Cirrhosis-associated immune dysfunction

Agustín Albillos*[1,2,3], Rosa Martin-Mateos[1,2,3], Schalk Van der Merwe[4,5], Reiner Wiest[6], Rajiv Jalan[7] and Melchor Álvarez-Mon[2,3,8]
[1]Department of Gastroenterology and Hepatology, Hospital Universitario Ramón y Cajal, Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain
[2]Departamento de Medicina y Especialidades Médicas, Universidad de Alcalá, Alcalá de Henares, Madrid, Spain
[3]Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD), Instituto de Salud Carlos III, Madrid, Spain.
[4]Laboratory of Hepatology, Department of Chronic Diseases, Metabolism and Aging (CHROMETA), University of Leuven, Leuven, Belgium
[5]Department of Gastroenterology and Hepatology, University Hospital Gasthuisberg, Leuven, Belgium.
[6]Department of Visceral Surgery and Medicine, University Inselspital, Bern, Switzerland.
[7]Liver Failure Group, UCL Institute for Liver and Digestive Health, UCL Medical School, Royal Free Hospital, London, UK.
[8]Department of Internal Medicine, Hospital Universitario Príncipe de Asturias, Alcalá de Henares, Madrid, Spain.

*email: agustin.albillos@uah.es

In this Review, Albillos and colleagues describe cirrhosis-associated immune dysfunction (CAID) and its components, systemic inflammation and immune deficiency, as well as the role of CAID in the pathogenesis of acute-on-chronic liver failure. Therapies that aim to modulate CAID are discussed.

Abstract

The term cirrhosis-associated immune dysfunction (CAID) comprises the distinctive spectrum of immune alterations associated with the course of end-stage liver disease. Systemic inflammation and immune deficiency are the key components of CAID. Their severity is highly dynamic and progressive, paralleling cirrhosis progression. CAID involves two different immune phenotypes: The low-grade systemic inflammatory phenotype can be found in patients with compensated disease or clinical decompensation with no organ failure. In this phenotype, there is an exaggerated immune activation, but the effector response is not markedly compromised. The high-grade systemic inflammatory phenotype is present in patients with acute-on-chronic liver failure, a clinical situation characterized by decompensation, organ failure and high short-term mortality. Along with high-grade inflammation, this CAID phenotype includes intense immune paralysis that critically increases the risk of infections and worsens prognosis. The intensity of CAID has important consequences on cirrhosis progression and correlates with the severity of liver insufficiency, bacterial translocation and organ failure. Therapies targeting the modulation of the dysfunctional immune response are currently being evaluated in preclinical and clinical studies

Key points

- Systemic inflammation and immune deficiency are the key components of cirrhosis-associated immune dysfunction (CAID), and their intensity varies according to the stage of cirrhosis and the presence of incidental events.
- The low-grade systemic inflammatory phenotype is present in patients with cirrhosis with no organ failure and contributes to worsening systemic circulatory dysfunction and precipitating complications and acute decompensation.
- The high-grade systemic inflammatory phenotype is the pathogenic driver of organ failure in acute-on-chronic liver failure. A key component of this phenotype is an intense immune paralysis that critically
- A crucial component of the high-grade systemic inflammatory phenotype is an intense functional paralysis of immune system cells that critically increases the risk of infections.
- An abnormal gut-liver axis, causing intestinal dysbiosis, disrupted intestinal barrier and increased bacterial translocation, has a leading pathogenic role in systemic inflammation.
- Treatment of CAID should involve strategies to modulate, rather than inhibiting, the immune response, as abrogation or stimulation of the inflammatory response could increase the infection risk or worsen immunopathology, respectively

[H1] Introduction

Cirrhosis is a progressive and dynamic liver disease that comprises two consecutive stages: an often-asymptomatic phase known as compensated disease, and a decompensated stage characterized by complications arising from portal hypertension and hepatic insufficiency. Cirrhosis is considered to be a systemic disease as it affects most organs and systems of the body, including the immune system. The term cirrhosis-associated immune dysfunction (CAID) refers to the wide spectrum of immune alterations present in cirrhosis[1,2]. CAID is characterized by two key components — systemic inflammation and immune deficiency — which show a variable intensity depending on cirrhosis stage and the presence of incidental events, such as bacterial infections [2]. The most severe immune alterations are found in patients with acute-on-chronic liver failure (ACLF), a syndrome characterized by acute decompensation of cirrhosis, hepatic and/or extrahepatic organ failures and high short-term mortality[3]. Patients with ACLF have the highest grade of systemic inflammation and severe immunodeficiency, which contributes not only to an increased risk of infections but also to organ failure pathogenesis[4].

According to the intensity of CAID, two different immune phenotypes can be characterized: a low-grade systemic inflammatory phenotype present in compensated and decompensated patients with no organ failure, and a high-grade systemic inflammatory phenotype present in patients with ACLF and characterized by severe systemic inflammation and immunodeficiency. These phenotypes represent both extremes of a continuous spectrum of immune alterations that are, however, potentially reversible, depending on the clinical situation. The low-grade systemic inflammatory phenotype includes an increased expression of surface activation antigens in circulating immune cells, and production of pro-inflammatory cytokines [5–8] . When cirrhosis progresses to a decompensated state, CAID is more pronounced and the effector immune response to the persistent bacterial challenge becomes increasingly impaired[9,10]. Finally, in ACLF, the high-grade systemic inflammation, along with the excessive compensatory anti-inflammatory response and the exhaustion and dysregulation of the immune effector cells, critically affects prognosis, leading to multi-organ failure and high short-term mortality[3,11–13].

The gut-liver axis has a key role in the pathogenesis of CAID. The intestinal epithelial and vascular barriers, the gut microbiota, the liver and the immune system establish complex interactions to both maintain tolerance to harmless stimuli while at the same time orchestrating an effective response against bacterial pathogens. However, cirrhosis is accompanied by detrimental changes to the gut, including dysbiosis and increased intestinal permeability[14,15]. These alterations promote bacterial translocation from the intestinal lumen to the portal and systemic circulation, which markedly contributes to the systemic inflammation[14,16] .

This Review summarizes the current knowledge about the immune alterations associated with cirrhosis at its different stages. We describe the contribution of the liver to the immune response, and its predominant role in innate immunity. Thereafter, we address in detail the distinctive abnormalities of the two components of CAID — systemic inflammation and immunodeficiency — and the pathogenic mechanisms of each one. The description of systemic inflammation also covers the stages of cirrhosis associated with the phenotypes of low-grade and high-grade inflammation. We explain in detail the contribution of CAID to

the pathogenesis of ACLF, in particular the roles of high-grade systemic inflammation in organ failure and that of immunodeficiency in the increased susceptibility to bacterial infections. This Review also describes abnormalities of the gut–liver axis, including failure of the intestinal barrier, and their contribution to the immunological disturbance of cirrhosis. Finally, we address the targets that are amenable to modulation to improve the dysfunctional immune response in cirrhosis.

[H1] Liver and immune system in health

The liver is strategically placed, receiving a dual blood supply through the portal vein and the hepatic artery. This blood supply provides a broad spectrum of systemic and gut-derived antigens that are continuously screened and classified as harmless dietary or commensal elements or as pathogenic bacteria and microbially-derived products. Depending on the pathogenic potential of the antigens, the liver must remain tolerant or activate an effective response, precisely balancing tolerance and immune activation.

[H2] Immune role of liver-resident cells

A number of specialized innate and adaptive immune cells are able to detect, present and clear pathogens arriving to the liver. Tissue distribution, gene expression and functional heterogeneity of liver-resident immune cells have been characterized by single-cell RNA sequencing[17]. Within the liver, antigen-presenting cells (APCs) (such as Kupffer cells, dendritic cells and liver sinusoidal endothelial cells), T cells, B cells, natural killer (NK) cells and monocytes have been identified. These populations control the local inflammatory response, thereby preventing the spread of inflammatory signals beyond the liver and, consequently, systemic inflammation. In addition, parenchymal cells such as hepatocytes have surveillance roles, acting as APCs by expressing MHC-I and MHC-II and costimulatory molecules[18]. They also produce critical immune components, such as acute-phase proteins (hepcidin, fibrinogen and proteinase inhibitors), complement proteins and soluble pattern recognition receptors (PRRs), that are involved in adaptive and innate immune responses[19] (**Figure 1**).

Despite continuous challenge with pathogen-associated molecular patterns (PAMPs), in basal conditions the balance between adaptive immunity and tolerance is regulated by a carefully controlled network of liver-resident APCs and the unique hepatic microenvironment, which has been reviewed elsewhere[20–22]. In sterile injury, release of damage-associated molecular patterns (DAMPs) from necrotic cells causes robust recruitment of innate immune cells. The subsequent release of toxic mediators from immune cells is thought to be damaging in non-resolving sterile injuries in which the dysregulated immune response leads to chronic inflammatory disease. In the case of bloodborne infection, the numerous innate and adaptive immune cells that specialize in detection and capture of pathogens from the blood participate in coordinated immune responses leading to pathogen clearance, leukocyte recruitment and antigen presentation to lymphocytes within the vasculature. The critical balance between immune activation and tolerance is disrupted in cirrhosis, correlating with the dysregulation and exhaustion of immune cells and the impaired synthesis of key immune proteins.

[H2] The gut-liver axis and gut immune system

The term gut-liver axis has been coined to highlight the close functional relationship between the intestine (particularly its microbiota and specific immune system) and the liver[14,15]. Anatomically, the portal vein transports gut-derived antigens towards the liver, which in turn provides bile acids, lipids and antibodies through the bile back to the intestine. This bidirectional communication is profoundly altered in cirrhosis due to damage to the epithelial, vascular and immune intestinal barriers at different levels[14,15,23].

The gut-associated lymphoid tissue (GALT) represents the largest immunological organ in the human body and constitutes the first defense against gut-derived antigens and pathogens[24] . GALT is composed of Peyer's patches, intestinal lymphoid follicles, intraepithelial lymphocytes, and mesenteric lymph nodes (MLN). In addition, colon patches and isolated lymphoid follicles in the large intestine have a role in intestinal immunity[24]. Peyer´s patches and MLN act as inducers of immunity and tolerance, whereas the effector sites are distributed along the lamina propria and mucosal epithelium[25,26].

An array of innate and adaptive immune cells orchestrates the intestinal response to luminal agents reaching and adhering to the epithelium. In particular, intestinal macrophages are effective in killing penetrated bacteria, whereas intestinal dendritic cells transport them alive to MLN for antigen presentation and modulation of adaptive T cell response (that is, induction of tolerance or priming)[27]. The GALT harbors nearly 95% of $CD4^+$ T cells[28]. Intraepithelial lymphocytes, in particular the γδ subpopulation, maintain a close relationship with the intestinal epithelial cells, acting as essential mediators balancing the host-microbial homeostasis[29]. Finally, intestinal B cells mediate protection against lethal spreading of commensal bacteria from the colon when epithelial integrity is disrupted[30]. Indeed, genetically modified mice with dysfunctional B cells (MYD88-depleted) present with an approximately 100 times higher culturable bacterial load in the liver in models of induced colitis without MYD88 depletion [31].

[H1] Systemic inflammation in cirrhosis

Inflammation is a physiological response initially designed to restore homeostasis following different insults such as bacterial infections or tissue injury [32]. Inflammation can be triggered by recognition of distinctive molecules derived from bacteria (for example, lipopolysaccharides (LPS)), known as PAMPs. In the absence of infection, systemic inflammation could also be activated by intracellular molecules released from injured or dying cells, known as DAMPs[33]. DAMPs, such as high-mobility group box 1 protein (HMGB1), are located inside cells, which prevents their recognition by the host immune system, thereby avoiding pathological inflammation and autoimmunity[33]. PAMPs and DAMPs bind to specific PRRs located on peripheral innate immune cells[34]. Receptor engagement activates downstream signaling pathways, leading to increased transcription and release of inflammatory cytokines and activation and recruitment of immune effector cells.

[H2] Evidence of systemic inflammation

Low-grade systemic inflammation is present in experimental models and patients with compensated and decompensated cirrhosis with no ACLF (such as patients with ascites) [2,5,35–37]. Systemic inflammation is evidenced by the increased plasma concentration of acute-phase proteins (such as C-reactive protein and LPS binding protein)[5], endothelial activation markers (such as intercellular adhesion molecule 1 (ICAM1), vascular cell

adhesion protein 1 (VCAM1), vascular endothelial growth factor (VEGF), von Willebrand factor (vWF), P-selectin, nitrates or nitrites)[38–40], cytokines (such as tumour necrosis factor (TNF), IL-1β, IL-6, interferon-γ (IFNγ) and IL-17)[6], and their soluble receptors (such as TNF soluble receptors I and II)[5,41] In addition, activation of circulating immune cells is supported by the increased respiratory burst in neutrophils of patients with cirrhosis[9], and the enhanced expression of CD11b receptor, which mediates neutrophil adhesion to the endothelium and is also involved in cytotoxicity and neutrophil-mediated tissue injury [42]. Systemic inflammation in cirrhosis is also supported by an increased expression of HLA-DR and co-stimulatory molecules in monocytes and B cells [7,43]. Further, there is a Th1 (pro-inflammatory) polarization of T cells, which produce increased levels of IFNγ[37,44] **(Table 1)**.

The dynamic nature of CAID is such that the intensity of systemic inflammation increases as cirrhosis progresses from the compensated to the decompensated stage[5,6,35]. In fact, it has been shown that inflammatory markers are only slightly increased in patients with compensated cirrhosis, different inflammatory grades and profiles are observed in acute decompensation with no ACLF, and certain cytokines are significantly increased in those with ACLF[45]. Mediators of adhesion and migration of leukocytes are particularly upregulated in patients with ACLF, and, interestingly, some of these markers (VCAM1, ICAM1 and granulocyte-macrophage colony-stimulating factor (GM-CSF)) positively correlate with 3-months mortality in these patients [46].

The extreme exacerbation of systemic inflammation in ACLF is evidenced by the increased levels of pro-inflammatory (that is, TNF, IL-6, IL-8) and anti-inflammatory (that is, IL-10, IL-1RA) cytokines[11], soluble markers of macrophage activation (sCD163 and mannose receptor)[47], C-reactive protein, and white blood cells in plasma of patients with ACLF [3]. The magnitude of this 'cytokine storm' defines and differentiates the high-grade and low-grade phenotypes. Interestingly, cytokines and immune cells of the innate response are predominantly involved, suggesting a greater contribution of the innate than the adaptive response to systemic inflammation in patients with ACLF[11]. Further, different cytokine profiles have been observed for different precipitating events. For instance, in patients with alcohol-induced ACLF, IL-8 is markedly augmented, whereas TNF, IL-6 and IL-1RA are predominantly increased in response to bacterial infections [11]. These patterns might indicate that specific signaling pathways are involved depending on the aetiology, which would have implications for the search for therapeutic targets and prognostic markers.

[H2] Mechanisms of systemic inflammation

Once in the circulation or in the liver, PAMPs and DAMPs are recognized by PRRs such as Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain-like receptors (NLRs), leading to the activation of pro-inflammatory signaling pathways[48]. Production of inflammatory cytokines requires the full activation of a cytosolic multiprotein complex known as the inflammasome, which can be accomplished via canonical and non-canonical pathways. Following recognition of PAMPs and DAMPs, canonical activation of inflammasomes requires the transcriptional upregulation of pro-inflammatory cytokines and inflammasome components, and assembly of the inflammasome, cleavage of caspase-1, and activation and secretion of cytokines[33] . This process leads to the conversion of pro-inflammatory cytokines IL1ß and IL-18 into their active forms and their

secretion into the extracellular compartment. In addition, human monocytes also display a non-canonical one-step pathway of inflammasome activation in response to LPS. Intracellular recognition of LPS is mediated by caspases 4 and 5 (caspase 11 in mice) and triggers a type of programmed pro-inflammatory cell death known as pyroptosis[44,45]. Further, IL1ß and IL1α are released upon non-canonical inflammasome activation[49,50]. Interestingly, it has been shown that IL-1α is an independent predictor of death in patients with ACLF and no previous clinical decompensations, whereas IL-1β is an independent predictor of death in those with previous episodes of ascites[51].

Depending on the CAID phenotype, PAMPs and DAMPs from different sources and in variable magnitudes lead to activation of the inflammasome. The low-grade systemic inflammation in compensated and decompensated cirrhosis with no ACLF is driven by translocation of bacteria and PAMPs, progressive loss of tolerance leading to an excessive pro-inflammatory response upon antigen recognition, and probably also to increased release of DAMPs from injured hepatocytes (**Figure 2**). PAMPs also have the driving role in the pathogenesis of the high-grade systemic inflammation of cirrhosis with organ failure, also known as ACLF. Additionally, in this setting there is more robust evidence of mechanisms that depend on the precipitating event that leads to DAMP release, and contribute to the high-grade inflammation, as explained below

[H2] Low-grade systemic inflammation

[H3] **Translocation of bacteria and PAMPs**. Intestinal permeability is increased in patients with cirrhosis due to functional and anatomical alterations that affect the mucus lining, intercellular junction proteins and endothelial cells [6,52]. Further, bacterial overgrowth and dysbiosis contribute to the migration of an increasing number of bacterial species and microbial-derived PAMPs from the intestinal lumen to the systemic circulation or the liver, following the lymphatic and portal vein routes, respectively[14,15].

