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REVIEW

Alcoholic, Nonalcoholic, and Toxicant-Associated Steatohepatitis: Mechanistic Similarities and Differences

Swati Joshi-Barve,[1,2,3] Irina Kirpich,[1,2,3] Matthew C. Cave,[1,2,3,4] Luis S. Marsano,[1,2,4] and Craig J. McClain[1,2,3,4]

[1]Division of Gastroenterology, Hepatology and Nutrition, [2]Department of Medicine, and [3]Department of Pharmacology and Toxicology, School of Medicine, University of Louisville; [4]Robley Rex Veterans Affairs Medical Center, Louisville, Kentucky

SUMMARY

This article reviews selected important mechanistic similarities and differences in alcoholic steatohepatitis, nonalcoholic steatohepatitis, and toxicant-associated steatohepatitis.

Hepatic steatosis and steatohepatitis are common histologic findings that can be caused by multiple etiologies. The three most frequent causes for steatosis/steatohepatitis are alcohol (alcoholic steatohepatitis, ASH), obesity/metabolic syndrome (nonalcoholic steatohepatitis, NASH), and environmental toxicants (toxicant-associated steatohepatitis, TASH). Hepatic steatosis is an early occurrence in all three forms of liver disease, and they often share common pathways to disease progression/severity. Disease progression is a result of both direct effects on the liver as well as indirect alterations in other organs/tissues such as intestine, adipose tissue, and the immune system. Although the three liver diseases (ASH, NASH, and TASH) share many common pathogenic mechanisms, they also exhibit distinct differences. Both shared and divergent mechanisms can be potential therapeutic targets. This review provides an overview of selected important mechanistic similarities and differences in ASH, NASH, and TASH. ***(Cell Mol Gastroenterol Hepatol 2015;1:356–367; http://dx.doi.org/10.1016/j.jcmgh.2015.05.006)***

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Hepatic steatosis and steatohepatitis are common histologic findings that can be caused by multiple etiologies (Figure 1). The three most frequent causes for steatosis/steatohepatitis are alcohol, obesity/metabolic syndrome, and environmental toxicants, as reviewed herein.

Alcohol remains one of the most common causes of both acute and chronic liver disease in the United States.[1] In Western countries, up to 50% of cases of end-stage liver disease have alcohol as a major etiologic factor.[2] Excessive alcohol consumption is the third leading preventable cause of death in the United States. Alcohol-related deaths, excluding accidents/homicides, accounted for 22,073 deaths in the United States in 2006, with 13,000 of those specifically attributed to alcoholic liver disease (ALD).[3] Cirrhosis from any cause represents the 12th leading cause of death in the United States, and 45.9% of all cirrhosis deaths are attributed to alcohol.[4] As shown by early studies involving controlled drinking with subsequent liver biopsies in volunteers, almost everyone who drinks heavily for 12 weeks will develop fatty liver.[5,6] This usually resolves with abstinence, but a subset of people who continue to drink heavily will develop alcoholic hepatitis, which may progress to cirrhosis or even hepatocellular carcinoma (HCC).

The progression of ALD is somewhat similar to nonalcoholic fatty liver disease (NAFLD) and toxicant-associated fatty liver disease (TAFLD) in that it generally occurs over several years. Importantly, studies from the Veterans Administration (VA) have shown that patients with cirrhosis and superimposed alcoholic hepatitis had >60% mortality over a 4-year period, with most of those deaths occurring in the first few months.[7] Thus, the prognosis for this aggressive stage of ALD is worse than for many common types of cancer, such as breast, prostate, and colon.

We have known that hepatic steatosis is associated with obesity since at least the 1950s. However, it was not until 1980, when Ludwig et al[8] coined the term "nonalcoholic steatohepatitis—NASH" to describe this previously unnamed condition that often occurred in cirrhotic patients, that its clinical importance became recognized. NAFLD encompasses a pathologic spectrum of liver disease that ranges from steatosis to steatohepatitis, cirrhosis, and hepatocellular carcinoma. NAFLD is by far the most common

Abbreviations used in this paper: **ALD, alcoholic liver disease; ALT, alanine aminotransferase; ASH, alcoholic steatohepatitis; AST, aspartate transaminase; BMI, body mass index; CYP2E1, cytochrome P450 isoform 2E1; ECM, extracellular matrix; ER, endoplasmic reticulum; HCC, hepatocellular carcinoma; HDAC, histone deacetylase; HSC, hepatic stellate cell; IL, interleukin; LA, linoleic acid; LPS, lipopolysaccharide; miR, microRNA; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NK, natural killer; NKT, natural killer T; OXLAM, oxidized linoleic acid metabolite; PAI-1, plasminogen activator inhibitor-1; PCB153, 2,2′,4,4′,5,5′-hexachlorobiphenyl; PPAR, peroxisome proliferator-activated receptor; RNS, reactive nitrogen species; SNP, single-nucleotide polymorphism; TASH, toxicant-associated steatohepatitis; TAFLD, toxicant-associated fatty liver disease; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; T_H, helper T cell; TLR, Toll-like receptor; TNF, tumor necrosis factor; VA, U.S. Department of Veterans Affairs/Veterans Administration.**

