World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v16.i11.1330

World J Gastroenterol 2010 March 21; 16(11): 1330-1336
ISSN 1007-9327 (print)

TOPIC HIGHLIGHT

Natalia A Osna, MD, PhD, *Series Editor*

Anti-inflammatory pathways and alcoholic liver disease: Role of an adiponectin/interleukin-10/heme oxygenase-1 pathway

Palash Mandal, Michele T Pritchard, Laura E Nagy

Palash Mandal, Michele T Pritchard, Laura E Nagy, Department of Pathobiology and Gastroenterology, Cleveland Clinic, Lerner Research Institute/NE-40, 9500 Euclid Avenue, Cleveland, OH 44195, United States
Author contributions: All authors contributed to the manuscript.
Supported by (in part) NIH Grants No. RO1AA0011975, No. R56AA001975 and No. RO1AA011876
Correspondence to: Laura E Nagy, PhD, Professor, Department of Pathobiology and Gastroenterology, Cleveland Clinic, Lerner Research Institute/NE-40, 9500 Euclid Avenue, Cleveland, OH 44195, United States. laura.nagy@case.edu
Telephone: +1-216-4444120 Fax: +1-216-6361493
Received: December 16, 2009 Revised: January 16, 2010
Accepted: January 23, 2010
Published online: March 21, 2010

Abstract

The development of alcoholic liver disease (ALD) is a complex process involving both the parenchymal and non-parenchymal cells in the liver. Enhanced inflammation in the liver during ethanol exposure is an important contributor to injury. Kupffer cells, the resident macrophages in liver, are particularly critical to the onset of ethanol-induced liver injury. Chronic ethanol exposure sensitizes Kupffer cells to activation by lipopolysaccharide *via* Toll-like receptor 4. This sensitization enhances production of inflammatory mediators, such as tumor necrosis factor-α and reactive oxygen species, that contribute to hepatocyte dysfunction, necrosis, apoptosis, and fibrosis. Impaired resolution of the inflammatory process probably also contributes to ALD. The resolution of inflammation is an active, highly coordinated response that can potentially be manipulated *via* therapeutic interventions to treat chronic inflammatory diseases. Recent studies have identified an adiponectin/interleukin-10/heme oxygenase-1 (HO-1) pathway that is profoundly effective in dampening the enhanced activation of innate immune responses in primary cultures of Kupffer cells, as well as in an *in vivo* mouse model of chronic ethanol feeding. Importantly, induction of HO-1 also reduces ethanol-induced hepatocellular apoptosis in this *in vivo* model. Based on these data, we hypothesize that the development of therapeutic agents to regulate HO-1 and its downstream targets could be useful in enhancing the resolution of inflammation during ALD and preventing progression of early stages of liver injury.

Key words: Liver disease; Alcohol; Macrophages; Heme-oxygenase-1; Inflammation

Peer reviewer: Wing-Kin Syn, MD, Division of Gastroenterology, GSRB-1, Suite 1073, DUMC 3256, 595 LaSalle Street, Durham, NC 27710, United States

Mandal P, Pritchard MT, Nagy LE. Anti-inflammatory pathways and alcoholic liver disease: Role of an adiponectin/interleukin-10/heme oxygenase-1 pathway. *World J Gastroenterol* 2010; 16(11): 1330-1336 Available from: URL: http://www.wjgnet.com/1007-9327/full/v16/i11/1330.htm DOI: http://dx.doi.org/10.3748/wjg.v16.i11.1330

INTRODUCTION

Chronic abuse of ethanol in humans leads to liver disease. An ordered progression of increasingly more serious liver injury, culminating in liver failure in 15%-20% of all alcohol abusers, has been described[1]. Fatty liver (steatosis) is the earliest stage of liver injury and is characterized by triglyceride accumulation in hepatocytes. If alcohol abuse continues, some patients with steatosis will develop inflammatory changes in their livers, a pathology described as steatohepatitis or simply hepatitis. Hepatitis is characterized by marked hepatomegaly and infiltration

of several different subtypes of leukocytes including neutrophils, monocytes/macrophages and both T and B lymphocytes. Of the patients who develop hepatitis, 50% will progress further and exhibit fibrotic changes in the liver. Of note, alcohol abuse is a leading cause of fibrosis in the USA[1,2]. Hepatic stellate cell activation is a major contributor to fibrosis though overproduction of excessive extracellular matrix components such as type I and type Ⅲ collagens and proinflammatory molecules[3]. Alcohol-induced steatosis and fibrosis can resolve, provided that the underlying stimulus, i.e. alcohol, is removed. Unfortunately, when the liver progresses to cirrhosis, a stage of liver injury characterized by significant hardening of the liver, decreased hepatocyte regeneration and significant loss of liver function, patients are likely to die as a result of liver failure unless they receive a liver transplant[4]. The medical costs associated with alcohol abuse in the United States is estimated to be $166 billion per year, of which more than $30 billion is for direct medical costs[5].

