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TOPIC HIGHLIGHT

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Toll-like receptor-mediated signaling cascade as a regulator of the inflammation network during alcoholic liver disease

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Abstract

Chronic abuse of alcohol leads to various histological abnormalities in the liver. These are conditions collectively known as alcoholic liver disease (ALD). Currently, ALD is considered to be one of the major causes of death worldwide. An impaired intestinal barrier with related endotoxemia is among the various pathogenetic factors. This is mainly characterized by circulating levels of lipopolysaccharide (LPS), considered critical for the onset of intra-hepatic inflammation. This in turn promotes hepatocellular damage and fibrosis in ALD. Elevated levels of LPS exert their effects by binding to Toll-like receptors (TLRs) which are expressed by all liver-resident cells. The activation of TLR signaling triggers an overproduction and release of some cytokines, which promote an autocatalytic cascade of other pro-inflammatory signals. In this review, we provide an overview of the mechanisms that sustain LPS-mediated activation of TLR signaling, reporting current experimental and clinical evidence of its role during inflammation in ALD.

Key words: Alcoholic liver disease; Toll-like receptors; Inflammation; Cirrhosis; Nuclear factor-κB; Cytokines

Core tip: Alcoholic liver disease (ALD) pathogenesis is quite complex and requires the activation/inhibition of several molecular pathways. The inflammatory storm caused by alcohol abuse on the gut-liver axis and consequent activation of Toll-like receptor (TLR) signaling is topical for researchers and physicians, both for understanding ALD pathophysiology and for translating novel clues into clinical practice. Here, we focus on the current evidence of TLR involvement in inflammation during ALD in experimental models and humans, offering readers with no first-hand knowledge of this topic a valuable tool to start novel studies.

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INTRODUCTION

Toll-like receptors (TLRs) belong to the family of pattern recognition receptors and are crucial sensors of the innate immune system committed to the recognition of both pathogen and damage-associated molecular patterns (PAMPs and DAMPs, respectively)[1-4]. TLRs are key molecules of the innate immune system which play a major role in the control of the inflammation process, promoting the production of several circulating inflammatory molecules, including cytokines, chemokines and other molecules that may participate in tissue repair or exacerbate tissue damage in several diseases[5]. It is noteworthy that TLRs have also been implicated in both liver physiology and in the pathophysiology of several liver diseases

as they are diffusely expressed in all types of liver cells[6-8].

The integrity of the intestinal barrier and appropriate gut permeability are crucial for maintaining the equilibrium of commensal and pathogenic microorganisms and for avoiding their translocation from the gut. Normally, only bacterial traces can pass the intestinal mucosa and reach the liver through the portal circulation where their clearance is accomplished. A large amount of the literature in animal models and humans has reported that excessive alcohol consumption increases intestinal permeability, disrupting the intestinal barrier and leading to a strong increase of portal and systemic levels of the most studied PAMP, lipopolysaccharide (LPS)[9-12]. However, only recently through novel metagenomic and metaproteomic approaches, the ability of acute and chronic ingestion of alcohol to alter gut microbiota composition by increased bacterial overgrowth and contributing to liver damage and inflammation has emerged[13-15].

Over the past decade, despite numerous prevention campaigns, alcohol consumption is still at alarming levels, particularly in industrialized countries[16]. Therefore, alcohol abuse is currently considered to be one of the major causes of chronic liver disease in Western countries, particularly in Europe, southern Europe and the United Kingdom[17]. Prevalently, heavy drinkers are susceptible to develop alcoholic liver disease (ALD) which may be characterized by different histological abnormalities, including steatosis, steatohepatitis and fibrosis, and evolving into more severe forms of liver injury, such as cirrhosis and hepatocellular carcinoma (HCC)[17,18].

During the last decade, the importance of research and clinical studies of the underlying molecular mechanisms that link TLRs and ALD have received increasing interest, particularly because of their therapeutic inference.

In the present review, we focus on the implication of TLRs and their role in inflammation in ALD pathogenesis and we provide an overview of their possible clinical impact in prevention and therapy.

