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REVIEW

Mechanisms of immunity in acutely decompensated cirrhosis and acute-on-chronic liver failure

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Abstract

The identification of systemic inflammation (SI) as a central player in the orchestration of acute-on-chronic liver failure (ACLF) has opened new avenues for the understanding of the pathophysiological mechanisms underlying this disease condition. ACLF, which develops in patients with acute decompensation of cirrhosis, is characterized by single or multiple organ failure and high risk of short-term (28-day) mortality. Its poor outcome is closely associated with the severity of the systemic inflammatory response. In this review, we describe the key features of SI in patients with acutely decompensated cirrhosis and ACLF, including the presence of a high blood white cell count and increased levels of inflammatory mediators in systemic circulation. We also discuss the main triggers (i.e. pathogen- and damage-associated molecular patterns), the cell effectors (i.e. neutrophils, monocytes and lymphocytes), the humoral mediators (acute phase proteins, cytokines, chemokines, growth factors and bioactive lipid mediators) and the factors that influence the systemic inflammatory response that drive organ failure and mortality in ACLF. The role of immunological exhaustion and/or immunoparalysis in the context of exacerbated inflammatory responses that predispose ACLF patients to secondary infections and re-escalation of end-organ dysfunction and mortality are also reviewed. Finally, several new potential immunogenic therapeutic targets are debated.

KEYWORDS
humoral mediators, immune cells, immunosuppression, metabolism, systemic inflammation

Abbreviations: ACLF, acute-on-chronic liver failure; AD, acute decompensation; CAID, cirrhosis-associated immune dysfunction; CRP, C-reactive protein; DAMP, damage-associated molecular pattern; G-CSF, granulocyte colony-stimulating factor; HLA, human leukocyte antigen; IL, interleukin; LT, leukotriene; MERTK, MER receptor tyrosine kinase; PAMP, pathogen-associated molecular pattern; PD1, programmed cell death 1; PG, prostaglandin; PRR, pattern recognition receptors; PUFA, polyunsaturated fatty acid; ROS, radical oxygen species; SI, systemic inflammation; Tim3, T cell immunoglobulin and mucin-domain-containing-3; TLR, toll-like receptor; TNF, tumour necrosis factor; WBC, white blood cell.

1 | SYSTEMIC INFLAMMATION AND IMMUNOSUPPRESSION IN PATIENTS WITH ACUTE DECOMPENSATION OF CIRRHOSIS AND ACUTE-ON-CHRONIC LIVER FAILURE

Liver cirrhosis is a progressive disease with a large spectrum of clinical presentations. In compensated cirrhosis, patients are asymptomatic but once they develop acute decompensation (AD), cirrhosis turns into a systemic disease with multiple complications such as ascites, variceal bleeding, hepatorenal syndrome and bacterial infections. In total, 30%–50% of patients with AD cirrhosis may develop acute-on-chronic liver failure (ACLF) characterized by hepatic and extrahepatic organ failures and high mortality.[1] AD and ACLF are mostly triggered by a precipitating event, of which active alcoholism and bacterial infections are the most common ones.[2]

The immune system is one of the central factors in a very complex network of pathomechanisms.[3] Cirrhosis-associated immune dysfunction (CAID) describes a spectrum of changes in the immune system, ranging from systemic inflammation (SI) to immune deficiency and paralysis.[4] Mild inflammation already exists in compensated cirrhosis[4,5] and it becomes an overarching pathomechanism once AD or ACLF develops.[6] In fact, less than 1% of patients with AD present without signs of SI in comparison to almost 50% of patients with compensated cirrhosis, emphasizing the relevance of inflammation during the process of decompensation.[7] Patients with AD cirrhosis and ACLF display increased white blood cell counts (WBC) and significantly higher serum C-reactive protein (CRP) levels compared to those with AD without ACLF.[8] In addition, patients with ACLF show increased levels of circulating pro-inflammatory cytokines such as interleukin (IL)-6, IL-8 and IL-1 receptor antagonist (IL-1ra), which further worsen with increasing disease severity and higher ACLF grades.[6] The type of precipitating event also defines the inflammatory response (see below) and the pattern of inflammatory markers allows to distinguish between different types of organ dysfunction in ACLF. Finally, ACLF patients present with high levels of intercellular adhesion molecule-1 (ICAM-1), vascular endothelial growth factor, von Willebrand factor and soluble cytokine receptors.[9–12]

The intensity of SI in AD is a valid marker for predicting the development of ACLF and bleeding episodes. Accordingly, a new prognosis score containing CRP (the Padua model) was validated in a bicentric European cohort where it proved to identify AD patients at risk of ACLF.[13] Other well-established scores of disease severity such as the CLIF-C ACLF score also include markers of inflammation such as WBC, reflecting the importance of inflammation in the network of ACLF pathomechanisms.[14] Interestingly, the degree of SI does not depend on the presence of bacterial infections, supporting the notion that the translocation of bacterial products triggers inflammation in the context of ACLF.[5] Besides organ failure, SI can also be linked with fibrogenesis and extracellular matrix (ECM) turnover in cirrhosis.[15,16]

CAID is a dynamic feature. In ACLF, SI coexists together with immune deficiency and paralysis indicated by high levels of anti-inflammatory IL-10.[6] IL-10 inhibits nuclear factor-κB (NF-κB), leading to a reduced secretion of pro-inflammatory cytokines. CAID is also characterized by high expression of MER receptor tyrosine kinase (MERTK), programmed cell death 1 (PD1) and T cell immunoglobulin and mucin-domain-containing-3 (Tim3), which are all known to comprise anti-inflammatory and immunosuppressant properties.[17] Immunosuppression develops as early as in compensated cirrhosis, but it is most prominent in severe and later phases of AD and ACLF.[18] In fact, ACLF mimics a sepsis-like state where ex vivo tumour necrosis factor (TNF) α production and human leukocyte antigen (HLA)-DR expression are severely decreased, while pro-inflammatory IL-6 levels remain high.[8]

Key points

- Acute-on-chronic liver failure (ACLF) is characterized by hepatic and extrahepatic organ failure and high mortality.
- Organ failure and mortality are closely associated with a systemic hyperinflammatory response.
- In ACLF patients, systemic inflammation coexists with persistent immunodeficiency that increases the risk of secondary infections.
- Novel therapies targeting immunosuppression and the hyperactivation of the immune system are currently under consideration.