INNATE IMMUNITY AND WOUND HEALING

Activation of innate immunity is an essential response to infection or injury; the ensuing inflammatory response protects from infection, and also limits cellular and organ damage to the host organism[6]. A well-controlled innate immune response is characterized by rapid initiation of an inflammatory response. However, this response is sustained only until the immune insult or injury is contained; at that point, the inflammatory response is terminated or resolved. The controlled and appropriate resolution of inflammation is an essential feature of the innate immune response. Despite the beneficial effects of innate immunity, a failure to appropriately regulate activation of innate immunity contributes to a number of chronic inflammatory diseases, including alcoholic liver disease (ALD)[7]. In addition to the clear role of innate immune responses to alcoholic hepatitis, activation of the innate immune response likely contributes to all stages of liver injury, including steatosis, hepatocellular injury and fibrosis[8].

Dysregulation in the initiation and/or resolution of the inflammatory process could contribute to the development of ALD. Indeed, there is a growing appreciation that the resolution of inflammation is an active, highly coordinated response that could potentially be manipulated *via* therapeutic interventions to enhance the resolution of inflammatory processes and treat chronic inflammatory diseases, such as ALD.

ROLE OF IMMUNE RESPONSES IN THE INITIATION AND PROGRESSION OF ALD

The innate and adaptive immune systems are 2 distinct branches of the immune response, yet these 2 components of immunity are intimately linked at many stages of an organism's response to injury or stress. Components of the innate immune response, including natural killer (NK) and NKT cells[9], Kupffer cells (resident hepatic macrophages)[10] and the complement system[11-13], as well as T-cells and antibody-dependent adaptive immune responses[14], are involved in the hepatic response to various types of injury, including bacterial and viral infections, exposure to toxins (including ethanol), partial hepatectomy and ischemia-reperfusion.

The localized hepatic response of the innate immune system to ethanol may be distinct from the systemic innate immune response and/or localized responses of other organs to ethanol. For example, chronic alcohol consumption generally increases the susceptibility of individuals to infections[5,15], suggesting that, despite increased inflammatory responses observed in the liver after chronic ethanol exposure, systemic immune responses are suppressed by chronic ethanol exposure. Even within the liver, there may be distinct responses of individual components of the immune response. One example is the interaction of ethanol and hepatitis C virus (HCV) infection. Chronic alcohol abuse is associated with an increased incidence of HCV infection[16]. This decreased ability to ward off viral infections contrasts with the increased response of the liver to endotoxins. Understanding the localized and specific effects of chronic ethanol on the immune system will help to develop intervention strategies specifically directed at targets of ethanol action leading to the progression of ALD.

Initiation of inflammation: chronic ethanol-dependent sensitization of Toll-like receptor 4 (TLR4)-dependent signal transduction and cytokine production

How does ethanol initiate inflammatory responses in the liver? An important working model for ethanol-induced liver injury proposes that lipopolysaccharide (LPS)-induced tumor necrosis factor-α (TNFα) production by Kupffer cells, the resident macrophage of the liver, is critical for development of ethanol-induced liver injury. Chronic ethanol exposure alters the jejunal microflora leading to an increase in Gram-negative bacteria and/or disrupts the barrier function of the small intestine allowing access of bacterial products to the portal circulation[17,18]. In support of this hypothesis, endotoxin or LPS, a component of the cell wall of Gram-negative bacteria, is increased in the circulation of alcoholics[19] and murine models of chronic ethanol exposure[20,21]. Further, if gut bacteria are diminished using antibiotics, ethanol-induced fatty liver and inflammation are attenuated[20].

Kuppfer cells and TLR4 in the initiation of inflammation: The liver contains the first capillary bed through which blood from the intestine flows. Kupffer cells are located in the hepatic sinusoids and, among other physiologic functions, clear endotoxins from the blood, but usually without discernable inflammation. Chronic ethanol exposure also increases the sensitivity of Kupffer cells to endotoxins[10]. Therefore, in the presence of persistent ethanol exposure and increased exposure to gut-derived endotoxins, Kupffer cells become activated and contribute to liver disease.

Kupffer cells are innate immune effectors that produce several proinflammatory mediators, including reactive oxygen species (ROS) and TNFα, which promote liver injury after chronic ethanol in response to LPS[17]. In rats depleted of Kupffer cells by gadolinium chloride treatment, ethanol-induced liver injury is attenuated[17]. Further evidence for a role of activated Kupffer cells and LPS in the development of ethanol-induced liver injury has been revealed, as mice deficient in LPS receptor complex components TLR4 and CD14, and NADPH oxidase function ($p47^{phox}$ -/- mice) are protected from ethanol-induced liver injury[22-24]. TLR4 signaling *via* its MyD88-independent pathway (TRIF-dependent) is a critical contributor to increased steatosis and inflammation during chronic ethanol exposure[25,26].