Although LPS is considered the prototypical PAMP, other molecules such as lipoteichoic acid from gram-positive bacteria, virus-derived double-stranded or single-stranded RNA, and bacterial-associated unmethylated CpG motifs, among others, can also bind to and activate PRRs[53]. In addition, the gut mycobiota might also be involved in the pathogenesis of inflammation in cirrhosis. Increased translocation of fungal β-glucan promotes inflammation via activation of the C-type lectin-like receptor CLEC7A on Kupffer cells. This induces IL-1β synthesis and secretion, which, interestingly, can be reversed with antifungal agents in mice with ethanol-induced steatohepatitis[48]. Moreover, patients with alcohol-induced cirrhosis have fungal dysbiosis, characterized by reduced diversity and by *Candida* overgrowth, and increased immune response to mycobiota, which correlate with mortality[54].

[H3] **Progressive loss of tolerance leading to an excessive pro-inflammatory response upon antigen recognition**. Under physiological conditions, the 'pro-inflammatory' hepatic environment (due to a persistent exposure to low levels of gut-derived antigens) is attenuated by mechanisms of tolerance that aim to dampen the ability of APCs to activate lymphocytes[55–57]. However, there is a progressive impairment of tolerance to antigen recognition in cirrhosis that leads to an augmented pro-inflammatory response and contributes to chronic inflammation. First, livers of cirrhotic rats are

abnormally sensitive to LPS-induced apoptosis due to an altered unfolded protein response that favors a lack of nuclear factor-κB (NF-κB)-dependent antiapoptotic proteins [58]. Second, monocytes of patients with cirrhosis show hyperproduction of TNF upon LPS stimulation due to decreased levels of IL-1R-associated kinase (an inducible suppressor of TNF)[59]. Further, defective production of the anti-inflammatory cytokine IL-10 by Kupffer cells has been observed in rats with CCl_4-induced liver fibrosis [60]. Finally, monocytes of patients with advanced cirrhosis show a defective phosphorylation of glycogen synthase kinase 3ß (which in normal circumstances would contribute to decrease pro-inflammatory cytokines and increase IL-10), resulting in unrestricted production of pro-inflammatory cytokines upon LPS stimulation [61].

[H3] **Increased release of DAMPs from injured hepatocytes**. Chronic liver diseases of many aetiologies (alcohol, viral, metabolic, etc.) progressively induce hepatocyte injury and death. The subsequent release of intracellular DAMPs has been proposed to contribute to sterile inflammation[33]. However, the potential contribution of DAMPs to systemic inflammation in cirrhosis without organ failure remains rather speculative.

Alcohol consumption promotes inflammation and has a deleterious effect on immune cells, which has been extensively reviewed elsewhere[62–64]. Alcohol alters both the innate and adaptive responses, leading to inflammation not only in the liver but also in other organs such as the gut, brain or the lungs, which suggests that common pathways underlie its proinflammatory effects. Other aetiologies of cirrhosis such as metabolic-associated fatty liver disease (MAFLD; also known as nonalcoholic fatty liver disease (NAFLD) also display specific pro-inflammatory sterile pathways[65]. In particular, dietary factors (such as fructose, saturated fat, trans fat or cholesterol) have been proven to trigger inflammation by lipotoxic effects, mitochondrial dysfunction, oxidative and endoplasmic reticulum stress and sterile cell death mechanisms in mice with high-fat and/or high-fructose-induced hepatic steatosis [66,67]. In addition, increased visceral adipose tissue is characterized by the infiltration of pro-inflammatory macrophages, which induce release of chemokines and cytokines that contribute to hepatic inflammation and insulin resistance in mice fed a high-fat and high-cholesterol diet [68].

[H2] High-grade systemic inflammation

[H3] **Bacterial infection-induced ACLF**. Bacterial infections are the most frequent identifiable trigger of ACLF[3]. LPS is characteristically recognized by TLR4[69] and, following receptor engagement, two different signaling pathways can be activated. The MYD88-dependent pathway involves nuclear translocation of the transcription factor NF-κB, which induces the synthesis and release of pro-inflammatory cytokines (such as TNF, IL-6 and pro-IL-1β). The MYD88-independent pathway, on the other hand, involves TRIF-mediated signaling in response to LPS, which occurs at the endosomal membrane after internalization of the TLR4. This results in the production of IFNβ and IP-10 (also known as CXCL10) via interferon regulatory factor 3 (IRF3) in liver parenchymal cells[70].

[H3] **Alcohol-induced ACLF**. Sterile and non-sterile mechanisms induce systemic inflammation in patients with alcohol-related liver disease and ACLF. On one hand, chronic alcohol consumption disrupts the intestinal barrier contributing to dysbiosis[71,72], impairing the tight junctions, reducing the mucus layer and decreasing the production of

antimicrobial peptides[73]. The increased bacterial translocation, which is exacerbated in the setting of ACLF, activates the pathogen-induced pro-inflammatory pathways. On the other hand, sterile mechanisms triggered by DAMPS released from apoptotic hepatocytes are also involved in the pathogenesis of alcohol induced liver injury. Specifically, ethanol contributes to mitochondrial cytochrome c release, which increases the expression of Fas ligand thus promoting hepatocyte apoptosis[74]. In addition, other experimental evidences suggest that cytochrome P450 2E1 contributes to the oxidative stress and macrovesicular fat accumulation induced by alcohol[75]. [3]. It is likely that the resultant hepatocyte injury and cell death results in the release of intracellular components (DAMPs), such as mitochondrial DNA, ATP and HMBG1, that further activate liver and circulating immune cells , further driving liver damage and systemic inflammation in ACLF. Caspase-cleaved keratin 18 and keratin 18 serum levels, reflecting apoptotic and mainly non-apoptotic cell death, respectively, are increased in patients with alcoholic hepatitis-related ACLF and their values correlate with markers of systemic inflammation, such as IL-6 and IL-8, hepatic failure and severity of ACLF, but not with markers of extrahepatic organ injury [76]. Similar findings have been reported in the context of acute deterioration of liver function in patients with chronic hepatitis B infection[77]. Intracellular components released by necrotic hepatocytes (such as HMGB1, cyclophilin A, gp60) contribute to exacerbating hepatic inflammation in experimental models of liver failure [78,79]. The clinical and experimental evidence supporting the contribution of liver-derived DAMPs to systemic inflammation in alcohol-induced ACLF is weaker than that for the driving-role of gut-derived PAMPs.

[H2] Consequences of systemic inflammation

Low-grade systemic inflammation influences the clinical expression of cirrhosis by modifying the function of somatic cells via the direct effects of proinflammatory cytokines and/or the recruitment of circulating activated immune cells by the peripheral tissues **(Figure 3).** Pro-inflammatory cytokines further stimulate the endothelial production of nitric oxide and ROS, worsening peripheral vasodilation, portal hypertension and cardiac dysfunction[5,80–84]. The intensity of splanchnic and peripheral vasodilation is, therefore, greater in patients and in experimental models of cirrhosis with bacterial translocation and severe systemic inflammation[5,81,83,84]. The magnitude of systolic dysfunction relates not only to the severity of systemic circulatory dysfunction but also to markers of systemic inflammation and bacterial translocation[80,82]. Indeed, sCD163, a marker of macrophage activation, has been shown to correlate with the risk of variceal bleeding and overall survival in patients with cirrhosis[85,86].

The influence of systemic inflammation on expression of cirrhosis is well-exemplified by acute kidney injury, including classical hepatorenal syndrome, which is driven by peripheral vasodilation and cardiac dysfunction aggravated by low-grade systemic inflammation[87] (**Figure 4**). Preliminary evidence suggests that inflammation-related tubular injury and apoptosis could contribute to renal dysfunction, as shown by increased renal expression and urinary excretion of TLR4 in patients with acute decompensation of cirrhosis[88]. Peripheral inflammation can modify brain signaling in patients with cirrhosis and contributes to regulate the onset and severity of hepatic encephalopathy[89,90] . Similar to other organs, pro-inflammatory cytokines can activate cerebral endothelial cells. Circulating immune cells can adhere to the activated endothelium and be recruited by brain parenchyma. This leads in turn to activation of resident immune cells (such as microglia and astrocytes) that produce mediators (such as cytokines) that alter

neurotransmission and behavior[91,92] . Inflammation in this setting is also driven by a dysbiotic microbiota, with a study showing a correlation between inflammatory cytokines in serum and certain microbial families (Enterobacteriacae, Veillonellaceae and Fusobacteriaceae) in faeces of patients with cirrhosis and encephalopathy [93]. The relationship between the gut microbiota, neuroinflammation and encephalopathy in cirrhosis is further supported by the fact that fecal microbiota transplantation ameliorates GABAergic, microglial and neuronal activation and inflammation in mice with cirrhosis [94].

In addition to modifying the clinical expression of cirrhosis, systemic inflammation, along with portal hypertension, drives the clinical course of decompensated cirrhosis, as elegantly shown in a study that analysed the course of patients with acute decompensation of cirrhosis [8]. In this study, patients with a stable course show low-grade inflammation that subsequently improves, whereas those with unstable course that required readmission had low-grade inflammation but severe portal hypertension, with a mortality at 1 year of 9.1% and 35.6%, respectively. In contrast, patients with pre-ACLF had the highest degree of inflammation at admission, which aggravated on follow-up, and had a mortality rate at 1 year of 67.4%. These data underscore the pathogenic role of systemic inflammation in driving organ failure in acutely decompensated cirrhosis and, therefore, the transition to ACLF.

Finally, chronic inflammation has also been suggested to be involved in the pathogenesis of hepatocellular carcinoma[95]. In particular, increased IL-6 levels and overactivated STAT3 have been observed in patients with hepatocellular carcinoma and in preclinical models[36,96]. TNF and its downstream targets, NF-κB and c-Jun N-terminal kinase, also promote hepatocellular carcinoma through inflammation, hepatocyte death and compensatory proliferation in mouse models [97,98]. Blocking tumour-promoting inflammatory signals and enhancing tumour immunity through immune checkpoint inhibitors could be an effective strategy for treating hepatocellular carcinoma[95].

High-grade systemic inflammation is the driver of disease in patients with ACLF, in whom there is a close correlation between the magnitude of inflammation and organ failure and survival[45,99]. This clinical correlation underlines the critical contribution of high-grade systemic inflammation to the pathogenesis of organ failure in ACLF. In fact, organ failure in this setting is not only the result of the haemodynamic derangement of cirrhosis, but mainly of the massive release of cytokines, activation of cell death mechanisms[100], exacerbation of oxidative stress, and high recruitment and activation of immune effector cells[101]. It has been hypothesized that high-grade systemic inflammation leads to organ failure by causing direct damage to tissues (immunopathology) or as a result of an energetic imbalance (immunometabolism).

The term ‘immunopathology’ has been coined to describe the collateral effects of the immune response in other cells and tissues[102]. The detrimental consequences of an exaggerated activation of effector cells is central to the pathogenesis of organ failure in critical conditions, such as sepsis: systemic inflammation promotes ischemic acute renal tubular necrosis, prompted by capillary leucocyte infiltration, microthrombosis, apoptosis, and mitochondrial injury, which substantially increases the risk of acute kidney failure [103,104]. In line with these findings, the greater the severity of ACLF the lower the therapeutic response to terlipressin and albumin in patients with hepato-renal syndrome–acute kidney

injury, which indicates an inflammatory rather than a haemodynamic pathogenesis of kidney injury in ACLF [105] (**Figure 4**). Inflammation also drives cerebral hypoperfusion and dysfunction, contributing to the development of hepatic encephalopathy in animal models and patients with advanced cirrhosis and ACLF [106,107].

On the other hand, the term 'immunometabolism' refers to the energetic imbalance that a highly metabolically active immune system provokes in cells of other tissues, contributing to organ dysfunction. An effective immune response is metabolically costly, as it requires the synthesis of many new proteins, including cytokines, chemokines and acute phase reactants[108]. Each population of immune cells has a distinct metabolism and nutrient usage. For example, metallothionein 3 has been suggested to control the phenotype and metabolic programming of macrophages by promoting mitochondrial respiration, suppressing glycolysis and promoting oxidative phosphorylation[109]. Consistent with these observations, metabolomic studies in the blood of patients with ACLF have shown that this condition is associated with a distinctive fingerprint of 38 metabolites, with an intensity that correlates with the severity of systemic inflammation[110]. The fingerprint represents increases in glycolysis and related pathways, indicating the presence of a marked inhibition of mitochondrial energy production (ATP-producing fatty acid ß-oxidation), which might contribute to the organ failure in ACLF[110]. Further, systemic inflammation induces changes in other bioactive molecules, such as tryptophan, which is degraded through the kynurenine pathway[111]. In fact, patients with ACLF have an increased serum concentration of metabolites of that route, which correlates with the intensity of systemic inflammation[112].

[H1] Immunodeficiency and cirrhosis

Although a standard definition of immunodeficiency is lacking, it can be arbitrarily identified as the presence of abnormalities of immune system cells that compromise their effector function and result in immune paralysis. These alterations include functional defects, expansion of immune inhibitory subsets or reduced expression of costimulatory molecules[12]. Immunodeficiency is also a dynamic feature of CAID that begins in compensated cirrhosis, increases with cirrhosis progression through the decompensated stage and peaks in ACLF[4] . Immunodeficiency in cirrhosis is the consequence of two main factors: the structural distortion of the liver parenchyma, which compromises its surveillance role, and the functional impairment of circulating immune cells.

[H2] Liver structural distortion in cirrhosis

The progression of cirrhosis alters the architecture of the liver, replacing parenchymal and non-parenchymal cells with scar tissue. Deposition of extracellular matrix and capillarization of sinusoids hinders the surveillance role of resident APCs. Activity of the mononuclear phagocyte system (previously known as the reticulo-endothelial system) is decreased in patients with cirrhosis, which is associated with a greater risk of bacterial infections and mortality [113,114]. In addition, intrahepatic shunting through vascularized septa prevents gut-derived portal and systemic bacteria to be 'filtered' and cleared by Kupffer cells[115]. Furthermore, hepatocyte loss decreases the synthesis of immune proteins and receptors, specifically the synthesis of complement components, soluble PRRs (LPS binding protein and soluble CD14), albumin, and acute phase proteins (mannose-binding Lectin, C-reactive protein, hepcidin, fibrinogen, and proteinase inhibitors), as demonstrated in different cohorts of patients with cirrhosis [116–118].

[H2] Damage of circulating immune cells

CAID entails not only local (that is, hepatic) but also systemic alterations that affect circulating innate and adaptive immune cells. Damage of circulating immune cells is evidenced by hyperactivation and upregulation of cell activation markers and a dysfunctional effector response that has been characterized in different immune system cell populations (**Table 2**).

[H3] Monocytes. Levels of circulating monocytes are increased in patients with decompensated cirrhosis, in particular the non-classical $CD14^+CD16^+$ proinflammatory and profibrotic subset [7,119]. These monocytes show signs of activation, such as increased human leucocyte antigen (HLA-DR) expression and increased spontaneous TNF production[7,119]. Indeed, circulating monocytes are one of the main sources of increased serum TNF in decompensated cirrhosis[7]. Remarkably, despite being increased in number, circulating monocytes decrease in function during cirrhosis progression . The reported defects in decompensated cirrhosis include altered phagocytosis, chemotaxis, superoxide generation, lysosomal enzyme production[120], and defective Fcγ receptors, which are key for the clearance of IgG-coated microorganisms [121]. In addition, expansion of an AXL-expressing monocyte population ($CD14^+CD16^{high}HLA\text{-}DR^{high}$) has been characterized. AXL^+ cells from patients with cirrhosis display attenuated TNF/IL-6 responses and T cell activation[122].

Monocyte function impairment worsens as cirrhosis progresses to ACLF. Monocytes of patients with ACLF display reduced HLA-DR isotype expression, elevated frequencies of IL-10-producing cells, an inability to produce TNF in response to LPS and impaired phagocytic and oxidative burst capacity [12,123–125]. This phenotype of circulating monocytes relies on altered transcriptional programs and epigenetic modifications that have been characterized in patients with severe alcoholic hepatitis[126]. Monocyte dysfunction represents the most distinctive feature of immune cell paralysis in ACLF, resembling what is observed in sepsis[12]. These abnormalities might be partially restored by metabolic reprograming of the cells using a pharmacological inhibitor of glutamine synthetase in *in vitro* generated- and in patients with ACLF-derived monocytes [13]. Additionally, the reduced response of cultured monocytes to LPS and impaired function of monocytes in ACLF could also be due to the concomitant increase in the levels of monocytes that express MER receptor tyrosine kinase (MERTK) [127]. MERTK is involved in downregulation of innate immune responses aimed at resolving inflammation in endotoxic shock [128]. Activation of MERK inhibits TLR activation and pro-inflammatory cytokine production. The number of MERK-expressing monocytes in ACLF has been found to positively correlate with disease severity and inflammation. Notably, the *ex vivo* addition of an inhibitor of MERTK rescued the production of inflammatory cytokines upon LPS stimulation in monocytes [127]. Finally, expansion of other monocytic cell subsets such as monocyte myeloid-derived suppressor cells also contributes to impair monocyte function in patients with ACLF[129].