TLR-MEDIATED SIGNALING

TLRs are regulators of innate immune response and sensors of both the pathogen signature bacteria, fungi and virus PAMPs and the endogenous components, DAMPs. They are highly conserved type I transmembrane proteins which comprise an extracellular leucine-rich ligand binding domain and an intracellular domain, Toll/interleukin (IL)-1 receptor (TIR) domain, responsible for their intracellular signal transduction[19]. TLRs have been classified based on their ligand specificity and selectivity, accounting for more than 13 members in mammals, of which 11 are expressed in humans. However, each human TLR exhibits differential activities, depending on its tissue expression and ligand specificity[20].

In the liver, TLRs are expressed in Kupffer cells (KCs), hepatocytes and hepatic stellate cells (HSCs) and they have been extensively studied in various chronic liver diseases[21]. In more detail, TLR2 and TLR4 expression is shared by hepatocytes, KCs, HSCs and biliary epithelial cells, while TLR4 is also expressed by sinusoidal endothelial cells. Moreover, KCs also express both TLR3 as biliary epithelial cells and TLR9, similar to HSCs and sinusoidal endothelial cells[22].

LPS, a component of Gram-negative bacteria walls, composed of a carbohydrate (O-antigen), an oligosaccharide region and a lipid part (called Lipid A), is the ligand of TLR4. TLR4 cannot bind directly as the LPS molecule requires a complex assembly composed by the CD14 co-receptor which facilitates the transfer of LPS to TLR4 complex and MD-2, an adapter molecule that modulates the LPS recognition. Another cofactor is LPS-binding protein (LBP) that shuttles LPS to the CD14 molecule. The association of these auxiliary molecules triggers the signal, resulting in the homodimerization of TLR4 molecules and consequent signaling[23,24].

Once TLRs have bound their specific ligands on the cell membrane, they transduce the downstream signal by means of the myeloid differentiation factor 88 (MyD88), a common molecule adaptor for all TLRs except TLR3[25]. Actually, the TLR downstream signaling can be distinguished as MyD88-dependent or MyD88-independent pathways with the alternative adapter molecule as TIR-domain-containing adapter-inducing interferon-β (TRIF). As a final effect, MyD88-dependent cascade leads to the activation of nuclear factor-κB and activating protein-1 (AP-1) by means of IκB kinase (IKK) complex and mitogen-activated protein kinases (MAPKs) respectively. NF-κB and AP-1, in turn, conduct the production of specific pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α, IL-6 and IL-1β[25]. In the liver, the MyD88 pathway also promotes the activation of the LPS-induced TNF-α factor (LITAF), another transcription factor that regulates the transcription of pro-inflammatory cytokines[26]. Conversely, MyD88-independent cascade predominantly induces the expression of Type I interferons by the activation of IRF-3 through the critical regulators TANK-binding kinase 1 (TBK1) and IKKs[27].

KC-dependent production and release of pro-inflammatory cytokines, such as TNF-α and IL-1β, will further increase production and release of other pro-inflammatory cytokines (*i.e.*, IL-6 and IL-8) and chemoattractant factors in a vicious cycle, leading to the activation of other immune cells, including neutrophils, monocytes and lymphocytes. These immune cells in turn also respond to TLR activation in KCs, generating reactive oxygen species (ROS), increasing phagocytosis and secreting anti-microbial peptides and a cascade of additional pro-inflammatory molecules[28].

In Figure 1 we have schematized the LPS-mediated TLR4 signaling pathways leading to intra-hepatic inflammation.

TLR ROLES IN ALD PATHOGENESIS

ALD pathogenesis

ALD is considered to be one of the major causes of death worldwide, accounting for half of alcohol-related fatalities[17]. Excessive alcohol consumption may lead to

