

Cirrhosis-associated immune dysfunction: Distinctive features and clinical relevance

Agustín Albillos^{1,2,3,*}, Margaret Lario¹, Melchor Álvarez-Mon^{1,2,4}

¹Department of Medicine, Universidad de Alcalá, Madrid, Spain; ²CIBERehd, Instituto de Salud Carlos III, Madrid, Spain;

³Service of Gastroenterology and Hepatology, Hospital Universitario Ramón y Cajal, IRYCIS, Madrid, Spain;

⁴Service of Immune Diseases and Oncology, Hospital Universitario Príncipe de Asturias, Alcalá de Henares, Madrid, Spain

Summary

The term cirrhosis-associated immune dysfunction refers to the main syndromic abnormalities of immune function, immunodeficiency and systemic inflammation that are present in cirrhosis. The course of advanced cirrhosis, regardless of its aetiology, is complicated by cirrhosis-associated immune dysfunction and this constitutes the pathophysiological hallmark of an increased susceptibility to bacterial infection, distinctive of the disease. Cirrhosis impairs the homeostatic role of the liver in the systemic immune response. Damage to the reticulo-endothelial system compromises the immune surveillance function of the organ and the reduced hepatic synthesis of proteins, involved in innate immunity and pattern recognition, hinders the bactericidal ability of phagocytic cells. Systemic inflammation, in form of activated circulating immune cells and increased serum levels of pro-inflammatory cytokines, is the result of persistent episodic activation of circulating immune cells from damage-associated molecular patterns, released from necrotic liver cells and, as cirrhosis progresses, from pathogen-associated molecular patterns, released from the leaky gut. Cirrhosis-associated immune dysfunction phenotypes switch from predominantly “pro-inflammatory” to predominantly “immunodeficient” in patients with stable ascitic cirrhosis and in patients with severely decompensated cirrhosis and extra-hepatic organ failure (e.g. acute-on-chronic liver failure), respectively. These cirrhosis-associated immune dysfunction phenotypes represent the extremes of a spectrum of reversible dynamic events that take place during the course of cirrhosis. Systemic inflammation can

affect the functions of tissue somatic cells and modify the clinical manifestation of cirrhosis. The best characterized example is the contribution of systemic inflammation to the haemodynamic derangement of cirrhosis, which correlates negatively with prognosis.

© 2014 European Association for the Study of the Liver. Published by Elsevier B.V. Open access under [CC BY-NC-ND license](#).

Introduction

The immune system plays a dual role in the pathogenesis of cirrhosis such that, besides the role of immune-mediated inflammatory mechanisms, cirrhosis itself also leads to immune system dysfunction. The immune system mediates hepatocyte damage due to alcohol, virus infection or autoimmunity, driving fibrogenesis through hepatic stellate cell activation. In addition, cirrhosis leads to impairment of the immune system with an inability to protect the host from bacterial infection and dysregulated immune cell activation.

This paper reviews the myriad of dynamic detrimental effects that cirrhosis has on the immune system that we have designated cirrhosis-associated immune dysfunction (CAID). This concept includes two main syndromic alterations: (i) immunodeficiency, due to an impaired response to pathogens at different levels of the immune system, and (ii) systemic inflammation, as a consequence of persistent and inadequate stimulation of cells of the immune system (Fig. 1). CAID should be considered a complication of cirrhosis of any aetiology. It accounts for many distinctive features of cirrhosis such as a predisposition to bacterial infection and a poor response to vaccination [1–3]. It may also play an important role in endothelial activation and the haemodynamic disturbance of cirrhosis and contributes to other clinical manifestations, such as asthenia.

Contribution of the liver to the systemic homeostasis of the immune system

The liver regulates homeostasis of the immune system through two mechanisms. First, it plays a role in immune surveillance, defending against blood-borne pathogens via its double blood

Keywords: Inflammation; Immunodeficiency; Immune response; Lipopolysaccharide; T-helper; T-cytotoxic.

Received 6 April 2014; received in revised form 27 July 2014; accepted 9 August 2014

* Corresponding author. Address: Departamento de Medicina, Facultad de Medicina-Campus Universitario, Universidad de Alcalá, Carretera Madrid-Barcelona km 33.600, 28805 Alcalá de Henares, Madrid, Spain. Tel.: +34 918854870.

E-mail address: agustin.albillos@uah.es (A. Albillos).

Abbreviations: CAID, cirrhosis-associated immune dysfunction; IL, interleukin; NK, natural killer; PRR, pattern recognition receptor; LPS, lipopolysaccharide; LBP, lipopolysaccharide binding protein; TLR, toll-like receptor; NLR, NOD-like receptor; Th cell, T helper cell; Tc cell, cytotoxic T cell; PAMP, pathogen-associated molecular pattern; DAMP, damage-associated molecular pattern; GALT, gut-associated lymphoid tissue; MLN, mesenteric lymph node.



Key Points

- Cirrhosis-associated immune dysfunction refers to both immunodeficiency and systemic inflammation that occur in cirrhosis
- Immunodeficiency in cirrhosis results from damage to the local immune surveillance function of the liver, reduced synthesis of pattern recognition receptors, and damage at the systemic level of immune response cell function
- Systemic inflammation accompanies immunodeficiency and is attributed to persistent immune cell stimulation as well as to PAMPs and DAMPs from a leaky gut and a damaged liver, respectively. Inflammation is reflected by an increased production of pro-inflammatory cytokines, their enhanced serum levels, and the upregulated expression of cell activation markers
- The cirrhosis-associated immune dysfunction phenotypes represent the extremes of a spectrum of reversible dynamic events that take place during the course of cirrhosis. Under constant PAMPs challenge, the immune response pattern in cirrhosis switches from a predominantly “pro-inflammatory” phenotype in patients with “stable” decompensated cirrhosis to a predominantly “immunodeficient” one in patients with severely decompensated cirrhosis and extra-hepatic organ failure (e.g. ACLF)
- Systemic inflammation can affect the function of tissue somatic cells and modify the clinical expression of cirrhosis. The best example is the contribution of systemic inflammation to the haemodynamic derangement of cirrhosis, which correlates negatively with prognosis

supply, thereby avoiding the systemic spread of microbial and dietary antigens arriving from the gut [4]. This function of the liver is offset by the local immune tolerance to non-pathogenic exogenous material [5]. The second mechanism, used by the liver to drive homeostasis of the immune system, is the synthesis of soluble molecules that are essential for an effective immune response [6].

Immune surveillance: Role of the liver

The liver exerts its antimicrobial surveillance function through different populations of resident antigen presenting cells and lymphocytes. These are organized in a manner specifically designed to maximize screening for both systemic and gut-derived pathogens. The liver antigen presenting cells include Kupffer and sinusoidal endothelial cells, which comprise the reticulo-endothelial system of the liver, and dendritic cells. Kupffer cells reside within the sinusoidal vascular space and represent the largest group of fixed macrophages in the body, and sinusoidal endothelial cells form a sieve-like, fenestrated endothelium. Unlike macrophage populations of other organs, Kupffer cells occur on the intraluminal side of the vasculature and can capture bacteria under flow conditions. Kupffer cells are specialized at eliminating insoluble waste by phagocytosis through a variety of receptors. As such, Kupffer cells are endowed with unique

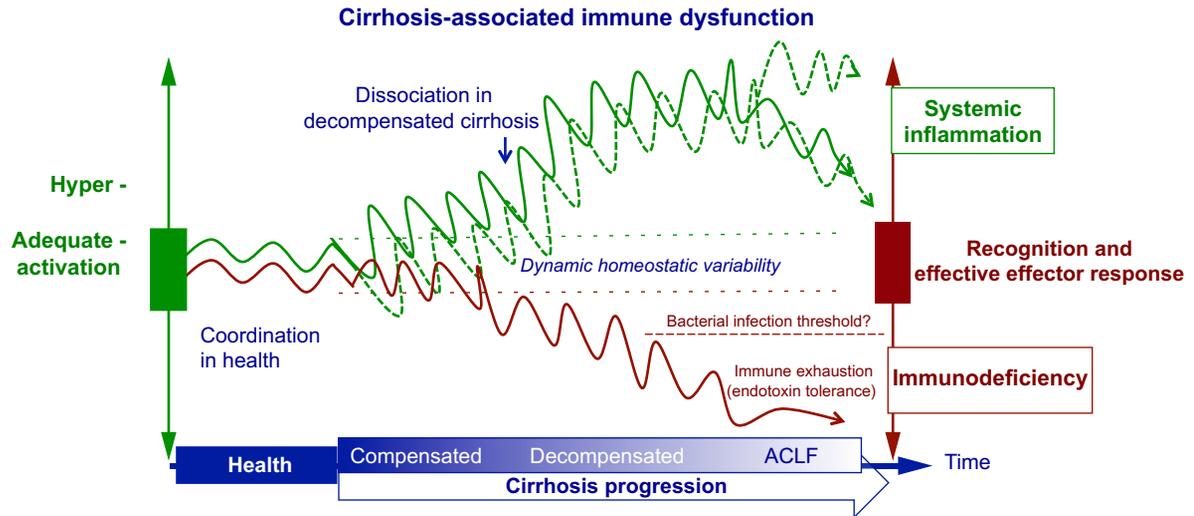
complement receptors that can bind avidly to complement component 3b (C3b) under shear conditions [7]. Sinusoidal endothelial cells are responsible for the elimination of soluble macromolecules and colloidal waste by endocytosis. Kupffer and sinusoidal endothelial cells are also antigen presenting cells, constitutively expressing MHC class I and II and co-stimulatory receptors in addition to molecules that promote antigen uptake, including mannose and scavenger receptors [8,9]. Despite Kupffer cells being critical for microbial capture, their role in microbial killing seems to be dependent on the nature of the pathogen and on the recruitment of additional immune cells to the liver [10]. The liver also contains several populations of dendritic cells, which characteristically have a reduced capacity to drive the activation of T cells, in part due to both, their “immature” development status, and to the local cytokine milieu of the liver, including high interleukin (IL)-10 and low IL-12 levels [11].

Additionally, the liver contains populations of both resident and transiting T and B lymphocytes scattered throughout the parenchyma and the portal tracts that are important in the defensive adaptive immune response. Further, the liver is enriched in natural killer (NK) cells and unconventional lymphocytes (natural killer T and $\gamma\delta$ T cells), which have roles in innate immune responses of the liver.