[H3] Neutrophils. In patients with decompensated cirrhosis, neutrophils are depleted due to splenic sequestration [10], and show an impaired phagocytic activity and reduced chemotaxis to the site of infection in patients with decompensated cirrhosis[130,131]. It has been suggested that hyperammonemia observed in cirrhosis induces neutrophil swelling, further impairing their phagocytic activity [131,132]. The $p38^{MAPK}$ signaling pathway is activated in response to the osmotic alteration; however, this in turn leads to an increase in the

neutrophil respiratory burst [131,132]. The respiratory burst refers to the massive release of ROS by neutrophils, monocytes or macrophages in order to degrade internalized particles and bacteria. Neutrophil priming and the excessive production of ROS induce tissue damage and fibrosis and contribute to the exhaustion of phagocytic cells in patients with cirrhosis [133,134]. Further mechanisms of neutrophil dysfunction include the formation of neutrophil extracellular traps, which are networks of extracellular fibers composed of DNA, citrullinated histones and antimicrobial peptides capable of directly killing bacteria. *Ex vivo* stimulation of neutrophils from patients with alcohol-related liver disease showed reduced formation of neutrophil extracellular traps compared with healthy individuals. In preclinical models, alcohol also markedly decreases formation of hepatic neutrophil extracellular traps and neutrophil clearance by macrophages[135]. Neutrophil dysfunction in patients with decompensated cirrhosis contributes to their increased susceptibility to bacterial infections[136,137], and, thus, the clinical correlation between neutrophil alterations and survival has been further explored. Specifically, it has been shown that neutrophil phagocytic capacity and the resting oxidative burst might predict the development of infection, organ dysfunction and 90-days survival in decompensated cirrhosis [138].

[H3] Lymphocytes. The lymphocyte compartment is also altered in patients with cirrhosis. Circulating $CD4^+$ T helper cells are depleted due to defective thymopoiesis, excessive splenic pooling and activation of cell death mechanisms by bacterial translocation[139]. Reduced numbers of blood mucosal-associated invariant T (MAIT) cells have been found in patients with alcohol-related liver disease. These cells were hyperactivated and displayed defective antibacterial cytokine and cytotoxic responses[140]. These alterations were exacerbated in patients with severe alcoholic hepatitis, which is in line with the dynamic course of CAID. Moreover, T cell responses in patients with ACLF are attenuated by the expansion of certain monocytic cells such as monocyte myeloid-derived suppressor cells that have reduced potential to activate T cells [129]. Other factors described in patients with decompensated cirrhosis that can contribute to impair T cell responses in this setting include the expansion of a subset of HLA-DR^+CD8^+ T cells with immune-modulatory properties through the expression of high levels of programmed cell death protein 1 (PD-1) and T cell immunoglobulin and mucin domain-containing protein 3 (TIM3)[141–143], and elevated IL-10 levels[144] (**Figure 5**) Moreover, circulating NK cells exhibit reduced cytolytic activity and poor response to cytokine stimulation in patients with alcohol-related cirrhosis [145] . Finally, profound abnormalities in B cells can be found as well. Memory B cells are depleted in the serum of patients with HCV-related cirrhosis , specifically the $CD27^+IgM^+$ subset, which are hyporesponsive upon CD40–TLR9 stimulation, showing an impaired production of cytokines and immunoglobulins and reduced $CD4^+$ T cell allostimulatory capacity [146].

[H2] Mechanisms of immunodeficiency in CAID

Immunodeficiency progresses from the compensated to the stable decompensated cirrhosis stage, culminating in ACLF. In patients with stable decompensated cirrhosis and with acute decompensations, immunodeficiency is exemplified by the previously described alterations in APCs (that is, monocytes and neutrophils), such as reduced phagocytic ability[9] . At the other end of the spectrum, immunodeficiency in ACLF is characterized by decreased HLA-DR expression and impaired TNF production in response to LPS by monocytes, severely impaired phagocytic and oxidative burst capacity by monocytes and

neutrophils, and by increased levels of the regulatory MERKT+ monocyte subset and of monocytes that produce anti-inflammatory cytokines (for example, IL-10)[9] (**Figure 6**).

There are other mechanisms that lead to immunodeficiency in cirrhosis:
[H3] Excessive immunosuppressive response to counteract systemic inflammation. This so-called compensatory anti-inflammatory response syndrome (CARS) was first described in critically ill patients with sepsis and trauma[147], and is characterized by increased lymphocyte apoptosis and anergy[148], decreased cytokine production and HLA expression upon monocyte stimulation[149,150], and upregulation of anti-inflammatory cytokines, such as IL-10[149–151].
The magnitude of this compensatory response at hospital admission, measured by the augmented serum levels of IL-10 or the number of circulating regulatory MERKT+ monocytes has been shown to predict a poor outcome in patients with ACLF[127,152].

[H3] Immune cell exhaustion. Immune cell exhaustion in ACLF is driven by an exacerbated translocation of bacteria and PAMPs from the gut, as well as by the release of DAMPs from injured hepatocytes. In patients with severe alcoholic hepatitis, activation of TLRs induces a pronounced impairment of neutrophil function (phagocytosis and oxidative burst)[10,131], reduced ability of T cells to produce IFNγ, and increased serum levels of immune inhibitory receptors, such as PD-1 and TIM3 [153] . Endotoxin removal or blockade of TLR restores the antimicrobial activity of neutrophils and T cells)[131,153], which is the rationale for the potential therapeutic role for endotoxin removal strategies in alcoholic hepatitis . In preclinical models of cirrhosis, long-term translocation of PAMPs induces the overexpression of type 1 interferons in the liver and IL-10 in myeloid cells, which impairs the bactericidal capacity of liver myeloid cells[154].

[H3] Reprogramming and dysfunction of immune cells by the metabolic abnormalities of cirrhosis.

Dysfunctional and decreased concentration of albumin[155,156] is relevant not only to the haemodynamic derangement but also to the immune cell damage in CAID. In healthy individuals, albumin can bind in serum to pro-inflammatory molecules and also to immunosuppressive mediators such as prostaglandin E2 (PGE2), whose serum concentrations are markedly elevated[156,157] . PGE2 contributes to alveolar macrophages dysfunction by inhibiting NADPH oxidase-mediated bacterial killing[158] and via upregulation of cAMP and inhibition of FcγR-mediated phagocytosis[159] . Human albumin infusion reduces circulating PGE2 levels, and attenuates suppressed macrophage proinflammatory cytokine secretion and bacterial killing in vitro in patients with acutely decompensated cirrhosis or ACLF and in mouse models of cirrhosis[157].

Other metabolic features contributing to immune dysfunction are hyperammonemia and hyponatremia, which act synergistically to induce neutrophil swelling and impair phagocytosis. Interestingly, neutrophil phagocytic dysfunction was abrogated by p38 mitogen-activated protein kinase signaling inhibition in ex vivo studies in neutrophils from rats and patients with cirrhosis [132]. Additionally, it has been suggested that the functional status of monocytes is determined by their underlying metabolic program. In comparison to non-classical human ($CD14^{dim}CD16^{+}$) monocytes, which utilize respiratory chain metabolism, classical monocytes ($CD14^{++}CD16^{+}$) exhibit a more pro-inflammatory

phenotype and depend on carbohydrate metabolism as their energy source[160]. Using cell culture experiments, it has been shown that ACLF-conditioned human monocytes had low glycolysis activity, with impaired expression of genes coding for glycolytic enzymes including *HK1, PGK1, PFKM* and *ENO2*. Redirecting glutamate metabolism by inhibiting glutamine synthetase in the tricarboxylic acid cycle restored phagocytosis[13]. Finally, it has been shown that the peritoneal cavity is particularly rich in glutamate, which is exploited by peritoneal tissue-resident macrophages to maintain respiratory burst activity during phagocytosis as demonstrated in human cultured cells[161]

Taken together, the evidence indicates that immune deficiency in cirrhosis is mainly the result of 'environmental' circulating factors that impair the function of immune system cells: an excessive regulatory immune response with increased inhibitory cytokines or immune cell subsets (e.g. IL10, MERKT), bacterial products from a disrupted intestinal barrier (e.g. endotoxin), and the metabolic abnormalities of liver insufficiency (e.g. ammonia, PGE2, dysfunctional albumin, catecholamines). In addition, transcriptional profiling of $CD14^{+}CD16^{-}$ monocytes from patients with ACLF revealed upregulation of an array of immunosuppressive genes associated with scavenger receptors (including *CD163, CD36, MARCO, MRC1*), suppressive cytokines (*IL10*), chemokines (*CCL22*) and *MERTK*, whereas monocytes in decompensated cirrhosis showed a preserved capacity to respond to inflammatory triggers, such as LPS [13]. Moreover, various key regulatory genes involved in antibacterial and oxidative burst responses, including *IRF8* and the gene encoding protein kinase C (*PRKCE*), were repressed in monocytes from patients with ACLF, which might contribute to the impaired ability to activate transcription and phosphorylation of the NADPH oxidase complex, respectively[13]. Culturing monocytes from healthy individuals in ACLF plasma induced an ACLF phenotype at the transcriptional level and impaired phagocytosis in these cells [13]. The potential pathogenic role of a circulating factor in immune deficiency in ACLF raises the possibility of restoring immune cell function by removing the toxins or immune inhibitory factors present in the circulation.

[H2] Consequences of immunodeficiency

This immunodeficiency increases the risk of bacterial infections at any stage of liver disease, even in compensated cirrhosis[162]. Bacterial infections are not only more frequent but also more severe than in healthy individuals, with an estimated overall mortality of 38% versus 10%, respectively [163,164]. In ACLF, bacterial infections are highly frequent and constitute the most identifiable triggers of this condition [3]. Bacterial infections are also highly detrimental if they occur during the clinical course of patients admitted with ACLF of any other aetiology, being independent predictors of 90-day mortality in ACLF[165].

[H1] Gut-associated lymphoid tissue in cirrhosis

Immune system dysfunction in cirrhosis affects the largest immunological organ in the human body, the GALT, which contains immune inducer sites such as interepithelial lymphocytes and Peyer's patches in the small intestine as well as cryptopatches, isolated lymphoid follicles in the large intestine, and immune effector sites such as the lamina propria and MLN.

[H2] Intestinal barrier dysfunction

Damage of the intestinal barrier associated to cirrhosis results in an increased passage of bacterial components such as such as LPS or bacterial DNA in experimental models and

patients [23,166–169] (**Figure 7**). Whereas bacterial components seem to reach the internal milieu by the paracellular route, whole viable bacteria could translocate via the transcellular route. The severity of intestinal barrier dysfunction and gut bacterial translocation correlate with the severity of cirrhosis, and they are more intense in patients with ascites. The severity of intestinal barrier dysfunction correlates with that of cirrhosis and especially with the development of ascites. There is a direct relationship between the frequency and severity of complications of decompensated cirrhosis, e.g. bacterial infections and encephalopathy, with dysbiosis and intestinal barrier dysfunction

Intestinal dysbiosis set the stage for intestinal barrier disruption in cirrhosis. Altered intestinal microbiota and bacterial overgrowth have been recognized in humans and experimental models of cirrhosis[170]. Metagenomic techniques have characterized the gut microbiome in cirrhosis as one of reduced diversity, significantly increased abundance of potentially pathogenic bacteria (such as Enterococcaceae, Staphylococcaceae and especially Enterobacteriaceae), and decreased relative abundance of potentially beneficial autochthonous bacteria (such as Lachnospiraceae and Ruminococcaceae)[171–174]. This microbiome profile in cirrhosis accompanies worsening disease, becomes more intense in the setting of decompensation, and is associated with poor outcomes[174,175]

The pathological translocation of viable bacteria from the intestinal lumen to the mesenteric lymph nodes and to the systemic circulation is well demonstrated in cirrhosis and is the pathogenetic basis of spontaneous bacterial infections and more specifically spontaneous bacterial peritonitis[16,176]. In cirrhosis, the access of intestinal bacteria that results in translocation occurs through the lymphatic and the vascular routes, the latter due to the breakdown of the intestinal vascular barrier[177] . The rupture of the gut vascular barrier facilitates the entry to the portal venous blood compartment, which is independent of the lymphatic pathway, since it is only present in experimental models of cirrhosis and not in those of portal hypertension without liver damage[177]. It is important to mention that in rats with cirrhosis obeticholic acid restored the reduced signaling of the farnesoid X receptor (FXR) in the ileum, improved the state of the mucous layer and stabilized the vascular barrier of the intestine, which supports the concept that the nuclear receptor FXR is involved, at least partially, in the regulation of the vascular and mucosal barriers of the intestine in cirrhosis[177]. The reduction in gut FXR signaling is likely to be a consequence of the luminal reduction of primary bile acids and the intestinal inflammation seen in experimental models of cirrhosis[177,178].

The functional abnormalities in the intestinal barrier described earlier have been linked to changes in intestinal mucosal immunity, deficiencies in secretory barrier function and disorganization of interepithelial tight junction proteins in humans and experimental models of cirrhosis. The persistent stimulation of intestinal immune cells by an abnormal gut microbiota with increased adherence to the muco-epithelial layer leads to a state of subclinical inflammation, which is evidenced by a higher number of activated monocytes, dendritic cells and T cells in the intestine and MLNs[23,37,179]. In experimental models of cirrhosis, intestinal $CD103^{+}$ dendritic cells become activated during ascitic decompensation, as shown by expansion of the pro-inflammatory CD4+ dendritic cell subpopulation, augmented TNF production and increased phagocytic and migratory capacity[179] Furthermore, intestinal macrophages that are normally anergic to low-level bacterial stimuli become activated, expressing innate immune receptors for bacterial

translocation and releasing nitric oxide and IL-6 in patients with cirrhosis[167]. As cirrhosis progresses to the ascitic stage, the levels of intraepithelial and lamina propria lymphocytes (T helper (including effector memory subset), cytotoxic, regulatory, B cells and NK cells) increase and switch to a Th1 regulatory pattern with expansion of TNF- and IFNγ-expressing cells in the lamina propria, with concomitant Th17 depletion[179,180]. Notably, bowel decontamination redistributes microbial composition, reduces pro-inflammatory activation of mucosal immune cells, and diminishes intestinal permeability and bacterial translocation, supporting the notion that dysbiosis has a major role in driving intestinal inflammation in cirrhosis [179].

Development of overt culturable and, thus, intense bacterial translocation into MLNs is associated with the absence of activated phenotype, lowered TNF production (even less than healthy individuals as controls) and relatively deficient phagocytosis and migration capacity of intestinal CD103+ dendritic cells in experimental models of decompensated cirrhosis[179]. Again, bowel decontamination with antibiotics eliminated bacterial DNA in MLNs and gut bacterial translocation, normalized the activation state and phagocytic function of intestinal CD103+ dendritic cells, and increased their TNF production. These data further reinforce the notion that the continuous pressure of gut dysbiosis in cirrhosis shapes the phenotypic and functional profile of intestinal immune cells to produce effects that range from their activation and enhanced function to their exhaustion and tolerance. Importantly, these changes appear to be specific to GALT, as monocyte-derived dendritic cells from peripheral blood from patients with cirrhosis are phenotypically and functionally indistinguishable from those derived from healthy donors[181]. This points to GALT being the primary site for derangement and deficiencies in innate immune function in advanced cirrhosis.

Disruption of the intestinal barrier in cirrhosis includes diminished secretion of anti-microbial peptides by intestinal Paneth cells, such as α-defensins, in particular α-defensins 5 and 7, as demonstrated in small intestinal tissue of human and experimental models of decompensated cirrhosis[178,182,183] Interestingly, upon intestinal microbiota-derived signals, such as LPS and muramyl dipeptide, Paneth cells also secrete pro-angiogenic molecules that promote intestinal and mesenteric angiogenesis and contribute to the development of portal hypertension[184]. Alterations in the secretory functions of the intestinal mucosa contribute to small intestinal bacterial overgrowth and translocation in models of cirrhosis but also affect intestinal dendritic cells and their migratory capacity, particularly into areas of inflammation[179]. Finally, dysfunctional mucus machinery, with diminished mucus thickness, reduced goblet cell numbers and reduced expression of mucin genes, has been observed in experimental models of advanced cirrhosis[177].

