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TOPIC HIGHLIGHT

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Immune dysfunction in cirrhosis

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Abstract

Innate and adaptive immune dysfunction, also referred to as cirrhosis-associated immune dysfunction syndrome, is a major component of cirrhosis, and plays a pivotal role in the pathogenesis of both the acute and chronic worsening of liver function. During the evolution of the disease, acute decompensation events associated with organ failure(s), so-called acute-on chronic liver failure, and chronic decompensation with progression of liver fibrosis and also development of disease specific complications, comprise distinct clinical entities with different immunopathology mechanisms. Enhanced bacterial translocation associated with systemic endotoxemia and increased occurrence of systemic bacterial infections have substantial impacts on both clinical situations. Acute and chronic exposure to bacteria and/or their products, however, can result in variable clinical consequences. The immune status of patients is not constant during the illness; consequently, alterations of the balance between pro- and anti-inflammatory processes result in very different dynamic courses. In this review we give a detailed overview of acquired immune dysfunction and its consequences for cirrhosis. We demonstrate the substantial influence of inherited innate immune dysfunction on acute and chronic inflammatory processes in cirrhosis caused by the pre-existing acquired immune dysfunction with limited compensatory mechanisms. Moreover, we highlight the current facts and future perspectives of how the assessment of immune dysfunction can assist clinicians in everyday practical decision-making when establishing treatment and care strategies for the patients with end-stage liver disease. Early and efficient recognition of inappropriate performance of the immune system is essential for overcoming complications, delaying progression and reducing mortality.

Key words: Cirrhosis; Immune dysfunction; Endotoxemia

Core tip: Innate and adaptive immune dysfunction, also referred to as cirrhosis-associated immune dysfunction syndrome, plays a pivotal role in the pathogenesis of cirrhosis in both acute and chronic disease progression. During progression, acute decompensation is associated with organ failure(s), the so-called acute-on chronic liver failure, and chronic decompensation with progression of liver fibrosis and development of disease specific complications comprise distinct clinical entities with different immunopathology mechanisms. Enhanced bacterial translocation associated with systemic endotoxemia and systemic bacterial infections have substantial impacts in both clinical situations. In this review the authors provide overview of immune dysfunction and its consequences in cirrhosis.

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INTRODUCTION

Cirrhosis is the final stage of chronic liver diseases from any cause and is associated with various levels of immune dysfunction, which are referred to as cirrhosis-associated immune dysfunction syndrome (CAIDS)[1]. Acquired alterations of both the innate and the adaptive immune functions are diverse, encompassing recognition, effector and regulatory mechanisms[2]. Paradoxically, depression and overstimulation exist concurrently in the system, and result in an enhanced susceptibility to acute inflammatory processes and their exaggerated courses, both locally and far from the portal of entry of the microbes or the non-microbial toxic agents. The worst consequence of the imbalance in the pro- and anti-inflammatory processes is the development of acute-on-chronic liver failure (ACLF). Subtle immune dysfunction, however, also favors a shift towards persistence of inflammation leading to progression of liver fibrosis and development of different complications (portal hypertension and hepatic encephalopathy). From a pathogenetic point of view, the predominant mechanisms are different during acute and chronic worsening of liver function in cirrhosis[3]. Enhanced bacterial translocation (BT)[4] associated with systemic endotoxemia and increased occurrence of systemic bacterial infections have substantial impacts on both clinical situations[5]. The other important feature is that the immune status of patients is not constant during the illness, and the extent of the acquired immune dysfunction is related to the severity and etiology of the liver disease. The more severe the liver disease, the more subtle is the immune dysfunction[6]. In the case of an alcoholic etiology, more profound alterations are generally expected[7]. Lastly, in cirrhosis, the clinical effect of functional variations of innate immunity-related genes are more pronounced compared to non-cirrhotic cases because of a pre-existing acquired immune dysfunction with limited compensatory mechanisms.

INNATE IMMUNE DYSFUNCTION

Pattern recognition receptors

Different classes of germ line-encoded pattern recognition receptors (PRRs) recognize invading pathogens, and monitor the extracellular and intracellular compartments of host cells for signs of microbes. Sequential detection of a pathogen by various PRRs in different subcellular compartments is essential and results in activation and the complex interplay of downstream, conserved signaling pathways[8]. PRRs are widely distributed in different forms with various functions all over the human body. They are abundant at the sites of possible entry for pathogenic microorganisms. PRRs are anchored in innate immune cells as surface or intracellular receptors, and are involved in signaling, resulting in an inflammatory response and subsequent cellular activation. The other type of PRRs includes various soluble receptors that move around freely and are considered as functional ancestors of the immunoglobulins (Ig). They act as phagocytic receptors, mediating direct non-opsonic uptake of pathogenic microbes and/or their products. On the basis of their function, scavenger receptors (SR), which are cell membrane-bound PRRs, also belong to this latter group[8]. These molecules recognize conserved structures, designated pathogen-associated molecular patterns (PAMPs), on microbes. Many of these molecules are present in commensals and opportunistic pathogens (MAMPs, microbial-associated molecular patterns)[9]. Moreover, PRRs interact not only with exogenous microbial molecules, but also with endogenous structures. Damaged or stressed cells that pose a "danger" to self-tissues are recognized through danger (or damage)-associated molecular patterns (DAMPs)[10]. A multifaceted interplay of different PRRs results in a complex spectrum of pro- and anti-inflammatory, immunogenic and suppressive responses induced within the host.