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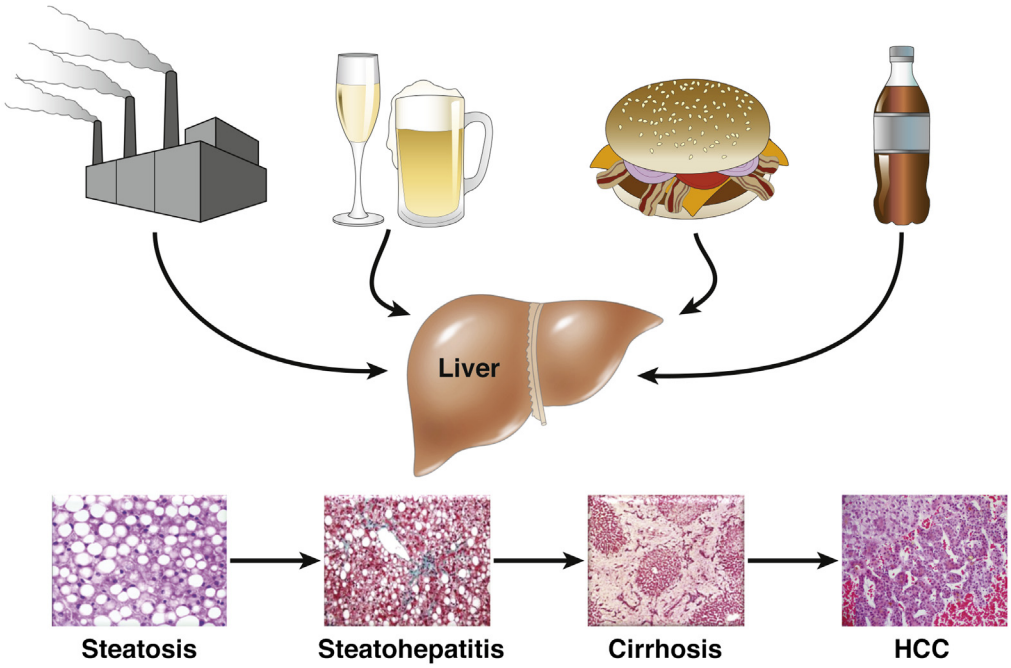

Figure 1. Multiple etiologic factors and metabolic pathways lead to the same histologic liver abnormalities.

cause of liver disease and abnormal enzymes in children and adults in the United States, with about one-third of adults thought to have NAFLD. The U.S. unselected prevalence of NASH is estimated to be 2% to 5%. Dietary factors, including high-fat and high-fructose diets, have been associated with the development of NASH.

Over 60 million unique chemicals were registered with the Chemical Abstracts Service Registry as of May 2011. With the rapid pace of new chemical discovery and commercialization, it is impossible to fully define the potential impact of these substances on the liver. However, the problem appears significant: 33% of the 677 most common workplace chemicals reported in the National Institute of Occupational Safety and Health Pocket Guide are associated with hepatotoxicity.[9] We first coined the term toxicant-associated steatohepatitis (TASH) in 2010, related to a cohort of patients with high vinyl chloride exposure who had classic steatohepatitis on liver biopsy but were not obese and did not drink alcohol.[10] Many classes of industrial chemicals have been associated with steatosis or steatohepatitis. These include (but are not limited to): solvents and other halogenated hydrocarbons, volatile organic mixtures, persistent organic pollutants, pesticides, and some nitro-organic compounds.[11] Recently enacted federal legislation (the Janey Ensminger Act of 2012) mandates medical coverage through the Department of Veteran's Affairs for hepatic steatosis in military personnel who were exposed to solvents in the drinking water at Marine Corps Base Camp Lejeune.[12] In addition to exposure level, an individual's susceptibility to chemical-induced liver disease is determined by polymorphisms in the genes of xenobiotic metabolism, concomitant use of alcohol or prescription medications, nutritional factors, and obesity—as many organic chemicals are lipid soluble.

This article reviews the mechanisms for the development of steatohepatitis, highlighting mechanistic differences as well as many common pathways between the three major etiologies (Table 1). For example, alcohol and the environmental toxicant vinyl chloride are both metabolized through cytochrome P450 2E1 (CYP2E1) to form toxic aldehyde intermediates. Moreover, fructose is also metabolized to an aldehyde. Thus, three divergent forms of steatohepatitis can have an aldehyde as an intermediate. On the other hand, dietary unsaturated fat may play a protective role in NASH, but n-6 unsaturated fat appears to augment ALD. There can also be major interactions between types of steatohepatitis. For example, high-fat feeding and subsequent NASH markedly reduces glutathione *S*-transferases, which play a protective role against a variety of environmental toxicants and alcohol. This review evaluates selected mechanistic similarities and differences in alcoholic steatohepatitis (ASH), NASH, and TASH.