On a cellular level, CAID involves a complex network of innate and adaptive immune cell subsets, which secrete cytokines and chemoattractants, provide a defence mechanism against invading pathogens, induce tissue injury and modulate the immune response by intercellular crosstalk during the transition from compensated cirrhosis to ACLF.[3,19,20] The current pathomechanistic paradigm for AD cirrhosis and ACLF focuses on the innate immunity and its potential role in mediating disease progression and organ injury. However, there is increasing evidence elaborating on the potential role of the adaptive immune system acting in concert with other immune subsets during disease progression. Therefore, this review aims for shedding light on the recent advances in deciphering the role of immunity in AD and ACLF and also for delineating the complex interaction among the individual components of the immune system. Figure 1 shows a schematic illustration of the main points addressed in this review.

2 | MECHANISMS

2.1 | Pathogen-associated molecular patterns, damage-associated molecular patterns and pattern recognition receptors

Activation of innate and adaptive immune responses requires the presence of danger signalling molecules, which can be classified into pathogen-associated molecular patterns (PAMPs) and

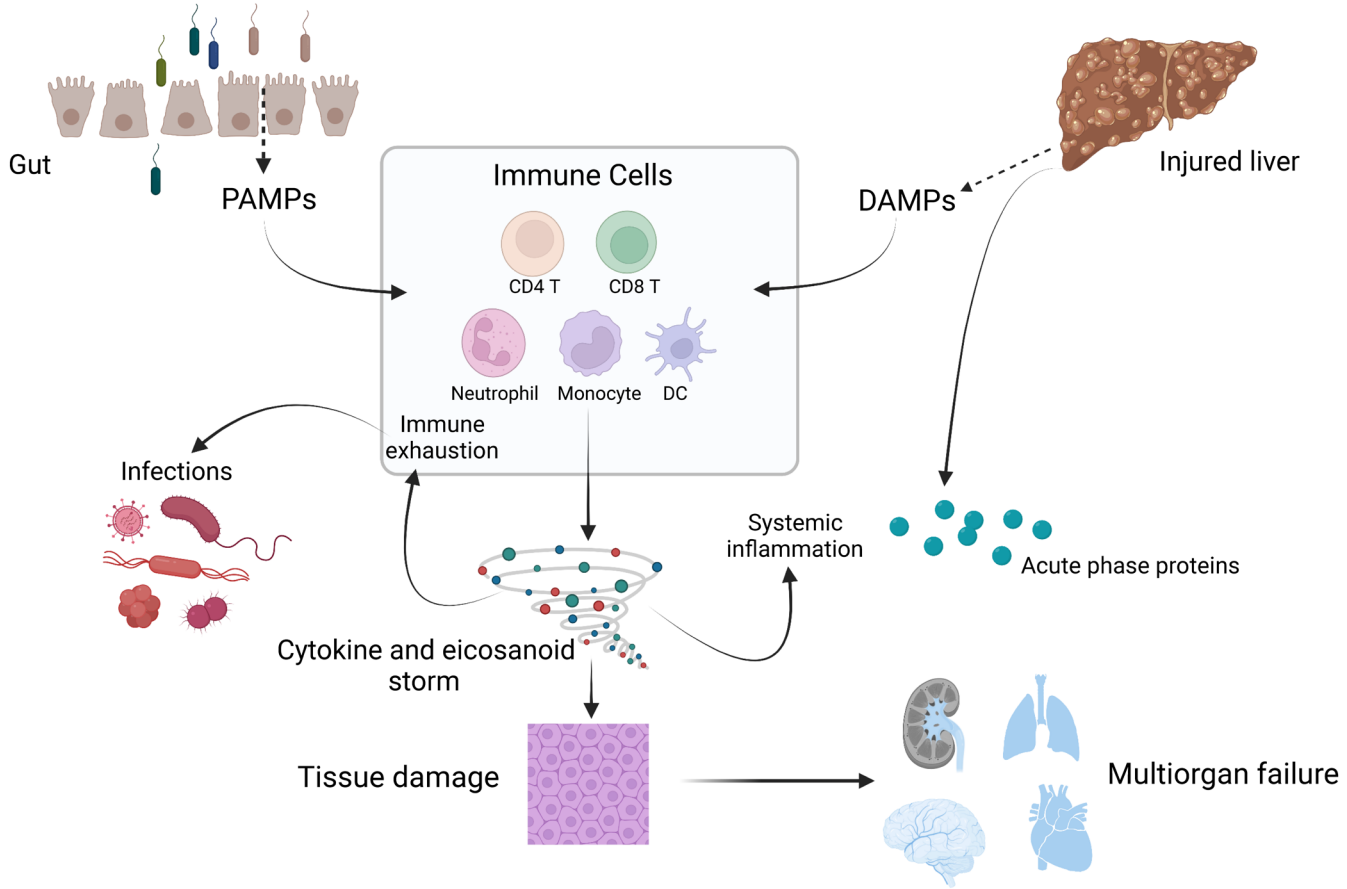

FIGURE 1 Schematic diagram summarizing the role of systemic inflammation in the progression of organ failure in patients with acutely decompensated cirrhosis. In this condition, systemic inflammation is likely due to the translocation of pathogen-associated molecular patterns (PAMPs) in the gut and/or the release of damage-associated molecular patterns (DAMPs) from dead, dying, injured or stressed nonapoptotic liver cells. PAMPs and DAMPs interact with specific receptors present in immune cells, especially monocytes and neutrophils resulting in the massive release of inflammatory cytokines and eicosanoids which cause tissue damage and organ failure. These inflammatory mediators producing systemic inflammation also lead to the release of acute-phase proteins by the liver. The persistence of this hyperinflammatory state induces the exhaustion of immune cells which are unable to properly respond to pathogens leading to impaired host defence and infection. This illustration was created using BioRender.com.