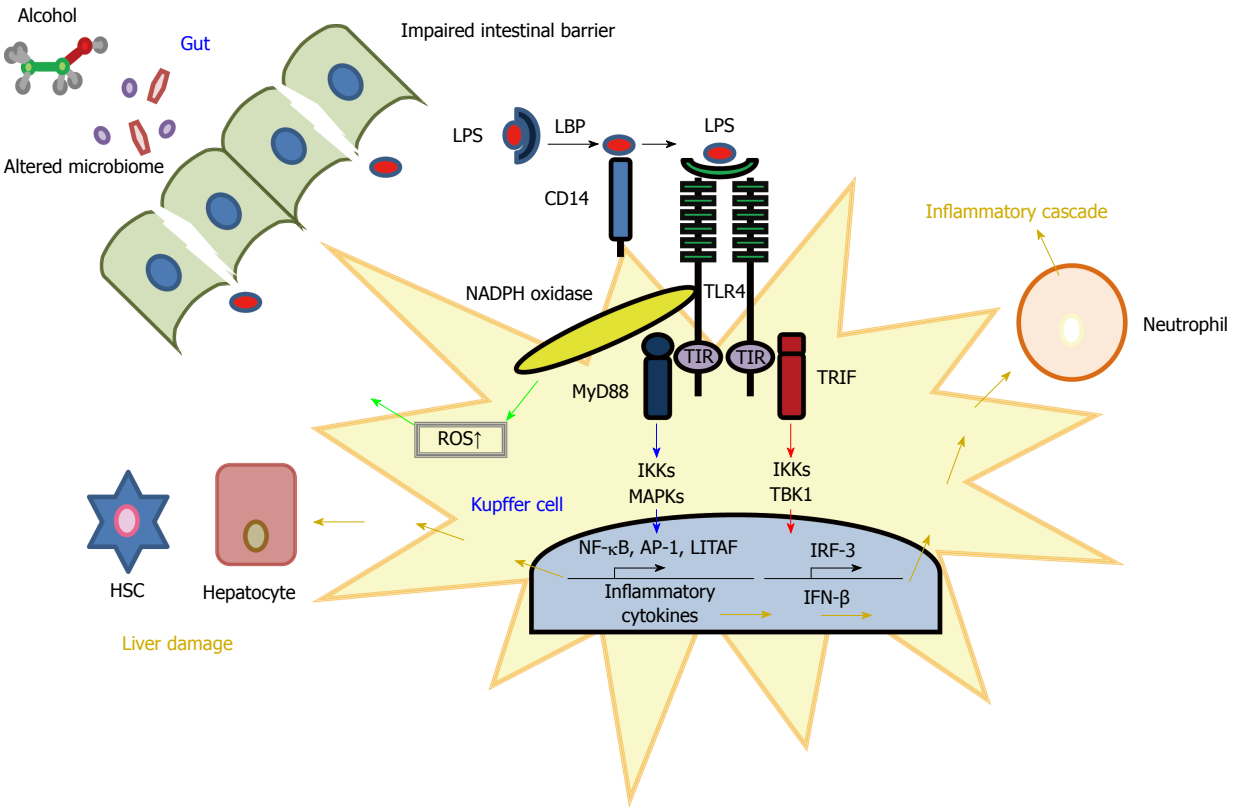

Figure 1 Schematic representation of lipopolysaccharide-mediated Toll-like receptor 4 signaling pathways. The disruption of intestinal integrity (*i.e.* caused by alcohol) results in migration of gut-derived microbial products, particularly LPS, through the portal circulation reaching the liver. LPS can prime KC-dependent production and release of pro-inflammatory cytokines (as TNF-α and IL-1β), which in turn, lead to release of other pro-inflammatory cytokines and chemokines recruiting and activating immune cells, affecting HSC and hepatocyte homeostasis. In the figure, the LPS-TLR4 interaction and consequent initiation of downstream signaling cascade distinguishing both the MyD88-dependent and independent pathways in KCs and their interplay with other cells involved in liver inflammation and damage is summarized. LPS: Lipopolysaccharide; LBP: LPS-binding protein; KC: Kupffer cell; TNF: Tumor necrosis factor; IL: Interleukin; HSC: Hepatic stellate cell; TLR: Toll-like receptor; TIR: Toll/interleukin-1 receptor; TRIF: TIR-domain-containing adapter-inducing interferon-β; NF-κB: Nuclear factor-κB; IKK: IKB kinase; MAPKs: Mitogen-activated protein kinases; LITAF: LPS-induced TNF-α factor; IFN: Interferon; ROS: Reactive oxygen species.

several liver tissue abnormalities. Therefore, histological features in ALD include steatosis, steatohepatitis and fibrosis, which may progress to cirrhosis and ultimately to HCC[29]. Although research interests in ALD pathogenetic mechanisms have increased during the last decades, some relevant pathways have only recently been characterized[30]. Studies on the direct effect of alcohol on intestinal permeability have shown that acetaldehyde, the most toxic metabolite of ethanol metabolism, has a major role in the increment of tight junction tyrosine phosphorylation, conducting altered localization of both tight junction (occludin and ZO1) and adherens junction (E-cadherin and β-catenin) molecules[31,32].