Mechanisms of Liver Disease

Nutritional Abnormalities

Moderate/severe alcoholic hepatitis is usually associated with malnutrition. In large VA cooperative studies, virtually every patient with alcoholic hepatitis had some degree of malnutrition.[13] Almost 50% of patients' energy intake came from alcohol. Although their calorie intake was frequently adequate, their intake of protein and critical micronutrients was often deficient. A classic example of micronutrient deficiency is zinc deficiency.[14,15] Alcoholics regularly have decreased dietary intake of zinc as well as poor absorption and increased excretion. Moreover, oxidative stress causes zinc to be released from critical zinc-finger proteins. The cumulative negative

Table 1. Clinical/Biochemical Characteristics

Liver Disease	Cell Death	Proinflammatory Cytokines	Adiponectin	Oxidative Stress	Nutrition/BMI	Dietary "Cofactor"	Mechanism-Targeted Treatments
ASH	Necrotic/ apoptotic ↑ AST	↑↑↑	↑↑	↑	BMI normal, malnutrition	n-6 unsaturated fat	Lifestyle modification Anticytokines (e.g., anakinra) Probiotics Anti-LPS
NASH	Apoptotic/ necrotic ↑ ALT	↑	↓	↑↑	BMI ↑, overnutrition	Saturated fat, fructose	Lifestyle modification Vitamin E Antioxidants Probiotics
TASH	Necrotic/ apoptotic Normal AST/ ALT	↑↑	↓	↑	BMI normal, normal nutrition	Unclear, possibly high-fat diet	Lifestyle modification Aldehyde/toxin scavengers Antioxidants

ALT, alanine aminotransferase; ASH, alcoholic steatohepatitis; AST, aspartate transaminase; BMI, body mass index; LPS, lipopolysaccharide; NASH, nonalcoholic steatohepatitis; TASH, toxicant-associated steatohepatitis.

impact on critical zinc-finger proteins can lead to liver injury, altered fat metabolism, and impaired liver regeneration as well as produce classic clinical manifestations of zinc deficiency in humans such as night blindness or skin lesions.

In the VA cooperative studies, the severity of liver disease correlated with malnutrition. Patients were given a balanced 2500-kcal hospital diet which was carefully monitored by a dietitian. Voluntary oral food intake correlated in a stepwise fashion with 6-month mortality data. Thus, patients who voluntarily consumed more than 3000 kcal/day had virtually no mortality, whereas those consuming less than 1000 kcal/day had greater than 80% 6-month mortality. Thus, global malnutrition and specific micronutrient depletion (eg, zinc) appear to play a role in alcoholic hepatitis.

The type of dietary fat consumed also appears to play an important role in the pathogenesis of ALD. Several studies have shown that dietary saturated fat protects against alcohol-induced liver disease in rodents, whereas dietary unsaturated fat enriched in linoleic acid (LA) promotes alcohol-induced liver damage.[16] The mechanism(s) by which the combination of LA and alcohol promotes liver injury are not fully understood. LA is the most abundant polyunsaturated fatty acid in human diets and in human plasma and membrane lipids. Dietary intake of LA has more than tripled over the past century. LA can be enzymatically converted to bioactive oxidation products—oxidized LA metabolites (OXLAMs)—primarily via the actions of 12/15-lipoxygenase or nonenzymatically via free-radical-mediated oxidation response to oxidative stress. OXLAMs (either alone or in conjunction with ethanol) can induce increased gut permeability and hepatic mitochondrial dysfunction in experimental ALD. OXLAMs are also postulated to play an etiologic role in NASH.

In contrast to ASH, NASH is commonly associated with overnutrition and obesity. This is especially true in the United States. However, it is important to note that in most well-performed studies, especially pediatric studies, the amount of total calories consumed is similar in obese patients with or without NAFLD/NASH. Thus, total caloric intake does not seem to be the discriminating factor in the development of fatty liver. Individual dietary components have been postulated to play a role. High-carbohydrate diets, especially those high in sugared drinks including fructose, have been implicated.[17] Moreover, endogenous production of fructose from high glucose intake has also been implicated in experimental NAFLD.[18] Similarly, high-fat diets likely play a role in NAFLD. However, diets high in saturated fats have generally been implicated in NAFLD (compared to unsaturated fats with ASH). These saturated fats are thought to cause hepatic lipotoxicity. Moreover, certain fatty acids may activate Toll-like receptors and induce inflammation/injury. There is increased visceral adiposity in human and experimental NAFLD, and the adipocytes are enlarged and inflamed. On the other hand, adipocytes from ALD patients are actually smaller than normal but still inflamed.

Although the vinyl chloride-exposed patients originally described with TASH were not overweight,[10] other chemicals are obesogens that disrupt endocrine signaling to cause steatosis.[19,20] Likewise, chemicals may modify the hepatic response to diet-induced obesity and mediate the transition from steatosis to steatohepatitis. This has recently been demonstrated for important food contaminants including polychlorinated biphenyls,[21] arsenic,[22] perfluorooctanoic acid,[23] and water disinfection byproducts.[24,25] High-fat diet

and chemical coexposures can impact hepatic inflammation,[22–25] oxidative stress,[23–26] fibrosis,[22,24] and aryl hydrocarbon receptor and/or nuclear receptor signaling,[21,23,27] Not only may ethanol worsen TASH,[28] solvents may also increase ethanol drinking behavior.[29,30] Thus, nutritional status modulates environmental liver disease, and environmental chemicals, in turn, influence the development and severity of both ALD and NAFLD. Dietary supplementation with oligofructose has been shown to improve steatohepatitis associated with arsenic and high-fat diet coexposures,[31] indicating a potential therapeutic role for nutrition in TASH.

