metabolism and alcohol consumption, HCV, and hepatitis B. The risk factors for cirrhosis appear to be more complex for women than for men, and more research is necessary to fully understand the risk factors.

THERAPY

Specific treatment of ALD remains to be resolved. Over the years, clinical trials have attempted to ameliorate different components of the pathogenesis of ALD, including increased metabolism, inflammation, oxidative stress, and nutritional abnormalities. Overall, however, little if any substantial improvement has been found in those studies, as reviewed in Rongey and Kaplowitz (2006).

One problem in investigating potential treatments for liver disease is that most of the clinical trials to date have been conducted in patients with severe acute alcoholic hepatitis, a condition that carries a nearly 50 percent mortality rate if untreated (Ceccanti et al. 2006). Scientists have attempted to overcome this challenge by assigning a specific statistical score (Maddrey discriminant factor
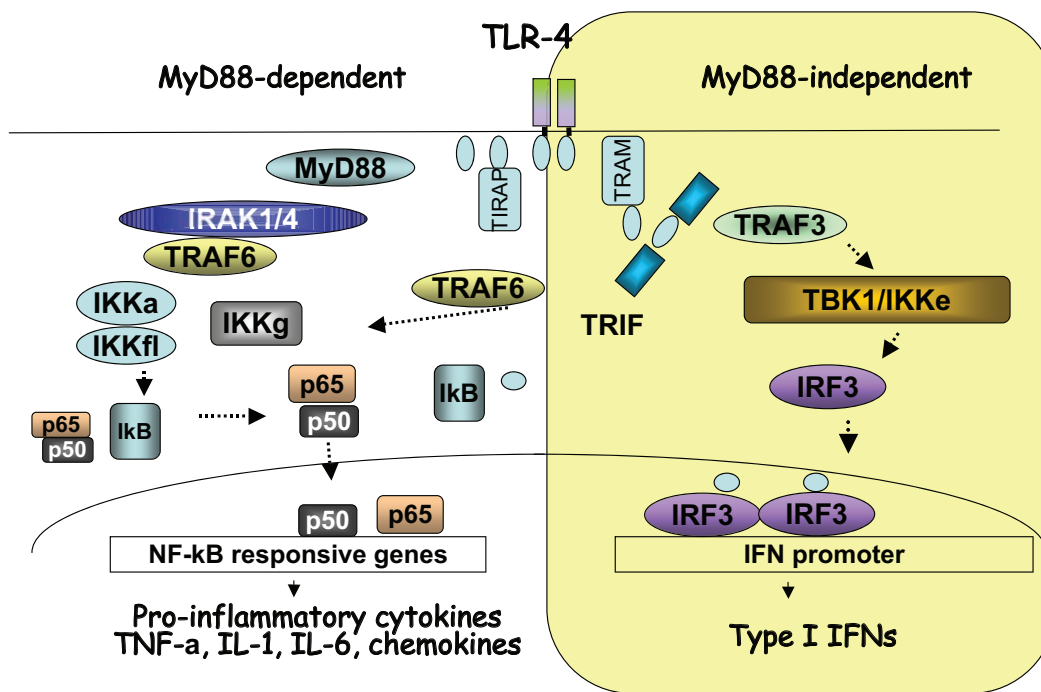

Figure 3 The Toll-like receptor (TLR)-4 signaling pathway in alcoholic liver disease. Engagement of the TLR-4 receptor results in activation of two distinct pathways: The myeloid differentiation primary response gene 88 (MyD88)-dependent pathway, which results in activation of nuclear factor (NF) κB and proinflammatory cytokines and the MyD88-independent pathway, which activates transcription factor interferon regulatory factor (IRF) 3 and induces type I interferons.

NOTE: Iκα, I B kinase α; I κβ, I B kinase β; p65, subunit 65 kD; p50, subunit 50 kD; Ikβ, Iκβ kinase; Ikkγ, I B kinase γ; IRAK1/4, interleukin 1/4 receptor–associated kinase; TRAF6, TNF receptor–associated factor 6; TRAF3, TNF receptor–associated factor 3; TBK, TANK-binding kinase; TRIF, TIR domain–containing adaptor–inducing IFNβ; TRAP, Toll-interleukin 1 receptor (TIR) domain–containing adaptor protein; TRAM, TRIF-related adaptor molecule; TBK/IKKε, TANK binding kinase.