Intra-hepatic lipid accumulation and steatosis in people who drink a toxic alcohol amount (40-80 g/d for men and 20-40 g/d for women for 10 years) is characterized by the impairment of the major pathways involved in alcohol metabolism, including its conversion to acetate by the activity of specific dehydrogenases which completes the conversion to acetate and mitochondrial fatty acid oxidation[33]. Furthermore, development of the progressive severe forms of ALD-related liver damage, such as steatohepatitis and fibrosis, results from a complex interplay between the oxidative stress due to release of ROS and the activation of several components of the innate immune system due to endotoxins such as LPS[34]. Besides, several studies suggested that LPS increases cytokine levels by NAPDH oxidase and ROS production in macrophages *via* the NF-κB pathway[35]. Although the complex upstream and downstream events and molecules that may explain the LPS-mediated mechanisms in ALD have been a subject of deep investigation for decades, they are only recently becoming clear[21]. Alcohol-induced LPS accumulation and consequent endotoxemia may occur by the alteration of gut-microbiome composition, which leads to endotoxin over-production and consequent disruption of intestinal barrier integrity. Therefore, the increased alcohol-mediated endotoxemia may reduce the detoxifying ability of KCs and the activation of the TLR4-mediated inflammatory response that promotes hepatocellular injury. Experimental and clinical evidence of this complex network of TLR4-associated upstream and downstream factors involved in ALD-associated inflammation are reported in the next paragraphs.

Evidence from animal models

The involvement of gut-derived microbial products, such as LPS, which can pass the systemic and portal circulation

due to the disruption of the intestinal barrier caused by ethanol was suggested several years ago[36]. Furthermore, Keshavarzian *et al*[37] demonstrated that intestinal barrier dysfunction and endotoxemia are early events preceding ethanol-dependent hepatic injury in rats. Particularly, serum LPS has been found to be increased in animal models resembling human ALD[38].

The relevance of TLR4-dependent KC activation followed by the initiation of the inflammatory cascade in the pathogenesis of alcohol-induced liver damage has been also proved in models of ALD[39,40]. Furthermore, studies performed on mice that have a functional mutation in the *TLR4* gene showed an impaired response to bacterial endotoxins[40]. Moreover, LBP knockout mice were significantly reduced in the pathological parameters characterizing ethanol-fed mice, such as endotoxin levels, steatosis, inflammation and liver injury[41]. Further, CD14 knockout mice were protected from alcohol-caused severe liver injury and from ethanol-induced NF-κB, transforming growth factor (TGF)-β and TNF-α increase[42]. As previously described, TLR4 signaling encompasses two distinct cascades: the MyD88-dependent and the TRIF-dependent (MyD88-independent).

These downstream pathways have been widely studied in animal models in order to clarify their respective involvement in ALD pathogenesis. Experimental evidence established that in ALD, the TLR4 down-stream signaling is principally regulated by the MyD88-independent cascade. In fact, while TLR4-knockout (KO) mice were protected from alcohol-induced liver damage, ROS production and inflammation, MyD88-KO mice were not, both being exposed to the Lieber-De-Carli diet[43]. Moreover, the disruption of MyD88-independent signaling, in studying TRIF-deficient mice, reported protection from alcohol-induced liver disease[44]. Additionally, the lack of IRF-3, a transcription factor downstream to TLR4/TRIF, resulted in preserving IRF-3-KO mice from alcohol-induced liver injury, steatosis and inflammation[45]. This evidence demonstrates the dispensable role of MyD88 adapter in TLR4-mediated liver injury.

Recently, the involvement of the protein kinase C (PKC) activity in the increase of intestinal permeability related to alcohol consumption has been described[46]. Specifically, both *in vitro* and *in vivo* evidence showed that alcohol, in a dose-dependent manner, increases TLR4 expression, which in turn results in augmented PKC activity. The consequent reduction of occludin phosphorylation alters the intercellular junctions, leading to the intestinal permeability increment[46].