Intestinal Barrier Dysfunction/Microbiota

Alcohol, and specifically acetaldehyde, disrupts tight junction proteins and increases gut permeability both in vitro and in vivo; increased endotoxin levels are regularly observed in rodent models of ALD. Elevated endotoxin levels in ALD may originate from 1) Gram-negative bacterial overgrowth in the intestine, 2) increased intestinal permeability, and/or 3) impaired hepatic clearance of endotoxin.[32] Endotoxin then stimulates the production of tumor necrosis factor (TNF) and other proinflammatory cytokines through Toll-like receptor 4 (TLR4) signaling, which plays a critical role in the development and progression of ALD (Figure 2). Other bacteria-derived toxins, such as peptidoglycan and flagellin, may also impact TLR signaling and proinflammatory cytokine production.[32] Indeed, injected peptidoglycan increases liver injury/inflammation in alcohol-fed compared with control-fed mice, and ethanol feeding increases peptidoglycan levels.[32,33] Moreover, chronic alcohol feeding increases hepatic TLRs and thus sensitizes hepatocytes to inflammation/injury induced by translocation of gut-derived bacteria/toxins. Endotoxin not only plays a role in the fatty liver and liver injury of experimental ALD, but it also appears to play a role in hepatic fibrosis. In vitro assays as well as in vivo mixed chimerism studies show that endotoxin primes stellate cells for transforming growth factor–stimulated collagen production.[34] Thus, lipopolysaccharide (LPS) also plays a role in fibrosis induction and progression.

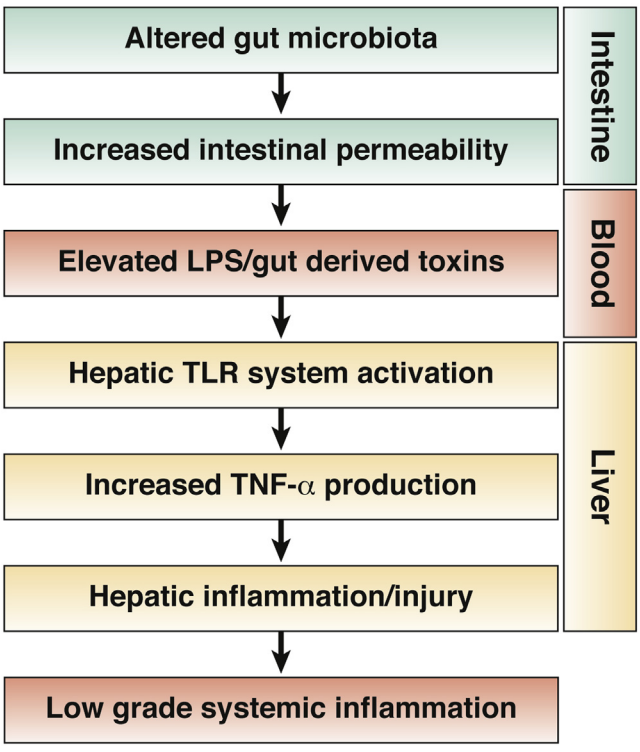

Figure 2. Alteration in gut-barrier function can lead to translocation of gut-derived products/toxins, which translocate and activate Toll-like receptors with subsequent production of inflammatory mediators, liver injury, and low-grade systemic inflammation.

Alterations in the gut microbiome likely play a major role in the development/progression of gut barrier dysfunction, endotoxemia, and liver injury/fibrosis of ALD.[35] We have shown that ethanol consumption can cause a time-dependent decline in the abundance of both *Bacteriodetes* and *Firmicutes*, which was accompanied by a proportional increase in *Actinobacteria* and *Proteobacteria;*[36] notably, the latter phylum encompasses pathogenic Gram-negative species such as *Escherichia*, *Salmonella*, *Vibrio*, and *Helicobacter*. These results strongly suggest that the increase in plasma endotoxin levels and hepatic inflammation are consequences of the expansion of the Gram-negative bacteria from the *Proteobacteria* phylum, which occurs in response to chronic ethanol consumption. Importantly, gut microbiome changes are important in the pathogenesis of human ALD (as well as human NAFLD/TAFLD), and probiotic therapy has improved liver enzymes in clinical trials in human ASH/NASH.[37–39]

The stability of the normal intestinal microbiome is influenced by several factors in the luminal environment, including gastric acidity, gut motility, bile salts, immunologic defense factors, colonic pH, and the competition between microorganisms for nutrients and intestinal binding sites. An altered luminal environment may lead to modifications in the microbial composition by supporting the growth of specific genera. Thus, a major increase of *Alcaligenes* (an alkaline-tolerant genus) correlates with an increase in fecal pH and a decrease in fecal short-chain fatty acids. Further, some short-chain fatty acids (eg, butyrate) have important signaling functions and epigenetic consequences, and they are a critical energy source for the intestine.[40] Increased luminal pH, leading to pathogenic alterations, has been implicated in diverse disease states ranging from infantile diarrhea to liver cirrhosis.