Altered expression and function of the PRRs are well-known features of cirrhosis. Of the acquired alterations in toll-like receptors (TLRs), PRRs are the most extensively studied and are reported to have a substantial impact on the pathogenesis and evolution of the disease[11,12]. Recently, interesting data has been revealed about other PRRs, such as the cluster of differentiation 14 (CD14)[13], macrophage SR, soluble(s) CD163[14], or C-type lectin receptors[15].

Altered TLR expression and functions

A wide range of TLRs is expressed to various extents in the liver parenchymal and non-parenchymal cells[12,16,17]. Acquired alterations in TLR signaling pathway have a major influence on the development of the disease and have been extensively studied in cirrhosis[18]. Previous experimental studies on animals, mostly rodent models of liver fibrosis mimicking different etiologies of chronic liver diseases (CLD)[17,19] and models with knock-outs of certain members of cell signaling molecules, delineated the most relevant signaling routes involved in the pathogenesis of fibrosis, namely the TLR2, 4 and 9 pathways[20]. TLR2 and TLR9 recognize their ligands, di- and triacyl lipoproteins and unmethylated CpG-DNA, respectively, while the lipid A component of lipopolysaccharide (LPS) triggers the activation of TLR4[11,12,21]. The above mentioned animal models also highlighted that hepatic stellate cells (HSCs) are the ultimate effectors of TLR ligand-mediated fibrogenesis in the liver[22] and that maintenance of liver homeostasis depends upon the summation of pro- and anti-fibrotic effects of various immune cells on HSCs. Profibrotic immune cells, like M1 macrophages, neutrophils, T helper (Th) 17 cells, $CD8^+$ T cells and nature killer T (NKT) cells, promote fibrosis *via* secretion

of proinflammatory cytokines and mediators activating HSCs, while secretion of interleukin (IL)-10 and IL-22, interferon gamma (IFNγ), tumor necrosis factor related apoptosis inducing ligand (TRAIL), and direct killing of HSCs by anti-fibrotic immune cells (M2 macrophages, $CD11b^+Gr1^+$ bone marrow cells, regulatory T cells (Treg), Th17 cells, NK cells and NKT cells) can negatively regulate HSCs. Interestingly, macrophages, NKT cells, Th17 cells and dendritic cells seem to possess dual functions in this regard[23]. Thus, NK cell-mediated elimination of activated HSCs is a key component of maintaining liver homeostasis and preventing fibrogenesis, principally in the early stages of liver fibrosis[24,25].

Changes in TLR signaling pathways are caused by the prolonged exposure to intestine-derived bacterial products (LPS, unmethylated CpG containing DNA and lipoteichoic acid), foreign toxic agents (ethanol and acetaldehyde derived adducts) and also damaged hepatocyte-derived endogenous TLR ligands[26], which are well-established components of CAIDS[1]. Intestinal bacterial overgrowth, altered composition of the gut microbiome, bowel dysmotility, impaired local intestinal mucosal immunity and multifactorial disruption of the intestinal mucosa barrier (increased oxidative stress, mucosal edema and consequential mucosal structural changes causing an enhanced intestinal permeability) ,together result in pathological BT in cirrhosis[4,27]. Moreover, the decreased capacity of the liver to filter these bacterial products by hepatic resident macrophages [Kuppfer cells (KC)] and reduced LPS scavenging capacity of albumin caused by oxidization[28] and low levels of high density lipoprotein (HDL) and apolipoprotein A- I [29], further assist the elevation of the above-mentioned, potentially immunogenic bacterial products in the systemic circulation. Attenuation or complete inhibition of LPS/TLR4 pathways by either intestinal decontamination (administration of a non-absorbable antibiotic, rifaximin) or the use of TLR4 mutant mice showed, significant reduction of HSC activation, angiogenesis, portal hypertension and fibrosis[30].

Changes in TLR expression in response to acute or chronic stimuli are shown by parenchymal and non-parenchymal hepatic cells, as well as peripheral blood mononuclear cells (PBMCs). Although LPS and other TLR ligands can activate different signaling pathways in various cell types (immune and non-immune), promoting a proinflammatory and profibrogenic cascade in acute circumstances, anti-inflammatory and anti-fibrogenic mechanisms are present concurrently to balance these processes and maintain liver homeostasis and immunotolerance. The phenomenon of LPS hyporesponsiveness or LPS tolerance has been observed in monocytes, KCs and liver sinusoidal endothelial cells (LSEC) in response to repetitive stimulation with low dose of LPS. LPS tolerance accompanied by reduced nuclear translocation of nuclear factor (NF)-κB is caused by alterations in the TLR-4 signaling pathway. In LSECs, this process is associated with surface expression of CD54 or other leukocyte adhesion molecules and chemokines [*e.g.*, monocyte chemotactic protein-1 (MCP-1)], while in rest of the above-mentioned cell populations it is associated with decreased TLR-4 expression[31].

Functional impairment of TLR2 and TLR4, the most important PRRs for bacterial recognition, caused by sustained LPS exposure, appears to play a significant role in the risk of infection in cirrhotic patients[32]. Studies on PBMCs collected from patients with cirrhosis clearly showed that there was dampened TLR2 function, even in the early stage of cirrhosis[33,34]. Moreover, at least in advanced cirrhosis, TLR4 impairment was also present[33,35-38], where TLR function was assessed by TNF-α production in culture. Antibiotic or probiotic treatment was able to relieve the TLR disruption by increasing TLR4 levels and restoring receptor function[35,38]. It must be noted, however, that there are also some contradictory results, probably reflecting the heterogeneity of the patient population and methodological differences. Decreased TLR levels are not sufficient to alter the TLR function, which also suggests probable intracellular dysfunction[32].