[H2] Abnormal gut-liver axis and CAID

An abnormal gut-liver axis characterized by an altered gut microbiome and a disrupted intestinal barrier is the main driver of low-grade and high-grade systemic inflammation in cirrhosis[2]. Indeed, in response to dysbiosis and pathological bacterial translocation, the GALT becomes a major cytokine-releasing organ, with lymphocytes from the epithelium and lamina propria as well as monocytes of MLNs displaying increased production of pro-inflammatory cytokines such as TNF and IL-6 in experimental models and patients with cirrhosis [23,37,185]. In advanced cirrhosis, systemic inflammation results from the sum of cytokines and immune cells activated in the gut and MLN and those produced by the

systemic immune system in response to the increased passage of bacteria and their products as a consequence of intestinal dysbiosis and barrier dysfunction[5,37]

The causal link between gut dysbiosis and systemic inflammation has been reinforced in a proof of concept study in patients with cirrhosis undergoing transjugular intrahepatic portosystemic shunt (TIPS), who display compartment-specific patterns of circulating bacteria, that is, different genera in central, hepatic, portal, and peripheral venous blood, and inflammatory cytokine clusters that are associated with to the abundance of blood microbiota genera in each patient[186]. Similarly, compartmentalization of microbiota composition and immune response is also observed between ascites and blood in patients with decompensated cirrhosis[187].

[H2] CAID and non-intestinal barriers

Dysbiosis, specifically a decrease in autochthonous bacteria and an increase in potentially pathogenic species, has been found in stool and colonic mucosa in patients with cirrhosis[188]. However, studies have also found changes in the salivary microbiota that were associated with significant inflammation related to Th1 and Th17 cell activation[189]. Interestingly, these alterations in bacterial salivary composition were associated with a higher risk of liver-related hospitalizations. The fact that the oral cavity is an additional source of inflammation was further validated with studies that demonstrated that periodontal care is associated with improved oral and gut dysbiosis, systemic inflammation, MELD score, and cognitive function in patients with cirrhosis [190]. This observation is particularly relevant as oral health problems and periodontitis are markedly increased in these individuals [191,192].

Another compartment with an emerging role in the immune dysfunction of cirrhosis is the peritoneal cavity. Spontaneous bacterial peritonitis (SBP) is the most frequent bacterial infection that occurs in patients with decompensated cirrhosis and is a common precipitant of ACLF[165] . The pathogenesis of SBP reflects failure of the intestinal and peritoneal barriers, enabling viable bacteria to gain access to the sterile peritoneal cavity. The healthy peritoneal niche is mainly populated by a heterogeneous population of macrophages that are crucial for immunological surveillance, resolution of inflammation, and recruitment and activation of other immune cells[193,194] In contrast to circulating MAIT cells, which are depleted and dysfunctional in cirrhosis, peritoneal MAIT cells of patients with cirrhosis are immunocompetent and migratory and accumulate in the peritoneal cavity during SBP, contributing to inflammation [195]. Furthermore, a study has identified in ascitic fluid of patients with cirrhosis an alternative phenotypically, transcriptionally and functionally distinct population of large CD14+ peritoneal macrophages that express CD206, exhibit features of inflammatory priming, and remain a substantial source of cytokine production after repeated exposure to bacterial products[196]. Similarly, peritoneal macrophages that express the complement receptor of the immunoglobulin superfamily (CRIg) display enhanced phagocytic and antimicrobial effector activity in patients with decompensated cirrhosis and ascites [197]. Finally, macrophages from sterile ascitic fluid show constitutive activation of caspase-1 and a marked increase expression of IL-1β and IL-18 compared with circulating monocytes, suggesting an activation of the inflammasome in the ascitic fluid of patients with cirrhosis that facilitates an exacerbated inflammatory response even in the absence of a priming signal[198]. In fact, SBP is characterized by a low microbial load that provokes an intense inflammatory peritoneal response, which correlates with systemic

inflammation and clinical outcomes, such as renal failure[56]. In addition, evidence indicates an association between alteration of peritoneal macrophage function, systemic inflammation and clinical outcomes in SBP. During SBP, levels of CD206 in ascitic fluid, but not in serum, correlate with mortality as well as with peritoneal and systemic inflammation [196]. Therefore, the dysregulated innate immune response of decompensated cirrhosis includes the presence in ascitic fluid of a subset of large peritoneal macrophages with an inflammatory phenotype that are resistant to tolerance and prone to massive release of proinflammatory cytokines upon TLR stimulation. Despite compartmentalization of the peritoneal immune response in cirrhosis, the massive release of pro-inflammatory cytokines during bacterial infection contributes to systemic inflammation and organ failure.

[H1] Modulation of CAID

Therapeutic approaches for CAID are extremely challenging because, on the one hand, systemic inflammation and organ immunopathology drive organ failure and increase risk of death. On the other hand, bacterial infections are the most important cause of organ failure and death in patients with decompensated cirrhosis[165]. Targeting systemic inflammation will clearly increase the risk of infection, whereas approaches to stimulate the inflammatory response likely worsen immunopathology. Therefore, treatment of CAID must involve strategies to modulate the immune response rather than directly inhibit or stimulate it. There are no specific therapies for CAID of proven benefit, but there is indirect evidence that some interventions might be beneficial and that provide clues to the development of novel approaches **(Table 3)**.

[H2] Targeting bacterial translocation

Decontamination with non-absorbable antibiotics ameliorates systemic vascular nitric oxide production, inflammation, and hemodynamic alterations in experimental models and in human cirrhosis[199,200]. It also helps normalize dendritic cell dysfunction and increase TNF production[179]. Studies have suggested temporal benefits of using poorly absorbed antibiotics for the prevention of further decompensation and reduction in mortality, and it is now the standard of care for patients with an episode of SBP[201]. In the first double-blind randomized controlled clinical trial of patients with a prior episode of SBP, 80 patients were randomized to norfloxacin or placebo. The recurrence of SBP was reduced from 68% to 20% in the norfloxacin group ($p=0.006$)[201] . The importance of targeting bacterial translocation with norfloxacin was confirmed in another study in which a statistically significant reduction in the risk of infection and mortality was observed in patients with decompensated cirrhosis[202]. In addition, intestinal decontamination with rifaximin has been shown to modulate the intestinal microbiome and improve cognitive function and endotoxemia in hepatic encephalopathy[203]. Ongoing clinical trials are further investigating the efficacy of rifaximin and simvastatin to prevent ACLF, reduce complications and hospital readmissions, and improve cost-effectiveness, quality-of-life and survival (LIVERHOPE project, EU H2020, NCT03780673). However, long-term use of antibiotics in patients with cirrhosis decreases gut bacterial diversity, and is an independent predictor of infection with antibiotic-resistant microorganisms[204]. Therefore, newer strategies are being developed that aim to reduce translocation without affecting microbial diversity. Probiotics have been studied in patients with cirrhosis but have not been found to affect immune function[205]. CARBALIVE is an orally administered, non-absorbable, highly engineered, activated charcoal with a wide range of porosities that adsorbs endotoxins and other products of bacterial metabolism, reduces translocation and influences severity

of immune dysfunction in a bile-duct ligation model of cirrhosis. The exact mechanism of how modulating the gut environment changes immune function is unknown [206]. This therapy is currently being evaluated in phase 2 clinical trials (NCT03202498). Another potential therapeutic strategy to reduce the effect of endotoxemia is inhibiting its receptor, TLR4. In sepsis, administration of TAK242, a TLR4 inhibitor, improved the outcome of patients with gram-negative infections[207]. This drug is being repurposed and is entering phase 2 clinical trials for patients with ACLF.

[H2] Targeting humoral factors in the circulation

Several lines of investigation indicate that incubation of normal monocytes or neutrophils with plasma from patients with cirrhosis and liver failure is associated with induction of cellular dysfunction, supporting the notion that circulating humoral factors, such as endotoxins, prostaglandins, catecholamines and products of cell death, might contribute to CAID [13,131,157]. It is possible that the beneficial effect of albumin on reducing the risk of recurrence of SBP and mortality is most likely due to its ability to bind to endotoxin and prostaglandins, both of which affect the function of immune cells[131,157,208–210]. This strategy of targeting circulating humoral factors is the basis of the newer modality of artificial extracorporeal liver support, DIALIVE, which incorporates two filters: one to exchange albumin, and the second to remove circulating factors such as endotoxins and products of cell death[211]. This device is currently being evaluated in phase 2 clinical trials in patients with ACLF (NCT03065699). In addition to binding circulating humoral factors, it has been proposed that albumin may internalize in leukocytes and reduce inflammation in patients with decompensated cirrhosis by inhibiting endosomal TLR signaling[212].

Another factor susceptible to modulation is increased sympathetic nervous system activity, a well-known feature of cirrhosis that is particularly excessive in the intestine [213]. Sympathetic nerve fibers are in close contact with GALT, and the mononuclear cells express ß- and α-adrenoreceptors[214,215]. Moreover, nearly all types of immune cells, whether local or circulating, express ß- and α-adrenoreceptors and are modulated by catecholamines. In cirrhosis, systemic noradrenaline levels are increased in early stages, correlate with severity of disease and are predictive for survival[216]. Sympathetic nervous system hyperactivity in cirrhosis has traditionally be seen as a compensatory mechanism counterbalancing arterial vasodilation. However, in addition to its hemodynamic actions, noradrenaline has been demonstrated to directly influence the microbiome[217,218], exert detrimental effects on enterocytes[219,220], increase intestinal permeability[221], facilitate various immunosuppressive actions[214,222], and contribute to the initiation and perpetuation of intestinal inflammation both in animal models and also in humans [223]. Indeed, in an animal model of cirrhosis, splanchnic sympathectomy increased the influx of neutrophils and monocytes into the peritoneal cavity in response to intraperitoneal *E. coli*, which improved *E. coli* phagocytosis, thereby preventing its systemic spreading [224]. Moreover, it is tempting to speculate whether noradrenaline levels contribute to driving the high-grade systemic inflammation in ACLF. In fact, noradrenaline levels are known to be up to three times higher in ACLF than in patients with acute decompensated cirrhosis, which strongly correlates with the severity of systemic inflammatory response[225]. In this context, a large-scale retrospective investigation in 349 patients indicates that non-selective β-blockers lower mortality in patients with ACLF[226]. Interestingly, 28-day and 3-month mortality were increased in patients who discontinued non-selective β-blockers at the time of ACLF onset.

[H2] Targeting the bone marrow

Patients with advanced cirrhosis, alcoholic hepatitis and ACLF typically display alterations in peripheral blood count, namely a reduction in the lymphocyte-to-neutrophil ratio[227]. This finding is probably due to increased granulopoiesis, which correlates with risk of infection and mortality in patients with advanced cirrhosis [227]. These observations are explained by the demonstration that hematopoetic stem cell niches in the bone marrow of patients with cirrhosis are shifted towards granulopoeisis , and the severity of this abnormality is associated with increased severity of liver disease and risk of infection[228]. Hence, strategies to improve immune function through the use of granulocyte colony-stimulating factor (GCSF) has been tried with some success. Although its use in patients with ACLF[229], long-term administration of GCSF was associated with increased mobilization of $CD34^+$ cells from the bone marrow, reduced risk of infection, and improved transplant-free survival[230], suggesting that targeting the bone marrow might have a beneficial role. However, in a placebo-controlled multicenter clinical trial in patients with ACLF, GCSF was not shown to reduce mortality, suggesting that it should be used only in the context of clinical trials[231]

[H2] Targeting immune cells directly

[H3] Immunometabolism. ACLF is characterised pathophysiologically by systemic inflammation and the increased production of cytokines, which are energy-consuming processes. Metabolomic data suggest that patients with ACLF have severe mitochondrial dysfunction, leading to a switch of metabolism to the cytosol, resulting in increased amino acid breakdown and production of toxic metabolites[110]. This metabolic switch has been shown to involve glutamine, which is an extremely important amino acid for maintaining gut and immune health. Inhibition of glutamine synthesis *in vitro* resulted in partial restoration of monocyte function in patients with ACLF, providing evidence of severely disrupted cellular energy metabolism [13]. However, these data do not necessarily provide evidence that inhibiting glutamine synthesis *in vivo* could be a possible therapeutic option, as this might negatively affect gut function by leading to hyperammonemia, which in itself might impair neutrophil function through the p38 MAPK pathway[132].

[H3] Cell signaling. In decompensated cirrhosis, cells of both the innate and adaptive immune system show intracellular signaling resulting in functional defects that provide potential therapeutic targets. Neutrophils from patients with ACLF were shown to have defective AKT-p38 MAPK pathway signalling, resulting in an impairment of N-formylmethionine-leucyl-phenylalanine-induced myeloperoxidase release and bactericidal activity[232]. Activation of the TLR7/TLR8 pathway using the agonist CL097 restored neutrophil function *ex vivo*, providing a potentially novel therapeutic approach[232]. Monocytes have been extensively studied in patients with ACLF, and show evidence of deficiencies in many pathways. Reduced phagocytic and bactericidal activity of monocytes in ACLF was shown to correlate with increased expression of MERTK . Pharmacological inhibition of MERTK with UNC56915 (Calbiochem/Millipore, UK) restored the functional status of the monocytes *ex vivo*, providing a possible future therapeutic target[127]. In addition, peripheral blood mononuclear cells have been shown to exhibit defective T cell responses in patients with alcohol-related ACLF, which was associated with increased expression of TIM3 and PD1. Inhibition of these proteins *ex vivo* restored T cell responses, indicating a potential novel therapy[153].

[H1] Conclusions

Immune system abnormalities are an intrinsic component of cirrhosis, and are responsible for some distinctive features of the disease. The key components of the so-called CAID are systemic inflammation and immune deficiency, which can be categorized as two phenotypes according to the intensity of inflammation: low-grade and high-grade. CAID is a dynamic process, and as cirrhosis progresses from the compensated to the decompensated stage and to ACLF so does the intensity of systemic inflammation and immune deficiency. Patients with ACLF have the highest grades of systemic inflammation ('high-grade phenotype') and of impairment of the effector functions of the immune system, which critically affect the risk of bacterial infections and survival. CAID contributes to modulation of the clinical course of cirrhosis: systemic inflammation worsens circulatory dysfunction and, along with increased portal hypertension, drive acute hepatic decompensations, whereas high-grade inflammation is the key conductor of organ failure and ACLF. The pathogenesis of systemic inflammation is mainly due to stimulation of immune system cells by PAMPS translocated into the circulation from a gut with dysbiosis and disruption of the intestinal barrier. Immunodeficiency in turn results from structural distortion of the liver parenchyma and mainly by the functional impairment and reprogramming of circulating immune cells due to the metabolic abnormalities of liver insufficiency. Treatment of CAID must involve strategies to modulate the immune response as targeting systemic inflammation will increase the infection risk, and approaches to stimulate the inflammatory response might worsen immunopathology and organ failure.

Table 1: Evidences of systemic inflammation in cirrhosis

Changes	**Compensated and decompensated cirrhosis**	**ACL**
Acute phase proteins	↑ C-Reactive protein and LBP [5]	↑↑↑
Cytokines	↑ Pro-inflammatory cytokines (TNFα, IL-1b, IL-6, IFNγ, IL-17, MCP-1, MIP-1b) and soluble receptors (TNF soluble receptors I and II, sTNFRI, sTNFRII, sgp130, suPAR)[233,234]	↑↑↑C inflar profil even
Neutrophil activation	↑ Respiratory burst and activation markers (CD11b)[9,42]	Incre
Monocyte activation	↑ HLA-DR expression and co-stimulatory molecules (CD80/CD86) ↑TNF secretion[7]	Incre recep
T cell activation	Th1 polarization and increased IFNγ production[37,44]	**Not**
B cell activation	↑ HLA-DR expression and co-stimulatory molecules[43]	**Not**
Endothelial activation molecules	↑ serum levels of ICAM-1, VCAM-1, VEGF, nitrates/nitrites [38,41]	↑↑ s

LBP, lipopolysaccharide binding protein; TNF, tumor necrosis factor; IFNγ, interferon-γ; sTNFRI, soluble tumor necrosis factor receptor I; sTNFRII, soluble tumor necrosis factor receptor II; sgp130, soluble gp130; suPAR, soluble urokinase plasminogen activator receptor; HLA-DR, human leukocyte antigen-DR; ICAM, intercellular adhesion molecule 1; VCAM-1, vascular cell adhesion protein 1; VEGF, vascular endothelial growth factor.

