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Pathways of liver injury in alcoholic liver disease

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Oxidative stress: a driving force in alcoholic liver injury

The development of ethanol-induced liver injury including liver cirrhosis and severe alcoholic steatohepatitis (ASH) is a complex process involving various liver cell types and mainly factors released under the control of the innate immune system. Chronic ethanol consumption induces oxidative stress and production of reactive oxygen species (ROS), cytokine release, mitochondrial dysfunction, endoplasmic reticulum stress, and others. ROS initiate lipid peroxidation that directly damages plasma and intracellular membranes and leads to the production of reactive aldehydes with potent pro-inflammatory and pro-fibrotic properties. Oxidative stress and ROS are predominantly generated through the induction of cytochrome P450-2E1 (CYP2E1). A key role for this enzyme in ethanol-induced liver injury has been demonstrated by its inhibition through chlormethiazole and by the finding that *CYP2E1* knock-out (KO) mice do not show evidence of ethanol-induced liver disease [1]. Furthermore, transgenic overexpression of human CYP2E1 in a mouse model results in more severe liver disease. Both the hydroxyethyl radical and acetaldehyde, the first products of ethanol metabolism, can bind glutathione (GSH), a tripeptide that acts as a direct free radical scavenger. The transcription factor nuclear factor (erythroid-derived 2)-like 2 (Nrf2) protects cells against xenobiotic and oxidative stress. *Nrf2* KO mice exhibit a dramatically increased mortality after ethanol feeding, highlighting the important role of oxidative stress in ethanol-induced injury [1] (Fig. 1).

Key role of pro-inflammatory cytokines

Kupffer cells are of particular importance in ethanol-induced liver injury. Chronic ethanol exposure sensitizes Kupffer cells to the activation by lipopolysaccharides (LPS) via toll-like receptor 4 (TLR4) [2]. This sensitization enhances the production of various pro-inflammatory mediators, such as tumor necrosis factor-α (TNFα) and ROS that contribute to hepatocyte dysfunction, necrosis and apoptosis of hepatocytes, and the generation of extracellular matrix proteins leading to fibrosis/cirrhosis [3]. Apoptosis, a major form of cell death, has been observed in a diverse spectrum of liver diseases. In experimental models and isolated hepatocytes, alcohol has been shown to induce liver cell apoptosis. Interestingly, the extent of apoptosis is correlated with clinical disease activity. TNFα has emerged as a key factor in various aspects of liver diseases being able to mediate many clinical aspects of severe ASH such as anorexia, fever, wasting, hypoalbuminemia, and neutrophilia. Some of the most definitive data about the importance of TNFα in the pathogenesis of liver disease have come from studies of alcoholic liver injury in animals whereas clinical studies blocking this cytokine in human studies of severe ASH have so far not proven successful. Plasma levels of both TNF and soluble TNF receptors are correlated with endotoxemia, impaired intestinal permeability, stage of liver disease, and mortality. Alcohol-associated liver injury is inhibited when the animals are treated with poorly absorbed oral antibiotics or lactobacillus to decrease endotoxemia, supporting the hypothesis that gut-derived bacterial products such as endotoxin might be important in activation of Kupffer cells and/or other cell types in the liver. This is in accordance with the recent observation that chronic ethanol feeding causes more severe liver injury in wild-type mice than in *CD14* knock-outs [4]. These results further support the notion that gut-derived endotoxin acting via its cellular receptor CD14 plays a major role in the development of early alcohol-induced liver injury. Other pro-inflammatory cytokines, however, might be of equal importance. Serum/plasma concentrations of IL-1, IL-6, and IL-8 have been shown to be increased initially in hospitalized patients with alcoholic steatohepatitis and to decline during recovery. Enhanced serum IL-1 activity has been demonstrated in patients with severe ASH more than 20 years ago. Hepatic expression of IL-8, the critical chemokine being responsible for neutrophil recruitment into the liver, correlates with progression of patients with alcoholic hepatitis further supporting the importance of cytokine circuits in those diseases [5]. IL-17, another pro-inflammatory cytokine affecting neutrophil recruitment, is also highly activated in human alcoholic liver disease and correlates with amount of liver inflammation [6] (Fig. 1).