Functional polymorphisms of PRR

Inherited variations of PRR gene functions have proven to underlie the risk of infection in cirrhosis. In a prospective study by Nischalke *et al*[39], a TLR2 GT microsatellite polymorphism and nucleotide-binding oligomerization domain (NOD) 2 variants were independent predictors of spontaneous bacterial peritonitis (SBP) (OR = 3.8, $P = 0.002$ and OR = 3.3, $P = 0.011$, respectively) in a multivariate analysis. Both the NOD2 variants[40] and the TLR2 microsatellite polymorphism[41] were associated with reduced levels of NF-κβ activation, suggesting a signaling defect *in vitro* and decreased release of pro-inflammatory cytokines, such as TNF-α, IL-12, IL-6 upon *in vitro* stimulation with bacterial lysates. Additionally, in a study by Bruns *et al*[42], patients carrying the *TLR2* polymorphism Arg753Gln (the GA genotype) had SBP more often than patients with the GG genotype (55.6% *vs* 18.2%, $P = 0.019$).

Genetic immune defects could also contribute to the high risk of systemic bacterial infections in cirrhosis beyond SBP. In a retrospective Spanish study[43], patients with ascites carrying the *TLR4* D299G polymorphism showed a trend towards a higher incidence of history of bacterial infections and a significantly higher number of infections per patient than wild-type patients. This single SNP has been shown to change the ligand-binding site of the receptor, because it is located close to the TLR-4-MD-2 binding areas[44] and is associated with blunted physiological response to LPS[45]. However, the functional impact of *TLR4* (D299G) polymorphisms on the LPS-induced cytokine response is controversial[46-48]. Mannose-binding lectin deficiency (MBL)[15] and haptoglobin (Hp) polymorphism type 1-1[49] have been found to confer a higher risk of systemic bacterial infections in patients with cirrhosis (OR = 2.14, $P = 0.04$ and OR = 2.74, $P = 0.015$, respectively) independently of disease severity. MBL, belonging to C-type lectin family, recog-

nizes surface carbohydrate sequences of a wide range of pathogens and stimulates direct opsonophagocytosis *via* the lectin pathway of the complement system. In case of MBL deficiency, both the recognition and the eradication of the pathogens are impaired. Hp is an acute phase plasma protein. Three phenotypes of the molecule exist, each with biologically important differences in their antioxidant, scavenging and immunomodulatory properties. These differences influence the course of subsequent inflammatory diseases. Hp1-1 has a weaker bacteriostatic effect than Hp2-2 and potentiates a Th2 immune response, thus predisposing subjects with Hp1-1 to develop bacterial infections. There is also a link between Hp polymorphisms and the body's iron store. Excessive iron accumulation has an adverse effect on immunity. Iron overload seems to exert a subtle effect on the immune system by altering the proliferation of T and B-lymphocytes. Furthermore, bacteria utilize the iron of the host organism as an important nutrient[50-52].

Though all the above-mentioned host genetic factors associated with significant ORs suggest an important role of single nucleotide polymorphisms (SNPs) in determining infection risk, a key question remaining is how these markers could be utilized in these clinical settings. Several points are worth considering. First, the frequencies of these polymorphisms in the population are relatively low (around 10%), limiting their efficacy as predictors. Second, ethnic and geographical differences in these functional polymorphisms exist. For example, the occurrence of the NOD2 risk alleles is highest in central Europe, but is absent from certain non-Caucasian populations, thus preventing their universal application[53]. One study[39] showed that the combination of the markers (simultaneous presence of both genetic variants, TLR2 GT microsatellite polymorphism and NOD2 risk variant) specifically improved the identification of patients with a high risk for SBP (OR = 11.3, $P < 0.001$). ORs of single clinical factors or laboratory markers were indeed inferior to ORs obtained using SNPs related to host immunity. In contrast, disease severity determined by a more complex way, using the Child-Pugh, score was superior to single SNPs to predict the infections, mainly in patients with advanced disease. However, this aspect was rarely examined in the above-mentioned studies. In one of our studies[49], the presence of the advanced disease (Child C) was associated with the highest risk of infection (HR = 4.43, $P < 0.001$) and was at least double the risk value of any other clinical or laboratory marker in a multivariate Cox regression model. The occurrence of the Child C disease stage was 29% in this population. There is no data regarding the added value of using host genetic risk factors to assess infection risk in combination with Child-Pugh stages. In earlier stages of the disease, combination of clinical score with genetic markers more likely enhances the risk assessment of the infections than in advanced stage of the disease. This approach could help to optimize patient care by identifying a high-risk population in which prophylactic antibiotic treatment might prevent SBP and other systemic infections and, therefore, mitigate the acute and chronic progression of the disease and prolong survival.