Besides conferring strong local innate immunity, the liver is a major site of induction of local and systemic adaptive immune responses, mediated by T lymphocytes, playing a critical role in the homeostatic regulation of the immune system. The delicate balance between immunity and tolerance, observed in the liver, is driven by several mechanisms. In the specific antigen challenging micro-environment of the liver, tolerance is maintained through: (i) the direct access of naive CD8+ T cells to antigen presenting cells in the absence of CD4+ T cell activation [12], (ii) the constitutively low abundance of MHC expression by liver-resident cells [13], and (iii) high IL-10 production by Kupffer and sinusoidal endothelial cells [14]. All these mechanisms promote the non-activation and/or apoptosis of CD4+ T lymphocytes. Further, the expression of adhesion molecules facilitates the sequestering of circulating activated T cells, particularly CD8+ T cells, by the liver endothelium [4].

Relevance of the liver in the systemic immune response

The liver, primarily through its hepatocytes, is a major source of proteins involved in innate and adaptive immune responses, including complement components and many secreted pattern-recognition receptors (PRRs) [15]. Complement proteins play roles in the regulation and effector stage of the immune response, and their activation gives rise to a wide range of opsonic, inflammatory and cytotoxic activities. The liver is also the main source of soluble PRRs (e.g. C reactive protein, lipopolysaccharide [LPS]-binding protein [LBP], peptidoglycan-recognition protein, soluble CD14), which activate complement, induce opsonization and regulate immune cell function [16,17]. The liver also produces other acute phase proteins, such as hepcidin, fibrinogen and proteinase inhibitors, which participate in the innate immune response and in controlling tissue damage and repair during inflammation. Hepatocytes synthesize and secrete most of these proteins in response to different pro-inflammatory cytokines (e.g. TNF α , IL-6), generated in the course of a systemic inflammatory responses.



Immune characteristic	Predominant CAID phenotypes	
	Pro-inflammatory	Immunodeficient
Pro-inflammatory cytokines (e.g., TNF- α , IL-6, IL-1 β)	↑↑	↑
Anti-inflammatory cytokines (e.g., IL-10, TGF β)	↑	↑↑
Phagocytosis (e.g., dendritic cells)	↑	↓
HLA-DR/co-stimulatory molecules expression on monocytes/macrophages	↑	↓
Expression of negative regulators (e.g., IRAK-M)	↓	↑

Fig. 1. Cirrhosis-associated immune dysfunction. Under bacterial challenge, in a healthy individual, antigen recognition (in red) and immune cell activation (in green) are tightly coordinated to mount an effective immune response against the pathogen. Cirrhosis progression from compensated to decompensated and then to further decompensated disease, i.e. acute-on-chronic liver failure (ACLF), is associated with mechanisms of mainly hepatic insufficiency and PAMPs- and DAMPs-driven stimulation. These mechanisms impair the regulatory and the effector immune response. In compensated cirrhosis, still in the absence of gut bacterial translocation, DAMPs released from necrotic hepatocytes may initiate activation of the immune system and sterile systemic inflammation (continuous green line). At the decompensated stage, the persistent episodic translocation of bacterial products, e.g. endotoxin, drives further the activation of the immune system, involving increased serum levels of pro-inflammatory cytokines and augmented surface expression of activation antigens on immune cells. The predominantly “pro-inflammatory” CAID phenotype occurs in response to continuous PAMPs signalling and downregulation of anti-inflammatory cytokines and negative feedback mechanisms (e.g. IL-10, IRAK-M, GSK3b) (dotted green line). In addition, progressive immunodeficiency occurs in “stable” decompensated cirrhosis, as a result of different mechanisms, including loss of the liver immune surveillance function, and impaired immune cell functions, such as phagocytosis ability (red line). At the terminal stage of cirrhosis, under persistent PAMPs pressure, the immune response is exhausted and the CAID phenotype switches to a predominantly “immunodeficient” phenotype of impaired innate and adaptive protective immune responses.

In addition, liver cells express different membrane-bound or cytoplasmic PRRs, which recognize different bacterial and viral molecules. These include cell surface and endosomal toll-like receptors (TLRs), cytoplasmic nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), and RNA helicases. Interactions of these PRRs with their ligands in immune cells cause regulatory signals and activation, which in the specific case of bacterial products promotes NF- κ B activation. The constitutive expression and low-level stimulation of these molecular systems is characteristic of the liver [18]. Specifically, TLR4 is expressed on all types of liver cells and is likely involved in the uptake and clearance of endotoxins, and the production of pro-inflammatory and anti-inflammatory cytokines.

Cirrhosis-induced immunodeficiency

Cirrhosis is associated with several abnormalities in innate and adaptive components of the immune system’s response to microbial challenge, leading to a state of acquired immunodeficiency (Fig. 2).

Damage to the liver’s immune surveillance function

The immune surveillance function of the liver is compromised by a reticulo-endothelial system, damaged by sinusoidal fibrosis and capillarization, septal fibrosis with portal-systemic shunts, and Kupffer cell loss or damage [4]. This structural derangement reduces the clearance of endotoxin and bacteria from the blood, leading to bacteremia, metastatic organ infection, and persistent immune system stimulation. A lack of Kupffer cells or of their complement receptors results in uncontrolled bacteraemia and increased host death in experimental models [19]. In agreement with these experimental findings, diminished reticulo-endothelial system function in cirrhosis has been associated with a greater risk of bacterial infection and lower survival [20].

Cirrhosis impairs the synthesis of innate immunity proteins and of PRRs, reducing the bactericidal capacity of phagocytic cells. Given the large functional reserve of the liver, lowered serum levels of these proteins are only evident in patients with advanced cirrhosis and ascites. Indeed, ascites due to cirrhosis increases the susceptibility to bacterial infection. This has been related to low opsonic activity as a result of reduced concentrations of C3,

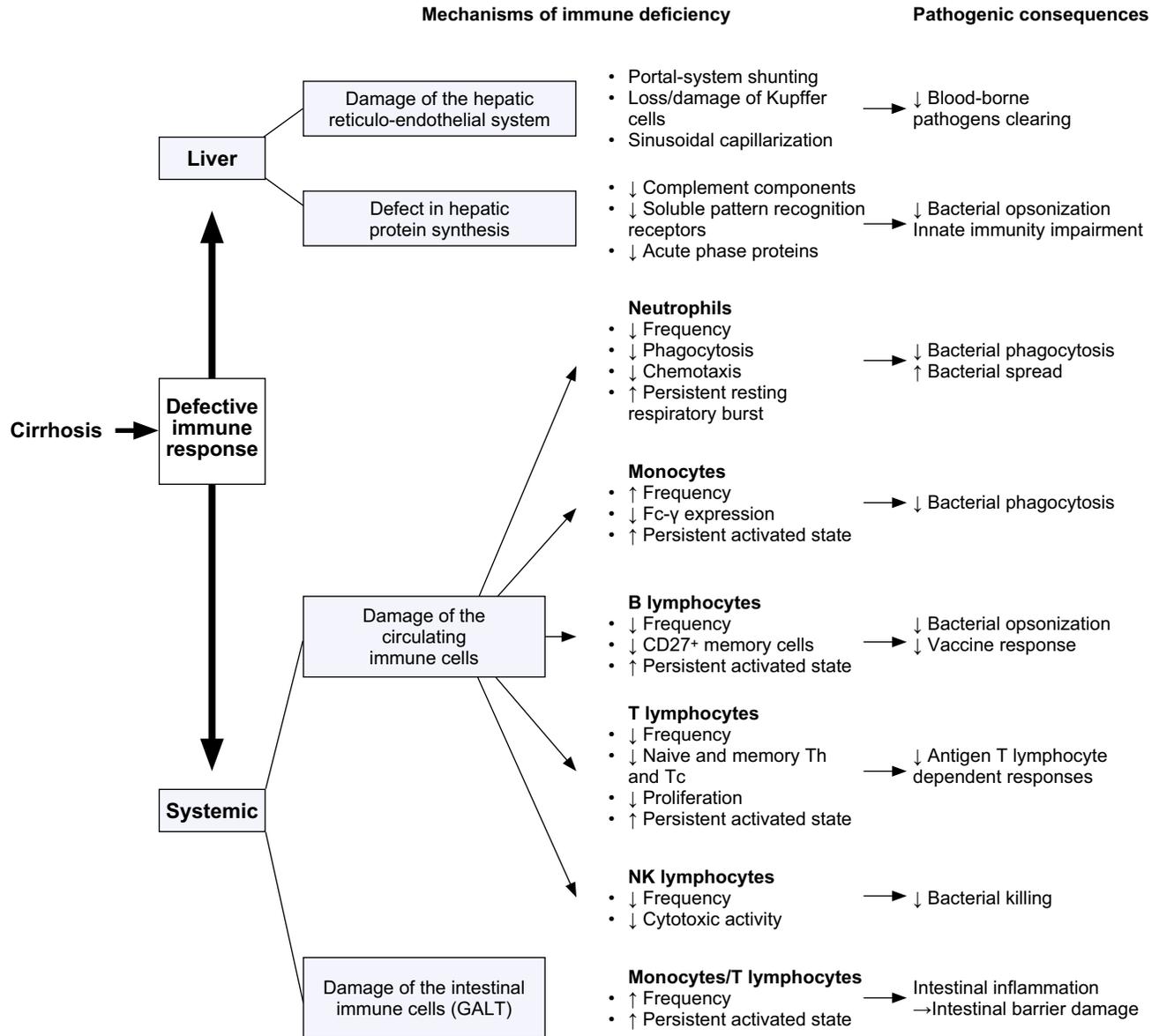


Fig. 2. Cirrhosis-induced immunodeficiency. Cirrhosis disrupts the architecture and cellular organization of the liver and diminishes the hepatic ability to synthesize proteins. These events compromise the immune surveillance function of the liver through damage of the reticulo-endothelial system and synthesis of innate immunity proteins and PRRs. Besides, cirrhosis affects the functions of circulating and intestinal populations of immune cells. Thus, immunodeficiency is the result of a panoply of abnormalities provoked by cirrhosis that affect cellular and soluble components of the immune response both at the liver and systemically.