Substantial data from experimental animal studies support the concept that the role of gut bacteria in NAFLD/NASH is multifactorial and includes regulation of energy homeostasis,[41] modulation of choline[42] and bile acid metabolism,[43] and/or the ability to generate bacteria-derived toxins such as LPS.[44] Small intestine bacterial overgrowth has also been linked to NASH pathogenesis.[45] Elevated representation of *Escherichia,* alcohol-producing bacteria, was observed in parallel with increased blood alcohol concentration in NASH patients, suggesting a novel mechanism for the pathogenesis of NASH: gut microbiota enriched in alcohol-producing bacteria (eg, *E. coli*) constantly produce

more alcohol, which in turn is known to play an important role in the disruption of intestinal tight junctions, hepatic oxidative stress, and liver inflammation.[46]

The gut bacteria may facilitate progression from the simple steatosis to NASH. Dysbiosis associated with the loss of NLRP3 (NOD-like receptor family, pyrin domain-containing 3), and NLRP6 inflammasomes resulted in increased influx of LPS and bacterial DNA to the liver; these bacterial products stimulate TLR4 and TLR9, respectively, leading to enhanced hepatic TNFα expression which drives NASH progression.[47] The gut microbiota may also contribute to hepatic fibrosis via stimulation of TLR9-dependent profibrotic pathways in hepatic Kupffer cells.[48] An exciting advance in the field has been the recent observation that gut microbiota transplantation from donor mice with NAFLD replicated the phenotype in wild-type recipients, demonstrating that NAFLD is a potentially transmissible process.[49]

Environmental toxicants can also alter the gut microbiome and its metabolic activity. Exposure to the environmental toxicant arsenic affects large populations both worldwide and in the United States, especially through contamination of drinking water. Recent studies by Lu et al[50] showed that arsenic exposure produces major alterations in the gut microbiome, and arsenic-treated mice were clearly delineated from control mice on metabolic profiles by principal component analysis. Moreover, clear-cut interactions between the high-fat diet and arsenic-induced steatohepatitis as well as altered fecal metabolites/microbiome have also been reported.[22,31,51] Likewise, polychlorinated biphenyl exposures have been associated with both intestinal dysbiosis[52] and increased gut permeability.[53]

Immune Alterations and Inflammatory Mediators

The immune system, including both innate—mediated by neutrophils, macrophages, natural killer (NK) and natural killer T (NKT) cells—and adaptive—mediated by T and B cells—immune responses, is an important pathogenic component of fatty liver diseases.[54] Macrophages and liver Kupffer cells are major producers of proinflammatory and anti-inflammatory cytokines (eg, TNFα, interleukin 8 [IL-8], and IL-10) and play a critical role, particularly in early fatty liver disease.[55] Moreover, Kupffer cells can activate the adaptive immune cells (T cells) and hepatic stellate cells (HSCs) via IL-6 and transforming growth factor-β. Kupffer cell depletion was shown to reduce hepatic damage and inflammation in alcohol-induced[56] and choline-deficient diet-induced steatohepatitis.[57]

Neutrophils are an initial inflammatory response to injury, and their infiltration into the liver in response to chemoattractant cytokines is a pathologic hallmark of fatty liver and most prominently ALD.[58,59] The contribution of neutrophils in NASH development is also becoming evident in mouse models[60,61] and human NASH,[62] with the occurrence of both apoptosis and necrosis shown in humans.[63]

Hepatic inflammation along with steatosis and hepatotoxicity is observed in TAFLD, but the data are scarce. Dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin [TCDD]) administration in immature, ovariectomized C57B/l/6 mice either alone or in combination with another environmental pollutant, PCB153 (2,2′,4,4′,5,5′-hexachlorobiphenyl), resulted in hepatic histologic changes that included lipid accumulation and inflammatory cell infiltration.[64] Subchronic exposure of mice to the pesticide malathion for 28 days resulted in hepatic steatosis and inflammation with neutrophil activation.[65] Further studies after exposures to various toxins will better establish the role of immune alterations in TAFLD.

The adaptive immune system is considered important in fatty liver, particularly in the advanced stages with a more progressive phenotype and significant fibrosis. Alcoholic hepatitis patients have an increased number of T cells in the liver and increased circulating antibodies against lipid peroxidation adducts, suggesting that adaptive immune activation may contribute to the ALD pathogenesis.[66–69] Adaptive immune responses also contribute to hepatic inflammation in NAFLD.[70]

Studies in humans and animals suggest that NKT cells may be significant contributors to inflammation, cell death, and fibrosis in NAFLD.[69,71,72] Moreover, evidence demonstrates a significant decrease of $CD4^+$ regulatory T cells in a high fat diet mouse model of fatty liver.[73,74] Recently, another subset of helper T cells, T_H17 cells, that secrete IL-17 and induce inflammation via neutrophils has been identified and is thought to play a role in fatty liver. T_H17 responses are involved in human ALD[75] and may be important for progression from simple steatosis to steatohepatitis in NAFLD.[76] Gadd et al[77] recently reported that in all stages of NAFLD the portal tracts were enriched by $CD68^+$ macrophages and $CD8^+$ lymphocytes. Further, Miyagi et al[78] demonstrated a protective role of invariant NKT cells in the progression from inflammation to fibrosis, without alteration of steatosis, in a high-fat mouse model.