Functional genetic variations of PRRs associated with stronger pro-inflammatory response, however, might pave the way to progression from the chronic inflammatory state to the definite breakdown of the liver tissue, resulting in the development of cirrhosis. Support for this concept comes from the study of Brun *et al*[13]. The authors reported enhanced progression of fatty liver disease according to -159C/T promoter polymorphism in the CD14 gene. This polymorphism was proved to influence the transcriptional activity, thus determining the expression level of CD14. Subjects carrying the TT genotype had the most prominent elevation in CD14[54] and TNF-α[13] levels. As previously mentioned, several hepatic cell populations involved in liver damage and fibrogenesis can directly respond to LPS. Thus, increased CD14 expression in patients carrying the TT genotype might enhance their sensitivity to intestinal LPS and so augment the pro-inflammatory responses and disease progression in obese subjects. Accordingly, the TT genotype distribution was significantly higher in non-alcoholic steatohepatitis (NASH) patients than in control subjects or non-alcoholic fatty liver disease patients[13]. In patients with chronic hepatitis C infection, the-399T/I *TLR4* polymorphism was one of seven SNPs that may predict the risk of cirrhosis (for CC genotype: OR = 3.11, $P < 0.001$), supposedly related to its functional impact on the LPS-induced cytokine response[55].

MONOCYTES

Impaired monocyte function, including defects in chemotaxis, superoxide generation[56], phagocytosis and killing activity, as well as a decrease in the production of lysosomal enzymes, are well-known components of CAIDS[57-59]. Numerous studies have investigated the role of monocytes in liver inflammation and fibrosis extensively[60-63], along with their indispensable involvement in "cirrhosis associated immunological dissonance"[37] and its clinical manifestation of increased susceptibility to bacterial infections or in ACLF. Zimmermann *et al*[61] found a significant increase in circulating monocytes, with a shift towards non-classical $CD14^{+}CD16^{++}$ monocyte subset in CLD patients. This non-classical monocyte subset possesses pro-inflammatory and pro-fibrogenic potentials; moreover, they express higher levels of CXCR3, MHC-Ⅱ (HLA-DR), FcγRⅡ and IL-2R (CD25) than the classical $CD14^{++}CD16^{-}$ monocyte subset[62,63]. Chemokine-mediated recruitment, accumulation and activation of $CD14^{+}CD16^{++}$ cells in the liver, along with consequent direct HSC activation, also contribute to the ongoing fibrogenetic processes[61-63]. Novel findings from Seidler *et al*[64] indicated that sIL-2R (sCD25) might be a potential biomarker of immune cells', especially $CD14^{+}CD16^{++}$ monocytes', activation in CLD. Independently of the underlying etiology, significantly elevated serum sIL-2R lev-

els were observed in established cirrhosis compared with controls and non-cirrhotic patients. sIL-2R levels were also correlated positively with total monocyte counts and subsets or non-invasive markers of fibrosis, and were inversely correlated with parameters reflecting the biosynthetic capacity of the liver. It should be noted that sIL-2R levels are influenced by renal function. Monocytes from ascitic patients with alcoholic cirrhosis, especially a subgroup with elevated LBP levels indicating enhanced BT, showed higher expressions of TNF-α, HLA-DR and CD80. Norfloxacin treatment *via* intestinal decontamination, and the consequential decrease of circulating bacteria and bacterial products, could normalize the number of circulating monocytes along with reduction of TNF-α expression and activated phenotype in these patients[65]. Intestinal decontamination with antibiotics, therefore, should be considered as a therapeutic weapon in restoring immune status and monocyte function in cirrhosis[66].

In contrast, functional monocyte deactivation, a phenomenon similar to *in vitro* LPS tolerance, is also described in patients with Child C cirrhosis and ACLF[36,37,67,68]. This phenomenon is presented as "immune paralysis" in the literature and is defined as downregulation of HLA-DR expression on monocytes. The etiological factor of "immune paralysis" was proven to be chronic endotoxemia by Lin *et al*[37]. Serum LPS levels correlated inversely with HLA-DR expression and positively with serum IL-10 levels, an anti-inflammatory cytokine. Supporting this observation, *in vitro* stimulation with LPS was able to suppress HLA-DR expression in monocytes derived from healthy volunteers in an IL-10-dependent manner. Monocytes from cirrhotic patients expressing low levels of HLA-DR showed a decreased ability to secrete TNF-α, accompanied by decreased expression of inducible nitric oxide synthase (iNOS) and co-stimulatory molecules (CD40, CD86). Furthermore, reduction in HLA-DR expression (< 40%) was associated with poor outcome in patients with ACLF[36,67], especially if monocytes were unable to show improvement in HLA-DR expression. The overall prognostic power, however, remains inferior to conventional markers. The sensitivity and specificity of reduced HLA-DR expression (< 40%) to predict the 90-d mortality were 59% and 80%, respectively[69]. In conclusion, "immune paralysis" is characterized by dominance of anti-inflammatory (elevated serum IL-6 and IL-10 levels) and suppression of pro-inflammatory processes (decreased TNF-α levels)[36,37,67,68]. In sepsis patients with reduced monocyte HLA-DR expression, the function of these cells could be restored with immunomodulatory agents like granulocyte-monocyte colony-stimulating factor (GM-CSF) and IFN-γ, thus their effect on monocyte function should be investigated in cirrhosis and ACLF[67].