Ethanol and the regulation of TNFα expression: TNFα is produced predominately by cells of monocyte/macrophage lineage and is particularly critical for ethanol-induced liver injury. TNFα concentration is enhanced in the blood of alcoholics[27] and in the blood of rats and mice exposed to chronic ethanol[17]. Additionally, if TNFα is blocked in mice using TNFα-specific antibodies or if TNFα signaling is prevented in TNFα receptor-deficient mice, ethanol induced liver injury is diminished[28].

Enhanced TLR4 signal transduction by ethanol

Production of inflammatory cytokines is a highly regulated process; regulation occurs at the level of transcription, translation and secretion[29,30]. Ethanol exposure impacts the molecular regulation of TNF-α expression at each level of control in macrophages, resulting in an enhanced initiation of inflammation in the liver[10]. Ethanol mediates these changes in the activation of Kupffer cells by a profound dysregulation in TLR4-initiated signal transduction[10]. Interestingly, many of the same signaling pathways targeted by ethanol in neurons, resulting in the complex behavioral effects of ethanol, are also involved in TLR4-mediated signal transduction in macrophages. Briefly, LPS binds to a cell surface receptor, CD14, which, *via* interactions with TLR4[31], stimulates a complex array of signal transduction cascades[32,33]. Stimulation of macrophages with LPS activates tyrosine kinases, protein kinase C, nuclear factor κB (NFκB), as well as members of the mitogen-activated protein kinase family, including extracellular signal-regulated kinase 1/2 (ERK1/2), p38 and c-jun N-terminal kinase[32]. Chronic ethanol feeding generally enhances these activation pathways, including increased LPS-stimulated phosphorylation of ERK1/2 and p38[34-36] and NFκB activation[37].

Role of ROS in increased cytokine expression

Increased production of ROS during chronic ethanol exposure, either by hepatocytes during ethanol metabolism[38] and/or from Kupffer cells during ethanol exposure[39] and in response to LPS[40], may contribute to this sensitization in LPS-dependent signal transduction. ROS play a critical role in the modulation/regulation of a number of signal transduction cascades[41], including LPS-stimulated signaling pathways both in cells of the innate immune system (monocytes/macrophages, neutrophils, *etc.*) and non-immune cells[42-44]. Indeed, we have specifically identified NADPH oxidase-derived ROS as an important contributor to LPS-stimulated ERK1/2 phosphorylation in rat Kupffer cells, particularly after chronic ethanol[45]. Taken together, these data suggest that the chronic ethanol-induced increase in ROS is an important contributor to the dysregulation of LPS-mediated signal transduction and inflammatory cytokine production in Kupffer cells.

Clinical implications of enhanced initiation of inflammation after chronic ethanol exposure

While the role of TNF-α in the development of ethanol-induced liver injury has been well characterized in animal models[17], clinical investigations of the therapeutic efficacy of antibodies to TNF-α (e.g. infliximab) to treat patients with acute alcoholic steatohepatitis have generated variable results[46,47]. There is particular concern about side effects of completely inhibiting TNF-α function. For example, since TNF-α is a critical component of immunity, infectious disease is a primary concern during anti-TNF-α therapy[47,48]. Moreover, TNF-α is required for normal liver regeneration; hepatocyte proliferation in response to injury is impaired in mice lacking TNF-α receptors[49]. Therefore, therapeutic strategies to normalize, rather than eliminate, TNF-α expression in ALD are more likely to be safe and effective. Interventions that re-establish normal homeostatic control of initiation and resolution of innate immune responses in liver would allow for maintenance of normal immune function and repair of hepatocyte injury during treatment to resolve ALD.

RESOLUTION OF INFLAMMATION: ROLE OF ANTI-INFLAMMATORY MEDIATORS

The controlled and appropriate resolution of inflammation is an essential feature of the innate immune response; failure to terminate an inflammatory response likely contributes to a number of chronic inflammatory diseases, including ALD[7]. Importantly, the resolution of inflammation is an active, highly coordinated response. Indeed, inflammatory signals initiate the induction of negative regulators. During ethanol exposure, despite high expression of inflammatory mediators, the resolution phase is not functionally appropriate. Understanding the mechanisms for the failure to resolve inflammation, and the identification of effective anti-inflammatory pathways that can be upregulated in the context of chronic ethanol exposure are likely to lead to therapeutic strategies to treat, as well as prevent, ALD.

The mechanisms for resolution of an inflammatory response are complex, requiring the elimination of infiltrating neutrophils, as well as a normalization of

macrophage numbers and activity[50]. Within the context of this review, we will focus our attention on the identification of specific intrinsic pathways that normalize the increased pro-inflammatory activity of Kupffer cells after chronic exposure to ethanol. In order for a particular anti-inflammatory pathway to have potential therapeutic value, it must remain functional after chronic ethanol exposure, must target the specific pro-inflammatory pathways enhanced by ethanol and must contribute to the prevention of hepatocellular injury. Finally, the pathway must be amenable to therapeutic intervention.