Table 2: Evidence of damage of circulating and tissue-resident immune cells in cirrhosis

Cell type	Cell/tissue	Compensated and decompensated cirrhosis
Circulating immune cells	**Monocytes**	-↑ non-classical CD14+CD16+ subset with ↑ HLA-DR express and TNF production [7,119] - ↓ chemotaxis, superoxide generation, phagocytosis a lysosomal enzyme production with cirrhosis severity [120,121] - ↑ AXL-expressing subset[122]
	Neutrophils	- ↓phagocytosis[9,10,132] - ↓ chemotaxis to the site of infection[130] - ↑ respiratory burst[132]
	Lymphocytes	- ↓ CD4+ T helper cells[139] - ↓ MAIT cells [140] - ↓ NK cells cytolytic activity[145] - Memory B cells: depleted, hyporesponsive, impaired product of cytokines and immunoglobulins[146]
Tissue-resident cells	**Liver**	-Kupffer cells: impaired and decreased activity[113] -Hepatocytes: replaced by scar tissue, ↓ synthesis of immu proteins and PPR [116,118]
	GALT and MLN	↑ activated monocytes[23,37,167,185] ↑ activated T cells with a Th1 switch (TNF, IFNγ)[23,37] ↑ activated dendritic cells with ↑phagocytosis and ↑TNF product (↓phagocytosis and ↓TNF in GBT)[179] ↑ proinflammatory cytokines (TNF, IL6)[23,37,167,185]
	Peritoneum (ascites)	- Competent MAIT cells that ↑ in SBP[195] - ↑pro-inflammatory CD206+ large peritoneal macrophag subset[196] - ↓ macrophages with high phagocytic and antimicrobial activ with cirrhosis severity [197] ↑ primed proinflammatory macrophages[198]

MERK, MER receptor tyrosine kinase; MAIT, mucosal-associated invariant T; HLA-DR, human leukocyte antigen-DR; CXCR, chemokine receptor; MLN, mesenteric lymph nodes; GBT, gut bacterial translocation; GALT, gut-associated lymphoid tissue.

Table 3: Potential strategies to modulate cirrhosis-associated immune dysfunction

Target	Mechanism	Therapy
Gut bacterial translocation	Reducing endotoxaemia and priming of immune cells	*Clinically available*: Poorly absorbed antibiotics[201–203] *Clinical trials*: CARBALIVE (NCT03202498), probiotics[205]
Circulating humoral factors	Endotoxin	*Clinical*: Albumin[208–210] *Clinical Trials*: DIALIVE (NCT03065699), TAK242[207] (NCT04620148)
	Products of cell death	*Clinical Trials*: DIALIVE (NCT03065699), TAK242[207] (NCT04620148)
	Prostaglandin E2	*Clinical*: Albumin[208–210] *Experimental*: COX-2 inhibitors[157]
	Catecholamines	*Clinical*: Non-selective beta-blockers[226]
Bone marrow	Reduced haematopoietic stem cell niche	*Clinical Trials*: Granulocyte colony stimulating factors[231]
Immunometabolism	Failure of cellular bioenergetics	*Experimental*: Inhibition of glutamine synthase[13]
	Ammonia	*Clinical*: Various[239]
Immune cell signalling	Neutrophils: AKT-p38 MAP kinase signalling	*Experimental*: TLR7/8 agonist, CL097[232]
	Monocytes: MERTK overexpression	*Experimental*: MERTK inhibitor UNC569[127]
	Lymphocytes	*Experimental*: PDI and TIM-3 inhibitors[153]

REFERENCES

1. Bonnel, A. R., Bunchorntavakul, C. & Reddy, K. R. Immune dysfunction and infections in patients with cirrhosis. *Clin Gastroenterol Hepatol* **9**, 727–38 (2011).
2. Albillos, A., Lario, M. & Álvarez-Mon, M. Cirrhosis-associated immune dysfunction: distinctive features and clinical relevance. *J Hepatol* **61**, 1385–96 (2014).
3. Moreau, R. *et al.* Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology* **144**, 1426–37 (2013).
4. Martin-Mateos, R., Alvarez-Mon, M. & Albillos, A. Dysfunctional Immune Response in Acute-on-Chronic Liver Failure: It Takes Two to Tango. *Front Immunol* **10**, 1–10 (2019).
5. Albillos, A. *et al.* Increased lipopolysaccharide binding protein in cirrhotic patients with marked immune and hemodynamic derangement. *Hepatology* **37**, 208–17 (2003).
6. Tilg, H. *et al.* Serum levels of cytokines in chronic liver diseases. *Gastroenterology* **103**, 264–274 (1992).
7. Albillos, A. *et al.* Tumour necrosis factor-alpha expression by activated monocytes and altered T-cell homeostasis in ascitic alcoholic cirrhosis: amelioration with norfloxacin. *J Hepatol* **40**, 624–631 (2004).
8. Trebicka, J. *et al.* The PREDICT study uncovers three clinical courses of acutely decompensated cirrhosis that have distinct pathophysiology. *J Hepatol* **73**, 842–854 (2020).
9. Tritto, G. *et al.* Evidence of neutrophil functional defect despite inflammation in stable cirrhosis. *J Hepatol* **55**, 574–581 (2011).
10. Rajkovic, I. A. & Williams, R. Abnormalities of neutrophil phagocytosis, intracellular killing and metabolic activity in alcoholic cirrhosis and hepatitis. *Hepatology* **6**, 252–262 (1986).
11. Clària, J. *et al.* Systemic inflammation in decompensated cirrhosis: Characterization and role in acute-on-chronic liver failure. *Hepatology* **64**, 1249–1264 (2016).
12. Wasmuth, H. E. *et al.* Patients with acute on chronic liver failure display 'sepsis-like' immune paralysis. *J Hepatol* **42**, 195–201 (2005).
13. Korf, H. *et al.* Inhibition of glutamine synthetase in monocytes from patients with acute-on-chronic liver failure resuscitates their antibacterial and inflammatory capacity. *Gut* **68**, 1872–1883 (2019).
14. Albillos, A., de Gottardi, A. & Rescigno, M. The gut-liver axis in liver disease: Pathophysiological basis for therapy. *J Hepatol* **72**, 558–577 (2020).
15. Wiest, R., Albillos, A., Trauner, M., Bajaj, J. S. & Jalan, R. Targeting the gut-liver axis in liver disease. *J Hepatol* **67**, 1084–1103 (2017).
16. Wiest, R., Lawson, M. & Geuking, M. Pathological bacterial translocation in liver cirrhosis. *J Hepatol* **60**, 197–209 (2014).
17. Zhao, J. *et al.* Single-cell RNA sequencing reveals the heterogeneity of liver-resident immune cells in human. *Cell Discov* **6**, 22 (2020).
18. Franco, A. *et al.* Expression of class I and class II major histocompatibility complex antigens on human hepatocytes. *Hepatology* **8**, 449–454 (1988).
19. Gao, B., Jeong, W.-I. & Tian, Z. Liver: An organ with predominant innate immunity. *Hepatology* **47**, 729–736 (2007).
20. Kubes, P., Jenne, C. & Snyder, J. Immune Responses in the Liver. *Annu Rev Immunol* **36**, 1–31 (2018).
21. Hossain, M. & Kubes, P. Innate immune cells orchestrate the repair of sterile injury in the liver and beyond. *Eur J Immunol* **49**, 831–841 (2019).
22. Knolle, P. A. Staying local-antigen presentation in the liver. *Curr Opin Immunol* **40**, 36–42 (2016).

23. Muñoz, L. *et al.* Intestinal Immune Dysregulation Driven by Dysbiosis Promotes Barrier Disruption and Bacterial Translocation in Rats With Cirrhosis. *Hepatology* **70**, 925–938 (2019).
24. Mörbe, U. M. *et al.* Human gut-associated lymphoid tissues (GALT); diversity, structure, and function. *Mucosal Immunol* **14**, 793–802 (2021).
25. Tripathi, A. *et al.* The gut–liver axis and the intersection with the microbiome. *Nat Rev Gastroenterol Hepatol* **15**, 397–411 (2018).
26. Mowat, A. M. Anatomical basis of tolerance and immunity to intestinal antigens. *Nat Rev Immunol* **3**, 331–341 (2003).
27. MacPherson, G. *et al.* Uptake of antigens from the intestine by dendritic cells. *Ann N Y Acad Sci* **1029**, 75–82 (2004).
28. Esplugues, E. *et al.* Control of TH17 cells occurs in the small intestine. *Nature* **475**, 514–518 (2011).
29. Ismail, A. S. *et al.* Gamma/Delta intraepithelial lymphocytes are essential mediators of host-microbial homeostasis at the intestinal mucosal surface. *Proc Natl Acad Sci* **108**, 8743–8748 (2011).
30. Slack, E., Balmer, M. L. & Macpherson, A. J. B cells as a critical node in the microbiota-host immune system network. *Immunol Rev* **260**, 50–66 (2014).
31. Kirkland, D. *et al.* B Cell-Intrinsic MyD88 Signaling Prevents the Lethal Dissemination of Commensal Bacteria during Colonic Damage. *Immunity* **36**, 228–238 (2012).
32. Medzhitov, R. Origin and physiological roles of inflammation. *Nature* **454**, 428–435 (2008).
33. Kubes, P. & Mehal, W. Z. Sterile Inflammation in the Liver. *Gastroenterology* **143**, 1158–1172 (2012).
34. Kumar, H., Kawai, T. & Akira, S. Pathogen Recognition by the Innate Immune System. *Int Rev Immunol* **30**, 16–34 (2011).
35. Úbeda, M. *et al.* Critical role of the liver in the induction of systemic inflammation in rats with preascitic cirrhosis. *Hepatology* **52**, 2086–95 (2010).
36. Wang, H. *et al.* Hepatoprotective versus Oncogenic Functions of STAT3 in Liver Tumorigenesis. *Am J Pathol* **179**, 714–724 (2011).
37. Muñoz, L. *et al.* Mesenteric Th1 polarization and monocyte TNF-α production: First steps to systemic inflammation in rats with cirrhosis. *Hepatology* **42**, 411–419 (2005).
38. Guarner, C. *et al.* Increased serum nitrite and nitrate levels in patients with cirrhosis: Relationship to endotoxemia. *Hepatology* **18**, 1139–1143 (1993).
39. La Mura, V. *et al.* Von Willebrand factor levels predict clinical outcome in patients with cirrhosis and portal hypertension. *Gut* **60**, 1133–1138 (2011).
40. Buck, M. *et al.* Novel inflammatory biomarkers of portal pressure in compensated cirrhosis patients. *Hepatology* **59**, 1052–1059 (2014).
41. Grünhage, F. *et al.* Elevated Soluble Tumor Necrosis Factor Receptor 75 Concentrations Identify Patients With Liver Cirrhosis at Risk of Death. *Clin Gastroenterol Hepatol* **6**, 1255–1262 (2008).
42. Fiuza, C., Salcedo, M., Clemente, G. & Tellado, J. M. Granulocyte Colony-Stimulating Factor Improves Deficient In Vitro Neutrophil Transendothelial Migration in Patients with Advanced Liver Disease. *Clin Vaccine Immunol* **9**, 433–439 (2002).
43. Girón, J. A. *et al.* Increased spontaneous and lymphokine-conditioned IgA and IgG synthesis by B cells from alcoholic cirrhotic patients. *Hepatology* **16**, 664–670 (1992).
44. Sun, H. Q. *et al.* Increased Th17 cells contribute to disease progression in patients with HBV-associated liver cirrhosis. *J Viral Hepat* **19**, 396–403 (2012).
45. Trebicka, J. *et al.* Addressing Profiles of Systemic Inflammation Across the Different Clinical Phenotypes of Acutely Decompensated Cirrhosis. *Front Immunol* **10**, (2019).

46. Solé, C. *et al.* Characterization of Inflammatory Response in Acute-on-Chronic Liver Failure and Relationship with Prognosis. *Sci Rep* **6**, 32341 (2016).
47. Grønbæk, H. *et al.* The soluble macrophage activation markers sCD163 and Mannose Receptor (sMR) predict mortality in patients with liver cirrhosis without or with acute-on-chronic liver failure (ACLF). *J Hepatol* **64**, 813–822 (2016).
48. Chen, P., Stärkel, P., Turner, J. R., Ho, S. B. & Schnabl, B. Dysbiosis-induced intestinal inflammation activates tumor necrosis factor receptor I and mediates alcoholic liver disease in mice. *Hepatology* **61**, 883–894 (2015).
49. Viganò, E. *et al.* Human caspase-4 and caspase-5 regulate the one-step non-canonical inflammasome activation in monocytes. *Nat Commun* **6**, 8761 (2015).
50. Christgen, S., Place, D. E. & Kanneganti, T.-D. Toward targeting inflammasomes: insights into their regulation and activation. *Cell Res* **30**, 315–327 (2020).
51. Monteiro, S. *et al.* Differential inflammasome activation predisposes to acute-on-chronic liver failure in human and experimental cirrhosis with and without previous decompensation. *Gut* **70**, 379–387 (2021).
52. Spadoni, I. *et al.* A gut-vascular barrier controls the systemic dissemination of bacteria. *Science (80-)* **350**, 830–834 (2015).
53. Jenne, C. N. & Kubes, P. Immune surveillance by the liver. *Nat Immunol* **14**, 996–1006 (2013).
54. Yang, A.-M. *et al.* Intestinal fungi contribute to development of alcoholic liver disease. *J Clin Invest* **127**, 2829–2841 (2017).
55. Crispe, I. N. Immune tolerance in liver disease. *Hepatology* **60**, 2109–17 (2014).
56. Navasa, M. *et al.* Tumor necrosis factor and interleukin-6 in spontaneous bacterial peritonitis in cirrhosis: relationship with the development of renal impairment and mortality. *Hepatology* **27**, 1227–1232 (1998).
57. Heller, J. *et al.* Effects of lipopolysaccharide on TNF-α production, hepatic NOS2 activity, and hepatic toxicity in rats with cirrhosis. *J Hepatol* **33**, 376–381 (2000).
58. Tazi, K. A. *et al.* In vivo altered unfolded protein response and apoptosis in livers from lipopolysaccharide-challenged cirrhotic rats. *J Hepatol* **46**, 1075–1088 (2007).
59. Tazi, K. A. *et al.* Upregulation of TNF-alpha production signaling pathways in monocytes from patients with advanced cirrhosis: Possible role of Akt and IRAK-M. *J Hepatol* **45**, 280–289 (2006).
60. Thompson, K. *et al.* Interleukin-10 expression and function in experimental murine liver inflammation and fibrosis. *Hepatology* **28**, 1597–1606 (1998).
61. Coant, N. *et al.* Glycogen synthase kinase 3 involvement in the excessive proinflammatory response to LPS in patients with decompensated cirrhosis. *J Hepatol* **55**, 784–793 (2011).
62. Szabo, G. & Saha, B. Alcohol's Effect on Host Defense. *Alcohol Res* **37**, 159–70 (2015).
63. Gao, B. *et al.* Innate immunity in alcoholic liver disease. *Am J Physiol Gastrointest Liver Physiol* **300**, G516-25 (2011).
64. Bajaj, J. S. Alcohol, liver disease and the gut microbiota. *Nat Rev Gastroenterol Hepatol* **16**, 235–246 (2019).
65. Schuster, S., Cabrera, D., Arrese, M. & Feldstein, A. E. Triggering and resolution of inflammation in NASH. *Nat Rev Gastroenterol Hepatol* **15**, 349–364 (2018).
66. Spruss, A. *et al.* Toll-like receptor 4 is involved in the development of fructose-induced hepatic steatosis in mice. *Hepatology* **50**, 1094–1104 (2009).
67. Liu, J. *et al.* Toll-like receptor-4 signalling in the progression of non-alcoholic fatty liver disease induced by high-fat and high-fructose diet in mice. *Clin Exp Pharmacol Physiol* **41**, 482–488 (2014).
68. Stanton, M. C. *et al.* Inflammatory signals shift from adipose to liver during high fat feeding and influence the development of steatohepatitis in mice. *J Inflamm* **8**, 8 (2011).
69. Guo, J. & Friedman, S. L. Toll-like receptor 4 signaling in liver injury and hepatic