MACROPHAGES

The resident macrophages in the liver are the KCs and account for approximately 80% of all macrophages in the body[70]. At the same time, KCs are the second most abundant non-parenchymal cell type populating liver tissue after LSEC[11]. Three major pathogenetic roles of KCs are relevant to cirrhosis: (1) as the main orchestrating immune cells in the liver; the KCs and their cross talk with HSCs, the ultimate effectors of fibrogenesis in the liver, are in the focus of attention for understanding fibrogenetic mechanisms. Activation of KCs by PAMPs or DAMPs *via* PRR signaling pathways results in activation of HSCs and recruitment of phagocytic cells through secretion of proinflammatory cytokines, chemokines (*i.e.*, MCP-1) and upregulation of adhesion molecules, thus contributing to fibrogenetic processes[71]; (2) in addition, activated KCs, along with recruited bone marrow (BM)-derived macrophages through production of vasoconstrictor agents like thromboxane A2 (TXA_2), seem to increase portal pressure in normal and in fibrotic animal models[72]. This concept is supported by recently published studies, which found a strong correlation between sCD163, a biomarker of macrophage activation, and the hepatic venous pressure gradient (HVPG)[14,73-75]; and (3) additionally, the deficient phagocytic capacity of KCs in advanced cirrhosis can also eventually lead to decreased elimination of blood-borne pathogens and mainly intestine-derived bacterial products, thereby contributing to an increased risk of bacterial infection[76].

Recently, CD163 has been proposed to be a specific marker of monocyte/macrophage cell populations[77]. The utility of this SR is not yet fully understood, but it supposedly functions as an innate immune sensor for bacteria[78] and has an essential role in the inflammatory processes. During the local activation of macrophages, the extracellular portion of CD163 is cleaved by metalloproteinases and enters the circulation as a soluble protein (sCD163)[79]. It is now evident that sCD163 is very useful as a biomarker of macrophage activation in various inflammatory diseases, as well as in chronic liver diseases. An elevated sCD163 level is related to portal hypertension, indicated by HVPG value in patients with cirrhosis[75]. In a very recent prospective clinical study by Waidmann *et al*[14] high sCD163 levels were shown to be associated with both the development of variceal bleeding and mortality in cirrhosis, independently of endoscopic risk factors and the disease severity, respectively.

MCP-1 is one of the most potent chemokines for monocytes/macrophages and activated lymphocytes during infections[80]. MCP-1 also plays a role in the recruitment and maintenance of the inflammatory infiltrate during liver injury[81]. Similar to *PRR* genes, a functional polymorphism of *MCP-1* gene (-2518 G/A) can also influence both the risk of bacterial infections and hepatic inflammation and fibrosis progression. In a small study by Gäbele *et al*[82], the 2518 MCP-1 genotype AA was identified as a risk factor for SBP in patients with alcoholic cirrhosis, supposedly caused by reduced MCP-1 protein level in ascites. Evaluating HCV patients, Mühlbauer *et al*[83] reported that carriers of the G allele were significantly more frequent among patients with more

advanced fibrosis and severe inflammation. In support of this, hepatic MCP-1 mRNA levels and cytokine-induced MCP-1 secretion of isolated HSC were significantly higher in patients carrying the G allele. Furthermore, there was a binding activity in nuclear extracts from activated HSCs specifically to the G allele, providing a potential mechanism for the differences observed.

The liver, *inter alia*, functions as a bacterial filter and the sinusoidal KCs play an important role in the elimination of intestinal bacteria and endotoxins translocated from the intestine. Patients with cirrhosis have impaired function of the reticuloendothelial system (RES), along with a decrease in the number and function of KCs[76,84]. Additionally, because of the formation of collateral circulation, a certain proportion of the blood-volume bypasses the liver, reaching the systemic circulation directly. Although limited data is available regarding RES dysfunction, Rimola *et al*[76] found that patients with decreased RES phagocytic activity developed bacterial infections more frequently compared with patients with normal RES function. Dysfunction of KCs was also proven in new studies with superparamagnetic iron oxide-magnetic resonance image (SPIO-MRI) in NASH and cirrhosis[85,86]. Furthermore, impairment of Fcγ-receptor function and consequential decrease in clearance capacity in macrophages also contributes to an increased incidence of bacterial infections in cirrhosis[87].

NEUTROPHILS

Polymorphonuclear leukocytes (PMNs) are present in a fully activated state in the peripheral blood in cirrhosis and alcoholic hepatitis, possibly because of sustained exposure to bacterial products, such as endotoxins[88]. This results in an energy depleted status of the PMNs, which have an inability to function properly (decreased chemotaxis, phagocytosis and bactericidal capacity)[66,89,90]. Removal of endotoxins *in vitro*[91] as well as attenuation of endotoxemia *in vivo* with probiotic[38] treatment can restore PMN function in cirrhosis, further supporting this hypothesis. Increased priming[92] and therefore "ready to act" status of PMNs is indicated by decreased L-selectin levels, overexpression of hydrogen peroxide and increased levels of neutrophil elastase)[93]. As a result of this preparedness to defeat bacteria and PMN activation with high resting respiratory burst activity[94], there is an elevation in harmful reactive oxygen species (ROS) in the circulation and the PMNs' microenvironment, establishing a platform for further potential cell and tissue injury. Necessarily, PMNs become energy depleted and unable to respond properly to further bacterial stimuli with phagocytosis[66,89]. Impaired tuftsin activity[95], hyponatremia and hyperammonemia[96,97], along with inadequate generation of superoxide anion caused by deficient phospholipase C (PLC) activity[98], all contribute to the aforementioned decrease of PMNs' phagocytic capacity. Elevated resting oxidative burst and the decreased phagocytic capacity appeared to correlate with the rate of infections and mortality[91]. These alterations can be restored *in vitro* by endotoxin removal[91] or GM-CSF incubation[99]. Analogous to other innate immune cells, dichotomy in PMN function (hyperactivity then dysfunction) manifests in different ways and contributes to the pathogenic processes in the distinct stages of cirrhosis. Recruitment of hyperactive PMNs to the liver can contribute to fibrogenesis, while exhausted PMNs defective in chemoattraction, enhanced adhesion to endothelial cells and deficient migration in later stage of cirrhosis can result in deficient influx into infected sites[90,100]. An *in vitro* study in cirrhotic patients demonstrated that G-CSF could enhance neutrophil transendothelial migration, despite having no effect on enhanced neutrophil adhesion[100]. Notably, in a randomized clinical trial, administration of G-CSF improved survival of patients with ACLF, partially through restoring PMN dysfunction. Though the exact mechanism of G-CSF improvement of PMN function has not yet been determined, increases in PMN surface antigen CD11b/CD18 expression, along with elevated plasma elastase-α1AT complex levels, were previously detected following G-CSF administration[101]. Apart from various functional impairments of PMNs, a decrease in cell volume as a result of hyponatremia and hyperammonemia[96,97] with reduced cell number (neutropenia) as a consequence of hypersplenia and shortened neutrophil survival *via* apoptosis[102], are also known features of CAIDS. The epidemiology[103], pathogenesis and clinical consequences of cirrhosis-associated neutropenia were reviewed in a recent publication by Kalambokis *et al*[104].