Cross-talk between various immune cells as well as interactions with other liver cells may also be critical determinants in fatty liver.[79] T cells are important activators of HSCs; while activated, HSCs can act as antigen-presenting cells to stimulate T cells (including NKT cells and $CD4^+$ and $CD8^+$ T cells).[80] Some studies have suggested that regulatory T cells may promote oncogenesis and tumor progression leading to HCC because they mediate immunosuppressive effects and inhibit NK and $CD8^+$ T cells.[81] In addition, hepatocytes are major producers of immunomodulatory cytokines such as IL-8,[82] and they are targets for cytokine toxicity. Thus, many liver cells and peripheral infiltrating immune cells are involved inflammation and damage in fatty liver.

Fat-derived products (such as acrolein, leukotrienes, and OXLAMs), and advanced glycation end products can also cause liver inflammation and injury.[83,84] Factors that activate the inflammasome, such as uric acid, can induce production of IL-1 and IL-18. The release of damage-associated molecular patterns from dying cells, is also believed to trigger sterile inflammation following tissue injury.[85] All these inflammatory/fibrotic mediators have been shown to be increased in fatty liver and are potential targets for therapeutic intervention.

Oxidative Stress and Lipid Peroxidation

Oxidative stress is an imbalance between pro-oxidants and antioxidants. Reactive oxygen species and reactive nitrogen species (RNS) are products of normal metabolism and can be beneficial to the host (eg, by contributing to bacterial killing).[86] Overproduction of reactive oxygen species and RNS, inadequate antioxidant defenses (eg, low levels of vitamins, selenium, or mitochondrial glutathione), or both can lead to liver injury. Oxidative stress in ASH, NASH, or TASH is usually documented by detection of one of several indirect markers: 1) protein oxidation (eg, protein thiol or carbonyl products), 2) lipid oxidation (eg, isoprostanes or malondialdehyde), 3) DNA oxidation (eg, oxodeoxyguanosine), or 4) depletion or induction of antioxidant defenses (eg, vitamin E, glutathione, or thioredoxin).[87]

The stimulus for oxidative stress in the liver comes from multiple sources. In hepatocytes, CYP2E1 activity increases after alcohol consumption—in part because of stabilization of messenger RNA (mRNA). Similarly, CYP2E1 activity is increased in NAFLD. The CYP2E1 system leaks electrons to initiate oxidative stress.[86] CYP2E1 is localized in the hepatic lobule in areas of alcohol-induced liver injury. Moreover, overexpression of CYP2E1 in mice and in HepG2 cells (a human hepatoma cell line) in vitro leads to enhanced alcohol hepatotoxicity. Nonparenchymal cells and infiltrating inflammatory cells (eg, polymorphonuclear neutrophils) are another major source of pro-oxidants that are used for normal cellular processes such as killing invading organisms. Infiltrating neutrophils use enzyme systems such as myeloperoxidase to generate hypochlorous acid ($HClO^-$, a halide species that causes oxidative stress) and RNS.

Hepatic steatosis in TAFLD caused by exposure to methyl mercury is associated with increased lipid peroxidation products in rat livers.[88] Inhibition of pyruvate dehydrogenase in the mitochondria, with resultant mitochondrial uncoupling and an increase in hydrogen peroxide production,[89] has been shown with elevated free radicals and lipid peroxidation products in multiple studies of arsenic-induced TAFLD.[90] Carbon tetrachloride is another well-studied hepatotoxicant that induces hepatic steatosis and injury after cleavage of CCl_4 by CYP2E1, which generates the trichloromethyl radical and leads to lipid peroxidation and membrane damage.[91] We recently demonstrated that PCB153 exposure causes TASH with hepatic antioxidant depletion.[26]

Oxidative stress can mediate liver injury through at least two major pathways: direct cell injury and cell signaling. Direct cell injury is indicated by markers such as lipid peroxidation and DNA damage. An even greater role is played by signaling pathways; for example, activation of transcription factors such as nuclear factor κB plays a critical role in the production of proinflammatory cytokines such as TNF.

Of all the mechanisms related to ASH, NASH, and TASH, oxidative stress has probably been the most widely studied. Antioxidant therapy offers potential as a clinical intervention for steatohepatitis. Importantly, there is no therapy approved by the U.S. Food and Drug Administration for any form of steatohepatitis. Moreover, vitamin E therapy (800 IU per day) is possibly the only widely accepted therapy for any form of steatohepatitis (in this case, NASH; see Table 1).[92] Unfortunately, therapy is beyond the scope of this article, but each of the listed mechanisms for steatohepatitis represents a potential therapeutic target.

Endoplasmic Reticulum Stress

Endoplasmic reticulum (ER) stress, or the unfolded protein response pathway, is activated by conditions of protein overload or increased unfolded proteins. Once triggered, this signaling pathway results in adaptation and recovery of homeostasis; however, severe or prolonged ER stress can ultimately result in cell death.