Keywords: Fatty liver disease; Alcoholic steatohepatitis; Cytokines; Tumor necrosis factor-alpha; Adiponectin.

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Abbreviations: A1,A2B, adenosine A1/2B receptors; ADH, alcohol dehydrogenase; ADP/ATP, adenine nucleotides; 2-AG, 2-arachidonoylglycerol; ALDH, aldehyde dehydrogenase; ARE, antioxidant response element; CB1, endocannabinoid receptor type B; CD14, cellular endotoxin receptor; CD39, nucleoside triphosphate phosphohydrolase; CD73, ecto-5′-nucleotidase; FAS, fatty acid synthase; GST, glutathione S-transferase; Hif-1, hypoxia-inducible factor 1; HO-1, heme oxigenase-1; HRE, hypoxia response element; HSC, hepatic stellate cells; IFNα/β, interferon-alpha/beta; IL, interleukin; IRF3, interferon regulatory factor 3; LSEC, liver sinusoidal endothelial cells; MDB, Mallory-Denk bodies; MEOS, microsomal ethanol oxidizing enzyme cytochrome P450-2E1 (CYP2E1); NQO1, NADPH quinine oxidoreductase-1; Nrf2, Nuclear factor-eythroid 2-related factor 2; ROS, reactive oxygen species; SREBP-1c, sterol-regulatory element-binding protein-1c; TLR, toll-like receptor; TGF-β, transforming growth factor-beta; TNF-α, tumor necrosis factor-alpha.