C4 and CH50 in the serum and ascitic fluid [21,22]. The defensive relevance of PRRs synthesized by the liver is also highlighted by the fact that cirrhotic patients with a gene polymorphism, conferring them low serum levels of the recognition molecule mannose-binding lectin, or transplant recipients of a liver with this polymorphism, show an increased risk of bacterial infection [23,24].

Circulating immune cell damage

Besides modifying the local immune surveillance function of the liver and PRR synthesis, cirrhosis also compromises immune cell functions at the systemic level. Characterizing immunodeficiency, associated with cirrhosis, requires the systematic study

of the main circulating populations of immune cells. The abnormalities in these populations described so far are:

1. Neutrophils. Besides being reduced in number due to sequestration by the spleen, the most commonly reported defect is the impaired phagocytosis of opsonized bacteria [25–28]. Accordingly, these cells show defective superoxide anion O₂⁻ production and myeloperoxidase activity and a lower response to the peptidoglycan recognition protein [25,29,30], which impairs their microbicidal capacity. These defects seem to be the result of intracellular signalling alterations, including impaired phosphorylation of a major NADPH oxidase 2 component, p47-phox (S345), by mitogen-activated protein kinases or defective phosphatidylinositol specific phospholipase C [29,30]. Neutrophils also show impaired chemotaxis to the infection focus, through

reduced adhesion to microvascular endothelial cells and decreased transendothelial migration [27,31]. Of note, and as for other circulating immune cells, neutrophil dysfunction has been linked to persistent *in vivo* stimulation, as shown by its increased resting respiratory burst, especially observed in patients with higher serum levels of pro-inflammatory cytokines [27].

2. Monocytes. Cirrhosis alters the number, subset distribution and function of circulating monocytes. In contrast to the frequently observed leukopenia, cirrhosis is associated with monocytosis, as the main increase is in a pro-inflammatory non-classical CD14⁺CD16⁺ subset of monocytes [32,33]. The expansion of these monocytes with limited phagocytic activity is observed regardless of the aetiology but not of the severity of cirrhosis [33,34]. A study has demonstrated the impaired function of the circulating monocyte Fc- γ receptor, which is needed for clearance of IgG coated bacteria in patients with cirrhosis, developing bacterial infection [35]. Remarkably, circulating monocytes are *in vivo* activated in cirrhosis, as further discussed below.

3. B lymphocytes are profoundly affected in cirrhosis. In patients with alcoholic or HCV cirrhosis, diminished B cell frequencies and peripheral blood absolute counts have been reported [36–39]. The most striking anomaly observed in the B cell compartment is memory B cell dysfunction. Specifically, cirrhosis leads to a loss of CD27⁺ memory B cells, a subset generated in response to T cell-independent antigens, which show hyporesponsiveness to CD40/TLR9 activation, impaired upregulation of co-stimulation markers as well as impaired TNF β and IgG production and T cell allostimulation [39].

4. T lymphocytes. Recent findings have suggested a disruption of the T cell compartment in cirrhosis. T cell lymphopenia is common in cirrhosis and affects T helper (Th) and cytotoxic T cells (Tc) [34,40–42]. T cell depletion is more pronounced in the naive than in the memory compartment, regardless of disease aetiology, and is evident since the early stages of cirrhosis [34,43,44]. Retraction of the T cell compartment results from: (i) impairment in the *de novo* production of new naive T cells due to accelerated aging and atrophy of the thymus, (ii) reduction of the T cell memory subset, due to spleen sequestration and cell consumption, related to activation-driven bacterial translocation and increased apoptosis, and (iii) impaired compensatory peripheral proliferation [43]. Additionally, circulating T lymphocytes are *in vivo* activated and show diminished proliferation [45–47].

5. Circulating NK cells are also defective in cirrhosis and show a poor response to cytokine stimulation [48]. These findings are also strikingly evident at the intrahepatic level, where NK cells play an important role in alleviating liver fibrogenesis [49].

Gut-associated lymphoid tissue (GALT) damage

Another immune system compartment that is also profoundly affected in cirrhosis is GALT, which constitutes the first barrier of defence against antigens and pathogens entering the organism from the intestine. The intestinal lymphoid tissue, distributed in Peyer's patches and mesenteric lymph nodes (MLN), acts by inducing immunity and tolerance, whereas its effector sites are scattered throughout the lamina propria and mucosal epithelium. In cirrhosis, GALT is under the constant pressure of pathological bacterial translocation and the increased passage of bacterial products that results from a leaky gut and an elevated enteric bacterial load. The consequence of this persistent stimulation is an increased number of activated monocytes, dendritic cells

and T lymphocytes at the intestine and MLN [50–53]. In turn, these activated cells cause the augmented expression of pro-/anti-inflammatory cytokines at the lamina propria, mucosal epithelium and MLN, as well as increased phagocytosis by intestinal dendritic cells [50–52]. Bowel decontamination with non-absorbable antibiotics reduces the number of activated immune cells in the intestinal lamina propria and MLN [50–53]. This supports the pathogenetic role of enteric bacteria in intestinal inflammation.

The first major consequence of intestinal and MLN inflammation in cirrhosis is systemic inflammation. As described later, as cirrhosis progresses, the gut becomes a major source of activated immune cells and pro-inflammatory cytokines, promoting and maintaining a systemic inflammation [34,50]. In addition, this intestinal inflammation might perpetuate intestinal barrier failure. It is tempting to speculate that the increased pro-inflammatory cytokine production (e.g. TNF α , IFN γ , IL-6) by intestinal immune cells disrupts epithelial tight-junctions and favours further increased translocation of bacteria and bacterial products, creating a vicious circle. Indeed, a recent study has correlated increased activated macrophages in the duodenal lamina propria, augmented intestinal permeability, and altered intestinal tight-junction protein expression in patients with decompensated cirrhosis [54]. Besides intestinal immune cell damage, the findings of several experimental models of chronic liver damage point to deficiencies in the production of intestinal antimicrobial peptides, such as α -defensins and RegIII proteins. These peptides are needed to maintain microbiota-host homeostasis, and their deficiency could induce intestinal dysbiosis and bacterial translocation [55,56].

Cirrhosis-induced systemic inflammation

A distinctive feature of CAID in cirrhosis is the dynamic coexistence of acquired immunodeficiency and systemic inflammation. The latter results from the persistent stimulation of immune cells and is defined by increased production and enhanced serum levels of pro-inflammatory cytokines and the upregulated expression of cell activation markers.

Evidence of systemic inflammation

As shown in Table 1, the *in vivo* activation of circulating immune cells in cirrhosis is supported by the presence of: (i) neutrophils, showing an increased respiratory burst and enhanced expression of CD11b [27,31], (ii) monocytes, featuring the enhanced surface expression of HLA-DR and activation/co-stimulatory molecules CD80 and CD86, as well as the upregulation of pathways and the increased production of pro-inflammatory cytokines (e.g. TNF α , IL-6) [34,50,52,57,58], (iii) T lymphocytes, showing an increased surface expression of activation antigens that are polarized to augmented IFN γ , TNF α , and IL-17 production [34,50,59,60], and (iv) B lymphocytes, showing an upregulation of the activation/co-stimulatory markers, HLA-DR and CD86, and an increased responsiveness to cytokines and hyperglobulinemia [61,62].

Activated circulating immune cells eventually become major contributors to increased serum concentrations of pro-inflammatory cytokines such as TNF α , TNF α soluble receptors I and II, IL-1 β , IL-6 and IFN γ , IL-17, as well as ICAM-1 and VCAM-1, present in experimental and human cirrhosis

Review

Table 1. Evidences supporting persistent systemic inflammation in cirrhosis.

Finding	[References]
Neutrophils with increased respiratory burst	[27]
Increased expression of surface antigens of activation/co-stimulation on circulating immune system cells (i.e., CD11b on neutrophils, HLA-DR or CD80/86 on antigen presentation cells, CD134 on T cells, loss of CD62L or of CD45RC on T cells)	[31,34,50-53]
Increased production of pro-inflammatory cytokines (TNF α , IFN γ , IL-17) by circulating immune system cells (monocytes, T cells, B cells)	[34,50,59-61,66]
Increased serum levels of pro-inflammatory cytokines (TNF α , IL-1 β , IL-6, IL-17, IL-18, IFN γ) or receptors (sTNFR1, IL1sRI, IL1Ra, sCD14, Fas-R)	[34,50,59,63-81]
Increased serum levels of acute phase reactants (LBP, CRP)	[34,63,64,69-71,76,77,80]
Increased serum levels of molecules of endothelial activation (ICAM-1, VCAM-1, VEGF, nitrates/nitrites)	[34,69,70,76,79,80,88,89]

[34,50,59,63–81]. Specifically, monocytes are a major source of circulating TNF α in cirrhosis, as shown by the direct correlation observed between serum levels of this critical effector cytokine and the TNF α production capacity of monocytes [34]. The severity of this state of systemic inflammation parallels that of cirrhosis itself, as assessed by the Child-Pugh score [63,65,71,78,80–83], and is particularly intense in cirrhosis with ascites [34,50,84]. It is important to point out that the final biological expression of the cytokine network depends on the balance between serum cytokine levels and those of their inhibitors, such as the soluble forms of cytokine receptors. The intense immune system dysregulation of cirrhosis involves a concomitant increase in serum cytokines (i.e. TNF α , IL-6) and in their soluble receptors (i.e. sTNFR1, sTNFR2, sgp130). In effect, cirrhosis has been associated with the enhanced serum expression of the soluble form of the IL-6 receptor, sgp130, which is a potent inhibitor of IL-6 signalling [85]. This could explain the resistance to IL-6, shown by patients with cirrhosis, and the resultant acute phase response to this cytokine [86,87].

Aetiopathogenesis of systemic inflammation

In advanced cirrhosis, the immune response leading to systemic inflammation is initiated when bacteria from the intestinal lumen reach the internal milieu (i.e. gut bacterial translocation). Pathogen-associated molecular patterns (PAMPs) from enteric bacterial organisms and/or damage-associated molecular patterns (DAMPs), originating from the host tissue upon injury, recognize PRRs, expressed on innate immune cells. Notably, not only live bacteria, but also the episodic, persistent inflow of PAMPs (including LPS, lipopeptides, glycopolymers, flagellin and bacterial DNA) into the hepato-splanchnic circulation contributes to the systemic inflammatory response [84,88–91] (Fig. 3). Immune recognition of bacteria and PAMPs in cirrhosis takes place both locally in the GALT and MLN and in the peripheral blood [50,52,92]. Furthermore, immune cells already activated in the GALT and MLN may enter the peripheral blood and spread the inflammatory response systemically [50,52].