Genetically determined enhanced myeloperoxidase (MPO) activity caused by an SNP in the promoter region of the enzyme (G-463-A MPO polymorphism) in patients with GG-MPO genotype was found to be independently associated with increased risk of hepatocellular carcinoma (HCC) and liver-related death with or without HCC in alcoholic cirrhosis (HR = 4.7 and 3.6, respectively, $P < 0.001$ for both)[105]. Activated KCs and liver-infiltrating neutrophils release MPO into the extracellular space and mediate oxidative processes by hypochlorus acid[106].

COMPLEMENT SYSTEM

Low opsonic activity and decreased complement levels, mainly C3, weaken the bacterial recognition and bactericidal capacity in cirrhosis[107,108], further contributing to an increased susceptibility to bacterial infections. One interesting feature of bacterial infections in the cirrhotic patient population is the extreme sensitivity to *Pneumococcus* pneumonia and the high mortality. The defect in early bactericidal activity of alveolar lining components (reduced levels of lysozyme and complement C3) is a probable explanation[109]. Overall, bacterial pneumonias are the third most frequent infections in cirrhosis, and comprise 15% of all systemic infections. In addition, the mortality rate of pneumonia is much higher than

that in any non-cirrhotic population[110]. Data concerning alterations of the lectin pathway of the complement system and their effect on the susceptibility to bacterial infection are scarce. Our group reported that MBL levels were significantly reduced in patients with the most advanced stages of cirrhosis and absolute MBL deficiency (< 100 ng/mL) was associated with higher probability and shorter time to develop bacterial infections in cirrhosis[15]. MBL antigen levels in the sera, estimated by a mannan-binding assay or complement activation in the C4b deposition assay, accurately indicated the function[111]. The serum levels of functional MBL also correlate well with underlying MBL2 genotypes. In this regard, other components of this third arm of the complement system (ficolins or MBL-associated serine protease-2) have not yet been studied.

ADAPTIVE IMMUNE DYSFUNCTION

B-cells and immunoglobulins

A broad defect of B-cells in patients with ALD and its association with the exposure to circulating antigens as a consequence of shunting, or KC abnormality, or both, has been known for a long time[112]. A very recent study of Doi *et al*[113] revealed novel information about the nature of the profound abnormalities in peripheral B-cell phenotype and function. B-cell dysfunction strongly implicated hepatic fibrosis and/or portal hypertension in the development of this phenotype, and it was independent of the etiology of the cirrhosis. Moreover, this study highlighted how these B-cell defects could explain, in part, the vaccine hyporesponsiveness and susceptibility to bacterial infection in this population. B-cell phenotypes were assessed by flow cytometry. $CD27^+$ memory B-cells and, more specifically, $CD27^+IgM^+$ B cells, were found to be markedly less frequent in cirrhotic patients. The frequency of $CD27^+/CD19^+$ B cells strongly correlated with several laboratory parameters related to progressive liver disease. Previously, peripheral B-cell CD27 expression was reported to be related directly to the capacity of B-cells activation by CD40 plus TLR9 ligation[114]. Accordingly, using isolated peripheral blood cells, the authors proved that B-cells were hyporesponsive to CD40/TLR9 activation, indicated by significantly reduced CD70 upregulation, less TNF-β secretion and IgG production. The allostimulatory capacity of cirrhotic B-cells on $CD4^+$ T-cell proliferation was also diminished. The presence of bacterial products in the circulation playing fundamental roles in driving B-cell changes in cirrhosis has been proposed. Soluble factors associated with BT, such as LPS[115,116] and bacterial DNA[117], can often be detected in cirrhotic plasma and are capable of activating B-cells *in vitro*. As a proof, Doi *et al*[113] found that blockade of TLR4 and TLR9 signaling abrogated the activation of healthy donor B-cells by cirrhotic plasma. The fate of lost $CD27^+$ B-cells remains incompletely defined.