KCs are the primary cells that respond to LPS *via* TLR4-dependent pro-inflammatory cytokines (as TNF-α, IL-1β, IL-8, IL-6) and leading to liver inflammation. In addition, in animal models of ethanol exposure, hepatocytes can be driven to accumulate lipid and augment TLR4 levels, altering their TLR4 sensitivity and LPS hepatotoxicity[47,48]. Moreover, damaged hepatocytes can release high-mobility group box 1 (HMGB1), an endogenous ligand that can be recognized by TLR4 and participate with a mechanism similar to that of non-alcoholic fatty liver disease in the promotion of ALD[7]. In fact, Wang *et al*[49] found that HMGB1 serum levels increased in chronic alcohol feeding of mice and its balance with milk fat globule-EGF factor 8 may control macrophage efferocytosis.

Interestingly, a recent study in TLR4 deficient mice transplanted with bone marrow (BM)-derived cells (KCs) or non-BM-derived cells (HSCs included) demonstrated that in both liver cell lineages, TLR4 is essential for the progression of alcohol-induced liver steatosis, inflammation, injury and fibrosis[50]. Besides, ROS production has been implicated in the alcohol-induced sensitization to LPS. In more detail, it has been shown that the direct interaction between TLR4 and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase isozyme (Nox)-4 leads to NF-κB activation and LPS-mediated ROS generation *in vitro*[51]. In a chronic alcohol-fed rat model, the inhibition of NADPH oxidase through diphenyleneiodonium (DPI) diminished the LPS-induced ERK1/2 phosphorylation and both ROS and TNF-α levels in KCs[52]. Furthermore, in C57Bl6/J mice fed a modified Lieber-De Carli diet, DPI protected from steatosis and liver inflammation induced by the usage of diverse bacterial components specific for different TLRs[48]. Previous evidence also showed that mice p47 phox -/-, depleted of the main cytosolic part of NADPH oxidase, were preserved from alcohol-induced liver injury, corroborating the crucial role of oxidative stress in TLR signaling along the ALD condition[53].

By the way, TLR4 is an important player in the development of fatty liver, although how it can influence lipid metabolism has not been completely clarified. Experimental studies on TLR4 mutant animal models have documented that TLR4 is involved in lipid accumulation and lipid peroxidation imbalance[40,54].

It is established that KC activation due to alcohol intake can lead to an increment in the production of prostaglandin (PG) E(2) which, interacting with prostanoid receptors and augmenting cAMP, can build up triglyceride in the liver. Furthermore, hepatic fat accumulation can be induced by the augmentation of the NADH/NAD(+) ratio, the sterol regulatory element-binding protein-1 (SREBP-1) activity and the decrease of peroxisome proliferator-activated receptor-alpha (PPAR-alpha) activity[55,56].

Also, microRNAs (miRNAs) have been implicated in the inflammation process in ALD and its complications. MiRNAs are conserved single-strand small noncoding RNAs which have a post-transcriptional gene-expression regulation function. They act on the 3'-untranslated mRNA target with whom miRNAs pair with different specificity. This match influences RNA stability and degradation or the translation and finally leads to protein repression[57]. Specifically, miR-155 is a ruler of inflammation and TLR signaling. It regulates TNF-α mRNA stability, as demonstrated in *in vitro* experiments performed on alcohol-treated macrophages[58]. Such evidence has been confirmed in a mouse model of ALD in which iso-

Table 1 Experimental evidence of Toll-like receptor involvement in inflammation during alcoholic liver disease