Upon interaction, PRRs trigger a transcriptional response leading to gene expression and to the synthesis of a broad range of molecules, including numerous pro- and anti-inflammatory cytokines, chemokines, cell adhesion molecules, and immunoreceptors that induce a panoply of cellular- and counter-responses, driving the adaptive immune response (Table 1). Further consequences of the PRRs' mediated immune cell activation responses include, but are not limited to, enhanced phagocytic activity [27],

vascular endothelial injury [34,69,70,76,79,80,88,89,92], synthesis of acute phase proteins by the liver [34,63,64,69–71,76,77,80], chemotaxis of leukocytes to the sites of inflammation, mainly the liver, and activation of leukocytes at the systemic level. The expression of PRRs, such as TLRs or NLRs, is distinctively upregulated on cells of the innate immune system in cirrhosis with ascites [27,93,94].

The increased translocation rate of enteric bacteria and/or their products is also a distinctive feature of advanced cirrhosis [95–97], arising from the breakdown of three interrelated levels of defence that constitute the intestinal barrier. Specifically, cirrhosis leads to increased intestinal permeability, due to compromised epithelial integrity, intestinal bacterial overgrowth and dysbiosis, caused by disruption of host microbiota homeostasis and intestinal and general immune defence impairment [97,98]. Concurrent damage to these three levels of defence explains the referred high rate of translocation of live bacteria and PAMPs from the gut that occurs in advanced human and experimental cirrhosis [34].

The contribution of enteric bacteria and PAMPs to the pathogenesis of immune cell activation and the inflammatory state of cirrhosis is supported by several findings: (i) the degree of expansion of activated monocytes, activated Th cells and memory B cells in the peripheral blood can be correlated with the presence of surrogate markers of bacterial translocation, such as increased LBP or sCD14 in the serum of patients with cirrhosis and ascites [34,39,84]. Similarly, the critical role of bacterial translocation, driving immune cell activation and inflammatory responses in cirrhosis, is supported by the correlation between bacterial growth in the MLN and the pro-inflammatory phenotype of circulating monocytes and T lymphocytes in cirrhotic rats with ascites [50]. In addition, elegant *ex vivo* experiments of TLR2 and TLR4 blockade or endotoxin removal abrogated B cell activation and neutrophil phagocytic dysfunction of cells from healthy donors, induced by plasma from patients with cirrhosis, respectively [27,39]. (ii) The expansion of B lymphocytes is restricted to clones that are responding to pathogens, as identified by the preferential increase of antibodies against *Saccharomyces cerevisiae*, Gala1-3Galb1-3GlcNAc, a glycan epitope found in the bacterial cell wall galactosamine, or complex gut bacterial protein lysates [99–102]. And finally, (iii) the suppression of enteric aerobic bacterial load and gut bacterial translocation by intestinal decontamination with antibiotics normalizes the expansion of circulating activated immune cells and attenuates the pro-inflammatory cytokine production in MLN and blood in experimental models and in patients with cirrhosis and ascites [34,50,84].

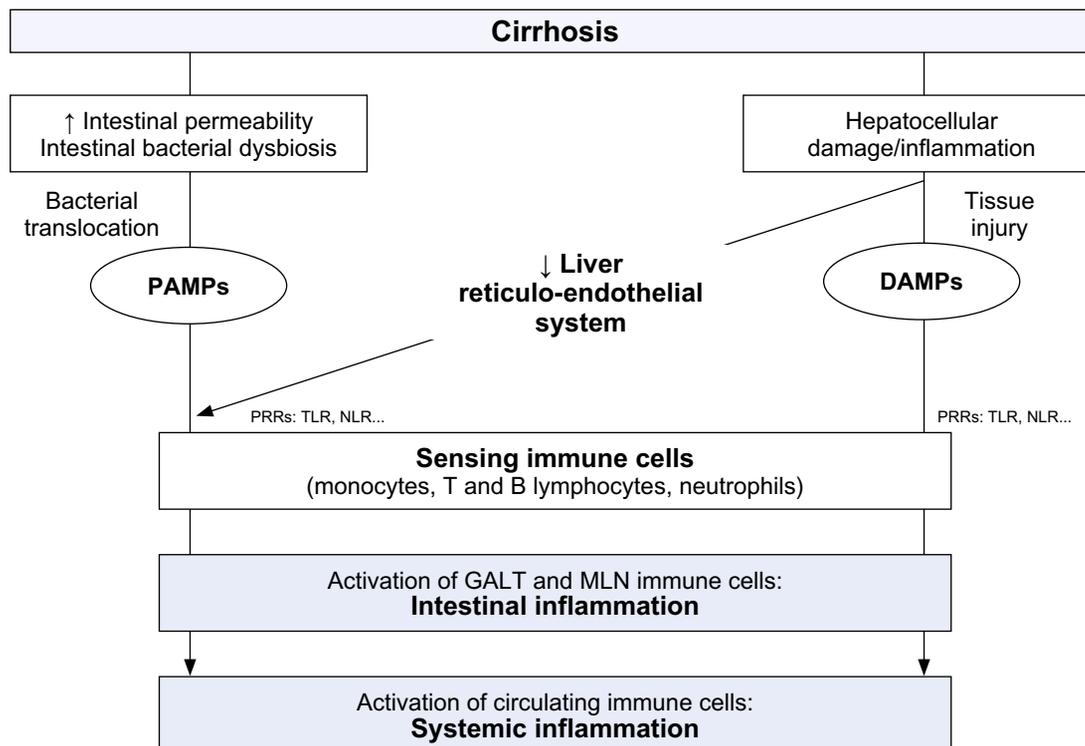


Fig. 3. Pathogenesis of systemic inflammation in cirrhosis. Host immune cells are stimulated by bacteria and pathogen-associated molecular patterns (PAMPs), produced and released from a leaky gut, and damage-associated molecular patterns (DAMPs), released from necrotic liver cells. These stimuli activate host pattern recognition receptors, such as the toll-like receptor (TLR) and the nucleotide-binding oligomerization domain-like receptors (NLRs). Activation of these receptors leads to expression of activation surface molecules (cytokine receptors, adhesion molecules) on immune cells and upregulation of cytokines (pro- and anti-inflammatory lymphokines and monokines), chemokines and growth factors, which are released to recruit and activate additional inflammatory cells. Pattern recognition receptor (PRR)-dependent immune cell activation in cirrhosis occurs locally in the gut-associated lymphoid tissue (GALT), mesenteric lymph nodes (MLN), and in peripheral blood. Immune cells already activated in the GALT and MLN may enter the peripheral blood and further spread systemically the inflammatory response.

DAMPs and sterile particulates, released from necrotic hepatocytes, might also contribute to elicit an inflammatory response in cirrhosis [103]. Sterile inflammation, induced by DAMPs, is evident in the acute hepatic injury by acetaminophen, during ischemia/reperfusion, and in the low-chronic hepatic injury by alcoholic and non-alcoholic steatohepatitis [104–106]. Due to the size of the liver and the extent of its damage in cirrhosis, it is tempting to speculate that a significant amount of DAMPs from necrotic hepatocytes could spill to the circulation in cirrhosis of any aetiology and contribute to immune cell activation (Fig. 3). Indeed, systemic inflammation is already present, albeit at a lower grade, in the pre-ascitic stage of human and experimental cirrhosis, when bacterial translocation is not yet critical [52,71,84]. In this regard, the hepatic lymph nodes, and not the gut, are the main source of activated circulating immune cells in rats with pre-ascitic cirrhosis, as shown by correlates between their frequencies in hepatic lymph nodes and peripheral blood [52].

As in other inflammatory conditions, genetic polymorphisms of cytokines and innate immunity pathways may lead to varying immune responses and bacterial infection susceptibilities in cirrhosis. Well-established correlates between intestinal and systemic immune responses and bacterial infections in cirrhosis have been demonstrated through variations in the genes coding for PRRs. Specifically, NOD2 and TLR2 variants impair innate host defence mechanisms, leading to increased SBP susceptibility and mortality in cirrhosis [107–111].

Dynamic pattern of the CAID phenotypes

The response of the immune system to antigens and/or activation signals is characterized by a dynamic pattern of cellular activation and differentiation, as well as amounts and types of cytokine and antibody secretion. Temporal variations exist in the internal mechanism of immune response regulation that are also modulated by environmental and activation challenge features. A well-known mechanism that is triggered to regulate the pro-inflammatory environment is endotoxin tolerance, whereby monocytes, persistently exposed to low endotoxin concentrations, enter into a transient state of unresponsiveness to further endotoxin challenge [112].

The dynamic immune response pattern is clearly manifested in CAID. Indeed, the immune disturbance of cirrhosis varies according to disease stage (compensated, decompensated, acute-on-chronic liver failure [ACLF]), the extent of liver injury, and the presence of environmental stimulation, induced by signalling from persistent episodic bacterial translocation.

The immune system in patients and experimental models of “stable” decompensated cirrhosis faces the frequent challenge of different types and intensities of PAMPs originated in the gut (Figs. 1 and 3). In this setting, the immune system exhibits a predominantly “pro-inflammatory” phenotype, with increased expression of activation antigens on immune cells, and augmented production and increased serum levels of pro- and anti-inflammatory cytokines. This unrestricted pro-inflammatory

Review

phenotype is present in spite of persistent immune cell stimulation, indicating that “endotoxin tolerance” is a late event in the natural history of cirrhosis (Fig. 2). Potential explanations for this CAID phenotype could be (i) defective production of the anti-inflammatory cytokine, IL-10 [75], (ii) the non-induction of negative feedback loops after TLR4 stimulation, such as IRAK-M [57] or the constitutively active glycogen synthase kinase GSK3b [113] in response to LPS, and (iii) reduced plasma high-density lipoprotein levels and abolition of LPS downregulation of the scavenger-receptor SR-BI in monocytes of patients with advanced cirrhosis, since high-density lipoproteins are able to bind and neutralize the bioactivity of LPS [114].