fibrogenesis. *Fibrogenesis Tissue Repair* **3**, 21 (2010).
70. Petrasek, J. *et al.* Interferon regulatory factor 3 and type I interferons are protective in alcoholic liver injury in mice by way of crosstalk of parenchymal and myeloid cells. *Hepatology* **53**, 649–660 (2011).
71. Yan, A. W. *et al.* Enteric dysbiosis associated with a mouse model of alcoholic liver disease. *Hepatology* **53**, 96–105 (2011).
72. Mutlu, E. A. *et al.* Colonic microbiome is altered in alcoholism. *Am J Physiol Liver Physiol* **302**, G966–G978 (2012).
73. Parlesak, A., Schäfer, C., Schütz, T., Bode, J. C. & Bode, C. Increased intestinal permeability to macromolecules and endotoxemia in patients with chronic alcohol abuse in different stages of alcohol-induced liver disease. *J Hepatol* **32**, 742–747 (2000).
74. Zhou, Z., Sun, X. & Kang, Y. J. Ethanol-Induced Apoptosis in Mouse Liver. *Am J Pathol* **159**, 329–338 (2001).
75. Lu, Y., Zhuge, J., Wang, X., Bai, J. & Cederbaum, A. I. Cytochrome P450 2E1 contributes to ethanol-induced fatty liver in mice. *Hepatology* **47**, 1483–94 (2008).
76. Macdonald, S. *et al.* Cell death markers in patients with cirrhosis and acute decompensation. *Hepatology* **67**, 989–1002 (2018).
77. Zheng, S.-J. *et al.* Prognostic value of M30/M65 for outcome of hepatitis B virus-related acute-on-chronic liver failure. *World J Gastroenterol* **20**, 2403–11 (2014).
78. Dear, J. W. *et al.* Cyclophilin A Is a Damage-Associated Molecular Pattern Molecule That Mediates Acetaminophen-Induced Liver Injury. *J Immunol* **187**, 3347–3352 (2011).
79. Guan, Z. *et al.* Extracellular gp96 is a crucial mediator for driving immune hyperactivation and liver damage. *Sci Rep* **10**, 12596 (2020).
80. Yotti, R. *et al.* Left ventricular systolic function is associated with sympathetic nervous activity and markers of inflammation in cirrhosis. *Hepatology* **65**, 2019–2030 (2017).
81. Tazi, K. A. *et al.* Norfloxacin Reduces Aortic NO Synthases and Proinflammatory Cytokine Up-Regulation in Cirrhotic Rats: Role of Akt Signaling. *Gastroenterology* **129**, 303–314 (2005).
82. Téllez, L. *et al.* Non-selective beta-blockers impair global circulatory homeostasis and renal function in cirrhotic patients with refractory ascites. *J Hepatol* **73**, 1404–1414 (2020).
83. Wiest, R. *et al.* Bacterial translocation in cirrhotic rats stimulates eNOS-derived NO production and impairs mesenteric vascular contractility. *J Clin Invest* **104**, 1223–1233 (1999).
84. Bellot, P. *et al.* Bacterial DNA translocation is associated with systemic circulatory abnormalities and intrahepatic endothelial dysfunction in patients with cirrhosis. *Hepatology* **52**, 2044–2052 (2010).
85. Waidmann, O. *et al.* Macrophage activation is a prognostic parameter for variceal bleeding and overall survival in patients with liver cirrhosis. *J Hepatol* **58**, 956–961 (2013).
86. Grønbæk, H. *et al.* Macrophage activation markers predict mortality in patients with liver cirrhosis without or with acute-on-chronic liver failure (ACLF). *J Hepatol* **64**, 813–822 (2016).
87. Arroyo, V. *et al.* The systemic inflammation hypothesis: Towards a new paradigm of acute decompensation and multiorgan failure in cirrhosis. *Journal of Hepatology* vol. 74 670–685 (2021).
88. Shah, N. *et al.* Increased renal expression and urinary excretion of TLR4 in acute kidney injury associated with cirrhosis. *Liver Int* **33**, 398–409 (2013).
89. Shawcross, D. L., Davies, N. A., Williams, R. & Jalan, R. Systemic inflammatory response exacerbates the neuropsychological effects of induced hyperammonemia in cirrhosis. *J Hepatol* **40**, 247–254 (2004).
90. Wright, G. *et al.* Reduction in hyperammonaemia by ornithine phenylacetate

prevents lipopolysaccharide-induced brain edema and coma in cirrhotic rats. *Liver Int* **32**, 410–9 (2012).
91. D'Mello, C., Le, T. & Swain, M. G. Cerebral microglia recruit monocytes into the brain in response to tumor necrosis factora signaling during peripheral organ inflammation. *J Neurosci* **29**, 2089–2102 (2009).
92. Kerfoot, S. M. *et al.* TNF-α-secreting monocytes are recruited into the brain of cholestatic mice. *Hepatology* **43**, 154–162 (2006).
93. Bajaj, J. S. *et al.* Linkage of gut microbiome with cognition in hepatic encephalopathy. *Am J Physiol Liver Physiol* **302**, G168–G175 (2012).
94. Liu, R. *et al.* Neuroinflammation in Murine Cirrhosis Is Dependent on the Gut Microbiome and Is Attenuated by Fecal Transplant. *Hepatology* **71**, 611–626 (2020).
95. Yang, Y. M., Kim, S. Y. & Seki, E. Inflammation and Liver Cancer: Molecular Mechanisms and Therapeutic Targets. *Semin Liver Dis* **39**, 26–42 (2019).
96. Naugler, W. E. *et al.* Gender Disparity in Liver Cancer Due to Sex Differences in MyD88-Dependent IL-6 Production. *Science (80-)* **317**, 121–124 (2007).
97. Inokuchi, S. *et al.* Disruption of TAK1 in hepatocytes causes hepatic injury, inflammation, fibrosis, and carcinogenesis. *Proc Natl Acad Sci* **107**, 844–849 (2010).
98. Pikarsky, E. *et al.* NF-κB functions as a tumour promoter in inflammation-associated cancer. *Nature* **431**, 461–466 (2004).
99. Jalan, R. *et al.* Development and validation of a prognostic score to predict mortality in patients with acute-on-chronic liver failure. *J Hepatol* **61**, 1038–1047 (2014).
100. Hotchkiss, R. S. *et al.* Apoptotic cell death in patients with sepsis, shock, and multiple organ dysfunction. *Crit Care Med* **27**, 1230–51 (1999).
101. Bernardi, M., Moreau, R., Angeli, P., Schnabl, B. & Arroyo, V. Mechanisms of decompensation and organ failure in cirrhosis: From peripheral arterial vasodilation to systemic inflammation hypothesis. *J Hepatol* **63**, 1272–1284 (2015).
102. Iwasaki, A. & Medzhitov, R. Control of adaptive immunity by the innate immune system. *Nat Immunol* **16**, 343–53 (2015).
103. Gomez, H. *et al.* A Unified Theory of Sepsis-Induced Acute Kidney Injury. *Shock* **41**, 3–11 (2014).
104. Alobaidi, R., Basu, R. K., Goldstein, S. L. & Bagshaw, S. M. Sepsis-Associated Acute Kidney Injury. *Semin Nephrol* **35**, 2–11 (2015).
105. Piano, S. *et al.* Association Between Grade of Acute on Chronic Liver Failure and Response to Terlipressin and Albumin in Patients With Hepatorenal Syndrome. *Clin Gastroenterol Hepatol* **16**, 1792–1800 (2018).
106. Rodrigo, R. *et al.* Hyperammonemia Induces Neuroinflammation That Contributes to Cognitive Impairment in Rats With Hepatic Encephalopathy. *Gastroenterology* **139**, 675–684 (2010).
107. Romero-Gómez, M., Montagnese, S. & Jalan, R. Hepatic encephalopathy in patients with acute decompensation of cirrhosis and acute-on-chronic liver failure. *J Hepatol* **62**, 437–447 (2015).
108. Ayres, J. S. Immunometabolism of infections. *Nat Rev Immunol* **20**, 79–80 (2020).
109. Chowdhury, D. *et al.* Metallothionein 3 Controls the Phenotype and Metabolic Programming of Alternatively Activated Macrophages. *Cell Rep* **27**, 3873–3886 (2019).
110. Moreau, R. *et al.* Blood metabolomics uncovers inflammation-associated mitochondrial dysfunction as a potential mechanism underlying ACLF. *J Hepatol* **72**, 688–701 (2020).
111. Badawy, A. A. B. Kynurenine Pathway of Tryptophan Metabolism: Regulatory and Functional Aspects. *Int J tryptophan Res* **10**, 1178646917691938 (2017).
112. Clària, J. *et al.* Orchestration of Tryptophan-Kynurenine Pathway, Acute

Decompensation, and Acute-on-Chronic Liver Failure in Cirrhosis. *Hepatology* **69**, 1686–1701 (2019).
113. Rimola, A. *et al.* Reticuloendothelial system phagocytic activity in cirrhosis and its relation to bacterial infections and prognosis. *Hepatology* **4**, 53–8 (1984).
114. Bolognesi, M. *et al.* Clinical significance of the evaluation of hepatic reticuloendothelial removal capacity in patients with cirrhosis. *Hepatology* **19**, 628–634 (1994).
115. Laleman, W., Claria, J., Van der Merwe, S., Moreau, R. & Trebicka, J. Systemic Inflammation and Acute-on-Chronic Liver Failure: Too Much, Not Enough. *Can J Gastroenterol Hepatol* **2018**, 1–10 (2018).
116. Altorjay, I. *et al.* Mannose-binding lectin deficiency confers risk for bacterial infections in a large Hungarian cohort of patients with liver cirrhosis. *J Hepatol* **53**, 484–491 (2010).
117. Papp, M. *et al.* Acute phase proteins in the diagnosis and prediction of cirrhosis associated bacterial infections. *Liver Int* **32**, 603–611 (2012).
118. Homann, C. *et al.* Acquired C3 deficiency in patients with alcoholic cirrhosis predisposes to infection and increased mortality. *Gut* **40**, 544–549 (1997).
119. Zimmermann, H. W. *et al.* Functional Contribution of Elevated Circulating and Hepatic Non-Classical CD14+CD16+ Monocytes to Inflammation and Human Liver Fibrosis. *PLoS One* **5**, e11049 (2010).
120. Nakagawara, A., Inokuchi, K., Ikeda, K., Kumashiro, R. & Tamada, R. Decreased superoxide (O2-)-generating activity of blood monocytes from patients with hepatic cirrhosis. *Hepatogastroenterology* **31**, 201–3 (1984).
121. Gomez, F., Ruiz, P. & Schreiber, A. D. Impaired Function of Macrophage Fcγ Receptors and Bacterial Infection in Alcoholic Cirrhosis. *N Engl J Med* **331**, 1122–1128 (1994).
122. Brenig, R. *et al.* Expression of AXL receptor tyrosine kinase relates to monocyte dysfunction and severity of cirrhosis. *Life Sci alliance* **3**, (2020).
123. Berres, M.-L. *et al.* Longitudinal monocyte Human leukocyte antigen-DR expression is a prognostic marker in critically ill patients with decompensated liver cirrhosis. *Liver Int* **29**, 536–543 (2009).
124. Berry, P. A. *et al.* Severity of the compensatory anti-inflammatory response determined by monocyte HLA-DR expression may assist outcome prediction in cirrhosis. *Intensive Care Med* **37**, 453–460 (2011).
125. 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* **147**, 184–8 (2007).
126. Weichselbaum, L. *et al.* Epigenetic basis for monocyte dysfunction in patients with severe alcoholic hepatitis. *J Hepatol* **73**, 303–314 (2020).
127. Bernsmeier, C. *et al.* Patients With Acute-on-Chronic Liver Failure Have Increased Numbers of Regulatory Immune Cells Expressing the Receptor Tyrosine Kinase MERTK. *Gastroenterology* **148**, 603–615 (2015).
128. Camenisch, T. D., Koller, B. H., Earp, H. S. & Matsushima, G. K. A novel receptor tyrosine kinase, Mer, inhibits TNF-alpha production and lipopolysaccharide-induced endotoxic shock. *J Immunol* **162**, 3498–503 (1999).
129. Bernsmeier, C. *et al.* CD14+ CD15- HLA-DR- myeloid-derived suppressor cells impair antimicrobial responses in patients with acute-on-chronic liver failure. *Gut* **67**, 1155–1167 (2018).
130. Fiuza, C., Salcedo, M., Clemente, G. & Tellado, J. M. In Vivo Neutrophil Dysfunction in Cirrhotic Patients with Advanced Liver Disease. *J Infect Dis* **182**, 526–533 (2000).
131. Mookerjee, R. P. *et al.* Neutrophil dysfunction in alcoholic hepatitis superimposed on cirrhosis is reversible and predicts the outcome. *Hepatology* **46**, 831–840 (2007).
132. Shawcross, D. L. *et al.* Ammonia impairs neutrophil phagocytic function in liver

disease. *Hepatology* **48**, 1202–1212 (2008).
133. Bruns, T., Peter, J., Hagel, S., Herrmann, A. & Stallmach, A. The augmented neutrophil respiratory burst in response to Escherichia coli is reduced in liver cirrhosis during infection§. *Clin Exp Immunol* **164**, 346–356 (2011).
134. Tranah, T. H. *et al.* Dysfunctional neutrophil effector organelle mobilization and microbicidal protein release in alcohol-related cirrhosis. *Am J Physiol Liver Physiol* **313**, G203–G211 (2017).
135. Bukong, T. N. *et al.* Abnormal neutrophil traps and impaired efferocytosis contribute to liver injury and sepsis severity after binge alcohol use. *J Hepatol* **69**, 1145–1154 (2018).
136. Moreau, R., Périanin, A. & Arroyo, V. Review of Defective NADPH Oxidase Activity and Myeloperoxidase Release in Neutrophils From Patients With Cirrhosis. *Front Immunol* **10**, 1–7 (2019).
137. Artru, F. *et al.* IL-33/ST2 pathway regulates neutrophil migration and predicts outcome in patients with severe alcoholic hepatitis. *J Hepatol* **72**, 1052–1061 (2020).
138. Taylor, N. J. *et al.* The severity of circulating neutrophil dysfunction in patients with cirrhosis is associated with 90-day and 1-year mortality. *Aliment Pharmacol Ther* **40**, 705–715 (2014).
139. Lario, M. *et al.* Defective thymopoiesis and poor peripheral homeostatic replenishment of T-helper cells cause T-cell lymphopenia in cirrhosis. *J Hepatol* **59**, 723–730 (2013).
140. Riva, A. *et al.* Mucosa-associated invariant T cells link intestinal immunity with antibacterial immune defects in alcoholic liver disease. *Gut* **67**, 918–930 (2018).
141. Arruvito, L. *et al.* Identification and Clinical Relevance of Naturally Occurring Human CD8 + HLA-DR + Regulatory T Cells. *J Immunol* **193**, 4469–4476 (2014).
142. Machicote, A., Belén, S., Baz, P., Billordo, L. A. & Fainboim, L. Human CD8+HLA-DR+ Regulatory T Cells, Similarly to Classical CD4+Foxp3+ Cells, Suppress Immune Responses via PD-1/PD-L1 Axis. *Front Immunol* **9**, 2788 (2018).
143. Lebossé, F. *et al.* CD8+ T cells from patients with cirrhosis display a phenotype that may contribute to cirrhosis-associated immune dysfunction. *EBioMedicine* **49**, 258–268 (2019).
144. Peter, J., Frey, O., Stallmach, A. & Bruns, T. Attenuated antigen-specific T cell responses in cirrhosis are accompanied by elevated serum interleukin-10 levels and down-regulation of HLA-DR on monocytes. *BMC Gastroenterol* **13**, 37 (2013).
145. Laso, F. J. *et al.* Decreased natural killer cytotoxic activity in chronic alcoholism is associated with alcohol liver disease but not active ethanol consumption. *Hepatology* **25**, 1096–100 (1997).
146. Doi, H. *et al.* Dysfunctional B-cell activation in cirrhosis resulting from hepatitis C infection associated with disappearance of CD27-Positive B-cell population. *Hepatology* **55**, 709–719 (2012).
147. Ward, N. S., Casserly, B. & Ayala, A. The Compensatory Anti-inflammatory Response Syndrome (CARS) in Critically Ill Patients. *Clin Chest Med* **29**, 617–625 (2008).
148. Bandyopadhyay, G. *et al.* Negative signaling contributes to T-cell anergy in trauma patients. *Crit Care Med* **35**, 794–801 (2007).
149. Monneret, G. *et al.* The anti-inflammatory response dominates after septic shock: Association of low monocyte HLA-DR expression and high interleukin-10 concentration. *Immunol Lett* **95**, 193–198 (2004).
150. Hynninen, M. *et al.* Predictive value of monocyte histocompatibility leukocyte antigen-DR expression and plasma interleukin-4 and -10 levels in critically ill patients with sepsis. *Shock* **20**, 1–4 (2003).
151. Oberholzer, A., Oberholzer, C. & Moldawer, L. L. Interleukin-10: A complex role in the pathogenesis of sepsis syndromes and its potential as an anti-inflammatory drug. *Crit Care Med* **30**, S58–S63 (2002).

