Review

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Roles of systemic inflammatory and metabolic responses in the pathophysiology of acute-on-chronic liver failure

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Summary

Acute-on-chronic liver failure (ACLF) is the most severe form of acutely decompensated cirrhosis and is characterised by the presence of one or more organ failures, intense systemic inflammation, peripheral blood lymphopenia, and a high risk of death without liver transplantation within 28 days. Herein, we propose the hypothesis that intense systemic inflammation may lead to organ failures through five different non-mutually exclusive mechanisms. First, pathogen-associated molecular patterns and inflammatory mediators (*i.e.* cytokines and lipid mediators) stimulate the production of the vasorelaxant nitric oxide in the walls of splanchnic arterioles, leading to enhanced splanchnic and systemic vasodilation which, in turn, induces enhanced activity of endogenous vasoconstrictor systems causing renal vasoconstriction and acute kidney injury. Second, neutrophils that reach the systemic circulation are prone to adhere to the vascular endothelium. Cytokines and lipid mediators act on the endothelium in microvessels of vital organs, an effect that favours the migration of neutrophils (and probably other leukocytes) to surrounding tissues where neutrophils can cause tissue damage and thereby contribute to organ failure. Third, cytokines and lipid mediators promote the formation of microthrombi that impair microcirculation and tissue oxygenation. Fourth, acute inflammation stimulates intense peripheral catabolism of amino acids whose products may be metabotoxins that contribute to hepatic encephalopathy. Fifth, acute inflammatory responses, which include the production of a broad variety of biomolecules (proteins and lipids), and an increase in biomass (*i.e.*, granulopoiesis requiring *de novo* nucleotide synthesis), among others, are energetically expensive processes that require large amounts of nutrients. Therefore, immunity competes with other maintenance programmes for energy. The brain stem integrates the energy demand of each organ system, with immunity considered a top priority. The brain stem may "decide" to make a trade-off which involves the induction of a dormancy programme that permits the shutdown of mitochondrial respiration and oxidative phosphorylation in peripheral organs. In the context of acutely decompensated cirrhosis, the consequence of a shutdown of mitochondrial respiration and ATP production would be a dramatic decrease in organ function.

Keywords: Decompensation of cirrhosis; ACLF; inflammation; inflammatory mediators; immune cells; metabolism

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Introduction

Acutely decompensated cirrhosis, which is the most frequent cause of non-elective hospital admission of patients with cirrhosis,[1] refers to the recent development of ascites, encephalopathy, gastrointestinal haemorrhage, or any combination of these disorders.[2,3] Acute-on-chronic liver failure (ACLF) is the most severe form of acutely decompensated cirrhosis. Compared to patients without ACLF, those with ACLF are characterised by a higher frequency of ongoing clinically apparent pro-inflammatory precipitants (bacterial sepsis, alcohol-related hepatitis, or both); more intense acute systemic inflammation (indicated by leukocytosis with neutrophilia, higher blood levels of C-reactive protein [CRP], as well as a variety of cytokines and chemokines); the presence of hepatic failure, extrahepatic organ failures, or both; and a much higher risk of short-term death, *i.e.* death without liver transplantation within 28 days after hospital admission.[2–5]

Several studies that have been performed since the description of ACLF in 2013 suggest that systemic inflammation plays a causal role in its development.[5–11] In parallel, important progress has been made by immunologists in their understanding of the general principles of inflammatory responses to different stimuli, primarily infections.[12–20] In addition, immunologists have uncovered the major role played by changes in the metabolism of nutrients (glucose, amino acids and fatty acids) in support of energy-demanding inflammatory responses.[17,21–23] Here, we first provide general information about principles of

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inflammatory responses and associated changes in metabolism. Then, we review the available data from the ACLF medical literature and finally propose a theory explaining how systemic inflammation drives the development of ACLF.