Finally, several lines of evidence indicate that immune response reprogramming occurs at the further decompensated stage of cirrhosis, after persistent LPS-driven stimulation. In the experimental setting, intestinal dendritic cells in rats with cirrhosis, ascites and intense gut bacterial translocation, thus subjected to high intestinal bacterial pressure, show decreased phagocytosis and TNF α production compared with rats without bacterial translocation, in which phagocytosis is clearly augmented [50]. In this model, bowel decontamination eliminates the bacterial stimulus and partially normalizes intestinal dendritic cell functions. This behaviour of dendritic cells could in part explain the fact that bowel decontamination in cirrhosis improves survival beyond mere infectious prophylaxis [115]. In the clinical setting, severe immune response reprogramming is maximally detectable at the very late stage of cirrhosis, i.e. in patients with acute-on-chronic liver failure (ACLF), who develop a state of immune paralysis that resembles that found in sepsis. This CAID phenotype is defined by defective HLA-DR monocyte expression, the inability of monocytes to produce TNF α in response to LPS, reduced T lymphocyte IFN γ production, and massive release of inflammatory and anti-inflammatory (e.g. IL-10) cytokines [116–119]. Importantly, this CAID phenotype, associated with defective HLA-DR monocyte expression, correlates with increased mortality, mostly owing to bacterial infection [116–118]. Thus, contrary to patients with “stable” decompensated cirrhosis, ACLF patients exhibit a predominantly “immunodeficient” CAID phenotype.

In summary, under persistent PAMPs challenge, the immune response pattern switches from a predominantly “pro-inflammatory” to a predominantly “immunodeficient” phenotype. CAID phenotypes represent the extremes of a spectrum of reversible dynamic events that take place during the course of cirrhosis, though the trend is that immunodeficiency predominates as the disease reaches its final stages.

Role of systemic inflammation in the clinical expression of cirrhosis

Immunodeficiency, coupled to systemic inflammation in CAID, is the pathophysiological basis for several of the clinical manifestations of cirrhosis. The clinical spectrum of CAID in cirrhosis spans from a poor response to the bacterial challenge, with increased susceptibility to bacterial infection accompanied by high mortality, to multi-organ inflammatory damage. The clinical expression of bacteria-dependent events during cirrhosis includes both chronic systemic and organ-specific damage and intercurrent acute insults (i.e. acute-on-chronic) [120,121]. It has been demonstrated that the greater the intensity of the cellular and molecular CAID, the greater the risk of severe bacterial

infection [84,122,123]. Specifically, the risk of bacterial infection is greater in patients with cirrhosis and ascites who show augmented serum level of molecules synthesized upon interaction of bacteria with the host immune system, such as LBP or IgA class anti-neutrophil cytoplasmic antibodies [122,123]. Further, the levels of some soluble immune mediators, such as TNFR-I and the adhesion molecule ICAM-1, are related to poor survival of patients with cirrhosis [76,78,81]. Another manifestation of CAID is also the non-protective response to vaccination [2,3,124].

The relevance of the systemic inflammation, distinctive of CAID, stems from the fact that circulating activated immune cells can be recruited by peripheral tissues and/or can produce soluble factors, such as pro-inflammatory cytokines. Via these mechanisms, inflammatory immune cells may damage somatic cells and contribute to the clinical expression of cirrhosis.

A well-characterized example of the consequence of systemic inflammation in the clinical expression of cirrhosis is the capacity of high pro-inflammatory cytokine levels to modulate the vascular tone. These pro-inflammatory cytokines worsen splanchnic and systemic vasodilation through nitric oxide overproduction [125]. Indeed, the severity of splanchnic and peripheral vasodilation is greater in patients and in experimental models of severe cirrhosis, associated with ascites and bacterial translocation, showing the most severe systemic inflammation [68,84,126]. The contribution of enteric bacteria-induced CAID to the haemodynamic derangement of cirrhosis is further supported by studies showing that selective bowel decontamination reduces nitric oxide and plasma renin activity, and improves peripheral vasodilation in patients with cirrhosis, ascites and high serum LBP levels, and attenuates endothelial activation and expression of inflammatory cytokines in the aorta of rats with biliary cirrhosis [84,92,127].

Besides, direct signalling in the brain by pro-inflammatory cytokines or recruitment of activated immune cells into brain tissue, subsequently activates resident macrophages to produce TNF α , modifies cerebral function and contributes to the pathogenesis of encephalopathy and the fatigue distinctive of cirrhosis [128–130].

Systemic inflammation as well as PAMPs and DAMPs release may also compromise kidney function [131]. Increasing evidence indicates that inflammation-mediated microvascular dysfunction can reduce glomerular filtration rate, whereas the oxidative stress that results from the organized interaction between PAMPs/DAMPs and the tubular epithelial cell can hurt tubular function and cause acute kidney injury in patients with severe bacterial infection or even sepsis in the absence of overt signs of renal hypoperfusion [132,133]. We hypothesize that pro-inflammatory cytokines as well as PAMPs/DAMPs originated in the gut or other sites of injury (i.e. liver) can gain access to the renal tubules by glomerular filtration or by proximity to the peritubular capillaries, and elicit an inflammatory response when actively recognized by tubular epithelial cells through TLR-4, and ultimately contribute to episodes of acute kidney injury in cirrhosis.

Hypothesis

Cirrhosis-associated immune dysfunction is the result of two concurrent and interlinked processes, namely systemic inflammation and damage of the immune system response that come into play as cirrhosis progresses. In the compensated pre-ascitic

stage, DAMPs from stressed and damaged tissue, mainly necrotic hepatocytes, activate circulating immune cells. These activation signals add to those of the aetiological agents of cirrhosis, such as alcohol or viruses. Cirrhosis progression distorts hepatic architecture and cellular organization and impairs its functional capacity. These events compromise the immune surveillance function of the liver, both locally by damaging the reticulo-endothelial system and systemically by impairing the bactericidal role of phagocytic cells through the reduced synthesis of proteins involved in the innate immune response and of PRRs. In the decompensated, ascitic stage of cirrhosis, gut bacterial translocation occurs at a high rate and PAMPs released from the leaky gut further activate the immune system and aggravate systemic inflammation. Immune response reprogramming occurs after constant PAMPs pressure and the predominantly “pro-inflammatory” CAID phenotype switches to the predominantly “immunodeficient” one of severely decompensated cirrhosis with extra-hepatic organ failure. CAID plays a critical pathogenetic role in several clinical manifestations of cirrhosis, including bacterial infections, haemodynamic derangement, and organ inflammatory damage, and as such has emerged as a potential therapeutic target in cirrhosis. Identification and grading of CAID is challenging and could be pursued by measuring the activity of phagocytic, cytotoxic and regulatory immune cells, their activation/anergic and differentiation stage, and serum levels of circulating cytokines.

Financial support

Agustín Albillos received grants from the Spanish Ministry of Health, Instituto de Salud Carlos III (Nos. PS09/00485, PI14/00876 and PI051871, Ciberehd) and Fundación Mutua Madrileña (AP100652012). Melchor Álvarez-Mon received grants from the Comunidad de Madrid (MITIC-CM S2010/BMD-2502) and the Spanish Ministry of Health, Instituto de Salud Carlos III (No. PI08/1890). Ciberhd is funded by the Instituto de Salud Carlos III.

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.

Acknowledgements

We thank Leticia Muñoz and María Úbeda from the Department of Medicine of the University of Alcalá for their useful comments to improve this manuscript.

References

[1] Jalan R, Fernandez J, Wiest R, Schnabl B, Moreau R, Angeli P, et al. Bacterial infections in cirrhosis: a position statement based on the EASL Special Conference 2013. *J Hepatol* 2014;60:1310–1324.
 [2] Keeffe EB, Iwarson S, McMahon BJ, Lindsay KL, Koff RS, Manns M, et al. Safety and immunogenicity of hepatitis A vaccine in patients with chronic liver disease. *Hepatology* 1998;27:881–886.
 [3] Keeffe EB, Krause DS. Hepatitis B vaccination of patients with chronic liver disease. *Liver Transpl Surg* 1998;4:437–439.
 [4] Jenne CN, Kubes P. Immune surveillance by the liver. *Nat Immunol* 2013;14:996–1006.