Increasing evidence has demonstrates that ER stress is a common feature of many liver diseases, including ASH and NASH.[93,94] Alcohol-induced ER stress is seen in experimental alcohol-feeding models in mice, micropigs, rats, and zebrafish.[95–98] ER stress has been also been reported in human patients with ALD,[99,100] with up-regulation of multiple ER stress markers, which correlated with dysregulated lipid metabolism and impaired insulin signaling. This suggests that ER stress is integral to ALD pathogenesis in human alcoholics. The induction of hepatic ER stress has been described in several genetic and diet-induced murine models of obesity, insulin resistance, and NAFLD, and in the livers of patients with NAFLD.[101–104]

Some upstream mechanisms that are demonstrated to cause ER stress in NAFLD include 1) hepatic steatosis/excess fatty acids, 2) oxidative stress and deficient nuclear factor erythroid 2-related factor 2 (Nrf2), 3) impaired hepatic autophagy in NAFLD patients and murine models of NAFLD, and 4) down-regulation of adiponectin.[105–107] Several alcohol-induced factors are also known to cause ER stress in ASH, including acetaldehyde and toxic lipid-derived aldehydes and metabolites, oxidative stress, dysregulated methionine metabolism, aberrant epigenetic modifications, altered interferon regulatory factor 3 (IRF3)/Sting signaling, and disruption of calcium homeostasis.[108,109]

Overall, hepatic ER stress occurs in both ASH and NASH in many species including humans, and is now accepted as an important mechanism in disease pathogenesis and progression. However, because ER stress appears to be both a cause and a consequence of other accompanying alterations, the question of association versus causality remains. The association of ER stress and TAFLD is highly likely but less well investigated. Thus, the exact role of ER stress in the pathogenesis of ALD, NAFLD, and TAFLD warrants further investigation to facilitate the development of therapies.

Fibrin/Extracellular Matrix

Fibrosis results from an imbalance between production and resorption of extracellular matrix (ECM) caused by a complex interplay between activation/transdifferentiation of HSCs, profibrogenic growth factors and cytokines, and alterations in the fibrin coagulation system. Fibrosis and the altered ECM subsequently provide a permissive setting for

the development of cellular dysplasia and HCC. HSCs are the dominant contributors to fibrosis in the liver driven by varied etiology, and upon activation they produce profibrogenic factors such as collagen and smooth muscle actin.[110] Although a mechanistic link between apoptosis or necroptosis and HSC activation has been suggested, it is not fully understood.

Hepatic injury in experimental models of liver disease often involves dysregulation of the fibrin cascade, resulting in the formation of fibrin clots that can cause hepatocellular death and induce inflammatory signaling in the liver. Inhibition of fibrinolysis by plasminogen activator inhibitor-1 (PAI-1) can cause fibrin-ECM to accumulate, even in the absence of enhanced fibrin deposition by the thrombin cascade. An imbalance in coagulation factors as well as elevated PAI-1 levels and hypofibrinolysis are common in patients with either NAFLD or ALD.[111] Indeed, it has been shown that circulating plasma PAI-1 levels in humans are closely related to the degree of liver steatosis.[112] Coagulation cascade activation has also been shown to be critical for liver inflammation and steatosis in Western-diet-induced NAFLD, and PAI-1 levels during disease development are a predictor of later severity.[113] Fibrosis is also commonly seen in TAFLD caused by exposure to drugs[114] and vinyl chloride.[10] Also, exposure of mice to the dioxin TCDD results in hepatic steatosis and fibrosis with up-regulation of profibrogenic genes.[115] However, the underlying mechanisms remain undetermined.

Genetics/Epigenetics

Recent studies have shown that both genetic and epigenetic factors are important for disease pathogenesis and progression in steatohepatitis. The genetic variations are often associated with conformational changes in protein structures and functions due to single-nucleotide polymorphisms (SNPs), whereas epigenetic changes are phenotypic changes resulting from altered gene expression without affecting the underlying DNA sequence.

Genomewide association studies have identified around 3.1 million SNPs that can contribute to disease states, and these SNPs may increase or decrease the function of encoded proteins. Specifically, a study conducted by Romeo et al[116] showed 9229 SNPs in NAFLD patients as compared with controls. Some of the more important ones appeared to be patatin-like phospholipase domain-containing 3 (PNPLA3), peroxisome proliferator-activated receptor-γ (PPAR-γ), and TNFα.[117] In NAFLD, TNFα-238, adiponectin-45, leptin 2548 PPAR-γ-161, and phosphatidylethanolamine *N*-methyltransferase-175 (PEMT-175) have an increased risk association, and adiponectin-276 and hepatic lipase-514 have a negative or decreased risk association.[118] In ALD, polymorphisms of alcohol metabolizing enzymes such as alcohol dehydrogenase and CYP2E1 as well as antioxidant enzymes and cytokine-coding genes have shown a strong correlation with the progression of ALD.[119]