damage-associated molecular patterns (DAMPs). Under healthy conditions, stimulation by and response to PAMPs and DAMPs are well-regulated processes which include a network of immune cell subtypes to ensure a quick resolution of the inflammatory response and to facilitate tissue repair.[4] Generally, inflammation is a physiological response, but in cirrhosis, its intensity is exaggerated, also because its resolution is severely impaired (see below). During decompensation and ACLF, increasing translocation of PAMPs, notably lipopolysaccharide (LPS), from the intestinal lumen into the systemic circulation exceeds the clearance capacity of the local gut-associated lymphoid tissue.[21] The subsequent persistent activation of the immune system surpasses the regulatory capacity leading to chronic inflammation.[4] Moreover, tissue injury releases DAMPs from dead cells and activates local immune cells, which promote SI and further tissue injury by secreting cytokines and chemoattractants.[4]

Circulating DAMPs and PAMPs bind to pattern recognition receptors (PRRs) such as toll-like receptor (TLR) subtypes and nucleotide-binding oligomerization domain-like receptors (NLRs), which are not only located on circulating innate immune cells but also on parenchymal cells.[22–24] There are several subtypes of TLRs and NLRs, which are distinctively specific for individual DAMPs and PAMPs. For example, TLR4, which is expressed on the cell membrane, represents the main PRR sensing LPS, whereas the endosomal TLR9 is activated by bacterial and mitochondrial DNA.[17,23,25] TLR stimulation prompts NF-κB-dependent rapid translation of cytokines and chemoattractants such as IL-6, TNFα and monocyte chemoattractant protein-1 (MCP-1). NLRP3 is besides other NLR subtypes one of the major components of the inflammasome and its activation leads to cleavage of pro-caspase-1 into caspase-1 which in turn promotes the release of IL-1β and IL-18.[3,26] The inflammasome senses multiple different types of DAMPs such as extracellular ATP, cholesterol and high mobility group box 1.[3]

PRR stimulation induces a response not only in circulating innate and adaptive immune cells but also in parenchymal organs such as the liver.[23–25] In fact, there is evidence suggesting that inflammasome activation in the liver is the actual source of circulating cytokines such

as IL-1β. In a rat model of ACLF, cytokines were higher expressed in the liver compared to circulating peripheral blood mononuclear cells (PBMCs). Additionally, blood IL-1α and IL-1β levels increased in liver veins compared to the portal vein, suggesting a net secretion of cytokines in the liver reaching finally the systemic circulation.[27] Cirrhosis seems to act as an organ sensitizer upregulating the expression of PRRs, notably TLR4, thereby enhancing the inflammatory response and cell injury after exposure to endotoxins.[24,28] The elevated PRR expression may be deleterious as it occurs in combination with elevated DAMPs and PAMPs. Accordingly, patients with cirrhosis and bacterial infections have a more than threefold higher likelihood of septic shock and death than patients with the same infections but no liver cirrhosis.[29]

2.2 | The gut-liver axis

The gut-associated lymphoid tissue is one of the largest immunological compartments that function as gatekeepers and first defence to prevent the spread of bacteria and their antigens into the systemic circulation. However, in liver cirrhosis, the gut becomes the major source of circulating PAMPs. The intestinal defence mechanisms are severely disrupted due to a loss of cell–cell contacts (e.g. tight junction proteins such as claudin-1 and zonulin-1) and subsequent loss of intestinal integrity as well as reduced clearance capacity of intestinal immunity.[7] Moreover, cirrhosis triggers qualitative and quantitative changes in the intestinal microbiota. Bacterial overgrowth develops early in compensated cirrhosis and correlates well with cirrhosis-related complications during disease progression.[30,31] There are additional changes to the bacterial composition. Cirrhosis is associated with a shift from healthy commensal taxa to pathological strains such as *Enterococcus*, *Streptococcus* and *Staphylococcus*. Translocation of these bacteria increases the risk for hepatic encephalopathy, spontaneous bacterial peritonitis and other cirrhosis-related complications.[30,32,33] SI and bacterial translocation do not seem to be a steady process, but rather follow periodic patterns with high peaks and rapidly declining plasma IL-6 levels within days to weeks.[34] The increasing translocation of PAMPs and bacteria in combination with additional triggers such as manifest bacterial infection or alcoholic hepatitis leads to a release of inflammatory mediators and nitric oxide which in turn damage intestinal tight junctions and induce vasodilation.[35] This uncontrolled vicious cycle is one of the main triggers for decompensation and organ failure in cirrhosis.[7]

2.3 | Cell effectors

Increased WBC in patients with AD cirrhosis and ACLF is mostly attributable to increased neutrophil counts without apparent quantitative changes in the monocyte population.[36] In contrast, these patients exhibit a remarkable reduction in the number of lymphocytes.[36] Therefore, the systemic immune cell landscape of patients with AD cirrhosis and ACLF is mostly characterized by neutrophilia and lymphopenia. At present, the relative role of each immune cell type and the relative contribution of the innate versus adaptive immune system in the pathogenesis of SI and immunosuppression in ACLF are largely unknown. In the following paragraphs, we provide an update on the currently available information about the role of lymphocytes, monocytes/macrophages and neutrophils in AD and ACLF. There is only very limited data on dendritic and natural killer cells, thus these subsets are not discussed in the current review.

2.3.1 | Lymphocytes

Many studies initially focused on effector cells of the innate immune system, although recent studies helped unveiling the relevance of the adaptive immune system. Cytokinomics and transcriptomics previously related signatures of activation and immunosuppression to innate immunity, while a predominantly exhausted response was ascribed to the cells of the adaptive immune system.[6,37,38] CD4+ and CD8+ T cells were not only reduced in the circulation of patients with AD and ACLF,[39] but also exhibited an immune-suppressive phenotype. Peripheral suppressor CD8+ T cells, characterized by high HLA-DR and Tim3 expression, are expanded in patients with cirrhosis. This observation was associated with an increased risk of infections and enhanced disease severity.[40] Similarly, the presence of an immunosuppressive CD4+ T cell population identified by expression of HLA-G (a non-classical HLA class I molecule), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and IL-35 has been linked with increasing disease severity, bacterial infections and poor outcome in AD patients.[41] In contrast to thymus-derived regulatory T cells (Tregs), the CD4 subgroup is formed in the periphery and exerts suppressive properties via CTLA-4 and HLA-G-driven inhibition of Th17-related cytokines.[42] IL-35, an anti-inflammatory cytokine that may be released by Kupffer cells challenged by PAMPs, was increased in AD patients and induced an expansion of regulatory T cells, illustrating the elaborate ties between innate and adaptive immune systems.[41] The classical $CD4^+CD25^+CD127^{low}$ Tregs are also increased within CD4+ T cells in AD and ACLF patients. They are marked by the upregulation of co-stimulatory molecules (HLA-DR, OX-40, GITR, TIM-1) and more variable expression of inhibitory molecules (PDPN, 2B4, CTLA-4, BTLA).[39] In the same study, in vitro production of interferon γ or TNFα by T cells was considerably attenuated, leading the authors to propose that impaired T cell immunity is an early event during the course of liver cirrhosis. T-cell phenotype changes were already evident at the early stages of cirrhosis and reached their full extent in AD patients.