[5] Thomson AW, Knolle PA. Antigen-presenting cell function in the tolerogenic liver environment. *Nat Rev Immunol* 2010;10:753–766.
 [6] Racaneli V, Rehmann B. The liver as an immunological organ. *Hepatology* 2006;43:S54–62.
 [7] Gregory SH, Cousens LP, van Rooijen N, Dopp EA, Carlos TM, Wing EJ. Complementary adhesion molecules promote neutrophil-Kupffer cell interaction and the elimination of bacteria taken up by the liver. *J Immunol* 2002;168:308–315.
 [8] Smedsrod B, Pertoft H, Gustafson S, Laurent TC. Scavenger functions of the liver endothelial cell. *Biochem J* 1990;266:313–327.
 [9] Willekens FL, Werre JM, Kruijt JK, Roerdinkholder-Stoelwinder B, Groenen-Dopp YA, van den Bos AG, et al. Liver Kupffer cells rapidly remove red blood cell-derived vesicles from the circulation by scavenger receptors. *Blood* 2005;105:2141–2145.
 [10] Gregory SH, Sagnimeni AJ, Wing EJ. Bacteria in the bloodstream are trapped in the liver and killed by immigrating neutrophils. *J Immunol* 1996;157:2514–2520.
 [11] Pillarisetty VG, Shah AB, Miller G, Bleier JL, DeMatteo RP. Liver dendritic cells are less immunogenic than spleen dendritic cells because of differences in subtype composition. *J Immunol* 2004;172:1009–1017.
 [12] Schildberg FA, Hegenbarth SI, Schumak B, Scholz K, Limmer A, Knolle PA. Liver sinusoidal endothelial cells veto CD8 T cell activation by antigen-presenting dendritic cells. *Eur J Immunol* 2008;38:957–967.
 [13] You Q, Cheng L, Kedl RM, Ju C. Mechanism of T cell tolerance induction by murine hepatic Kupffer cells. *Hepatology* 2008;48:978–990.
 [14] Ellett JD, Atkinson C, Evans ZP, Amani Z, Balish E, Schmidt MG, et al. Murine Kupffer cells are protective in total hepatic ischemia/reperfusion injury with bowel congestion through IL-10. *J Immunol* 2010;184:5849–5858.
 [15] Gao B, Jeong WI, Tian Z. Liver: an organ with predominant innate immunity. *Hepatology* 2008;47:729–736.
 [16] Ramadori G, Christ B. Cytokines and the hepatic acute-phase response. *Semin Liver Dis* 1999;19:141–155.
 [17] Liu C, Xu Z, Gupta D, Dziarski R. Peptidoglycan recognition proteins: a novel family of four human innate immunity pattern recognition molecules. *J Biol Chem* 2001;276:34686–34694.
 [18] Crispe IN. The liver as a lymphoid organ. *Annu Rev Immunol* 2009;27:147–163.
 [19] Helmy KY, Katschke Jr KJ, Gorgani NN, Kljavin NM, Elliott JM, Diehl L, et al. C1Rlg: a macrophage complement receptor required for phagocytosis of circulating pathogens. *Cell* 2006;124:915–927.
 [20] Rimola A, Soto R, Bory F, Arroyo V, Piera C, Rodes J. Reticuloendothelial system phagocytic activity in cirrhosis and its relation to bacterial infections and prognosis. *Hepatology* 1984;4:53–58.
 [21] Runyon BA, Morrissey RL, Hoefs JC, Wyle FA. Opsonic activity of human ascitic fluid: a potentially important protective mechanism against spontaneous bacterial peritonitis. *Hepatology* 1985;5:634–637.
 [22] Runyon BA. Patients with deficient ascitic fluid opsonic activity are predisposed to spontaneous bacterial peritonitis. *Hepatology* 1988;8:632–635.
 [23] Bouwman LH, Roos A, Terpstra OT, de Knijff P, van Hoek B, Verspaget HW, et al. Mannose binding lectin gene polymorphisms confer a major risk for severe infections after liver transplantation. *Gastroenterology* 2005;129:408–414.
 [24] Altorjay I, Vitalis Z, Tornai I, Palatka K, Kacska S, Farkas G, et al. Mannose-binding lectin deficiency confers risk for bacterial infections in a large Hungarian cohort of patients with liver cirrhosis. *J Hepatol* 2010;53:484–491.
 [25] Rajkovic IA, Williams R. Abnormalities of neutrophil phagocytosis, intracellular killing and metabolic activity in alcoholic cirrhosis and hepatitis. *Hepatology* 1986;6:252–262.
 [26] Fiuza C, Salcedo M, Clemente G, Tellado JM. In vivo neutrophil dysfunction in cirrhotic patients with advanced liver disease. *J Infect Dis* 2000;182:526–533.
 [27] Tritto G, Bechlis Z, Stadlbauer V, Davies N, Frances R, Shah N, et al. Evidence of neutrophil functional defect despite inflammation in stable cirrhosis. *J Hepatol* 2011;55:574–581.
 [28] Ono Y, Watanabe T, Matsumoto K, Ito T, Kunii O, Goldstein E. Opsonophagocytic dysfunction in patients with liver cirrhosis and low responses to tumor necrosis factor- α and lipopolysaccharide in patients' blood. *J Infect Chemother* 2004;10:200–207.
 [29] Rolas L, Makhezer N, Hadjoudj S, El-Benna J, Djerdjouri B, Elkrief L, et al. Inhibition of mammalian target of rapamycin aggravates the respiratory burst defect of neutrophils from decompensated patients with cirrhosis. *Hepatology* 2013;57:1163–1171.

Review

- [30] Garfia C, Garcia-Ruiz I, Solis-Herruzo JA. Deficient phospholipase C activity in blood polymorphonuclear neutrophils from patients with liver cirrhosis. *J Hepatol* 2004;40:749–756.
- [31] Fiuza C, Salcedo M, Clemente G, Tellado JM. Granulocyte colony-stimulating factor improves deficient in vitro neutrophil transendothelial migration in patients with advanced liver disease. *Clin Diagn Lab Immunol* 2002;9:433–439.
- [32] Zimmermann HW, Seidler S, Nattermann J, Gassler N, Hellerbrand C, Zerneck A, et al. Functional contribution of elevated circulating and hepatic non-classical CD14CD16 monocytes to inflammation and human liver fibrosis. *PLoS One* 2010;5:e11049.
- [33] Seidler S, Zimmermann HW, Weiskirchen R, Trautwein C, Tacke F. Elevated circulating soluble interleukin-2 receptor in patients with chronic liver diseases is associated with non-classical monocytes. *BMC Gastroenterol* 2012;12:38.
- [34] Albillos A, Hera Ad Ade L, Reyes E, Monserrat J, Munoz L, Nieto M, et al. Tumour necrosis factor- α expression by activated monocytes and altered T-cell homeostasis in ascitic alcoholic cirrhosis: amelioration with norfloxacin. *J Hepatol* 2004;40:624–631.
- [35] Gomez F, Ruiz P, Schreiber AD. Impaired function of macrophage Fc gamma receptors and bacterial infection in alcoholic cirrhosis. *N Engl J Med* 1994;331:1122–1128.
- [36] Cook RT, Waldschmidt TJ, Cook BL, Labrecque DR, McLatchie K. Loss of the CD5+ and CD45RAhi B cell subsets in alcoholics. *Clin Exp Immunol* 1996;103:304–310.
- [37] Laso FJ, Madruga JI, Lopez A, Ciudad J, Alvarez-Mon M, San Miguel J, et al. Distribution of peripheral blood lymphoid subsets in alcoholic liver cirrhosis: influence of ethanol intake. *Alcohol Clin Exp Res* 1996;20:1564–1568.
- [38] Massonnet B, Delwail A, Ayrault JM, Chagneau-Derrode C, Lecron JC, Silvain C. Increased immunoglobulin A in alcoholic liver cirrhosis: exploring the response of B cells to Toll-like receptor 9 activation. *Clin Exp Immunol* 2009;158:115–124.
- [39] Doi H, Iyer TK, Carpenter E, Li H, Chang KM, Vonderheide RH, et al. Dysfunctional B-cell activation in cirrhosis resulting from hepatitis C infection associated with disappearance of CD27-positive B-cell population. *Hepatology* 2012;55:709–719.
- [40] Perrin D, Bignon JD, Beaujard E, Cheneau ML. Populations of circulating T lymphocytes in patients with alcoholic cirrhosis. *Gastroenterol Clin Biol* 1984;8:907–910.
- [41] Morita K, Fukuda Y, Nakano I, Katano Y, Hayakawa T. Peripheral lymphocyte subsets vary with stage of hepatitis C virus-associated liver disease. *Hepatogastroenterology* 2005;52:1803–1808.
- [42] McGovern BH, Golan Y, Lopez M, Pratt D, Lawton A, Moore G, et al. The impact of cirrhosis on CD4+ T cell counts in HIV-seronegative patients. *Clin Infect Dis* 2007;44:431–437.
- [43] Lario M, Munoz L, Ubeda M, Borrero MJ, Martinez J, Monserrat J, et al. Defective thymopoiesis and poor peripheral homeostatic replenishment of T-helper cells cause T-cell lymphopenia in cirrhosis. *J Hepatol* 2013;59:723–730.
- [44] Yonkers NL, Sieg S, Rodriguez B, Anthony DD. Reduced naive CD4 T cell numbers and impaired induction of CD27 in response to T cell receptor stimulation reflect a state of immune activation in chronic hepatitis C virus infection. *J Infect Dis* 2011;203:635–645.
- [45] Deviere J, Denys C, Schandene L, Romasco F, Adler M, Wybran J, et al. Decreased proliferative activity associated with activation markers in patients with alcoholic liver cirrhosis. *Clin Exp Immunol* 1988;72:377–382.
- [46] Giron-Gonzalez JA, Alvarez-Mon M, Menendez-Caro JL, Manzano L, Abreu L, Yebra M, et al. T lymphocytes from alcoholic cirrhotic patients show normal interleukin-2 production but a defective proliferative response to polyclonal mitogens. *Am J Gastroenterol* 1994;89:767–773.
- [47] Morishima C, Di Bisceglie AM, Rothman AL, Bonkovsky HL, Lindsay KL, Lee WM, et al. Antigen-specific T lymphocyte proliferation decreases over time in advanced chronic hepatitis C. *J Viral Hepat* 2012;19:404–413.
- [48] Laso FJ, Madruga JI, Giron JA, Lopez A, Ciudad J, San Miguel JF, et al. Decreased natural killer cytotoxic activity in chronic alcoholism is associated with alcohol liver disease but not active ethanol consumption. *Hepatology* 1997;25:1096–1100.
- [49] Tian Z, Chen Y, Gao B. Natural killer cells in liver disease. *Hepatology* 2013;57:1654–1662.
- [50] Munoz L, Albillos A, Nieto M, Reyes E, Lledo L, Monserrat J, et al. Mesenteric Th1 polarization and monocyte TNF- α production: first steps to systemic inflammation in rats with cirrhosis. *Hepatology* 2005;42:411–419.
- [51] Munoz L, Borrero MJ, Ubeda M, Lario M, Diaz D, Frances R, et al. Interaction between intestinal dendritic cells and bacteria translocated from the gut in rats with cirrhosis. *Hepatology* 2012;56:1861–1869.
- [52] Ubeda M, Munoz L, Borrero MJ, Diaz D, Frances R, Monserrat J, et al. Critical role of the liver in the induction of systemic inflammation in rats with preascitic cirrhosis. *Hepatology* 2010;52:2086–2095.
- [53] Muñoz L, Borrero MJ, Ubeda M, Lario M, Diaz D, Aguado-Fraile E, et al. Commensal gut flora drives the expansion of proinflammatory T cells in the small intestinal mucosa in rats with CCl4 cirrhosis. *Hepatology* 2013;58:985A.
- [54] Du Plessis J, Vanheel H, Janssen CE, Roos L, Slavik T, Stivaktas PI, et al. Activated intestinal macrophages in patients with cirrhosis release NO and IL-6 that may disrupt intestinal barrier function. *J Hepatol* 2013;58:1125–1132.
- [55] Teltschik Z, Wiest R, Beisner J, Nuding S, Hofmann C, Schoelmerich J, et al. Intestinal bacterial translocation in rats with cirrhosis is related to compromised Paneth cell antimicrobial host defense. *Hepatology* 2012;55:1154–1163.
- [56] Yan AW, Fouts DE, Brandl J, Starkel P, Torralba M, Schott E, et al. Enteric dysbiosis associated with a mouse model of alcoholic liver disease. *Hepatology* 2011;53:96–105.
- [57] Tazi KA, Quioc JJ, Saada V, Bezeaud A, Lebrec D, Moreau R. Upregulation of TNF- α production signaling pathways in monocytes from patients with advanced cirrhosis: possible role of Akt and IRAK-M. *J Hepatol* 2006;45:280–289.
- [58] Gandoura S, Weiss E, Rautou PE, Fasseu M, Gustot T, Lemoine F, et al. Gene- and exon-expression profiling reveals an extensive LPS-induced response in immune cells in patients with cirrhosis. *J Hepatol* 2013;58:936–948.
- [59] 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.
- [60] Sun HQ, Zhang JY, Zhang H, Zou ZS, Wang FS, Jia JH. Increased Th17 cells contribute to disease progression in patients with HBV-associated liver cirrhosis. *J Viral Hepat* 2012;19:396–403.
- [61] Giron JA, Alvarez-Mon M, Menendez-Caro JL, Abreu L, Albillos A, Manzano L, et al. Increased spontaneous and lymphokine-conditioned IgA and IgG synthesis by B cells from alcoholic cirrhotic patients. *Hepatology* 1992;16:664–670.
- [62] Deviere J, Content J, Denys C, Vandenbussche P, Schandene L, Wybran J, et al. High interleukin-6 serum levels and increased production by leucocytes in alcoholic liver cirrhosis. Correlation with IgA serum levels and lymphokines production. *Clin Exp Immunol* 1989;77:221–225.
- [63] Lee FY, Lu RH, Tsai YT, Lin HC, Hou MC, Li CP, et al. Plasma interleukin-6 levels in patients with cirrhosis. Relationship to endotoxemia, tumor necrosis factor- α , and hyperdynamic circulation. *Scand J Gastroenterol* 1996;31:500–505.
- [64] Zhang W, Yue B, Wang GQ, Lu SL. Serum and ascites levels of macrophage migration inhibitory factor, TNF- α and IL-6 in patients with chronic virus hepatitis B and hepatitis cirrhosis. *Hepatobiliary Pancreat Dis Int* 2002;1:577–580.
- [65] Giron-Gonzalez JA, Martinez-Sierra C, Rodriguez-Ramos C, Macias MA, Rendon P, Diaz F, et al. Implication of inflammation-related cytokines in the natural history of liver cirrhosis. *Liver Int* 2004;24:437–445.
- [66] Deviere J, Content J, Denys C, Vandenbussche P, Schandene L, Wybran J, et al. Excessive in vitro bacterial lipopolysaccharide-induced production of monokines in cirrhosis. *Hepatology* 1990;11:628–634.
- [67] Le Moine O, Soupison T, Sogni P, Marchant A, Moreau R, Hadengue A, et al. Plasma endotoxin and tumor necrosis factor- α in the hyperkinetic state of cirrhosis. *J Hepatol* 1995;23:391–395.
- [68] Wiest R, Das S, Cadelina G, Garcia-Tsao G, Milstien S, Groszmann RJ. Bacterial translocation in cirrhotic rats stimulates eNOS-derived NO production and impairs mesenteric vascular contractility. *J Clin Invest* 1999;104:1223–1233.
- [69] Genesca J, Marti R, Gonzalez A, Torregrosa M, Segura R. Soluble interleukin-6 receptor levels in liver cirrhosis. *Am J Gastroenterol* 1999;94:3074–3075.
- [70] Lopez-Talavera JC, Levitzki A, Martinez M, Gazit A, Esteban R, Guardia J. Tyrosine kinase inhibition ameliorates the hyperdynamic state and decreases nitric oxide production in cirrhotic rats with portal hypertension and ascites. *J Clin Invest* 1997;100:664–670.
- [71] Tiig H, Wilmer A, Vogel W, Herold M, Nolchen B, Judmaier G, et al. Serum levels of cytokines in chronic liver diseases. *Gastroenterology* 1992;103:264–274.
- [72] Ludwiczek O, Vannier E, Moschen A, Salazar-Montes A, Borggraefe I, Gabay C, et al. Impaired counter-regulation of interleukin-1 by the soluble IL-1