Ref.	Experimental model	Upstream factor	Involved TLRs	Effects
Enomoto *et al*[39], 2000	Ethanol-fed rats	LPS	TLR4	Kupffer cell activation and consequent inflammation
Uesugi *et al*[40], 2001	TLR4 non-functional mice fed ethanol	LPS	TLR4	Decreased: Steatosis Inflammation Focal necrosis
Park *et al*[51], 2004	HEK293T cells	Nox4	TLR4	NF-κB activation LPS-mediated ROS generation
Gustot *et al*[48], 2006	Mice fed a modified Lieber-DeCarli diet			Increased:
		LTA, PGN	TLR2	Steatosis
		LPS	TLR4	Liver weight -aminotransferase levels
		flagellin	TLR5	Liver mRNA expression of different TLRs.
	Addition of multiple bacterial products			Increased:
		loxoribine	TLR7	TNF-α mRNA expression
		CpG	TLR9	Liver inflammatory infiltrate
Hritz *et al*[43], 2008	TLR4-(KO) mice feed Lieber-De-Carli diet		TLR4	Protection from: Alcohol-induced liver damage ROS production Inflammation
Bala *et al*[58], 2011	ALD mouse model	LPS	TLR4	MiRNA-155 increase and TNF-α levels in isolated KCs
Bala *et al*[59], 2012	ALD mouse model administered with CpG + LPS	CpG	TLR9	Plasma increase of: miR-155, miR-122 and miR-146a
	TLR4-KO			Protection from:
		LPS	TLR4	Alcohol-induced liver disease Increase of both miR-155 and miR-122
Byun *et al*[61], 2013	Mice feed high-fat diet plus binge ethanol TLR3 (KO) mice	poly I:C	TLR3	Protection from alcoholic liver damage Preservative action of poly I:C abrogated

LPS: Lipopolysaccharide; TLR: Toll-like receptor; LTA: Lipoteichoic acid; PGN: Peptidoglycan; TNF: Tumor necrosis factor; NADPH: Nicotinamide adenine dinucleotide phosphate; Nox4: NADPH oxidase 4; ROS: Reactive oxygen species; ALD: Alcoholic liver disease; KCs: Kupffer cells; poly I:C: Polyinosinic-polycytidylic acid.

lated KCs exhibited increased levels of both miRNA-155 and TNF-α. The same author showed that in alcohol-fed mice there are augmented serum/plasma miR-155 levels and an increased quantity of TNF-α in the liver[59]. In the same model, the authors evidenced an increase of serum/plasma miR-122 which is known to exert multiple functions in hepatocytes as it is important in the regulation of cholesterol metabolism[59,60]. Furthermore, the increase of both miR-155 and miR-122 was absent in TLR4-deficient mice that were preserved from ALD[59]. It is also noteworthy that treatment with TLR9 ligands (that is CpG) leads to plasma increase of both miR-155 and miR-122 and increased levels of TLR9 and serum endotoxin have been reported in alcohol-fed mice[48,59]. Actually, to date, diverse TLRs have been extensively studied and found to be involved in ALD pathogenesis. In the Lieber-DeCarli chronic alcohol feeding model, expression of TLR1, 2, 4, 6, 7, 8 and 9 liver mRNA resulted from increased enteral administration concomitantly to steatosis induction. It has been also demonstrated that the alcohol treatment sensitizes hepatic inflammation and damage since the triggering of the TLR1, 2, 4, 6, 7, 8 and 9 with their specific ligands resulted in TNF-α augmentation. Moreover, TLR expression remained unaffected after antibiotic treatment which, however, ameliorated the alcoholic fatty liver condition[48]. Recently, Byun *et al*[61] studied the TLR3 involvement in alcohol liver injury both in HSCs and KCs *in vivo*. The authors demonstrated, by means of TLR3-KO and IL-10-KO mice fed a high-fat diet with added ethanol, that TLR3 is protective for alcoholic liver injury and exerts this function by stimulating IL-10 production. In more detail, polyinosinic-polycytidylic acid (poly I:C, that is TLR3 ligand) administration ameliorated alcoholic liver damage and diminished the amount of TNF-α, IL-6 and monocyte chemoattractant protein-1 (MCP-1) in control mice. The preservative action of poly I:C was abrogated in TLR3-KO and IL-10-KO murine models. Coherently, HSCs and KCs isolated from poly I:C-treated animals had higher levels of IL-10 than the controls. IL-10 was also over-expressed *in vitro* both in HSCs and KCs in the presence of poly I:C[61].

The most relevant experimental studies on the involvement of TLRs in inflammation during ALD are summarized in Table 1.