A significant reduction in both blood and liver naïve B cells in ACLF patients has been described,[43] combined with an increase in antigen-experienced B cells in the liver. In addition, significant phenotype changes within the B cell compartment have been reported. For instance, atypical $CD27^-CD21^-$ memory B cells, also known as inflammatory or age-associated B cells, were increased in the periphery and in the liver of ACLF patients, where they expressed higher levels of CD11c and CXCR5 compared to cirrhotic patients without ACLF,[43] suggesting once again chronic and persistent antigen stimulation.[44]

2.3.2 | Mononuclear phagocytes (monocytes/macrophages)

Seminal evidence that monocyte function (i.e. chemotaxis, bacterial phagocytosis and killing and monocyte spreading) was markedly reduced in cirrhosis was provided by Holdstock et al., Hassner et al. and Clària et al.[45–47] Later on, evidence of impaired antigen-presentation capacities and functional deactivation of monocytes from patients with AD cirrhosis and ACLF was provided by Wasmuth et al.[8] More recently, Bernsmeier et al. provided evidence that impaired monocyte function was associated with the expansion of monocytes positive for MERTK, which is a potent negative regulator of innate immune responses.[36] In fact, MERTK+ monocytes have an impaired response to LPS stimulation and possibly contribute to secondary infections. These authors also demonstrated the expansion of immunosuppressive CD14+HLA-DR- monocytic myeloid-derived suppressor cells in patients with ACLF.[48] These cells suppress T cell function and attenuate antimicrobial innate immune responses, thereby increasing the susceptibility to secondary infection. Functional alterations have also been demonstrated in classical $CD14^{++}CD16^{-}$ monocytes from ACLF patients. This highly phagocytic subset of monocytes exhibits a reduced expression of TLR2 and TLR4 in AD patients with ACLF and more severely impaired phagocytic capacity and oxidative burst response compared to AD patients without ACLF.[49] In this study, the classical monocyte subset showed upregulation of genes associated with immunosuppressive responses such as scavenger receptors (CD163, MRC and CD36), suppressive cytokines (IL-10) and chemokines (CCL22) as well as MERTK. Of note, the authors demonstrated that the phagocytic capacity of monocytes from ACLF patients can be partially restored by targeting immunometabolism via inhibition of glutamine synthetase.[49] Another investigation has provided evidence of downregulation of Tim3, most probably elicited by endotoxemia, on monocytes from AD patients.[50] This downregulation resulted in decreased HLA-DR expression and reduced phagocytic activity. This monocyte alteration is clinically relevant since persistently reduced HLA-DR expression as a surrogate marker of monocyte dysfunction is associated with a high risk of secondary infection and mortality.[48,51] Information on macrophages, which are monocyte-derived immune cells residing in tissue and organs, is scarce in ACLF. However, an expansion of hepatic macrophages expressing high levels of MERTK and CD163 in ACLF patients undergoing liver transplantation has been reported.[36,49]

2.3.3 | Neutrophils

The presence of defective chemoattractant activity, decreased production of superoxide and hydrogen peroxide and impaired degranulation, and therefore depressed phagocytic and bactericidal properties in neutrophils from patients with cirrhosis was first observed by Clària et al., Laffi et al., Maderazo et al. and Rajkovic et al.[47,52–54] Similarly, the response of neutrophils to bacterial stimuli of radical oxygen species (ROS) production such as *N*-formylmethionine-leucyl-phenylalanine is dampened in patients with alcoholic cirrhosis, most likely due to a dysregulation in the components of the NADPH oxidase complex.[55] Nevertheless, the reduced phagocytic capacity of neutrophils is independent of cirrhosis aetiology.[56]

Impaired neutrophil function conflicts with increased neutrophil counts which are present in patients with AD cirrhosis and ACLF. Neutrophil release is likely related to emergency granulopoiesis and the presence of high plasma levels of granulocyte colony-stimulating factor (G-CSF). Despite impaired phagocytic and bacterial killing capacity, activated blood neutrophils from patients with ACLF overexpress CD177, which mediates their firm adhesion to endothelial cells.[38] The increased adhesion of neutrophils to the endothelium may be the first step in the migration of neutrophils to tissues, which is otherwise favoured by the endothelial dysfunction combined with the overproduction of the master neutrophil-attracting chemokine IL-8 by inflamed tissues. Moreover, neutrophils of patients with ACLF are hyperreactive towards parenchymal cells, such as hepatocytes, inducing their death.[56] These observations might provide a direct link between the alterations in neutrophil function and immunopathology (a term coined by pathologists to refer to the collateral tissue damage produced by the exacerbated activation of the immune system).

3 | HUMORAL MEDIATORS OF INFLAMMATION AND IMMUNOSUPPRESSION

SI in ACLF is characterized by increased circulating levels of humoral factors that signal for a vigorous inflammatory response including acute-phase proteins, cytokines, chemokines, growth factors and bioactive lipid mediators. The following paragraphs describe the current knowledge on the most important humoral effectors of inflammation identified in the circulation of patients with AD cirrhosis and ACLF.