- receptor type II in patients with chronic liver disease. *Scand J Gastroenterol* 2008;43:1360–1365.
- [73] Ludwiczek O, Kaser A, Novick D, Dinarello CA, Rubinstein M, Vogel W, et al. Plasma levels of interleukin-18 and interleukin-18 binding protein are elevated in patients with chronic liver disease. *J Clin Immunol* 2002;22:331–337.
- [74] von Baehr V, Docke WD, Plauth M, Liebenthal C, Kupferling S, Lochs H, et al. Mechanisms of endotoxin tolerance in patients with alcoholic liver cirrhosis: role of interleukin 10, interleukin 1 receptor antagonist, and soluble tumour necrosis factor receptors as well as effector cell desensitisation. *Gut* 2000;47:281–287.
- [75] Byl B, Roucloux I, Crusiaux A, Dupont E, Deviere J. Tumor necrosis factor alpha and interleukin 6 plasma levels in infected cirrhotic patients. *Gastroenterology* 1993;104:1492–1497.
- [76] Giron-Gonzalez JA, Martinez-Sierra C, Rodriguez-Ramos C, Rendon P, Macias MA, Fernandez-Gutierrez C, et al. Adhesion molecules as a prognostic marker of liver cirrhosis. *Scand J Gastroenterol* 2005;40:217–224.
- [77] Kaser A, Ludwiczek O, Waldenberger P, Jaschke W, Vogel W, Tilg H. Endotoxin and its binding proteins in chronic liver disease: the effect of transjugular intrahepatic portosystemic shunting. *Liver* 2002;22:380–387.
- [78] Trebicka J, Krag A, Gansweid S, Schiedermaier P, Strunk HM, Fimmers R, et al. Soluble TNF-alpha-receptors I are prognostic markers in TIPS-treated patients with cirrhosis and portal hypertension. *PLoS One* 2013;8:e83341.
- [79] Buck M, Garcia-Tsao G, Groszmann RJ, Stalling C, Grace ND, Burroughs AK, et al. Novel inflammatory biomarkers of portal pressure in compensated cirrhosis patients. *Hepatology* 2014;59:1052–1059.
- [80] Wiese S, Mortensen C, Gotze JP, Christensen E, Andersen O, Bendtsen F, et al. Cardiac and proinflammatory markers predict prognosis in cirrhosis. *Liver Int* 2013;34:e19–30.
- [81] Grunhage F, Rezori B, Neef M, Lammert F, Sauerbruch T, Spengler U, et al. Elevated soluble tumor necrosis factor receptor 75 concentrations identify patients with liver cirrhosis at risk of death. *Clin Gastroenterol Hepatol* 2008;6:1255–1262.
- [82] Tilg H, Vogel W, Wiedermann CJ, Shapiro L, Herold M, Judmaier G, et al. Circulating interleukin-1 and tumor necrosis factor antagonists in liver disease. *Hepatology* 1993;18:1132–1138.
- [83] Eriksson AS, Gretzer C, Wallerstedt S. Elevation of cytokines in peritoneal fluid and blood in patients with liver cirrhosis. *Hepatogastroenterology* 2004;51:505–509.
- [84] Albillos A, de la Hera A, Gonzalez M, Moya JL, Calleja JL, Monserrat J, et al. Increased lipopolysaccharide binding protein in cirrhotic patients with marked immune and hemodynamic derangement. *Hepatology* 2003;37:208–217.
- [85] Lemmers A, Gustot T, Durnez A, Evrard S, Moreno C, Quertinmont E, et al. An inhibitor of interleukin-6 trans-signalling, sgp130, contributes to impaired acute phase response in human chronic liver disease. *Clin Exp Immunol* 2009;156:518–527.
- [86] Le Moine O, Deviere J, Devaster JM, Crusiaux A, Durand F, Bernuau J, et al. Interleukin-6: an early marker of bacterial infection in decompensated cirrhosis. *J Hepatol* 1994;20:819–824.
- [87] Mackenzie I, Woodhouse J. C-reactive protein concentrations during bacteraemia: a comparison between patients with and without liver dysfunction. *Intensive Care Med* 2006;32:1344–1351.
- [88] Guarner C, Soriano G, Tomas A, Bulbena O, Novella MT, Balanzo J, et al. Increased serum nitrite and nitrate levels in patients with cirrhosis: relationship to endotoxemia. *Hepatology* 1993;18:1139–1143.
- [89] Campillo B, Bories PN, Benvenuti C, Dupeyron C. Serum and urinary nitrate levels in liver cirrhosis: endotoxemia, renal function and hyperdynamic circulation. *J Hepatol* 1996;25:707–714.
- [90] Lin RS, Lee FY, Lee SD, Tsai YT, Lin HC, Lu RH, et al. Endotoxemia in patients with chronic liver diseases: relationship to severity of liver diseases, presence of esophageal varices, and hyperdynamic circulation. *J Hepatol* 1995;22:165–172.
- [91] Gonzalez-Navajas JM, Bellot P, Frances R, Zapater P, Munoz C, Garcia-Pagan JC, et al. Presence of bacterial-DNA in cirrhosis identifies a subgroup of patients with marked inflammatory response not related to endotoxin. *J Hepatol* 2008;48:61–67.
- [92] Tazi KA, Moreau R, Herve P, Dauvergne A, Cazals-Hatem D, Bert F, et al. Norfloxacin reduces aortic NO synthases and proinflammatory cytokine up-regulation in cirrhotic rats: role of Akt signaling. *Gastroenterology* 2005;129:303–314.
- [93] Riordan SM, Skinner N, Nagree A, McCallum H, McIver CJ, Kurtovic J, et al. Peripheral blood mononuclear cell expression of toll-like receptors and relation to cytokine levels in cirrhosis. *Hepatology* 2003;37:1154–1164.
- [94] Manigoff T, Bocker U, Hanck C, Gundt J, Traber P, Antoni C, et al. Differential expression of toll-like receptors 2 and 4 in patients with liver cirrhosis. *Eur J Gastroenterol Hepatol* 2003;15:275–282.
- [95] Cirera I, Bauer TM, Navasa M, Vila J, Grande L, Taura P, et al. Bacterial translocation of enteric organisms in patients with cirrhosis. *J Hepatol* 2001;34:32–37.
- [96] Garcia-Tsao G, Lee FY, Barden GE, Cartun R, West AB. Bacterial translocation to mesenteric lymph nodes is increased in cirrhotic rats with ascites. *Gastroenterology* 1995;108:1835–1841.
- [97] Perez-Paramo M, Munoz J, Albillos A, Freile I, Portero F, Santos M, et al. Effect of propranolol on the factors promoting bacterial translocation in cirrhotic rats with ascites. *Hepatology* 2000;31:43–48.
- [98] Wiest R, Lawson M, Geuking M. Pathological bacterial translocation in liver cirrhosis. *J Hepatol* 2014;60:197–209.
- [99] Mehta AS, Long RE, Comunale MA, Wang M, Rodemich L, Krakover J, et al. Increased levels of galactose-deficient anti-Gal immunoglobulin G in the sera of hepatitis C virus-infected individuals with fibrosis and cirrhosis. *J Virol* 2008;82:1259–1270.
- [100] Papp M, Norman GL, Vitalis Z, Tornai I, Altorjay I, Foldi I, et al. Presence of anti-microbial antibodies in liver cirrhosis – a tell-tale sign of compromised immunity? *PLoS One* 2010;5:e12957.
- [101] Vallat L, Benhamou Y, Gutierrez M, Ghillani P, Hercher C, Thibault V, et al. Clonal B cell populations in the blood and liver of patients with chronic hepatitis C virus infection. *Arthritis Rheum* 2004;50:3668–3678.
- [102] Fazi C, Dagklis A, Cottini F, Scarfo L, Bertilaccio MT, Finazzi R, et al. Monoclonal B cell lymphocytosis in hepatitis C virus infected individuals. *Cytometry B Clin Cytom* 2010;78:S61–68.
- [103] Kubes P, Mehal WZ. Sterile inflammation in the liver. *Gastroenterology* 2012;143:1158–1172.
- [104] Martin-Murphy BV, Holt MP, Ju C. The role of damage associated molecular pattern molecules in acetaminophen-induced liver injury in mice. *Toxicol Lett* 2010;192:387–394.
- [105] Petrasek J, Bala S, Csak T, Lippai D, Kodys K, Menashy V, et al. IL-1 receptor antagonist ameliorates inflammasome-dependent alcoholic steatohepatitis in mice. *J Clin Invest* 2012;122:3476–3489.
- [106] Zhu P, Duan L, Chen J, Xiong A, Xu Q, Zhang H, et al. Gene silencing of NALP3 protects against liver ischemia-reperfusion injury in mice. *Hum Gene Ther* 2011;22:853–864.
- [107] Appenrodt B, Grunhage F, Gentemann MG, Thyssen L, Sauerbruch T, Lammert F. Nucleotide-binding oligomerization domain containing 2 (NOD2) variants are genetic risk factors for death and spontaneous bacterial peritonitis in liver cirrhosis. *Hepatology* 2010;51:1327–1333.
- [108] Bruns T, Peter J, Reuken PA, Grabe DH, Schuldes SR, Brenmoehl J, et al. NOD2 gene variants are a risk factor for culture-positive spontaneous bacterial peritonitis and monomicrobial bacterascites in cirrhosis. *Liver Int* 2012;32:223–230.
- [109] Reiberger T, Ferlitsch A, Payer BA, Mandorfer M, Heinisch BB, Hayden H, et al. Non-selective betablocker therapy decreases intestinal permeability and serum levels of LBP and IL-6 in patients with cirrhosis. *J Hepatol* 2013;58:911–921.
- [110] Nischalke HD, Berger C, Aldenhoff K, Thyssen L, Gentemann M, Grunhage F, et al. Toll-like receptor (TLR) 2 promoter and intron 2 polymorphisms are associated with increased risk for spontaneous bacterial peritonitis in liver cirrhosis. *J Hepatol* 2011;55:1010.
- [111] Bruns T, Reuken PA, Fischer J, Berg T, Stallmach A. Further evidence for the relevance of TLR2 gene variants in spontaneous bacterial peritonitis. *J Hepatol* 2012;56:1207–1208, [author reply 1208–1209].
- [112] Biswas SK, Lopez-Collazo E. Endotoxin tolerance: new mechanisms, molecules and clinical significance. *Trends Immunol* 2009;30:475–487.
- [113] Coant N, Simon-Rudler M, Gustot T, Fasseu M, Gandoura S, Ragot K, et al. Glycogen synthase kinase 3 involvement in the excessive proinflammatory response to LPS in patients with decompensated cirrhosis. *J Hepatol* 2011;55:784–793.
- [114] Galbois A, Thabut D, Tazi KA, Rudler M, Mohammadi MS, Bonnefont-Rousselot D, et al. Ex vivo effects of high-density lipoprotein exposure on the lipopolysaccharide-induced inflammatory response in patients with severe cirrhosis. *Hepatology* 2009;49:175–184.
- [115] Fernandez J, Ruiz del Arbol L, Gomez C, Durandez R, Serradilla R, Guarner C, et al. Norfloxacin vs ceftriaxone in the prophylaxis of infections in patients with advanced cirrhosis and hemorrhage. *Gastroenterology* 2006;131:1049–1056, [quiz 1285].