Ethanol and adiponectin

Recent studies have explored the potential of adiponectin, an abundant 30 kDa adipokine with potent anti-inflammatory properties, as a useful anti-inflammatory to exploit in the treatment of ALD. Treatment of mice with supra-physiological concentrations of adiponectin during chronic ethanol exposure prevented the development of liver injury, decreasing both steatosis and TNF-α expression in the liver[51]. You *et al*[52] have recently reviewed the molecular mechanisms for the anti-steatotic effects of adiponectin in liver. In hepatocytes, activation of a SIRT1-AMPK (sirtuin 1-AMP-activated protein kinase) pathway appears to be critical in decreasing ethanol-induced lipid accumulation in the liver[52].

Adiponectin also normalizes LPS-induced TNF-α production in primary cultures of Kupffer cells after chronic ethanol exposure[53]. Interestingly, the anti-inflammatory effects of adiponectin appear to be mediated by either globular adiponectin (gAcrp) or full-length adiponectin[53]. This may be due, at least in part, to the ability of macrophages to cleave full-length adiponectin to its globular form[54]. gAcrp signals primarily *via* adiponectin receptor 1 in Kupffer cells[55]. In contrast to the predominant role of AMPK in mediating the effects of adiponectin in hepatocytes[52], gAcrp activates multiple signaling pathways in macrophages, including the mitogen-activated protein kinase family members, NFκB and protein kinase A[56]. Together, activation of these signaling pathways culminates in the expression of anti-inflammatory mediators, including interleukin (IL)-10[56].

Despite the efficacy of adiponectin in decreasing LPS-mediated responses, both in mouse models and primary cultures of Kupffer cells, the development of adiponectin for therapeutic interventions in patients with ALD is likely of limited utility, because of the high concentration of adiponectin in the circulation, as well as the complex oligomeric structure of adiponectin. Therefore, investigators have begun to focus either on mechanisms to increase endogenous adiponectin expression, such as treatment of mice with rosiglitazone[57], as well as on the identification of downstream molecular targets of adiponectin in the liver. Recent studies in Kupffer cells have identified an adiponectin-mediated IL-10/heme oxygenase-1 (HO-1) pathway involved in the anti-inflammatory effects of adiponectin in macrophages that may be more amenable to pharmacological intervention.

IL-10/HO-1 pathway

IL-10 is an immunomodulatory cytokine with potent anti-inflammatory properties. IL-10 decreases production of pro-inflammatory cytokines, including TNF-α and IL-1β[58]. While little is known about the regulation of IL-10 expression and activity in the liver in response to chronic ethanol, studies show that impaired expression of IL-10 contributes to inflammation in alcoholic cirrhotics[59] and IL-10 deficient mice are more sensitive to ethanol-induced liver injury[60]. Drechsler *et al*[61] found that treatment with IL-10 reduced the acute effects of ethanol in monocytes in an HO-1 dependent mechanism.

We have recently identified an IL-10/HO-1 mediated pathway that can be activated in Kupffer cells after chronic ethanol feeding and suppresses LPS-stimulated TNF-α expression[55]. IL-10 mediates its anti-inflammatory functions *via* induction of IL-10-inducible genes, including HO-1, suppressor of cytokine signaling 3 and BCL3[58]. There is a growing appreciation that HO-1, in particular, is an important downstream mediator of the anti-inflammatory effects of IL-10 in macrophages[58]. Interestingly, induction of HO-1 by Kupffer cells after chronic ethanol is particularly robust[55]; siRNA knockdown or chemical inhibition of HO-1 in Kupffer cells demonstrates that HO-1 is critical to the suppression of TLR4-stimulated TNF-α expression in response to either adiponectin or IL-10 after chronic ethanol exposure[55].

HO-1 plays an important anti-inflammatory role in a number of chronic inflammatory diseases, and also has anti-apoptotic and anti-proliferative properties[62]. HO-1 is a 288 amino acid protein that is anchored in the endoplasmic reticulum, although localization of a cleaved form of HO-1 has also been observed in the nucleus under some conditions[63]. HOs catalyze the initial and rate limiting step in the oxidative degradation of heme, yielding equimolar amounts of biliverdin IXα, carbon monoxide (CO), and free iron[64]. Three isoforms of the HO protein have been identified, which are encoded by separate genes. HO-2 and HO-3 are constitutive forms, while HO-1 (also known as heat shock protein 32) is an inducible isozyme, with high expression levels in spleen and Kupffer cells[65]. HO-1 is a stress-responsive protein whose expression is upregulated by a broad spectrum of inducers, including heme, heavy metals, nephrotoxins, cytokines, endotoxins and oxidative stress.

Surprisingly, acute and chronic ethanol exposure do not increase HO-1 expression in Kupffer cells or in the liver[55]. However, this may be dependent on the age of the animals studied[66,67]. While ethanol does not increase HO-1 expression, several recent studies suggested that induction of HO-1, as well as increasing concentrations of its downstream mediator CO, prevents ethanol-induced inflammation in the intestine[68] and liver[55], as well as oxidative damage to hepatocytes[69] and hepatocyte apoptosis. Importantly, induction of HO-1 in mice after chronic ethanol exposure by treatment with cobalt-protoporphyrin-IX normalizes LPS-induced TNFα expression in the liver[55].