3.1 | Cytokines, chemokines and growth factors

The first indication of high plasma levels of cytokines in patients with cirrhosis was described decades ago.[57,58] However, the finding that cytokine levels are strikingly high in patients with ACLF—of similar magnitude to those commonly reported in patients with sepsis and sepsis-like diseases—was not reported until recently.[6] Specifically, the plasma levels of 29 cytokines/chemokines/growth factors were measured in 237 AD patients with ACLF and 285 AD patients without ACLF derived from CANONIC and in 40 healthy subjects. At hospital admission, AD patients without ACLF showed higher levels of inflammatory cytokines in comparison to healthy subjects. These levels further increased in AD patients presenting ACLF.[6] Of interest, cytokines

such as IL-6 and IL-8 increased in parallel to ACLF severity from ACLF grade 1 to ACLF grades 2 and 3. Furthermore, different cytokine profiles were identified according to the type of ACLF precipitating event. TNFα, IL-1ra and particularly IL-6 were higher in ACLF precipitated by bacterial infection than ACLF precipitated by active alcoholism or acute alcoholic hepatitis or other.[6] In contrast, IL-8 was significantly elevated in ACLF precipitated by active alcoholism or acute alcoholic hepatitis compared to ACLF precipitated by bacterial infection or other. In line with these findings, IL-6 strongly correlated with the development of renal impairment and mortality in patients with cirrhosis and bacterial peritonitis.[58] Similarly, IL-8, which is produced by liver cells, is a predictor of ACLF mortality.[56] Another chemokine, CXCL10, was shown to predict ACLF development and survival in cirrhotic patients with portal hypertension receiving transjugular intrahepatic portosystemic shunt (TIPS).[59] Furthermore, a significant association between changes in cytokine levels during hospitalization and the course of ACLF was described, in which reductions of both IL-6 and IL-8 were linked to the improvement of ACLF, whereas increases in these cytokines were associated with worsening.[6]

The hypersecretion of cytokines in patients with AD cirrhosis developing ACLF is likely to induce a dysfunctional innate immune system, leading to immunosuppression and higher vulnerability to recurrent infections. Indeed, Bernsmeier et al. provided evidence that inflammatory cytokines produce an expansion of immunosuppressed monocytes expressing MERTK.[36] On the contrary, the hypersecretion of cytokines and other inflammatory mediators in patients with AD cirrhosis developing ACLF is also likely to reflect an intense inflammatory tissue reaction. In this regard, Solé et al. described that most of the inflammatory markers altered in patients with ACLF including vascular cell adhesion molecule-1 (VCAM-1) and ICAM-1 are functionally related to adhesion, chemotaxis and migration of leukocytes, particularly monocytes and macrophages, into tissues.[60] Finally, Weiss et al. confirmed increased levels of markers of macrophage activation, coagulation/platelet function and endothelial dysfunction.[38]

3.2 | Lipid mediators

Lipid mediators are small bioactive molecules rapidly produced upon cell activation either by enzymatic process or by oxidative fragmentation of larger structural components of membrane phospholipids.[61] Most lipid mediators are derived from polyunsaturated fatty acids (PUFAs).[61] Arachidonic acid is an omega-6-PUFA released on demand in response to an inflammatory stimulus from the cell membrane into the cytosol by the action of phospholipase A2.[61–63] In the cytosol, arachidonic acid is readily converted by cyclooxygenase, lipoxygenase and cytochrome P450 enzymatic pathways into an array of biologically active lipid mediators, which are released from the cell to exert their actions as autacoids.[63] The arachidonic acid-derived lipid mediators are known as eicosanoids and include prostaglandins (PGs), thromboxane A_2 (TXA_2), leukotrienes (LTs), lipoxins (LXs) and epoxyeicosatrienoic acids (EETs). Except for LXs, most eicosanoids have pro-inflammatory properties and, in fact, PGs and TXA_2 are the primary targets for non-steroidal anti-inflammatory drugs.[64] Like cytokines, eicosanoids are massively released by leukocytes in response to infection or tissue injury originating from the so-called 'eicosanoid storm'.[61] Recent studies have unveiled that in addition to producing eicosanoids from arachidonic acid, the same cyclooxygenase, lipoxygenase and cytochrome P450 can also generate bioactive lipid mediators from the omega-3-PUFAs eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids.[65] These lipid mediators are termed resolvins, protectins and maresins and are generically known as 'specialized pro-resolving mediators' because of their potent anti-inflammatory and pro-resolving activities. These lipid mediators have attracted much attention in recent years because they not only act as 'braking signals' of unremitting inflammation but also play critical roles in the dynamic resolution of inflammation.[65,66]

Unlike cytokines and chemokines, much less information is currently available on the role of lipid mediators in AD and ACLF. The formation and actions of eicosanoids in patients with AD cirrhosis have been extensively studied in the context of renal dysfunction,[67] but their role in SI and immunosuppression has not been tackled until recently. O'Brien and collaborators have provided evidence that increased PGE_2 levels might drive immunosuppression and increase the risk of infection in patients with AD cirrhosis.[68] These authors have also described in a feasibility study of the ATTIRE trial that the immunosuppressive effects of PGE_2 in patients with AD cirrhosis and ACLF can be counteracted by albumin infusion.[69] However, in a more recent targeted lipidomic analysis of more than 100 lipid mediators in 200 patients with AD cirrhosis with and without ACLF, López-Vicario et al. found no evidence of increased PGE_2 levels and no association with the risk of infection during hospitalization.[70] This finding could be related to upregulated expression of 15-hydroxy-PG-dehydrogenase, the enzyme degrading PGE_2 recently described by Casulleras et al. in patients with AD cirrhosis.[71] In addition to PGE_2, López-Vicario et al. also provided evidence of elevated levels of pro-inflammatory and vasoconstrictor eicosanoids in ACLF patients, mainly members of the leukotriene family such as LTE_4.[70] In these patients, LTE_4 shaped a minimal plasma fingerprint that discriminated AD patients with ACLF from those without, correlated strongly with IL-8, distinguished ACLF grades and followed the clinical course of the disease (increased in parallel to worsening and decreased with improvement).[70] On the contrary, neutrophils from patients with AD cirrhosis are known to exhibit defective chemotactic responses to LTB_4, another member of the leukotriene family, an alteration that likely contributes to the characteristic bacterial killing dysfunction existent in this condition.[47] Of interest, some lipid mediators derived from linoleic acid, an omega-6-PUFA precursor of arachidonic acids, such as 9(10)-epoxy-9Z-octadecenoic acid (EpOME) and 12(13)-EpOME, which are indicators of effective bactericidal activity, were remarkably suppressed in ACLF patients.[70]