Evidence from human studies

The role of gut dysbiosis and increased gut permeability and endotoxemia into the portal circulation has also been extensively explored in patients with ALD[9,62]. Consequently, the critical role exerted by TLR signaling in hu-

man ALD has been investigated. Schäfer *et al*[63] found that circulating CD14 levels were higher in serum from patients with severe alcoholic hepatitis than in those from healthy controls, suggesting the crucial role of this TLR4 co-receptor in ALD. Furthermore, it has been reported that the CD14-159C>T polymorphism in patients with ALD may significantly correlate with the severity of disease and be associated with the risk for alcoholic cirrhosis[64,65]. However, no correlation has been found between TLR4, TNF-α and IL-1β gene variants and ALD in different populations to date[66,67].

Clinical evidence of the TLR involvement in intrahepatic inflammatory response in ALD is still scarce but reinforces the experimental data reported above. The role of TLRs on neutrophils in patients with ALD was reported by Stadlbauer *et al*[68]. These authors demonstrated that LPS-induced over-expression of TLR2, 4 and 9 may play a pivotal role in the neutrophil dysfunction observed in patients with alcoholic hepatitis and suggested that the use of TLR antagonists was unable to prevent this dysfunction, while the use of an endotoxin scavenger might reduce inflammatory response and improve clinical outcome[69]. Interestingly, other authors found an impairment of TLR2 but not TLR4-mediated innate immune response in peripheral blood monocytes from patients with stable chronic ALD, explaining their susceptibility to immunodeficiency and disease worsening[70].

Interestingly, it has been reported that TLR-dependent priming of B cells might explain the increased circulating levels of immunoglobulins in patients with alcoholic liver cirrhosis[71].

As mentioned above, most experimental studies reported that NF-κB transcriptional activity is crucial for the TLR signal and Stärkel *et al*[72] demonstrated that a persistent activation of this transcription factor, an up-regulation of TLR3 and TLR7 expression and high pro-inflammatory cytokine levels were associated with end-stage ALD and involved in disease progression in humans.

Additional proof of the activation of the TLR-dependent inflammatory network in ALD was reported by the analysis of circulating levels of cytokines and chemokines. In fact, several authors found high levels of pro-inflammatory cytokines, including TNF-α and IL-6, in the serum of actively drinking patients with ALD, predicting their outcome and long-term survival[73,74]. Interestingly, fibroblast growth factor-inducible 14 (Fn14), a member of the TNF receptor superfamily (member 12A), was found to be over-expressed in the liver, primarily by hepatic progenitors, in patients with alcoholic hepatitis and it correlated with disease severity[75]. In addition, it has been reported that several chemokines, including IL-8, correlated with a worse prognosis in patients with alcoholic hepatitis[76].

Finally, a recent study demonstrated that increased serum levels of chemokine-ligand-1 (CXCL1), an inflammatory chemokine mainly expressed by mononuclear cells in response to LPS-dependent TLR activation, coupled with a polymorphism in the gene encoding for this protein are risk factors for alcoholic cirrhosis[77].

CONCLUSION

ALD remains one of the major causes of hepatic-associated morbidity and mortality worldwide. Furthermore, the current therapeutic options for patients with ALD are alcohol abstinence and liver transplantation for end-stage liver disease. The liver inflammatory response, which is generated early in ALD, is probably triggered by bacteria and their products, particularly LPS. Due to increased intestinal permeability, they translocate into circulation and activate TLR-dependent production and secretion of pro-inflammatory cytokines and chemokines. There is a body of experimental evidence of the crucial role of TLR-mediated inflammatory network in ALD development and progression. However, to date, these findings have little translational proof in patients. More studies in humans are urgently needed to advance the field and to translate experimental/informative findings into novel therapies.

Furthermore, as qualitative and quantitative changes of the gut microbiome play a major role in the TLR-dependent liver inflammatory response and ALD pathogenesis, further metagenomic, transcriptomic and metabolomic studies may help, not only to explain the "gut-liver axis" in ALD, but also to identify novel potential therapeutic targets. In fact, according to previous studies, treatment with probiotics inhibits alcohol-induced TLR4 and TLR5 activation of TNF-α production, reducing hepatic inflammation in experimental models, and restores neutrophil phagocytic ability in patients with alcoholic cirrhosis, probably by changing TLR4 expression and IL-10 secretion[78,79].

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