Key points

- Acute-on-chronic liver failure (ACLF) is characterised by the presence of systemic inflammation and high risk of death within 28 days. In this review, we propose a hypothesis linking systemic inflammation with organ failure through the following mechanisms.
- Pathogen-derived molecules and inflammatory mediators (*i.e.* cytokines and lipid mediators) stimulate the production of the vasorelaxant nitric oxide which induces splanchnic and systemic vasodilation that in turn activate endogenous vasoconstrictors causing renal vasoconstriction and acute kidney injury.
- Inflammatory mediators activate neutrophils to adhere to vascular endothelium and migrate to tissues, causing tissue damage and contributing to organ failure.
- Inflammatory mediators promote formation of microthrombi that impair microcirculation and tissue oxygenation.
- Acute inflammation stimulates intense peripheral catabolism of amino acids whose products may be metabotoxins that contribute to hepatic encephalopathy.
- Acute inflammatory responses, which are energetically demanding compete with other maintenance energy programmes. The brain stem prioritises the immune system over the peripheral organs which enter a dormancy state that leads to organ dysfunction.

General principles of inflammatory responses

We will first comment on paradigmatic inflammatory responses to acute infections which are the most common triggers of acute inflammation and have been extensively investigated. As with any acute inflammatory response, the response elicited by infections generally involves four components that are successively and coordinately involved.[14,15] The four components include the pathogen byproducts (including pathogen-associated molecular patterns [PAMPs; which are unique molecular structures associated with the pathogens] and virulence factors) that trigger the response, receptors located in immune cells (sensor cells) that detect them, inflammatory signals secreted by sensor cells, and effector cells that are targets of inflammatory signals. Inflammatory signals elicit activation, recruitment, and differentiation of effector immune cells capable of eliminating the causal pathogen. Therefore, the inflammatory response is an immune response whose objective is to eliminate the causal pathogen. Immunologists distinguish three types of inflammatory responses (type 1, type 2, and type 3) depending on the nature of the triggers of these responses[15] (Fig. 1). Each type of immune response is generally a two-tiered process in which tissue-resident innate immune cells (dendritic cells [DCs], macrophages) are activated by pathogens to secrete cytokines that act on tissue-resident differentiated lymphocytes of both innate classes (*e.g.*, natural killer [NK] cells) and adaptive classes (tissue-resident memory T cells). These lymphocytes, in turn, secrete cytokines to activate effector cells.[14] There is a cytokine signature for each of the three types of immune responses and accordingly, cytokines are classified into type 1, type 2, and type 3 cytokines[15] (Fig. 1).

Triggers of the inflammatory response in ACLF

A recent prospective study (PREDICT study) revealed that among 420 patients with ACLF, 273 (65%) had clinically apparent triggers of systemic inflammation, including proven bacterial infection, severe alcohol-related hepatitis, and gastrointestinal haemorrhage with shock.[10] Importantly, compared to patients with no clinically apparent trigger or those with a single apparent trigger, patients with two triggers or more had greater white cell, neutrophil and monocyte blood counts as well as more elevated CRP levels, suggesting a cumulative effect of triggers on systemic inflammation. More details are available in the original publication by Trebicka *et al.*.[10] While bacterial infections or active alcoholism are the most common precipitants of ACLF in Western countries, hepatotropic viral infections, *e.g.* hepatitis B virus reactivation and super-infection with hepatitis A or hepatitis E viruses, are more commonly responsible in Asian countries.[24] Extrahepatic viral infections, such as influenza virus infection, have also been associated with the development of organ failure, secondary infections and death in patients with cirrhosis.[25]

Bacterial infections that trigger inflammatory responses in patients with ACLF are primarily caused by extracellular bacteria, including Gram-negative and Gram-positive bacteria.[26,27] The most common Gram-negative bacteria are *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumanii*. Gram-positive bacteria are *Staphyloccocus aureus*, *Enterococcus faecium*, and *Enterococcus faecalis*. Infections caused by fungi such as *Candida albicans* can also trigger inflammation. The mechanisms by which these bacteria and fungi induce inflammation have been reviewed elsewhere.[14,28] Of note, the prevalence of Gram-positive or Gram-negative bacteria that are resistant to antibiotics is increasing in patients with ACLF.[26] Resistance to antibiotics contributes to the intensity, prolongation and severity of inflammatory responses to bacteria.[10] Finally, it is important to mention the potential role of changes in the gut microbiome as a source of bacterial products that might trigger systemic inflammation in patients with ACLF. It is widely known that intestinal bacterial overgrowth and dysbiosis in conjunction with impaired gut barrier function and increased bacterial translocation are central mechanisms by which bacterial products entering the systemic circulation trigger systemic inflammation (reviewed in ref.[29]). In fact, in the PREDICT study, 35% of the patients with ACLF did not have clinically apparent triggers of inflammatory responses.[10] However, these patients had features of systemic inflammation, although less intense than those in patients with clinically apparent inflammatory triggers.[6,10] Intestinal translocation of bacterial PAMPs has been documented in patients with cirrhosis,[3,4] and therefore it has been suggested that translocated PAMPs may induce inflammatory responses in some patients with ACLF, in the absence of clinically apparent infection.[4]