HO-1: downstream anti-inflammatory mediators

Induction of HO-1 plays a key role in mediating cellular protection against the insult of oxidants both *in vitro* and *in vivo*[62]. *In vivo* expression of HO-1 has potent protective effects against atherogenesis, acute cardiac ischemic failure, ischemia/reperfusion injury in liver, as well as hyperoxia-induced liver injury[62]. The exact mechanisms involved in the anti-inflammatory effects of HO-1 are poorly understood. Recent studies suggested that 2 of the break-down products of heme generated by HO-1, i.e. CO and biliverdin (and its breakdown product bilirubin), are each potential mediators of HO-1 activity[62]. Release of free iron by HO-1 typically induces the expression of ferritin, which can also have protective properties. In Kupffer cells from ethanol-fed rats, we find that treatment with the CO donor, CORM-2 (CO-releasing molecule), was sufficient to decrease LPS-stimulated TNF-α expression[55], suggesting CO has strong anti-inflammatory effects in Kupffer cells after chronic ethanol feeding.

CO is also an important regulator of a number of signaling pathways that regulate hepatic metabolism and inflammatory responses, including MAPK family members, peroxisome proliferator-activated receptor-γ, Egr-1 and adenosine 2A receptor[70-72]. Each of these signaling pathways is also a target of chronic ethanol in hepatocytes and/or Kupffer cells.

CONCLUSION

Considering the strong association between ethanol-mediated activation of the innate immune system and ALD, it is critical to understand the mechanism(s) by which ethanol disrupts the intricately regulated innate immune response. Characteristically, the innate immune response involves both a rapid, yet transient, activation; the transient nature of the innate immune response contributes to an appropriate resolution of inflammation[50,73]. In the past, studies have focused primarily on the impact of ethanol on activation of the innate immune response *via* production of pro-inflammatory mediators. However, recent studies suggest that ethanol must also impair the "anti-inflammatory" responses elicited during inflammation, thus delaying and/or impairing the resolution of the inflammatory response. Impaired resolution of inflammation contributes to chronic inflammatory states[6]. Recent studies have demonstrated that induction of HO-1 in primary cultures of Kupffer cells isolated from rats after chronic ethanol exposure normalizes chronic ethanol-induced sensitization to LPS[55]. Importantly, HO-1 induction also normalizes LPS-stimulated TNF-α expression, as well as hepatocyte apoptosis, in an *in vivo* mouse model of chronic ethanol exposure[55]. We hypothesize that the development of therapeutic agents to regulate these HO-1-activated pathways will enhance the resolution of inflammation during ALD and prevent progression of early stages of liver injury.