The plasma lipid mediator landscape of patients with ACLF is also characterized by a lower content of anti-inflammatory/

pro-resolving lipid mediators. This is consistent with the presence of higher omega-6 to omega-3 ratio in AD patients with ACLF, which is a surrogate marker of SI and/or a deficit of resolution.[72] Indeed, a deficit in LXA_4 formation and reduced levels of the pro-resolving lipid mediator LXA_5, which is generated from the omega-3-PUFA EPA, has been described in these patients.[47,70] In AD patients at risk of developing ACLF, the deficit in the circulating levels of pro-resolving lipid mediators is paralleled by a reduced content of anti-inflammatory/pro-resolving lipid mediators in the circulating albumin molecule, which has avidity to bind lipid mediators and avoid their degradation.[71] Moreover, a recent work by Sánchez-Rodríguez et al. has demonstrated low levels of the macrophage anti-inflammatory protein CD5L and the pro-resolving lipid mediator resolvin E1 in extracellular vesicles from patients with ACLF, which were associated with circulatory, brain and respiratory failures.[73] Finally, Becares et al. recently described in ACLF patients from ATTIRE that their survival was associated with a shifted profile in the levels of pro-resolving lipid mediators.[74] Together, these findings suggest that SI in ACLF can be driven by a loss of anti-inflammatory and pro-resolving molecules involved in the control of acute inflammation.

4 | FACTORS INFLUENCING SYSTEMIC INFLAMMATION AND IMMUNOSUPPRESSION

In the following, we will discuss inherent host factors that influence the immune response in patients with AD and ACLF. Early follow-up of a subgroup of the CANONIC study revealed that patients with a severe early course, defined as ACLF grade 2 or 3 assessed between Day 3 and 7 after ACLF diagnosis, were younger than those with the non-severe early course and characterized by more intense SI in line with a higher immune responsiveness at younger age.[75,76] Furthermore, many of those with severe early course had not experienced prior decompensations, and there was a higher incidence of active alcoholism and bacterial infections as precipitating events in this group.[75] However, age is an independent predictor of mortality included in the CLIF-C ACLF score, a composite score that also comprises CLIF-C OF (organ failure) score and WBC.[77] The higher the score, the greater the risk of death. The inconsistency regarding age, that is, more severe ACLF grade at a younger age, but increased mortality risk in the elder, could be reconciled under the hypothesis that SI prevails in younger patients, while immune exhaustion might become more preponderant with age. Nevertheless, further research is needed to study the impact of age on the disparate immune responses exhibited by patients with AD cirrhosis and ACLF.

ACLF is particularly prevalent in men.[78–80] The explanations for sex disparity are multifaceted, but oestrogens as modulators of the immune system and metabolism most probably play a role. For instance, they regulate fibrosis through decreasing lipid peroxidation and production of collagen,[81] which might explain the lower levels of C4M and PRO-C4, markers of collagen type IV remodelling, in females.[82]

An individual's immune response is also influenced by genetic factors. Variants in genes coding for receptors of the innate immune system such as nucleotide-binding oligomerization domain 2 (NOD2) or members of the lectin pathways such as mannan-binding lectin (MBL) and MBL-associated serine proteases (MASP) 2 are associated with increased risk of short-term mortality in AD and ACLF patients.[83] However, there is inconclusiveness regarding whether the NOD2-G908R variant is associated with bacterial infections, with two studies reporting opposite findings.[83,84] Interestingly, two polymorphisms belonging to the IL-1 gene cluster (IL-1β and IL-1ra) that confer reduced risk of ACLF development have been described. The functional role of the IL-1β variant (rs1143623) was confirmed by reduced plasma levels of IL-1β in parallel with decreased IL-6, CRP and WBC,[85] confirming that the levels of cytokines related to the IL-1 network modulate the degree of SI and the susceptibility to develop ACLF.

The risk of decompensation in cirrhosis is not only determined by genetic variants in immunity, but also by variants traditionally linked with liver fibrosis. A recent study found that individuals who are heterozygous for the Pi*Z allele (Pi*MZ) in *SERPINA1*, the gene which is mutated in alpha-1 antitrypsin deficiency, are at risk of accelerated hepatic decompensation and severe liver disease defined as transplantation or liver-related death.[86] Moreover, Praktiknjo et al. have shown that AD boosts collagen type III deposition in experimental and human cirrhosis, possibly contributing to the worsened outcome of these patients.[87]

Interestingly, the genetic variant rs7175922 in the aromatase gene (cytochrome P450 family 19 subfamily A member 1, CYP19A1) is associated with elevated circulating oestrogen levels and the presence of portopulmonary hypertension in patients with advanced liver disease, independently of sex.[88,89] This finding emphasizes that sex disparities with regard to the prevalence of cirrhosis and its complications including ACLF and death[90] are by no means unequivocal. The significance of the finding that higher level of the oestrogen-derived estrone-3-sulfate was linked with the occurrence of ACLF and death also needs further clarification.[91] A partial explanation for the oestrogen paradox might be the existence of two receptors, ERα and ERß, with differences in their anti-fibrotic properties.[92]

4.1 | Metabolism

Activation of the immune system requires the integrated coordination of the metabolism of glucose, amino acids and one-carbon metabolism, which converge in mitochondria as the cellular energy hub. Based on the conspicuous metabolic changes detected by untargeted approaches using metabolomics and transcriptomics,[37,93] it can be postulated that metabolic derangement in immune cells and the circulation is the second major pathophysiological process. Very strikingly, a specific composition of the metabolic fingerprint which was described for CANONIC could also be detected in PREDICT,[94] even though these two observational

studies were designed for different purposes (CANONIC included consecutive patients hospitalized for AD or ACLF at inclusion, whereas PREDICT followed up on AD patients without ACLF at enrolment[78,80]).