Epigenetic changes occurring in response to various environmental signals can produce diverse tissue-specific effects. These epigenetic modifications include microRNAs (miR), DNA methylation, and histone modifications. The role of microRNA is well established in both NASH and ASH. Some of these microRNAs play a causal role, as they have targets that are important for the development of disease, but others likely are seen only as associations. A decrease in miR-122 and induction in miR-155 expression has been reported in models of NASH and ASH. Mice deficient in miR-122 develop steatohepatitis and fibrosis. TNF and CEBP are the targets of miR-155. Additionally, other microRNAs such as miR-320, miR-486, miR-705, miR-1224, miR-27b, miR-214, miR-199a, miR-192, and miR-183 likely contribute to both diseases.[120] Importantly, there is a strong association between HCC, which can arise from ALD, NAFLD, or TAFLD, and hepatic microRNA alterations.[121,122]

The potential role of alcohol induced histone modifications in the development of ALD has been observed in several in vivo and in vitro studies. Increases in histone H3 acetylation have been documented resulting from increased histone acetyltransferase activity and histone deacetylase (HDAC) inhibition. Reduced expression of sirtuin 1 (SIRT 1), a class III HDAC, has been shown in alcohol-exposed hepatocytes and is known to regulate the lipid metabolism pathway. Our own studies support this notion. We have shown that dysregulation of hepatic HDAC expression plays a major role in the binge alcohol-induced hepatic steatosis and liver injury by affecting lipogenesis and fatty acid β-oxidation.[123] In DNA methylation studies, hypermethylation has been observed in PPARGC1A (PPAR-γ coactivator 1α) and TFAM (mitochondrial transcription factor A) promoters in NAFLD livers. In ALD, decreased *S*-adenosyl-L-methionine levels seem to influence DNA methylation. Decreased *S*-adenosyl-L-methionine is known to induce global hypomethylation and regional hypermethylation of various promoters, and such changes are hypothesized to contribute to ASH.

Our analysis of environmental chemicals associated with TAFLD showed that exposures to chemicals that cause hepatic steatosis also can lead to multigenerational toxic effects in offspring, strongly suggesting that epigenetic alterations may be responsible.[124] Long-term exposure of humans to high concentrations of arsenic is hepatotoxic and associated with an increased risk of cancer. Arsenic-induced fatty liver and hepatotoxicity are closely associated with both DNA damage (genetic changes) and DNA methylation (epigenetic changes), and such alterations may lead to the development of liver cancer.[125] Interestingly, long-term arsenic exposure has been shown to down-regulate p16 (INK4a) by targeting recruitment of G9a and H3K9 dimethylation without changing DNA methylation in the normal mouse liver, and these changes occurred in the absence of tumorigenesis, suggesting that they may be precursors.[126]

Cell Death

Defining mechanisms for hepatocyte cell death is a critical area of interest for liver injury of all etiologies, and the common modes of cell death relevant to this article are apoptosis, necrosis, and necroptosis.[127] Apoptotic death has been demonstrated in ALD and NAFLD in both animal models and humans; moreover, apoptosis and necrosis

frequently coexist in liver pathology. We have observed coexistent apoptosis and necrosis in human ASH and NASH, with necrosis tending to be more dominant in ASH and apoptosis in NASH. Our work has shown that hepatocyte necrosis (rather than apoptosis) is seen in TASH;[128] this is also a primary death mechanism with other hepatotoxins such as carbon tetrachloride. Acetaminophen-induced liver injury evokes both necrosis and necroptosis.[129,130] Interestingly, unlike ALD and NAFLD, which are typically associated with elevated aspartate transaminase (AST) and alanine aminotransferase (ALT) activities, TAFLD is commonly associated with normal liver enzymes.[10] Alcoholic liver disease classically has AST>ALT whereas NAFLD usually has ALT predominance. These liver enzyme profiles are sometimes helpful in identifying/suggesting an underlying cause of liver injury (eg, alcohol abuse in someone who does not provide a reliable alcohol history, NAFLD in a nonobese patient). Dying hepatocytes, particularly during necrosis/necroptosis, can release pathogenic mediators or damage-associated molecular patterns such as lipid-derived metabolites (eg, aldehydes), HMGB1 (high-mobility group box 1), formyl peptides, and mitochondrial DNA, which trigger inflammation and cell death in neighboring hepatocytes and exacerbate liver damage.

Conclusions

Fatty liver disease (ASH, NASH, TASH) occurs as a result of varied etiologies (see Figure 1) and can progress to histologically identical, more severe liver disease. Disease progression is a result of both direct effects on the liver as well as indirect alterations in other organs/tissues such as intestine, adipose tissue, and the immune system. Although the three diseases share many common pathogenic mechanisms, they also exhibit distinct differences. Both shared and divergent mechanisms can be potential therapeutic targets. Better biomarkers for ASH, NASH, and TASH and improved model systems that more closely resemble human disease will promote future mechanistic investigations and therapeutic development.

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Correspondence
Address correspondence to: Craig J. McClain, MD, University of Louisville, 505 South Hancock Street, Louisville, Kentucky 40292. e-mail: cjmccl01@louisville.edu; fax: (502) 852-8927.

Conflicts of interest
The authors declare no conflicts.

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