REFERENCES

1 **Lieber CS**. Alcoholic liver disease: new insights in pathogenesis lead to new treatments. *J Hepatol* 2000; **32**: 113-128
2 **Bataller R**, North KE, Brenner DA. Genetic polymorphisms and the progression of liver fibrosis: a critical appraisal. *Hepatology* 2003; **37**: 493-503
3 **Gressner OA**, Weiskirchen R, Gressner AM. Evolving concepts of liver fibrogenesis provide new diagnostic and therapeutic options. *Comp Hepatol* 2007; **6**: 7
4 **Iredale JP**. Hepatic stellate cell behavior during resolution of liver injury. *Semin Liver Dis* 2001; **21**: 427-436
5 **Nelson S**, Kolls JK. Alcohol, host defence and society. *Nat Rev Immunol* 2002; **2**: 205-209
6 **Han J**, Ulevitch RJ. Limiting inflammatory responses during activation of innate immunity. *Nat Immunol* 2005; **6**: 1198-1205
7 **Vidali M**, Stewart SF, Albano E. Interplay between oxidative stress and immunity in the progression of alcohol-mediated liver injury. *Trends Mol Med* 2008; **14**: 63-71
8 **Schwabe RF**, Seki E, Brenner DA. Toll-like receptor signaling in the liver. *Gastroenterology* 2006; **130**: 1886-1900
9 **Minagawa M**, Deng Q, Liu ZX, Tsukamoto H, Dennert G. Activated natural killer T cells induce liver injury by Fas and tumor necrosis factor-alpha during alcohol consumption. *Gastroenterology* 2004; **126**: 1387-1399
10 **Nagy LE**. Recent insights into the role of the innate immune system in the development of alcoholic liver disease. *Exp Biol Med* (Maywood) 2003; **228**: 882-890
11 **Strey CW**, Markiewski M, Mastellos D, Tudoran R, Spruce LA, Greenbaum LE, Lambris JD. The proinflammatory mediators C3a and C5a are essential for liver regeneration. *J Exp Med* 2003; **198**: 913-923
12 **Markiewski MM**, Mastellos D, Tudoran R, DeAngelis RA, Strey CW, Franchini S, Wetsel RA, Erdei A, Lambris JD. C3a and C3b activation products of the third component of complement (C3) are critical for normal liver recovery after toxic injury. *J Immunol* 2004; **173**: 747-754
13 **Pritchard MT**, McMullen MR, Stavitsky AB, Cohen JI, Lin F, Medof ME, Nagy LE. Differential contributions of C3, C5, and decay-accelerating factor to ethanol-induced fatty liver in mice. *Gastroenterology* 2007; **132**: 1117-1126
14 **Tuma DJ**, Casey CA. Dangerous byproducts of alcohol breakdown--focus on adducts. *Alcohol Res Health* 2003; **27**: 285-290
15 **Szabo G**. Consequences of alcohol consumption on host defence. *Alcohol Alcohol* 1999; **34**: 830-841
16 **Siu L**, Foont J, Wands JR. Hepatitis C virus and alcohol. *Semin Liver Dis* 2009; **29**: 188-199
17 **Thurman RG**. II. Alcoholic liver injury involves activation of Kupffer cells by endotoxin. *Am J Physiol* 1998; **275**: G605-G611
18 **Vallabhaneni R**, Kaczorowski DJ, Yaakovian MD, Rao J, Zuckerbraun BS. Heme oxygenase 1 protects against hepatic hypoxia and injury from hemorrhage via regulation of cellular respiration. *Shock* 2010; **33**: 274-281
19 **Fukui H**, Brauner B, Bode JC, Bode C. Plasma endotoxin concentrations in patients with alcoholic and non-alcoholic liver disease: reevaluation with an improved chromogenic assay. *J Hepatol* 1991; **12**: 162-169
20 **Adachi Y**, Moore LE, Bradford BU, Gao W, Thurman RG. Antibiotics prevent liver injury in rats following long-term exposure to ethanol. *Gastroenterology* 1995; **108**: 218-224
21 **Keshavarzian A**, Farhadi A, Forsyth CB, Rangan J, Jakate S, Shaikh M, Banan A, Fields JZ. 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
22 **Kono H**, Rusyn I, Yin M, Gäbele E, Yamashina S, Dikalova A, Kadiiska MB, Connor HD, Mason RP, Segal BH, Bradford BU, Holland SM, Thurman RG. NADPH oxidase-derived free radicals are key oxidants in alcohol-induced liver disease. *J Clin Invest* 2000; **106**: 867-872
23 **Uesugi T**, Froh M, Arteel GE, Bradford BU, Thurman RG.