The metabolic perturbations in AD and ACLF can be summarized as extreme upregulation of catabolic pathways, as evidenced by intense glycolysis, lipolysis and proteolysis including ketogenic amino acids, reflecting the tissue and immune cell response to acute SI and strong engagement of the hypothalamic–pituitary–adrenal axis.[93,95] The caveat with blood metabolomics is that it might reflect tissue-specific metabolism to some extent, but it may not capture the complete metabolic activity of individual organs. Based on metabolic flux analyses, we know that extreme catabolism with a preference for glycolytic substrates is present in peripheral mononuclear cells from AD and ACLF patients, the main cellular effectors of SI.[96]

Apart from a generalized increased metabolite turnover, more specific alterations of metabolic pathways located in mitochondria have been reported. For instance, increased blood levels of medium-chain hexanoyl- and octanoyl-carnitines in patients with ACLF indicate impaired mitochondrial ß-oxidation.[93] More importantly, hexanoyl-carnitine was among the top metabolites predicting short-term outcomes in CANONIC and PREDICT.[94] Hexanoyl-carnitine is part of the CLIF-C MET score, a new prognostic model which outperformed MELD and MELDNa.[94] Metabolic flux profiling also uncovered perturbations of the tricarboxylic acid (TCA) cycle in PBMCs with breakpoints at the isocitrate and succinate dehydrogenase level.[96] Comparable breakpoints in the TCA cycle have been described in other models of acute inflammation, and it was proposed that they serve the purpose to redirect intermediates towards the production of immunometabolites, that is, small molecules with the potential to shape immune cell function.[97] Relevant immunometabolites that have been identified in ACLF patients are intermediates of the kynurenine pathway as a result of increased tryptophan degradation, which has context-dependent anti- or pro-inflammatory properties.[98]

Transcriptomics performed on PBMCs from patients with HBV-ACLF mostly confirm the seminal findings from the metabolomics study. Dysregulation of metabolic processes progressed from chronic hepatitis B to HBV-ACLF,[37] indicating that metabolic perturbations in immune cells is a common trait in the disease course of cirrhosis, regardless of aetiology and precipitating event.

4.2 | Mitochondrial function

Metabolic dysregulation is intricately linked to mitochondrial function. Mitochondrial impairment can involve altered ultrastructure, dysfunctional mitophagy, reduced bioenergetic output and increased production of ROS. Mitochondria of mononuclear cells in ACLF patients are reduced in size but increased in number, which, together with decreased hepatic mitofusin 2 expression indicate an imbalance between fusion and fission processes.[96,99] Cristae rarefication and predominance of fission is associated with reduced ATP production as a consequence of reduced oxidative phosphorylation,[100] leading to bioenergetic failure. Reduced generation of ATP in ACLF patients might also be a consequence of processes that are upstream of the electron transport chain, such as reduced TCA cycle activity and mitochondrial ß-oxidation. Dysfunction of mitochondria, which are the cell's main producers of ROS, is also evidenced by a marked increase in human nonmercaptalbumin 1, an oxidized form of albumin that acts as an oxidative stress marker.[101] Importantly, mitochondrial impairment is present not only in immune cells but also in failing organs of patients, for instance in the context of hepatic encephalopathy.[102] In line with this, fibroblast growth factor 21, a biomarker of mitochondrial dysfunction that is expressed by several metabolically active organs and the brain,[103] is increased in the circulation of patients with AD and ACLF.[96]

5 | FUTURE PERSPECTIVES BASED ON IMMUNOGENIC APPROACHES

Recent advances in understanding the disease progression in AD and ACLF deciphered a complex network of disease mechanisms with the immune system acting as a superordinate coordinator. An exaggerated immune response in concert with immune paralysis facilitates organ injury and secondary complications such as bacterial infections leading to the dismal prognosis in end-stage liver cirrhosis. The role of innate immunity and how metabolic changes may affect its functional status underwent a paradigm shift in the recent years since there is an increasing awareness of the importance of lymphocytes in that context. In the following paragraphs, we present novel therapeutic strategies that might rebalance the immunological dysregulation existing in patients with AD cirrhosis and ACLF. Figure 2 summarizes in a schematic illustration some of the therapeutic approaches that are currently under evaluation.

5.1 | Targeting the exhausted immunophenotype

In a patient with predominant immunoparalysis, blockade of immune checkpoints might boost the immune system to fight infections. For instance, anti-CTLA-4 treatment reduced the suppressive effect of HLA-G+ T cells on responder T cell proliferation.[41] The advantage of exploring checkpoint inhibitors as novel therapeutic strategies is that safety data already exist for cirrhotic patients since they are being widely used for the treatment of hepatocellular carcinoma. MERTK presents another potential druggable target to overcome immune paralysis in ACLF patients. Inhibition of MERTK with UNC569 restored the production of inflammatory cytokines in vitro.[36] Currently, a Phase 1 clinical trial (NCT04458259) is investigating a small-molecule inhibitor of MERTK and AXL in patients with advanced or metastatic solid tumours. Results of this trial might also open new avenues for the treatment of ACLF patients. G-CSF was considered to restore immune function in ACLF, but recent