Stimulation of B-cells by TLR ligands can lead to polyclonal activation and Ig production. Notably, in humans, TLR-2, TLR-4 and TLR-8 are expressed strongly by monocytes/macrophages, but are expressed poorly by B-cells. In contrast, TLR-7 and TLR-9 are expressed mainly by B lymphocytes and plasmacytoid dendritic cells[118,119]. In cirrhosis, there is an enhanced serum IgA level, mainly in those with an etiology of ALD. However, the mechanisms leading to the increase of IgA levels are not fully understood[120]. Previously, it was attributed, at least partially, to a defective clearance of IgA and IgA-immune complexes *via* altered monocytes, Fc receptor expression, and subsequent defective Fcα receptor-triggered endocytosis[121]. For a long while, it was hypothesized that the increase in Ig synthesis in alcoholic cirrhosis might be associated with bacterial stimulation[112]. Several reports now support this hypothesis. Massonnet *et al*[122] found significantly enhanced absolute IgA production by TLR-9 ligand CpG-activated B-cells in alcoholic cirrhosis compared to healthy subjects, which correlated with their intrinsic ability to produce spontaneously more IgA than healthy subjects. Relative TLR-9 ligand CpG-induced IgA production by purified B-cells from alcoholic cirrhotic patients was, however, less prominent, which corresponded to the lower TLR-9 expression on their B-cells compared to B-cells from healthy subjects. Such downregulation of TLR-9 expression by B-cells has been reported after *in vitro* CpG treatment, suggesting that the decrease in TLR-9 expression by B-cells from patients suffering in alcoholic cirrhosis could reflect *in vivo* priming by bacterial DNA during sustained BT[123].

Concerning IgA production, cirrhosis has another characteristic feature, namely the increased occurrence of various antibodies against gut bacterial proteins[97,124-127] or host proteins having cross-reactive epitopes with bacterial constituents[120,128,129] in the sera of the patients. These specific antibodies are present mainly in those patients with advanced diseases and portal hypertension. Moreover, positivity for anti-*Saccharomyces cerevisiae* antibody (ASCA)[97] was an independent risk factor for the development of clinically significant bacterial infections (OR = 1.63, $P = 0.018$). Similarly, presence of anti-neutrophil cytoplasmic antibody (ANCA) IgA was identified as an independent predictor for a shorter time to develop an infectious complication in multivariate Cox-regression analysis (HR = 1.74, $P = 0.006$), suggesting that serological response to various microbial components might be the consequence of sustained exposure to microbial antigens[129]. In non-vasculitic disorders, the presence of ANCA has been considered a sign of immunological response to enteric bacterial antigens[130,131]. Pathogen-induced inflammation might result in enhanced presentation of self-antigens because of molecular mimicry and the known pathogenic feature of *Helicobacter pylori*-associated human autoimmune gastritis[132]. In autoimmune liver disorders, atypical perinuclear-ANCA (atypical P-ANCA) has been reported to be directed against human β tubulin isotype-5 (TBB-5)

and cross-react with the bacterial protein FtsZ because of their extraordinarily high structural homology[133]. In the development of the enhanced IgA production, not only systemic overproduction, but also a contribution by the gut mucosal compartment is very probable. The composition and extent of the bacterial load in the gut have a very clear effect on IgA production. Sustained exposure to bacterial antigens during BT derived from the mucosal compartment might play a central role in the enhanced IgA class antibody formation in cirrhosis. Determination of the ratio of IgA1 and IgA2 subtypes, and detection of the secretory component (SC) on IgA molecules in sera, can help identify the location of antibody formation (bone marrow or mucosal compartment). An increase in the proportion of IgA2 subtype and the presence of SC are concurrently considered as confirmatory evidence for the mucosal origin of IgA secretion[134,135]. The proportion of IgA2 is about 10% of total IgA in human sera, while IgA1 is 90%, and they largely exist in the monomeric(m) form. The proportion of SC-containing IgA antibodies from the total IgA pool is no more than 1%, because SC is attached to di- or polymeric IgA (pIgA) *via* its transport through the epithelial cells into the gut lumen or to other mucosal surfaces[136].

Thus, in a recent work by our group[129], a detailed characterization of IgA type ANCAs revealed that the proportion of the ANCA IgA2 subtype was markedly elevated (46%), and SCs were present in the majority of ANCA IgA positive samples (87%) of our patients with cirrhosis. Moreover, high levels of total serum sIgA in alcoholic cirrhosis were reported in a study by Pelletier *et al*[137]. Both studies support significant gut involvement in IgA production. IgA has traditionally been regarded as a non-inflammatory antibody. Serum IgA, however, potently triggers (pro)-inflammatory activity upon binding to the myeloid IgA receptor, FcαRI[138]. Whether the elevated IgA has any harmful effect on disease progression remains to be determined. Parallel to specific IgA overproduction, there is a diminished IgG production. The more severe the liver disease, the more subtle the decrease in the specific IgG level in patients with cirrhosis[129]. The alcoholic etiology has an obvious negative impact on specific IgG production. These alterations in the ANCA IgA and IgG response clearly reflect those tendencies known from vaccination studies in this patient population, and presumably reflect the impaired adaptive immune system in cirrhosis, mainly in the advanced stage, and the direct inhibitory effect of alcohol on T-cell-mediated immunity[66]. After pneumococcal vaccination, anti-PPS (pneumococcal polysaccharide) IgA antibody levels were significantly higher than in control subjects, whereas IgG levels were reduced[139]. Considerably lower immunogenicity and faster decline of specific, protective IgG responses were reported in individuals with cirrhosis, particularly in the alcohol-induced form, after hepatitis B vaccination compared with CLD[140]. Patients with compensated cirrhosis were five times more likely to respond to hepatitis A vaccination compared with cirrhotic patients in the decompensated stage[141].