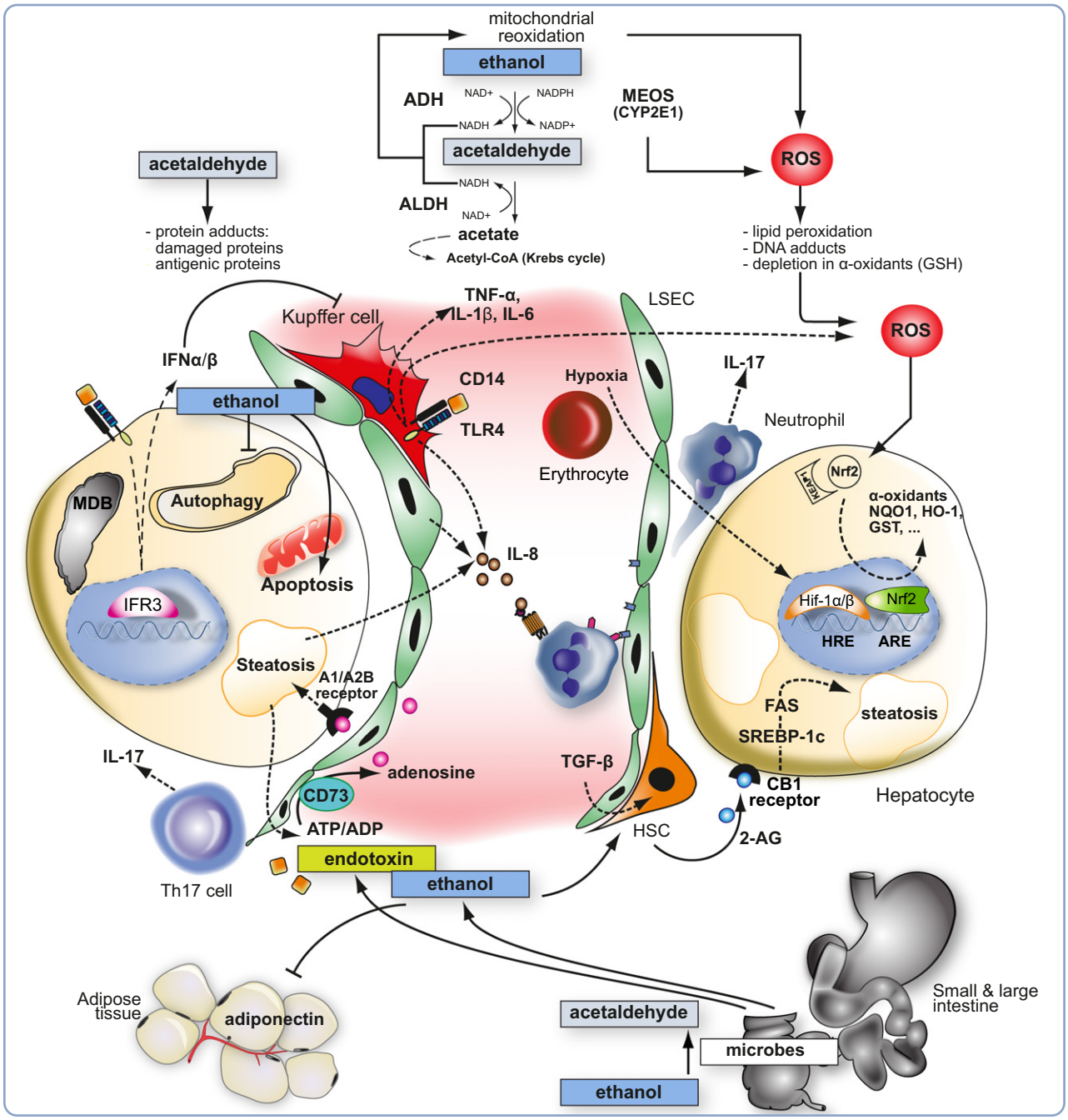

Fig. 1. Pathways of liver injury in alcoholic liver disease: central role of the innate immune system. After ingestion, ethanol is metabolized to acetaldyhyde by alcohol dehydrogenases (ADH), by the microsomal ethanol oxidizing enzyme cytochrome P450-2E1 (CYP2E1), and by bacterial enzymes of the intestinal microbiota. Acetaldehyde is then oxidized to acetate by aldehyde dehydrogenase (ALDH) and introduced into the citric acid (Krebs) cycle as acetyl-CoA. Chronic ethanol exposure increases the production of reactive oxygen species (ROS). ROS are generated at different stages of ethanol metabolism and are highly toxic when surpassing the antioxidative capacity of the hepatocyte. ROS activate redox-sensitive pro-inflammatory signaling pathways, they cause lipid peroxidation and lead to formation of protein and lipid adducts. Oxidative stress is counterbalanced by induction of anti-oxidative transcription factors such as nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and/or hypoxia-inducible factor 1 (Hif-1) that bind to antioxidant (ARE) and hypoxia response element (HRE) and induce the transcription of important detoxifying enzymes such as NADPH quinine oxidoreductase-1 (NQO1), heme oxigenase-1 (HO-1), and glutathione S-transferase (GST). It has been suggested that pericentral hypoxia contributes to hepatic injury in alcohol-induced liver injury. Chronic alcohol exposure increases the permeability of the gut mucosa resulting in translocation of bacterial products such as endotoxin into the portal circulation. The gut-derived endotoxin activates its cellular receptors CD14 and Toll-like receptor 4 (TLR4), mainly on Kupffer cells but also on hepatocytes. *CD14* KO mice are protected from the deleterious effects of chronic alcohol feeding. Activated liver cells then produce large amounts of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6. Other chemokines/cytokines such as IL-8 or IL-17 play a major role in neutrophilic infiltration of the liver. Adiponectin is a major anti-inflammatory mediator that originates mainly from the adipose tissue. Adiponectin production is significantly impaired in patients with chronic ethanol exposure. Interferon regulatory factor 3 (IRF3)-mediated production of type I interferons by parenchymal cells has been demonstrated to suppress Kupffer cell activation via induction of the anti-inflammatory cytokine IL-10 in ethanol-induced liver injury. Adiponectin is also a potent inducer of IL-10. Several other mechanisms have been demonstrated to be involved in ethanol-induced liver injury such as the adenosine receptor type B or the endocannabinoid system. Adenine nucleotides (ADP and ATP) are released from