[DF]: a score based on laboratory testing for prediction of mortality in hepatitis patients) to aid in these investigations. For example, patients with a Maddrey DF greater than 32 and no gastrointestinal bleeding who were given corticosteroid treatment improved their outcomes (Ceccanti et al. 2006). Modification of this formula by evaluating the effectiveness of corticosteroids after 7 days of therapy resulted in better clinical outcomes and reduced the infectious side effects of corticosteroid treatment. The rationale for steroid use is to decrease the immune and the proinflammatory cytokine responses. Most investigators agree that if steroids are to be used they should be reserved for patients with severe liver disease (i.e., DF greater than 32). Steroids have well-documented side effects, including enhancing the risk for infection, which already is substantial in patients with alcoholic hepatitis.

Another compound that has been investigated for the treatment of alcoholic hepatitis is pentoxiphylline (PTX). PTX has anti-inflammatory properties. In patients with severe alcoholic hepatitis (DF score higher than 32), PTX reduced the development of hepatorenal syndrome and mortality compared with placebo (Wheeler at al. 2001; Whitfield et al. 2009). The same trend was found in another, double-blind, placebo-controlled study (Akriviadis et al. 2000). Interestingly, unlike corticosteroids, PTX treatment did not improve liver function. Instead, PTX's beneficial effects were related to the decreased hepatorenal syndrome (Mathurin 2005).

Given the important role of TNF-α in inflammation and its association with acute alcoholic hepatitis, clinical trials also have evaluated the efficacy of TNF-α antibodies. The use of infliximab, an antibody that binds TNF-α in the serum and in membranes, improved survival, Maddrey score, and laboratory parameters in a clinical trial of patients with alcoholic hepatitis (Tilg et al. 2003). However, the study was discontinued because of high number of deaths resulting from infections in the treatment group (Tilg et al. 2003). Another antibody, eternacept, which targets soluble TNF-α, also has been associated with increased infections in patients with alcoholic hepatitis (Boetticher et al. 2008).

As noted above, the antioxidant GSH may have a protective role in alcoholic liver injury (Fernández-Checa et al. 2002). GSH cannot be administered directly, because the molecule cannot penetrate directly into liver cells. The amino acid cysteine, which ensures adequate GSH, also cannot be used as a supplement, because it too cannot enter liver cells. Clinicians have tried using a precursor of cysteine, SAMe, which can reach cells where it is converted into cysteine.

In a 2001 review, SAMe administration showed no obvious benefit, although these were not patients with alcoholic hepatitis (Rambaldi and Gluud 2001). In a study of patients with alcoholic cirrhosis, 2 years of administration of SAMe resulted in an overall decline in mortality compared with placebo, but no statistical significance was achieved (Mato et al. 1999). Overall, SAMe as a therapeutic agent in ALD is attractive from the standpoint of correction of biochemical abnormalities, but its clinical benefit must be carefully assessed in future clinical trials.

The best outcomes from ALD are achieved in patients who had lower cumulative doses of alcohol consumption and achieved long-term abstinence from alcohol. The survival of patients with end-stage decompensated alcohol- related cirrhosis can only be improved by liver transplantation, which has an excellent patient and graft survival provided that recipients remain alcohol-free in the posttransplant period.

The Impact of Discoveries From ALD on the Entire Field of Hepatology

The impact of NIAAA-supported research on the field of hepatology reaches far beyond ALD. Novel concepts that emerged from studies on ALD have significantly advanced our basic understanding of liver pathobiology. Some of the highlights include the concept of Kupffer cell activation and the role of these cells in liver inflammation, oxidative stress–related injury, stellate cell activation, and liver fibrosis. The concept of the gut–liver axis, which emerged from ALD, revolutionized our thinking about inflammation in other liver diseases, including nonalcoholic steatohepatitis and HCV infection. In this way, discoveries in ALD have led to the rapidly increasing body of knowledge that exists today in nonalcoholic liver disease.

Summary

There have been numerous advances in our understanding of alcohol-related liver disease since NIAAA was created 40 years ago. We now know that ALD, a serious and potentially fatal consequence of drinking alcohol, encompasses three conditions—steatosis, alcoholic hepatitis, and cirrhosis.

Scientists also have a much clearer understanding of the role that alcohol plays in the development and progression of liver disease, especially with the advent of new tools in molecular biology, such as genetically engineered rodent models. Although there are no Food and Drug Administration–approved therapies for ALD, lifestyle changes, nutritional support, and "off-label" therapies such as PTX can improve outcomes. Future discoveries on the mechanisms in the liver affected by chronic alcohol consumption will provide further insight into therapeutic targets to combat this disease. ∎

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The authors declare that they have no competing financial interests.

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