Toll-like receptor 4 is involved in the mechanism of early alcohol-induced liver injury in mice. *Hepatology* 2001; **34**: 101-108
24 **Yin M**, Bradford BU, Wheeler MD, Uesugi T, Froh M, Goyert SM, Thurman RG. Reduced early alcohol-induced liver injury in CD14-deficient mice. *J Immunol* 2001; **166**: 4737-4742
25 **Hritz I**, Mandrekar P, Velayudham A, Catalano D, Dolganiuc A, Kodys K, Kurt-Jones E, Szabo G. 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
26 **Zhao XJ**, Dong Q, Bindas J, Piganelli JD, Magill A, Reiser J, Kolls JK. TRIF and IRF-3 binding to the TNF promoter results in macrophage TNF dysregulation and steatosis induced by chronic ethanol. *J Immunol* 2008; **181**: 3049-3056
27 **McClain CJ**, Cohen DA. Increased tumor necrosis factor production by monocytes in alcoholic hepatitis. *Hepatology* 1989; **9**: 349-351
28 **Yin M**, Wheeler MD, Kono H, Bradford BU, Gallucci RM, Luster MI, Thurman RG. Essential role of tumor necrosis factor alpha in alcohol-induced liver injury in mice. *Gastroenterology* 1999; **117**: 942-952
29 **Papadakis KA**, Targan SR. Tumor necrosis factor: biology and therapeutic inhibitors. *Gastroenterology* 2000; **119**: 1148-1157
30 **Watkins LR**, Hansen MK, Nguyen KT, Lee JE, Maier SF. Dynamic regulation of the proinflammatory cytokine, interleukin-1beta: molecular biology for non-molecular biologists. *Life Sci* 1999; **65**: 449-481
31 **Poltorak A**, He X, Smirnova I, Liu MY, Van Huffel C, Du X, Birdwell D, Alejos E, Silva M, Galanos C, Freudenberg M, Ricciardi-Castagnoli P, Layton B, Beutler B. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* 1998; **282**: 2085-2088
32 **Sweet MJ**, Hume DA. Endotoxin signal transduction in macrophages. *J Leukoc Biol* 1996; **60**: 8-26
33 **Ulevitch RJ**, Tobias PS. Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. *Annu Rev Immunol* 1995; **13**: 437-457
34 **Shi L**, Kishore R, McMullen MR, Nagy LE. Chronic ethanol increases lipopolysaccharide-stimulated Egr-1 expression in RAW 264.7 macrophages: contribution to enhanced tumor necrosis factor alpha production. *J Biol Chem* 2002; **277**: 14777-14785
35 **Kishore R**, McMullen MR, Nagy LE. Stabilization of tumor necrosis factor alpha mRNA by chronic ethanol: role of A + U-rich elements and p38 mitogen-activated protein kinase signaling pathway. *J Biol Chem* 2001; **276**: 41930-41937
36 **Cao Q**, Mak KM, Lieber CS. Dilinoleoylphosphatidylcholine decreases LPS-induced TNF-alpha generation in Kupffer cells of ethanol-fed rats: respective roles of MAPKs and NF-kappaB. *Biochem Biophys Res Commun* 2002; **294**: 849-853
37 **Mandrekar P**, Bala S, Catalano D, Kodys K, Szabo G. The opposite effects of acute and chronic alcohol on lipopolysaccharide-induced inflammation are linked to IRAK-M in human monocytes. *J Immunol* 2009; **183**: 1320-1327
38 **Arteel GE**. Oxidants and antioxidants in alcohol-induced liver disease. *Gastroenterology* 2003; **124**: 778-790
39 **Wheeler MD**, Kono H, Yin M, Nakagami M, Uesugi T, Arteel GE, Gäbele E, Rusyn I, Yamashina S, Froh M, Adachi Y, Iimuro Y, Bradford BU, Smutney OM, Connor HD, Mason RP, Goyert SM, Peters JM, Gonzalez FJ, Samulski RJ, Thurman RG. The role of Kupffer cell oxidant production in early ethanol-induced liver disease. *Free Radic Biol Med* 2001; **31**: 1544-1549
40 **Spolarics Z**. Endotoxemia, pentose cycle, and the oxidant/antioxidant balance in the hepatic sinusoid. *J Leukoc Biol* 1998; **63**: 534-541
41 **Thannickal VJ**, Fanburg BL. Reactive oxygen species in cell signaling. *Am J Physiol Lung Cell Mol Physiol* 2000; **279**: L1005-L1028
42 **Iles KE**, Forman HJ. Macrophage signaling and respiratory burst. *Immunol Res* 2002; **26**: 95-105
43 **Finkel T**. Oxidant signals and oxidative stress. *Curr Opin Cell Biol* 2003; **15**: 247-254
44 **Nagy LE**. Molecular aspects of alcohol metabolism: transcription factors involved in early ethanol-induced liver injury. *Annu Rev Nutr* 2004; **24**: 55-78
45 **Thakur V**, Pritchard MT, McMullen MR, Wang Q, Nagy LE. Chronic ethanol feeding increases activation of NADPH oxidase by lipopolysaccharide in rat Kupffer cells: role of increased reactive oxygen in LPS-stimulated ERK1/2 activation and TNF-alpha production. *J Leukoc Biol* 2006; **79**: 1348-1356
46 **Tilg H**, Jalan R, Kaser A, Davies NA, Offner FA, Hodges SJ, Ludwiczek O, Shawcross D, Zoller H, Alisa A, Mookerjee RP, Graziadei I, Datz C, Trauner M, Schuppan D, Obrist P, Vogel W, Williams R. Anti-tumor necrosis factor-alpha monoclonal antibody therapy in severe alcoholic hepatitis. *J Hepatol* 2003; **38**: 419-425
47 **Naveau S**, Chollet-Martin S, Dharancy S, Mathurin P, Jouet P, Piquet MA, Davion T, Oberti F, Broët P, Emilie D. A double-blind randomized controlled trial of infliximab associated with prednisolone in acute alcoholic hepatitis. *Hepatology* 2004; **39**: 1390-1397
48 **Keane J**, Gershon S, Wise RP, Mirabile-Levens E, Kasznica J, Schwieterman WD, Siegel JN, Braun MM. Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. *N Engl J Med* 2001; **345**: 1098-1104
49 **Yamada Y**, Kirillova I, Peschon JJ, Fausto N. Initiation of liver growth by tumor necrosis factor: deficient liver regeneration in mice lacking type I tumor necrosis factor receptor. *Proc Natl Acad Sci USA* 1997; **94**: 1441-1446
50 **Serhan CN**, Savill J. Resolution of inflammation: the beginning programs the end. *Nat Immunol* 2005; **6**: 1191-1197
51 **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
52 **You M**, Rogers CQ. Adiponectin: a key adipokine in alcoholic fatty liver. *Exp Biol Med* (Maywood) 2009; **234**: 850-859
53 **Thakur V**, Pritchard MT, McMullen MR, Nagy LE. Adiponectin normalizes LPS-stimulated TNF-alpha production by rat Kupffer cells after chronic ethanol feeding. *Am J Physiol Gastrointest Liver Physiol* 2006; **290**: G998-G1007
54 **Waki H**, Yamauchi T, Kamon J, Kita S, Ito Y, Hada Y, Uchida S, Tsuchida A, Takekawa S, Kadowaki T. Generation of globular fragment of adiponectin by leukocyte elastase secreted by monocytic cell line THP-1. *Endocrinology* 2005; **146**: 790-796
55 **Mandal P**, Park PH, McMullen MR, Pratt BT, Nagy LE. The anti-inflammatory effects of adiponectin are mediated via a heme oxygenase-1-dependent pathway in rat Kupffer cells. *Hepatology* 2009; Epub ahead of print
56 **Park PH**, Huang H, McMullen MR, Bryan K, Nagy LE. Activation of cyclic-AMP response element binding protein contributes to adiponectin-stimulated interleukin-10 expression in RAW 264.7 macrophages. *J Leukoc Biol* 2008; **83**: 1258-1266
57 **Ajmo JM**, Liang X, Rogers CQ, Pennock B, You M. Resveratrol alleviates alcoholic fatty liver in mice. *Am J Physiol Gastrointest Liver Physiol* 2008; **295**: G833-G842
58 **O'Shea JJ**, Murray PJ. Cytokine signaling modules in inflammatory responses. *Immunity* 2008; **28**: 477-487
59 **Le Moine O**, Marchant A, De Groote D, Azar C, Goldman M, Devière J. Role of defective monocyte interleukin-10 release in tumor necrosis factor-alpha overproduction in alcoholics cirrhosis. *Hepatology* 1995; **22**: 1436-1439
60 **Hill DB**, D'Souza NB, Lee EY, Burikhanov R, Deaciuc IV, de Villiers WJ. A role for interleukin-10 in alcohol-induced liver sensitization to bacterial lipopolysaccharide. *Alcohol*