152. Berry, P. A. *et al.* Admission levels and early changes in serum interleukin-10 are predictive of poor outcome in acute liver failure and decompensated cirrhosis. *Liver Int* **30**, 733–740 (2010).
153. Markwick, L. J. L. *et al.* Blockade of PD1 and TIM3 Restores Innate and Adaptive Immunity in Patients With Acute Alcoholic Hepatitis. *Gastroenterology* **148**, 590-602.e10 (2015).
154. Hackstein, C.-P. *et al.* Gut microbial translocation corrupts myeloid cell function to control bacterial infection during liver cirrhosis. *Gut* **66**, 507–518 (2017).
155. Jalan, R. *et al.* Alterations in the functional capacity of albumin in patients with decompensated cirrhosis is associated with increased mortality. *Hepatology* **50**, 555–564 (2009).
156. Arroyo, V., García-Martinez, R. & Salvatella, X. Human serum albumin, systemic inflammation, and cirrhosis. *J Hepatol* **61**, 396–407 (2014).
157. O'Brien, A. J. *et al.* Immunosuppression in acutely decompensated cirrhosis is mediated by prostaglandin E2. *Nat Med* **20**, 518–523 (2014).
158. Serezani, C. H. *et al.* Prostaglandin E2 suppresses bacterial killing in alveolar macrophages by inhibiting NADPH oxidase. *Am J Respir Cell Mol Biol* **37**, 562–570 (2007).
159. Aronoff, D. M., Canetti, C. & Peters-Golden, M. Prostaglandin E 2 Inhibits Alveolar Macrophage Phagocytosis through an E-Prostanoid 2 Receptor-Mediated Increase in Intracellular Cyclic AMP . *J Immunol* **173**, 559–565 (2004).
160. Schmidl, C. *et al.* Transcription and enhancer profiling in human monocyte subsets. *Blood* **123**, 90–9 (2014).
161. Davies, L. C. *et al.* Peritoneal tissue-resident macrophages are metabolically poised to engage microbes using tissue-niche fuels. *Nat Commun* **8**, 2074 (2017).
162. Villanueva, C. *et al.* Bacterial infections in patients with compensated cirrhosis and clinically significant portal hypertension: implications on the risk of developing decompensation and on survival. *Hepatology* **70**, 36A-37A (2019).
163. Arvaniti, V. *et al.* Infections in patients with cirrhosis increase mortality four-fold and should be used in determining prognosis. *Gastroenterology* **139**, 1246–56 (2010).
164. Gustot, T. *et al.* Impact of infection on the prognosis of critically ill cirrhotic patients: Results from a large worldwide study. *Liver Int* **34**, 1496–1503 (2014).
165. Fernández, J. *et al.* Bacterial and fungal infections in acute-on-chronic liver failure: Prevalence, characteristics and impact on prognosis. *Gut* **67**, 1870–1880 (2018).
166. Rao, R. K. Acetaldehyde-Induced Increase in Paracellular Permeability in Caco-2 Cell Monolayer. *Alcohol Clin Exp Res* **22**, 1724–1730 (1998).
167. Du Plessis, J. *et al.* Activated intestinal macrophages in patients with cirrhosis release NO and IL-6 that may disrupt intestinal barrier function. *J Hepatol* **58**, 1125–32 (2013).
168. Assimakopoulos, S. F. *et al.* Altered intestinal tight junctions' expression in patients with liver cirrhosis: a pathogenetic mechanism of intestinal hyperpermeability. *Eur J Clin Invest* **42**, 439–446 (2012).
169. Pijls, K. E., Jonkers, D. M. A. E., Elamin, E. E., Masclee, A. A. M. & Koek, G. H. Intestinal epithelial barrier function in liver cirrhosis: an extensive review of the literature. *Liver Int* **33**, 1457–1469 (2013).
170. Shah, A. *et al.* Systematic Review and Meta-Analysis: Prevalence of Small Intestinal Bacterial Overgrowth in Chronic Liver Disease. *Semin Liver Dis* **37**, 388–400 (2017).
171. Chen, Y. *et al.* Characterization of fecal microbial communities in patients with liver cirrhosis. *Hepatology* **54**, 562–72 (2011).
172. Qin, N. *et al.* Alterations of the human gut microbiome in liver cirrhosis. *Nature* **513**, 59–64 (2014).
173. Bajaj, J. S. *et al.* Altered profile of human gut microbiome is associated with cirrhosis and its complications. *J Hepatol* **60**, 940–947 (2014).

174. Solé, C. *et al.* Alterations in Gut Microbiome in Cirrhosis as Assessed by Quantitative Metagenomics: Relationship With Acute-on-Chronic Liver Failure and Prognosis. *Gastroenterology* **160**, 206–218 (2021).
175. Bajaj, J. S. *et al.* Diet affects gut microbiota and modulates hospitalization risk differentially in an international cirrhosis cohort. *Hepatology* **68**, 234–247 (2018).
176. Garcia-Tsao, G., Lee, F.-Y., Barden, G. E., Cartun, R. & Brian West, A. Bacterial translocation to mesenteric lymph nodes is increased in cirrhotic rats with ascites. *Gastroenterology* **108**, 1835–1841 (1995).
177. Sorribas, M. *et al.* FXR modulates the gut-vascular barrier by regulating the entry sites for bacterial translocation in experimental cirrhosis. *J Hepatol* **71**, 1126–1140 (2019).
178. Úbeda, M. *et al.* Obeticholic acid reduces bacterial translocation and inhibits intestinal inflammation in cirrhotic rats. *J Hepatol* **64**, 1049–57 (2016).
179. Muñoz, L. *et al.* Interaction between intestinal dendritic cells and bacteria translocated from the gut in rats with cirrhosis. *Hepatology* **56**, 1861–1869 (2012).
180. Inamura, T. *et al.* Alteration of intestinal intraepithelial lymphocytes and increased bacterial translocation in a murine model of cirrhosis. *Immunol Lett* **90**, 3–11 (2003).
181. Tanoue, S., Chang, L.-Y., Li, Y. & Kaplan, D. E. Monocyte-derived dendritic cells from cirrhotic patients retain similar capacity for maturation/activation and antigen presentation as those from healthy subjects. *Cell Immunol* **295**, 36–45 (2015).
182. Kaliannan, K. Compromise of α-Defensin Function in Liver Cirrhosis Facilitates the Toxic Relationship Between Gut Permeability and Endotoxemia. *Dig Dis Sci* **63**, 2492–2494 (2018).
183. Teltschik, Z. *et al.* Intestinal bacterial translocation in rats with cirrhosis is related to compromised paneth cell antimicrobial host defense. *Hepatology* **55**, 1154–1163 (2012).
184. Hassan, M. *et al.* Paneth cells promote angiogenesis and regulate portal hypertension in response to microbial signals. *J Hepatol* **73**, 628–639 (2020).
185. Genescà, J. *et al.* Increased tumour necrosis factor alpha production in mesenteric lymph nodes of cirrhotic patients with ascites. *Gut* **52**, 1054–9 (2003).
186. Schierwagen, R. *et al.* Circulating microbiome in blood of different circulatory compartments. *Gut* **68**, 578–580 (2019).
187. Alvarez-Silva, C. *et al.* Compartmentalization of Immune Response and Microbial Translocation in Decompensated Cirrhosis. *Front Immunol* **10**, 1–11 (2019).
188. Qin, N. *et al.* Alterations of the human gut microbiome in liver cirrhosis. *Nature* **513**, 59–64 (2014).
189. Bajaj, J. S. *et al.* Salivary microbiota reflects changes in gut microbiota in cirrhosis with hepatic encephalopathy. *Hepatology* **62**, 1260–1271 (2015).
190. Bajaj, J. S. *et al.* Periodontal therapy favorably modulates the oral-gut-hepatic axis in cirrhosis. *Am J Physiol Liver Physiol* **315**, G824–G837 (2018).
191. Grønkjær, L. L. *et al.* Periodontitis in patients with cirrhosis: a cross-sectional study. *BMC Oral Health* **18**, 22 (2018).
192. Grønkjær, L. L. & Vilstrup, H. Oral health in patients with liver cirrhosis. *Eur J Gastroenterol Hepatol* **27**, 834–839 (2015).
193. Kubicka, U. *et al.* Normal human immune peritoneal cells: Subpopulations and functional characteristics. *Scand J Immunol* **44**, 157–163 (1996).
194. Ruiz-Alcaraz, A. J. *et al.* Characterization of human peritoneal monocyte/macrophage subsets in homeostasis: Phenotype, GATA6, phagocytic/oxidative activities and cytokines expression. *Sci Rep* **8**, (2018).
195. Ibidapo-Obe, O. *et al.* Mucosal-Associated Invariant T Cells Redistribute to the Peritoneal Cavity During Spontaneous Bacterial Peritonitis and Contribute to Peritoneal Inflammation. *Cell Mol Gastroenterol Hepatol* **9**, 661–677 (2020).
196. Stengel, S. *et al.* Peritoneal Level of CD206 Associates With Mortality and an Inflammatory Macrophage Phenotype in Patients With Decompensated Cirrhosis

and Spontaneous Bacterial Peritonitis. *Gastroenterology* **158**, 1745–1761 (2020).
197. Irvine, K. M. *et al.* CRIg-expressing peritoneal macrophages are associated with disease severity in patients with cirrhosis and ascites. *JCI Insight* **1**, 1–15 (2016).
198. Lozano-Ruiz, B. *et al.* Absent in melanoma 2 triggers a heightened inflammasome response in ascitic fluid macrophages of patients with cirrhosis. *J Hepatol* **62**, 64–71 (2015).
199. Rasaratnam, B., Kaye, D., Jennings, G., Dudley, F. & Chin-Dusting, J. The Effect of Selective Intestinal Decontamination on the Hyperdynamic Circulatory State in Cirrhosis. *Ann Intern Med* **139**, 186 (2003).
200. Chin-Dusting, J. P. F., Rasaratnam, B., Jennings, G. L. R. & Dudley, F. J. Effect of fluoroquinolone on the enhanced nitric oxide-induced peripheral vasodilation seen in cirrhosis. *Ann Intern Med* **127**, 985–988 (1997).
201. Ginés, P. *et al.* Norfloxacin prevents spontaneous bacterial peritonitis recurrence in cirrhosis: Results of a double-blind, placebo-controlled trial. *Hepatology* **12**, 716–724 (1990).
202. Moreau, R. *et al.* Effects of Long-term Norfloxacin Therapy in Patients with Advanced Cirrhosis. *Gastroenterology* **155**, 1816–1827 (2018).
203. Bajaj, J. S. *et al.* Modulation of the Metabiome by Rifaximin in Patients with Cirrhosis and Minimal Hepatic Encephalopathy. *PLoS One* **8**, e60042 (2013).
204. Fernández, J. *et al.* Prevalence and risk factors of infections by multiresistant bacteria in cirrhosis: A prospective study. *Hepatology* **55**, 1551–1561 (2012).
205. Horvath, A. *et al.* Randomised clinical trial: the effects of a multispecies probiotic vs. placebo on innate immune function, bacterial translocation and gut permeability in patients with cirrhosis. *Aliment Pharmacol Ther* **44**, 926–935 (2016).
206. Macnaughtan, J. *et al.* Oral therapy with non-absorbable carbons of controlled porosity (YAQ-001) selectively modulates stool microbiome and its function and this is associated with restoration of immune function and inflammasome activation. *J Hepatol* **62**, S240 (2015).
207. Rice, T. W. *et al.* A randomized, double-blind, placebo-controlled trial of TAK-242 for the treatment of severe sepsis. *Crit Care Med* **38**, 1685–1694 (2010).
208. Sort, P. *et al.* Effect of Intravenous Albumin on Renal Impairment and Mortality in Patients with Cirrhosis and Spontaneous Bacterial Peritonitis. *N Engl J Med* **341**, 403–409 (1999).
209. Caraceni, P. *et al.* Long-term albumin administration in decompensated cirrhosis (ANSWER): an open-label randomised trial. *Lancet* **391**, 2417–2429 (2018).
210. Fernández, J. *et al.* Efficacy of Albumin Treatment for Patients with Cirrhosis and Infections Unrelated to Spontaneous Bacterial Peritonitis. *Clin Gastroenterol Hepatol* **18**, 963-973.e14 (2020).
211. Lee, K. C. L. *et al.* Extracorporeal liver assist device to exchange albumin and remove endotoxin in acute liver failure: Results of a pivotal pre-clinical study. *J Hepatol* **63**, 634–642 (2015).
212. Casulleras, M. *et al.* Albumin internalizes and inhibits endosomal TLR signaling in leukocytes from patients with decompensated cirrhosis. *Sci Transl Med* **12**, (2020).
213. Dietrich, P. *et al.* Dysbalance in sympathetic neurotransmitter release and action in cirrhotic rats: impact of exogenous neuropeptide Y. *J Hepatol* **58**, 254–61 (2013).
214. Brinkman, D. J., ten Hove, A. S., Vervoordeldonk, M. J., Luyer, M. D. & de Jonge, W. J. Neuroimmune Interactions in the Gut and Their Significance for Intestinal Immunity. *Cells* **8**, 670 (2019).
215. Felten, D. L., Felten, S. Y., Carlson, S. L., Olschowka, J. A. & Livnat, S. Noradrenergic and peptidergic innervation of lymphoid tissue. *J Immunol* **135**, 755s-765s (1985).
216. Henriksen, J. H., Ring-Larsen, H., Kanstrup, I. L. & Christensen, N. J. Splanchnic

and renal elimination and release of catecholamines in cirrhosis. Evidence of enhanced sympathetic nervous activity in patients with decompensated cirrhosis. *Gut* **25**, 1034–43 (1984).
217. Freestone, P. P. *et al.* Growth Stimulation of Intestinal Commensal Escherichia coli by Catecholamines: A Possible Contributory Factor in Trauma-Induced Sepsis. *Shock* **18**, 465–470 (2002).
218. Chen, C., Brown, D. R., Xie, Y., Green, B. T. & Lyte, M. Catecholamines Modulate Escherichia coli O157:H7 Adherence to Murine Cecal Mucosa. *Shock* **20**, 183–188 (2003).
219. Piton, G. *et al.* Catecholamine Use is Associated With Enterocyte Damage in Critically Ill Patients. *Shock* **43**, 437–442 (2015).
220. Habes, Q. L. M., van Ede, L., Gerretsen, J., Kox, M. & Pickkers, P. Norepinephrine Contributes to Enterocyte Damage in Septic Shock Patients. *Shock* **49**, 137–143 (2018).
221. Green, B. T., Lyte, M., Kulkarni-Narla, A. & Brown, D. R. Neuromodulation of enteropathogen internalization in Peyer's patches from porcine jejunum. *J Neuroimmunol* **141**, 74–82 (2003).
222. Hart, A. & Kamm, M. A. Review article: mechanisms of initiation and perpetuation of gut inflammation by stress. *Aliment Pharmacol Ther* **16**, 2017–28 (2002).
223. Straub, R. H., Wiest, R., Strauch, U. G., Harle, P. & Scholmerich, J. The role of the sympathetic nervous system in intestinal inflammation. *Gut* **55**, 1640–1649 (2006).
224. Worlicek, M. *et al.* Splanchnic sympathectomy prevents translocation and spreading of E coli but not S aureus in liver cirrhosis. *Gut* **59**, 1127–1134 (2010).
225. Mehta, G., Mookerjee, R. P., Sharma, V. & Jalan, R. Systemic inflammation is associated with increased intrahepatic resistance and mortality in alcohol-related acute-on-chronic liver failure. *Liver Int* **35**, 724–34 (2015).
226. Mookerjee, R. P. *et al.* Treatment with non-selective beta blockers is associated with reduced severity of systemic inflammation and improved survival of patients with acute-on-chronic liver failure. *J Hepatol* **64**, 574–82 (2016).
227. Forrest, E. H. *et al.* Baseline neutrophil-to-lymphocyte ratio predicts response to corticosteroids and is associated with infection and renal dysfunction in alcoholic hepatitis. *Aliment Pharmacol Ther* **50**, 442–453 (2019).
228. Bihari, C. *et al.* Bone marrow stem cells and their niche components are adversely affected in advanced cirrhosis of the liver. *Hepatology* **64**, 1273–1288 (2016).
229. Garg, V. *et al.* Granulocyte Colony–Stimulating Factor Mobilizes CD34+ Cells and Improves Survival of Patients With Acute-on-Chronic Liver Failure. *Gastroenterology* **142**, 505–512 (2012).
230. Verma, N. *et al.* Outcomes after multiple courses of granulocyte colony-stimulating factor and growth hormone in decompensated cirrhosis: A randomized trial. *Hepatology* **68**, 1559–1573 (2018).
231. Engelmann, C. *et al.* Granulocyte-Colony Stimulating Factor (G-CSF) to Treat Acute-on-Chronic Liver Failure (Graft Trial): Interim Analysis of the First Randomised European Multicentre Trial. *Hepatology* **70**, 17 (2019).
232. Boussif, A. *et al.* Impaired intracellular signaling, myeloperoxidase release and bactericidal activity of neutrophils from patients with alcoholic cirrhosis. *J Hepatol* **64**, 1041–1048 (2016).
233. Lemmers, A. *et al.* An inhibitor of interleukin-6 trans-signalling, sgp130, contributes to impaired acute phase response in human chronic liver disease. *Clin Exp Immunol* **156**, 518–527 (2009).
234. Zimmermann, H. W. *et al.* Soluble urokinase plasminogen activator receptor is compartmentally regulated in decompensated cirrhosis and indicates immune activation and short-term mortality. *J Intern Med* **274**, 86–100 (2013).
235. Lehmann, J. M. *et al.* Circulating CXCL10 in cirrhotic portal hypertension might reflect systemic inflammation and predict ACLF and mortality. *Liver Int* **38**, 875–