T cells

Different T cell populations could possess either pro-, anti-fibrogenetic or dual properties regarding their relationship with HSCs. Elevated numbers of $CD8^+$ T cells and the consequential decrease in the $CD4^+/CD8^+$ ratio was associated with promotion of fibrogenetic processes in mice and humans. IL-17 producing $CD4^+$ T cells (Th17), along with NKT cells, seemed to be involved in fibrosis; however, their role in fibrogenesis is cytokine profile-dependent. Production of IL-17, IL-4 and IL-13 is somewhat pro-fibrogenetic, while secretion of IFN-γ, TRAIL and IL-22 is anti-fibrogenetic. In contrast, regulatory T cells ($CD4^+$ $CD25^+$ forkhead box P3 [FoxP3]) in the close vicinity of HSCs *via* secretion of IL-10 represent anti-fibrogenetic properties[23].

Similar to the changes in B-cell function, broad defects of T cells were also reported in an early publication of Nouri-Aria *et al*[112]. A recent study by Márquez *et al*[142] depicted an intensive derangement of T cell compartments of the immune system in patients with cirrhosis. High antigen load as a consequence of enhanced BT, indicated by elevated LBP levels, can contribute to prolonged activation and subsequent "exhaustion" of T lymphocytes. Significant reduction in the total number of peripheral blood T cells ($CD3^+$ cells) was observed in cirrhotic patients with ascites. The proportions of activated $CD4^+$ T cells (indicated by expression of CD25 and CD122 antigens) and senescence $CD8^+$ T cells ($CD8^+CD45RO^+CD57^+$ cells) significantly increased. Additionally, the proportion of memory $CD4^+$ and $CD8^+$ populations expressing apoptosis markers ($CD95^+$) was also higher in cirrhotic patients compared with healthy controls. Increased proportion of regulatory T cells [$CD4^+$ $CD25^+$ forkhead box P3 (FoxP3)] was also observed, and a significant correlation was found with LBP levels. Downregulation of lymphocyte co-stimulatory molecules, such as CD28, was also detected. Therefore, it can be speculated that these changes in adaptive immunity could play a role in the immunosupression seen in cirrhosis, leading to increased susceptibility to bacterial infections.

RISK ASSESSMENT OF CIRRHOSIS RELATED BACTERIAL INFECTIONS IN THE CLINICAL PRACTICE

Standard clinical factors, and serological and genetic markers associated with immune dysfunction in cirrhosis all have their potentials, but they also have limitations to predict bacterial translocation, infections and disease progression in cirrhosis. The biological pathways involved in these processes, however, are multiple. It is most likely that these markers will be used for effective

risk assessment in combination, providing complementary information, rather than used singly. Clinical factors are easily accessible without cost, but may change during the long natural history and in certain cases are subjective, suffering from recall bias and inaccuracy. Laboratory tests have several advantages over clinical factors, such as objectivity, consistency during the disease course (for serological markers only in definite clinical circumstances) and higher odds ratio. However, they are not always widely available, and their costs could represent a drawback. Prospective clinical studies must be initiated to build up and validate composite score (CS) for risk assessment covering clinical factors and biomarkers.

ACLF

ACLF is an increasingly recognized entity encompassing an acute deterioration of liver function in patients with cirrhosis, which is usually associated with a hepatic or extrahepatic precipitating event and results in the failure of one or more organs and has high short-term mortality. During evolution of cirrhosis, this condition comprises a distinct clinical entity from acute decompensation (AD)[3]. The recently published CANONIC study[142] established diagnostic criteria for ACLF and provided valuable data about its development and progression. The occurrence of ACLF is not rare, with approximately one-third of AD being associated with ACLF. From the immunological aspect, inappropriate regulation of the host inflammatory response to injury and infection plays an important role in the development of the disease. Exaggerated pro- and anti-inflammatory responses and their imbalance relative to each other are hypothesized to be the most important determinants in the disorder. In cirrhosis, both the systemic inflammatory response and the compensatory anti-inflammatory response (CARS) are more pronounced compared with those in normal subjects. It is likely that those patients that do not resolve the CARS are the ones that have highest mortality rates. The state of unresolved CARS (the so-called prolonged "immunoparalysis" state) may predispose patients to acquire infection that would further aggravate a pro-inflammatory response, resulting in a vicious circle[3,143]. In this acute situation, the presence of bacterial infection and/or enhanced BT trigger quite different processes compared with those relevant to the chronic progression of liver disease. The development of ACLF and multiorgan failure is characterized by significant alteration in systemic and hepatic hemodynamics, and worsening of the liver and the other organs' functions[3].

CONCLUSION

In cirrhosis, the precise exploration of immune dysfunction has resulted in a more accurate understanding of the processes, leading to recognition of the development of complications in both the acute and the chronic progression of the disease. Considering the significant role of BT and bacterial infections in these processes, recognition how the host defense mechanisms are disrupted against invading microorganisms is of distinct clinical relevance. Early and efficient assessment of immune dysfunction using methods routinely available can assist clinicians in everyday practical decision-making when establishing treatment and care strategies for the patients with end-stage liver disease. The biological pathways involved in hepatic fibrogenesis and bacterial infections are multiple, suggesting that this goal can only be achieved by applying combinations of different markers. In the clinical setting, the establishment and validation of a composite score comprising clinical, serological and genetic markers could help to identify efficiently those patients at high-risk for progression and development of bacterial infections, even at an early disease stage. This would therefore lead to a decreased risk of complications, delayed progression of the disease and reduced mortality. Individually tailored steps for prophylaxis will enable clinicians to optimize patient care and expenditure.

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