Clin Exp Res 2002; **26**: 74-82
61 **Drechsler Y**, Dolganiuc A, Norkina O, Romics L, Li W, Kodys K, Bach FH, Mandrekar P, Szabo G. Heme oxygenase-1 mediates the anti-inflammatory effects of acute alcohol on IL-10 induction involving p38 MAPK activation in monocytes. *J Immunol* 2006; **177**: 2592-2600
62 **Otterbein LE**, Soares MP, Yamashita K, Bach FH. Heme oxygenase-1: unleashing the protective properties of heme. *Trends Immunol* 2003; **24**: 449-455
63 **Morse D**, Lin L, Choi AM, Ryter SW. Heme oxygenase-1, a critical arbitrator of cell death pathways in lung injury and disease. *Free Radic Biol Med* 2009; **47**: 1-12
64 **Maines MD**. Heme oxygenase: function, multiplicity, regulatory mechanisms, and clinical applications. *FASEB J* 1988; **2**: 2557-2568
65 **Bauer I**, Wanner GA, Rensing H, Alte C, Miescher EA, Wolf B, Pannen BH, Clemens MG, Bauer M. Expression pattern of heme oxygenase isoenzymes 1 and 2 in normal and stress-exposed rat liver. *Hepatology* 1998; **27**: 829-838
66 **Patriarca S**, Furfaro AL, Cosso L, Pesce Maineri E, Balbis E, Domenicotti C, Nitti M, Cottalasso D, Marinari UM, Pronzato MA, Traverso N. Heme oxygenase 1 expression in rat liver during ageing and ethanol intoxication. *Biogerontology* 2007; **8**: 365-372
67 **Zheng J**, Tian Q, Hou W, Watts JA, Schrum LW, Bonkovsky HL. Tissue-specific expression of ALA synthase-1 and heme oxygenase-1 and their expression in livers of rats chronically exposed to ethanol. *FEBS Lett* 2008; **582**: 1829-1834
68 **Li X**, Schwacha MG, Chaudry IH, Choudhry MA. Heme oxygenase-1 protects against neutrophil-mediated intestinal damage by down-regulation of neutrophil p47phox and p67phox activity and O2- production in a two-hit model of alcohol intoxication and burn injury. *J Immunol* 2008; **180**: 6933-6940
69 **Liu LG**, Yan H, Zhang W, Yao P, Zhang XP, Sun XF, Nussler AK. Induction of heme oxygenase-1 in human hepatocytes to protect them from ethanol-induced cytotoxicity. *Biomed Environ Sci* 2004; **17**: 315-326
70 **Otterbein LE**, Bach FH, Alam J, Soares M, Tao Lu H, Wysk M, Davis RJ, Flavell RA, Choi AM. Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway. *Nat Med* 2000; **6**: 422-428
71 **Bilban M**, Bach FH, Otterbein SL, Ifedigbo E, d'Avila JC, Esterbauer H, Chin BY, Usheva A, Robson SC, Wagner O, Otterbein LE. Carbon monoxide orchestrates a protective response through PPARgamma. *Immunity* 2006; **24**: 601-610
72 **Haschemi A**, Wagner O, Marculescu R, Wegiel B, Robson SC, Gagliani N, Gallo D, Chen JF, Bach FH, Otterbein LE. Cross-regulation of carbon monoxide and the adenosine A2a receptor in macrophages. *J Immunol* 2007; **178**: 5921-5929
73 **Khabar KS**. Rapid transit in the immune cells: the role of mRNA turnover regulation. *J Leukoc Biol* 2007; **81**: 1335-1344

S- Editor Tian L **L- Editor** Cant MR **E- Editor** Ma WH