Review

- [116] Wasmuth HE, Kunz D, Yagmur E, Timmer-Stranghoner A, Vidacek D, Siewert E, et al. Patients with acute on chronic liver failure display "sepsis-like" immune paralysis. *J Hepatol* 2005;42:195–201.
- [117] Berres ML, Schnyder B, Yagmur E, Inglis B, Stanzel S, Tischendorf JJ, et al. Longitudinal monocyte human leukocyte antigen-DR expression is a prognostic marker in critically ill patients with decompensated liver cirrhosis. *Liver Int* 2009;29:536–543.
- [118] Berry PA, Antoniadis CG, Carey I, McPhail MJ, Hussain MJ, Davies ET, et al. Severity of the compensatory anti-inflammatory response determined by monocyte HLA-DR expression may assist outcome prediction in cirrhosis. *Intensive Care Med* 2011;37:453–460.
- [119] Xing T, Li L, Cao H, Huang J. Altered immune function of monocytes in different stages of patients with acute on chronic liver failure. *Clin Exp Immunol* 2007;47:184–188.
- [120] Arvaniti V, D'Amico G, Fede G, Manousou P, Tsochatzis E, Pleguezuelo M, et al. Infections in patients with cirrhosis increase mortality four-fold and should be used in determining prognosis. *Gastroenterology* 2010;139, 1246–1256, 1256 e1241–1245.
- [121] Moreau R, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, et al. CANONIC Study Investigators of the EASL–CLIF Consortium. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology* 2013;144, 1426–1437, 1437 e1421–1429.
- [122] Albillos A, de-la-Hera A, Alvarez-Mon M. Serum lipopolysaccharide-binding protein prediction of severe bacterial infection in cirrhotic patients with ascites. *Lancet* 2004;363:1608–1610.
- [123] Papp M, Sipeki N, Vitalis Z, Tornai T, Altorjay I, Tornai I, et al. High prevalence of IgA class anti-neutrophil cytoplasmic antibodies (ANCA) is associated with increased risk of bacterial infection in patients with cirrhosis. *J Hepatol* 2013;59:457–466.
- [124] McCashland TM, Preheim LC, Gentry MJ. Pneumococcal vaccine response in cirrhosis and liver transplantation. *J Infect Dis* 2000;181:757–760.
- [125] Iwakiri Y, Groszmann RJ. The hyperdynamic circulation of chronic liver diseases: from the patient to the molecule. *Hepatology* 2006;43:S121–131.
- [126] Bellot P, Garcia-Pagan JC, Frances R, Abalades JG, Navasa M, Perez-Mateo M, et al. Bacterial DNA translocation is associated with systemic circulatory abnormalities and intrahepatic endothelial dysfunction in patients with cirrhosis. *Hepatology* 2010;52:2044–2052.
- [127] Rasaratnam B, Kaye D, Jennings G, Dudley F, Chin-Dusting J. The effect of selective intestinal decontamination on the hyperdynamic circulatory state in cirrhosis. A randomized trial. *Ann Intern Med* 2003;139:186–193.
- [128] Wright G, Davies NA, Shawcross DL, Hodges SJ, Zwingmann C, Brooks HF, et al. Endotoxemia produces coma and brain swelling in bile duct ligated rats. *Hepatology* 2007;45:1517–1526.
- [129] Jover R, Rodrigo R, Felipe V, Insausti R, Saez-Valero J, Garcia-Ayllon MS, et al. Brain edema and inflammatory activation in bile duct ligated rats with diet-induced hyperammonemia: a model of hepatic encephalopathy in cirrhosis. *Hepatology* 2006;43:1257–1266.
- [130] Kerfoot SM, D'Mello C, Nguyen H, Ajuebor MN, Kubes P, Le T, et al. TNF-alpha-secreting monocytes are recruited into the brain of cholestatic mice. *Hepatology* 2006;43:154–162.
- [131] Kalakeche R, Hato T, Rhodes G, Dunn KW, El-Achkar TM, Plotkin Z, et al. Endotoxin uptake by S1 proximal tubular segment causes oxidative stress in the downstream S2 segment. *J Am Soc Nephrol* 2011;22:1505–1516.
- [132] Murugan R, Karajala-Subramanyam V, Lee M, Yende S, Kong L, Carter M, et al. Acute kidney injury in non-severe pneumonia is associated with an increased immune response and lower survival. *Kidney Int* 2010;77: 527–535.
- [133] Gomez H, Ince C, De Backer D, Pickkers P, Payen D, Hotchkiss J, et al. A unified theory of sepsis-induced acute kidney injury: inflammation, microcirculatory dysfunction, bioenergetics, and the tubular cell adaptation to injury. *Shock* 2014;41:3–11.