884 (2018).
236. Khanam, A. *et al.* Blockade of Neutrophil's Chemokine Receptors CXCR1/2 Abrogate Liver Damage in Acute-on-Chronic Liver Failure. *Front Immunol* **8**, 464 (2017).
237. Xiang, X. *et al.* Interleukin-22 ameliorates acute-on-chronic liver failure by reprogramming impaired regeneration pathways in mice. *J Hepatol* **72**, 736–745 (2020).
238. Shubham, S. *et al.* Cellular and functional loss of liver endothelial cells correlates with poor hepatocyte regeneration in acute-on-chronic liver failure. *Hepatol Int* **13**, 777–787 (2019).
239. Rose, C. F. *et al.* Hepatic encephalopathy: Novel insights into classification, pathophysiology and therapy. *J Hepatol* **73**, 1526–1547 (2020).
240. Lee, W.-Y. *et al.* An intravascular immune response to Borrelia burgdorferi involves Kupffer cells and iNKT cells. *Nat Immunol* **11**, 295–302 (2010).
241. Diehl, L. *et al.* Tolerogenic maturation of liver sinusoidal endothelial cells promotes B7-homolog 1-dependent CD8+ T cell tolerance. *Hepatology* **47**, 296–305 (2007).
242. Wang, Y. & Zhang, C. The Roles of Liver-Resident Lymphocytes in Liver Diseases. *Front Immunol* **10**, 1582 (2019).
243. McNamara, H. A. *et al.* Up-regulation of LFA-1 allows liver-resident memory T cells to patrol and remain in the hepatic sinusoids. *Sci Immunol* **2**, 1996 (2017).
244. Shoukry, N. H. *et al.* Memory CD8+ T Cells Are Required for Protection from Persistent Hepatitis C Virus Infection. *J Exp Med* **197**, 1645–1655 (2003).
245. Pallett, L. J. *et al.* IL-2high tissue-resident T cells in the human liver: Sentinels for hepatotropic infection. *J Exp Med* **214**, 1567–1580 (2017).
246. Artis, D. & Spits, H. The biology of innate lymphoid cells. *Nature* **517**, 293–301 (2015).
247. Jeffery, H. C. *et al.* Biliary epithelium and liver B cells exposed to bacteria activate intrahepatic MAIT cells through MR1. *J Hepatol* **64**, 1118–1127 (2016).
248. Clarembeau, F., Bale, G. & Lanthier, N. Cirrhosis and insulin resistance: current knowledge, pathophysiological mechanisms, complications and potential treatments. *Clin Sci* **134**, 2117–2135 (2020).
249. Bhanji, R. A., Montano-Loza, A. J. & Watt, K. D. Sarcopenia in Cirrhosis: Looking Beyond the Skeletal Muscle Loss to See the Systemic Disease. *Hepatology* **70**, 2193–2203 (2019).
250. Angeli, P., Garcia-Tsao, G., Nadim, M. K. & Parikh, C. R. News in pathophysiology, definition and classification of hepatorenal syndrome: A step beyond the International Club of Ascites (ICA) consensus document. *J Hepatol* **71**, 811–822 (2019).
251. Yin, M. *et al.* Essential role of tumor necrosis factor alpha in alcohol-induced liver injury in mice. *Gastroenterology* **117**, 942–952 (1999).

Acknowledgements

Authors are supported by grant from the Spanish Ministry of Science and Innovation (SAF 2017-86343-R, and Instituto de Salud Carlos III PI20/01302). CIBEREHD is funded by the Instituto de Salud Carlos III with grants co-financed by the European Development Regional Fund "A way to achieve Europe" (EDRF).

Author contributions

A.A. researched data for article, made a substantial contribution to discussion of content, wrote the article, and reviewed/edited the manuscript before submission. R.M.-M. made a substantial contribution to discussion of content, wrote the article, and reviewed/edited the

manuscript before submission. S.V.d.M., R.W. and R.J. researched data for article, made a substantial contribution to discussion of content, and wrote the article. M.A.-M. researched data for article and made a substantial contribution to discussion of content.

Competing interests

The authors declare no competing interests.

Peer review information

Nature Reviews Gastroenterology & Hepatology thanks [Referee#1 name], [Referee#2 name] and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Figure 1. Immune cells in the liver. Kupffer cells are highly specialized tissue-resident macrophages that can detect, bind and internalize apoptotic neutrophils and pathogens. Activation of Kupffer cells might also lead to the production of cytokines involved in the inflammatory response. As antigen-presenting cells (APCs), Kupffer cells express MHC-I, MHC-II, and costimulatory molecules needed for activation of T and invariant natural killer (NK) T cells[240]. Liver sinusoidal endothelial cells (LSECs) line the sinusoidal capillary channels and are endowed with fenestrations that allow them to filter the blood in search of pathogens. Similarly to Kupffer cells, LSECs act as scavengers and APCs and contribute to maintaining immunotolerance by expressing inhibitory molecules[241]. Dendritic cells enter the liver as immature cells, and mature as they move from the portal to the systemic circulation. Dendritic cells are poor activators of T cells in the liver in contrast to other tissues. Hepatocytes also display surveillance roles, acting as APCs by expressing MHC-I and II and costimulatory molecules[18]. Finally, multiple types of lymphocytes can be found within the liver[242]: $CD8^+$ liver-resident memory T cells patrol and reside within the hepatic sinusoids[243]. They can activate stronger anti-viral immune responses than circulating memory CD8+ T cells in chronic viral hepatitis[244,245]. Innate lymphoid cells (ILCs) present a classic lymphoid cell morphology, but lack the expression of antigen-specific receptors. This subgroup of lymphocytes includes classic cytotoxic NK cells, lymphoid tissue-inducer cells, and other non-cytotoxic ILC populations[246]. Mucosal-associated invariant T (MAIT) cells are unconventional T cells that can be found not only in the liver but also in the intestinal mucosa and peripheral blood. They recognize riboflavin metabolites of bacterial or fungal origin, and perform antibacterial functions by secreting interferon-γ (IFNγ), tumor necrosis factor (TNF) and IL-17, and killing infected cells[247]. Invariant NKT cells patrol the sinusoids by displaying a T cell receptor that recognizes glycolipid antigens (mainly α-galactosylceramide)[242]. Upon activation, they produce both pro-inflammatory and anti-inflammatory cytokines and further activate other immune effectors such as dendritic cells, NK, B, and T cells.

Figure 2. Low-grade systemic inflammation in cirrhosis. Several factors contribute to low-grade systemic inflammation. **A)** Increased translocation of bacteria and pathogen-associated molecular patterns (PAMPs) due to intestinal dysbiosis, bacterial overgrowth and altered tight junction proteins. This also activates pattern recognition receptors, contributing to the overproduction of cytokines[2,5,14,23,37,185]. **B)** Increased damage-associated molecular patterns (DAMPs) released from injured hepatocytes bind to pattern recognition receptors, activating pro-inflammatory sterile signaling pathways. **C)** Dysfunctional and decreased levels of albumin also contribute to systemic inflammation because, under physiological conditions, albumin has anti-inflammatory properties by binding and neutralizing PAMPs[155]. **D)** The loss of tolerance is related to an altered unfolded protein response at the endoplasmic reticulum and to an increased production of tumor necrosis factor (TNF) upon lipopolysaccharide (LPS) stimulation[58,59]. Although LPS is the most characteristic ligand of Toll-like receptor 4 (TLR4), other PAMPs and DAMPs can bind and activate this receptor. TLR4 is mainly expressed in sentinel cells such as macrophages and dendritic cells, but it has been also identified in other cell subtypes such as hepatic stellate cells and endothelial cells. Depending on the specific ligand and the target cell expressing TLR4, two downstream signaling pathways can be activated. The MYD88-dependent pathway involves the nuclear translocation of the transcription factor nuclear factor κB (NF-κB), which induces the release of pro-inflammatory cytokines. On

the other hand, the MYD88-independent pathway involves the nuclear translocation of interleukin regulatory factor 3 (IRF3) and leads to the induction of type 1 interferons.

Figure 3. Pathogenesis and consequences of low-grade systemic inflammation in cirrhosis. Low-grade systemic inflammation is a distinctive feature of compensated and stable decompensated cirrhosis[2,5,8,35,37]. (**A**) Systemic inflammation is characterized by an increased production of proinflammatory cytokines and the upregulation of cell activation markers in circulating immune cells. Systemic inflammation mainly results from the chronic and episodic passage of bacterial products (that is, pathogen-associated molecular patterns (PAMPs)) from a leaky gut to the internal milieu, and to a minor extent from the release of damage-associated molecular patterns (DAMPs) from damaged hepatocytes undergoing necroptosis or apoptosis[5,8,35,37].Circulating activated immune cells can be recruited by peripheral tissues and, in addition, the pro-inflammatory cytokines can alter the function of somatic cells. Via these two mechanisms, the chronic low-grade inflammation contributes to the clinical expression of cirrhosis. (**B**) Pro-inflammatory cytokines activate endothelial nitric oxide synthase and endothelial cells that release tissue factor increasing von Willebrand factor, which worsen peripheral vasodilation and promote portal vein thrombosis[5,39,80–84]. Chronic inflammation is also accompanied by sarcopenia, frailty, insulin resistance and osteopenia[248,249]. IL-6 and other cytokines stimulate the hepatic synthesis of hepcidin leading to anemia, and of C-reactive protein and other acute phase reactants. Fatigue and depression are related to inflammation-induced tryptophan metabolism via the kynurenine pathway. Pro-inflammatory cytokines and immune system cells recruited by the brain lead to microglial activation, which alters neurotransmission, behavior and encephalopathy[89–94].

Figure 4. Pathogenetic contribution of low-grade and high-grade systemic inflammation to hepatorenal syndrome. Acute kidney injury (AKI), which is one of the most frequent complications of cirrhosis, exemplifies the different pathogenic roles of low-grade and high-grade systemic inflammation. (**A**) Peripheral arterial vasodilatation related to portal hypertension leads to effective hypovolemia, which is the pathogenic driver of classical hepatorenal syndrome (HRS), a functional form of AKI[250].In this context, low-grade systemic inflammation contributes to worsen peripheral vasodilation by further increasing nitric oxide overproduction. (**B**) Kidney is the most frequent failing organ in acute-on-chronic liver failure (ACLF). High-grade inflammation is the pathogenic driver of kidney failure in ACLF, having a lesser role in hemodynamic changes[11,105] In this setting, kidney tubular and parenchymal (and other organs) failure are the consequence of apoptotic and non-apoptotic cell death mechanisms, mitochondrial dysfunction and microthrombosis[88,110]. Additionally, further tissue injury is the consequence of 1) immunopathology, that stands for the collateral damage secondary to the massive cytokine release and immune tissue infiltration by activated immune system cells, and 2) immunometabolic alterations, specifically an energetic switch aiming to counteract the nutrient shortage by diminishing mitochondrial oxidative phosphorylation and ATP production. **C**) Progression of cirrhosis from the compensated to the decompensated stage and to ACLF is characterized by the development of acute complications, such as ascites, variceal bleeding or bacterial infections, and by tissue injury leading to organ failure, respectively. Portal hypertension and systemic cardiocirculatory dysfunction are the drivers of the transition from compensated to decompensated cirrhosis, whereas

systemic inflammation drives the tissue injury that leads to extrahepatic organ failure and results in acute-on-chronic liver failure.

Figure 5. Cirrhosis-associated immune dysfunction in ACLF. High-grade systemic inflammation and immune cell paralysis characterize the dysfunctional immune response in acute-on-chronic liver failure (ACLF). A) High-grade systemic inflammation. The overproduction of pro- and anti-inflammatory cytokines as well as activation and recruitment of neutrophils to the site of organ failure are a consequence of the activation of Toll- and Nod-like receptors in peripheral monocytes and dendritic cells, as a consequence of the increased levels of damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs). [11,73,76,78,79,251]. **B)** Immune cell paralysis. In ACLF, there is an acquired paralysis of the immune system that affects innate and acquired immune responses and is secondary to an excessive compensatory anti-inflammatory response, exhaustion of immune effectors, and metabolic and neuroendocrine disturbances [such as increased prostaglandin E2 (PGE2), hyperammonemia and hyponatremia] [12,13,123–129]. Increased IL-10 is involved in the paralysis of cells of the immune system, since it inhibits nuclear factor-κB (NF-kB) activity, which results in a reduction in monocyte secretion of tumor necrosis factor (TNF), IL-1, IL-6, IL-8 and IL-12. IL10 levels inversely correlate with the expression of inhibitory receptors in monocytes (MERTK) and lymphocytes [programmed cell death 1 (PD1) and T cell immunoglobulin and mucin domain-containing 3 (TIM3)]. IL10 expression also correlates with decreased interferon-γ (INFγ) production, which contribute to immune cell dysfunction. Monocyte antigen presentation ability and TNF production in response to LPS are compromised due to decreased monocyte HLA-DR expression. The augmented expression of CXCR1 and CXCR2 receptors by neutrophils contributes to hepatocyte death through early apoptosis and necrosis. In addition, they show an impaired phagocytic activity, reduced chemotaxis to the site of infection and an increased respiratory burst, which generates reactive oxygen species (ROS) that contribute to tissue damage and to the exhaustion of phagocytic cells. MERTK: tyrosine-protein kinase MER.

Figure 6. Dynamics of cirrhosis-associated immune dysfunction. Systemic inflammation and immune deficiency are the two components of the cirrhosis-associated immune dysfunction (CAID). CAID should be viewed as a continuum of immunological abnormalities thorough the spectrum of cirrhosis severity. CAID can be arbitrarily divided into two phenotypes according to the intensity of inflammation: low-grade and high-grade systemic inflammation. The severity of systemic inflammation parallels the course of cirrhosis, beginning in the compensated stage, becoming more intense in the stable decompensated stage, peaking during episodes of acute decompensation, and culminating in acute decompensation with acute-on-chronic liver failure (ACLF). Peaks of inflammation due to infectious (such as bacterial infections) or non-infectious (such as alcohol) triggers worsen systemic circulatory dysfunction and contribute to the precipitation of episodes of acute decompensation (such as ascites, acute kidney injury, and encephalopathy) in stable decompensated cirrhosis. Immunodeficiency is characteristically present in ACLF, where it contributes to the extremely high susceptibility to bacterial infections. However, it is already present in patients with stable

decompensated cirrhosis, contributing to episodes of acute decompensation triggered by bacterial infection.

Figure 7. Disruption of the gut-liver axis in cirrhosis: contribution to cirrhosis-associated immune dysfunction. Intestinal dysbiosis sets the stage for the disruption of the gut-liver axis in patients with cirrhosis[14]. Dysbiosis in cirrhosis is the consequence of intestinal hypomotility and reduced bile acid flow with decreased luminal levels of bile acids. Dysbiosis and overabundance of 7-α-dehydroxylating bacteria increase secondary bile acids in the intestinal lumen. This leads to reduced intestinal farnesoid X receptor (FXR) signaling, which compromises the synthesis of mucous and antimicrobial peptides and damage of the integrity of the gut vascular barrier. The final consequence is loosening of the epithelial tight junctions, mucous layer thinning and reduced innate defenses, which facilitate penetrability of bacteria and the interaction of pathobionts with mucosal immune system cells. The persistent stimulation of intestinal immune cells by an abnormal gut microbiome leads to a state of subclinical inflammation, which is evidenced by a higher number of activated monocytes, dendritic cells and T lymphocytes in the intestine and mesenteric lymph nodes. Intraepithelial, lamina propria and mesenteric lymph node inflammation features a Th1 regulatory pattern of immune cell activation along with an increased synthesis of interferon-γ (INF-γ) and tumor necrosis factor (TNF), which further contributes to an increase in intestinal permeability. Additionally, activated immune system cells convert gut-associated lymphoid tissue into a cytokine-releasing organ that majorly contributes to systemic inflammation in cirrhosis. Disruption of the intestinal barrier is extreme in patients with advanced decompensated cirrhosis with the concurrence of profound dysbiosis and an overabundance of Enterobacteriaceae on top of markedly damaged epithelial, immune and vascular intestinal barriers. The result is the massive passage to the circulation of PAMPs and viable bacteria, causing systemic and liver inflammation as well as spontaneous bacterial infections.

Glossary

Cirrhosis-associated immune dysfunction
Dysfunctional immune response associated with cirrhosis and characterized by systemic inflammation and immune paralysis.

Systemic inflammation:
Increased expression of surface activation antigens in circulating immune cells, and production of pro-inflammatory cytokines.

Acute-on-chronic liver failure:
Acute decompensation of cirrhosis associated with organ failure and high short-term mortality.

Phenotype with low-grade systemic inflammation
Increased immune activation and mild to moderate compromise of the immune effector response in patients with compensated cirrhosis or acute decompensation with no organ failure.

Phenotype with high-grade systemic inflammation
Extreme activation with massive release of cytokines along with functional impairment of circulating immune system cells in patients with acute-on-chronic liver failure.

Gut-liver axis
Bidirectional functional connection between the liver and the intestine, particularly its microbiota and immune system.

Pathogen-associated molecular patterns
Sets of microbial molecular patterns that can be recognized by specific receptors of immune system cells.

Damage-associated molecular patterns
Intracellular molecules released by injured or dying cells that can be recognized by specific receptors of immune system cells.

Gut-associated lymphoid tissue
Lymphoid tissue lining the intestine and composed of Peyer’s patches, intestinal lymphoid follicles, intraepithelial lymphocytes and mesenteric lymph nodes.

Immunopathology
Immune-mediated tissue damage due to an excesive immune activation.