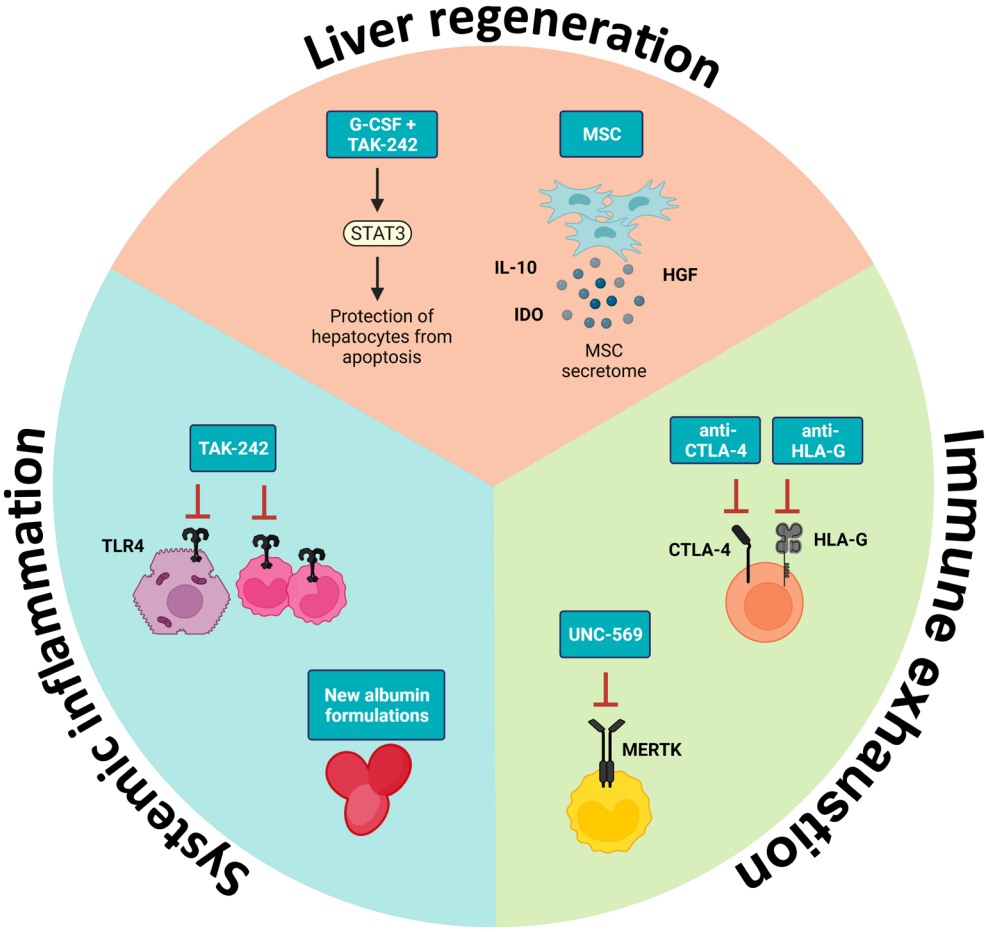

FIGURE 2 Immunogenic approaches for ACLF treatment. Treatment strategies aimed at boosting liver regeneration and counterbalancing systemic inflammation or immune exhaustion are depicted. Combinatorial therapies might be needed, since ACLF patients typically present features of both hyperinflammation and immunoparalysis. ACLF, acute-on-chronic liver failure; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; G-CSF, granulocyte colony-stimulating factor; HGF, hepatocyte growth factor; IDO, indoleamine 2,3-dioxygenase; IL-10, interleukin-10; MERTK, MER receptor tyrosine kinase; TLR4, toll-like receptor 4. This illustration was created using BioRender.com.

studies indicated a lack of efficacy and an exaggerated immune activation.[25,104] Intriguingly, addressing both immune activation and exhaustion by combining G-CSF with a TLR4-inhibitor reduced inflammation and organ failure while facilitating tissue repair in murine models of ACLF.[25]

5.2 | Allogeneic bone marrow-derived mesenchymal stem cell transplantation in acute-on-chronic liver failure

Mesenchymal stem cells (MSCs) are multipotent progenitor cells with paracrine, cell-repairing and immunomodulatory properties, which can migrate to injured sites and repair damaged hepatocytes.[105,106] Two randomized trials in Asia explored infusion of allogeneic MSCs derived from bone marrow and umbilical cord in patients with HBV-ACLF as a treatment option.[107,108] The incidence of infection and short-term outcome were improved in both studies. Future studies need to address the questions of type and source of stem cells, way of administration (peripheral vein, hepatic artery), number of infused MSCs, long-term outcome, side effects and efficacy in non-HBV related ACLF.

5.3 | Targeting systemic inflammation

Recent studies have uncovered the anti-inflammatory properties of blocking the TLR4 pathway. Specifically, inhibition of TLR4 signalling by TAK-242 has been shown to prevent inflammation and cell death leading to a reduced mortality which underlines that PRRs may represent a promising therapeutic target in the future.[23] Also, recent studies have unearthed the ability of albumin to modulate the immune cell response also through modification of PRRs.[17] In fact, in leukocytes from patients with AD cirrhosis with and without ACLF, albumin abolished leukocyte cytokine production induced by bacterial DNA, an effect that was related to its internalization into the endosomal compartment where bacterial DNA interacts with its endosomal cognate receptor TLR9.[17] These anti-inflammatory activities are not observed when albumin is highly oxidized.[101] The presence of a systemic pro-oxidant environment which favours the

occurrence of posttranslational modifications of circulating albumin in patients with AD cirrhosis and ACLF provides a solid argument for the design and synthesis of novel oxidative-resistant albumin formulations that maintain stability and functionality to further improve the anti-inflammatory efficacy of the infused albumin. Alternatively, patients with AD cirrhosis and ACLF could benefit from therapies promoting an antioxidant environment. Plasma exchange with albumin or fresh frozen plasma replacement has proven to be an efficacious anti-inflammatory therapy in critically ill patients with coronavirus infection, in patients with acute liver failure and AD patients with ACLF.[109–111] In all these studies, plasma exchange has been shown to reduce the levels of inflammatory cytokines. The ongoing APACHE study (ClinicalTrials ID: NCT03702920) is currently analysing the short-term survival of subjects with ACLF after plasma exchange with human serum albumin 5%.

5.4 | Addressing mitochondrial dynamics

An extensive overview of potential pharmacological approaches to target different aspects of mitochondrial function can be found in Singh et al.[112] Among those, mdivi-1, a quinazoline derivative, might be of interest for the treatment of ACLF. It inhibits mitochondrial fission by inhibiting dynamin-related protein 1. So far, mdivi-1 has been studied in preclinical models of acute liver failure, Alzheimer's disease and myocardial ischemia/reperfusion injury with favourable outcomes.[113–115] Therefore, mdivi-1 might be a worthy candidate to be studied in preclinical models of ACLF.

Some of the novel therapies targeting metabolism and inflammation described in the preceding paragraphs have shown promising results in pre-clinical studies but require to be evaluated in early-phase human trials. In addition, individualized and combinatorial therapies might be considered in future to target a significant spectrum of the pathomechanistic complexity to modify the disease course and prevent fatalities. These future therapeutic approaches need to ponder the varying degrees of exhaustion and hyperactivation of the immune system, that might coexist in an individual.

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CONFLICT OF INTEREST STATEMENT

JC and IWZ have nothing to declare; CE is a shareholder of Hepyx Ltd. He received speaker fees from Albireo and Novartis. CE received advisory fees from Albireo.

DATA AVAILABILITY STATEMENT

Please contact the corresponding author of each manuscript referenced in this review for the availability of the data.

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