Protective pathways impaired by chronic ethanol administration

Anti-inflammatory protective strategies may be of critical importance to protect the liver against ethanol toxicity. Adiponectin, one of the most relevant anti-inflammatory adipocytokines in humans, circulates at high concentrations in healthy humans and chronic ethanol feeding decreases adiponectin secretion by subcutaneous adipocytes. Adiponectin improves both alcoholic and nonalcoholic fatty liver diseases in mice [7]. Alcoholic fatty liver is associated with decreased adiponectin levels, decreased expression of hepatic adiponectin receptors, and impaired adiponectin liver signaling in animals. Decreased adiponectin availability may contribute not only to hepatic steatosis but also to development of liver inflammation. Kupffer cells from ethanol-fed rats are highly sensitive to inhibition by adiponectin and this effect involves an IL-10/Stat3/heme oxygenase-1 pathway. Importantly, via such a mechanism, TLR4-dependent cytokine expression in rat Kupffer cells and in mouse livers after chronic ethanol exposure is suppressed. However, besides these pathways, others may be of relevance such as adenosine. Blockade of adenosine receptors type 2B decreases TNFα and IL-6 release in sepsis models. Indeed, adenosine signaling via this receptor contributes to ethanol-induced fatty liver in animals and targeting this receptor may be effective in treatment of ethanol-associated liver inflammation [8]. Evidence from rat studies indicates that ethanol-induced gut leakiness/hyperpermeability and endotoxemia precede liver inflammation suggesting that, indeed, ethanol-induced gut damage comes first [9] (Fig. 1). Even though the described cytokine dysregulations in fatty liver diseases may reflect a secondary phenomena, blockade of these highly pro-inflammatory molecules remains an attractive treatment concept.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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References

[1] Lu Y, Zhuge J, Wang X, Bai J, Cederbaum AI. Cytochrome P450 2E1 contributes to ethanol-induced fatty liver in mice. Hepatology 2008;47: 1483–1494.
[2] Hritz I, Mandrekar P, Velayudham A, Catalano D, Dolganiuc A, Kodys K, et al. The critical role of toll-like receptor (TLR) 4 in alcoholic liver disease is independent of the common TLR adapter MyD88. Hepatology 2008;48:1224–1231.
[3] Tilg H, Diehl AM. Cytokines in alcoholic and nonalcoholic steatohepatitis. N Engl J Med 2000;343:1467–1476.
[4] Yin M, Bradford BU, Wheeler MD, Uesugi T, Froh M, Goyert SM, et al. Reduced early alcohol-induced liver injury in CD14-deficient mice. J Immunol 2001;166:4737–4742.
[5] Dominguez M, Miquel R, Colmenero J, Moreno M, Garcia-Pagan JC, Bosch J, et al. Hepatic expression of CXC chemokines predicts portal hypertension and survival in patients with alcoholic hepatitis. Gastroenterology 2009;136:1639–1650.
[6] Lemmers A, Moreno C, Gustot T, Marechal R, Degre D, Demetter P, et al. The interleukin-17 pathway is involved in human alcoholic liver disease. Hepatology 2009;49:646–657.
[7] Xu A, Wang Y, Keshaw H, Xu LY, Lam KS, Cooper GJ. The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. J Clin Invest 2003;112:91–100.
[8] Peng Z, Borea PA, Varani K, Wilder T, Yee H, Chiriboga L, et al. Adenosine signaling contributes to ethanol-induced fatty liver in mice. J Clin Invest 2009;119:582–594.
[9] Keshavarzian A, Farhadi A, Forsyth CB, Rangan J, Jakate S, Shaikh M, et al. Evidence that chronic alcohol exposure promotes intestinal oxidative stress, intestinal hyperpermeability and endotoxemia prior to development of alcoholic steatohepatitis in rats. J Hepatol 2009;50:538–547.

damaged cells and are dephosphorylated by nucleoside triphosphate phosphohydrolase (CD39) and ecto-5′-nucleotidase (CD73) to adenosine. Adenosine promotes hepatic steatosis through its G-protein-coupled receptors A1 and A2B. Endogenous cannabinoids are lipid mediators that act through two types of cannabinoid receptors (CB1 and CB2). Ethanol feeding upregulates the endocannabinoid 2-arachidonoylglycerol (2-AG) selectively in hepatic stellate cells. 2-AG paracrinely activates CB1 on hepatocytes promoting hepatic steatosis. TGF-β, which is induced by many inflammatory events, activates stellate cells and promotes hepatic fibrosis. Furthermore, ethanol impairs autophagy by disrupting vesicle elongation in hepatocytes. This leads to aggregation of damaged proteins and cell organelles in inclusion bodies called Mallory-Denk bodies (MDB). Diverse processes such as autophagy, apoptosis, and necrosis, mainly under the control of the mediators discussed here, finally contribute to disease phenotype.