A broad variety of mechanisms have been identified as potential triggers of acute inflammation in patients with severe alcohol-related hepatitis. Features of liver injury that characterise this disease include hepatocyte death (which is indicated, for example, by the release of keratin 18, a “passive” marker of cell death),[30] resulting in the release of high-mobility group protein 1, a DAMP that triggers inflammatory responses through inflammasome activation.[31] In addition, bulk liver transcriptome analysis in patients with severe alcohol-related hepatitis revealed RNA features related to senescent cells,[32] which are known to secrete inflammatory mediators, including various cytokines, chemokines, extracellular matrix proteins and growth factors, collectively referred to as the senescence-associated secretory phenotype.[33] Of note, excessive alcohol consumption

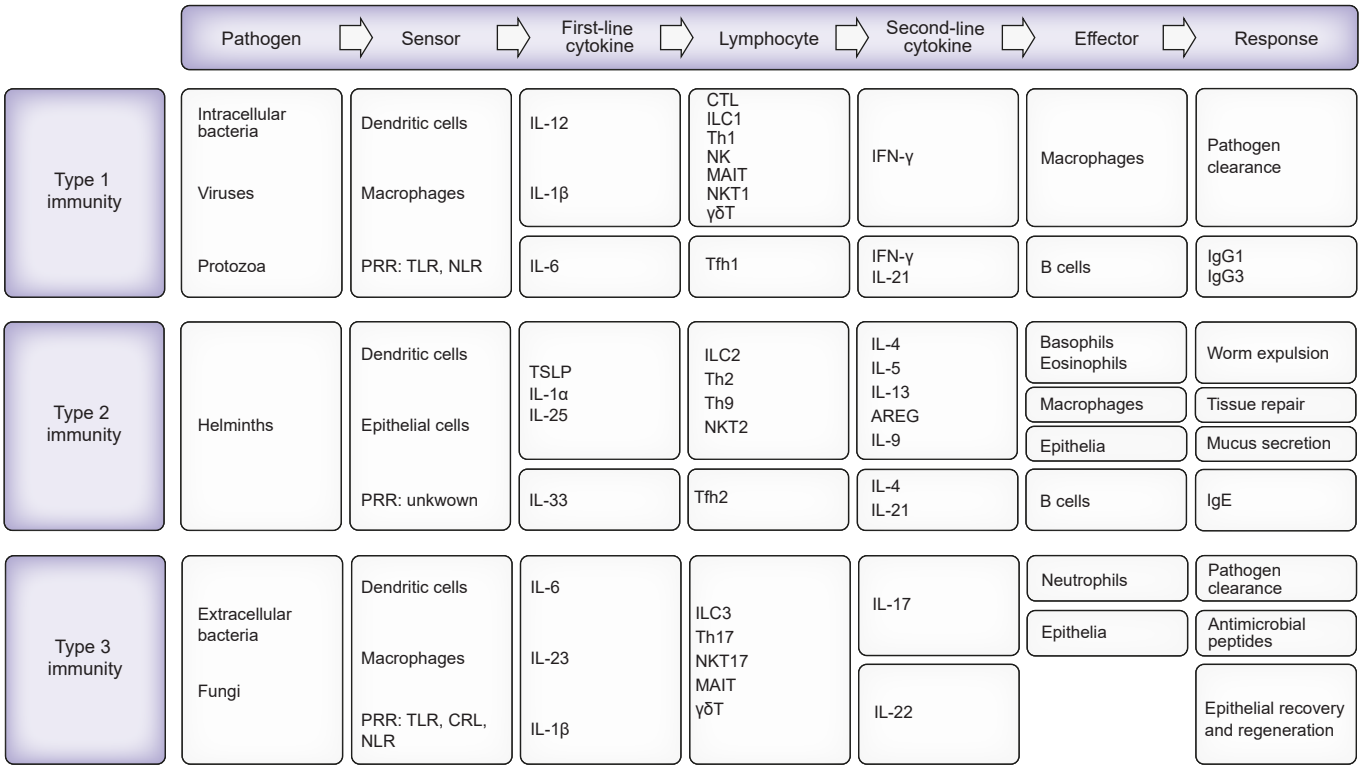

Fig. 1. Three types of immune responses to infections have been well documented. Type 1 immune responses are triggered by intracellular microbes (bacteria, protozoa, and some viruses) whose PAMPs are sensed by dendritic cells (DCs) and macrophages through specific pattern recognition receptors (PRRs; which are germ-line encoded receptors), such as TLRs and nucleotide-binding oligomerisation domain [Nod]-, leucine-rich repeat-containing receptors (NLRs). These cells secrete first-line cytokines (here IL-12, IL-1β or IL-18) which act on different lymphocytes including innate lymphocytes (here group 1 innate lymphoid cells [ILC1 cells] and NK cells), innate-like lymphocytes (also known as unconventional T cells including type 1 natural killer (NKT1) cells, mucosal-associated invariant T [MAIT cells] cells, γδ T cells) and adaptive lymphocytes such as T helper type 1 (Th1) memory cells and CD8 memory T cells in the tissue, to produce the second-line cytokine INF-γ. INF-γ activates effector cells (here macrophages) to kill pathogens. Cytotoxic T cells and NK cells can also kill virus-infected cells. IL-12 can also stimulate follicular helper T cells (Tfh1 cells) to secrete INFγ which drives IgG responses in B cells. Type 2 immune responses are triggered by helminths. Although the mechanisms by which helminths are detected are still unclear, some components of the immune response to these multicellular parasites have been identified. First, the damage of epithelial cells and endothelial cells caused by helminths results in the release of first-line cytokines (here IL-33, IL-1α, IL-25 [also known as IL-17E], thymic stromal lymphopoietin [TSLP]). Second, a specific subset of DCs seems to be indispensable to mount a type 2 inflammatory response. Third, first-line cytokines stimulate ILC2 cells, NKT2 cells, γδ T cells, and Th2 memory cells to secrete second-line cytokines IL-5, IL-13, and amphiregulin (AREG). IL-4, another second-line cytokine, is secreted by memory Th2 cells in response to engagement of T-cell antigen receptors (TCRs). First-line cytokines also stimulate tissue-resident Th9 cells to produce IL-9. Second-line cytokines activate effector cells including mast cells, basophils, eosinophils, and macrophages, as well as IgE antibody production (which is driven by IL-4-producing Tfh2 cells), and therefore promote tissue repair and immunity against helminths. Type 3 immune responses are triggered by extracellular bacterial and fungi whose PAMPs are sensed by DCs and macrophages through PRRs including TLRs, NLRs and C-type lectin receptors (CLRs). These cells secrete first-line cytokines (here IL-1β, IL-23, IL-21) which activate ILC3 cells, NKT3 cells, MAIT cells, γδ T cells, and memory Th17 cells to secrete second-line cytokines IL-17 and IL-22. These cytokines, in turn, stimulate effector cells (here neutrophils) to kill extracellular pathogens. In addition, IL-17 and IL-22 activate endothelial cells, epithelial cells, fibroblasts and tissue-resident macrophages to produce matrix metallopeptidases, nitric oxide, cytokines, antimicrobial peptides and CXCL8 (a neutrophil-attractant chemokine also known as IL-8). ILC3-produced IL-22 also stimulates epithelial proliferation.

causes major alterations in the intestinal barrier and changes in the microbiome, including increased pathogenic bacteria (in particular *E. faecalis*). A virulence factor (*i.e.*, the exotoxin cytolysin) from *E. faecalis* can promote alcohol-induced liver injury.[34] In addition, the case has been made that alterations in intestinal viruses[35] and fungi[36] may be associated with poor outcomes in patients with alcohol-related liver disease, suggesting that viral and fungal byproducts may play a role in the induction of inflammation in these patients. As mentioned earlier, patients with severe alcohol-related hepatitis often present with bacterial infection, which thereby contributes to inflammatory responses in these patients. In addition, a high incidence of infections caused by the saprophytic fungus *Aspergillus fumigatus* has been reported among patients with severe alcohol-related hepatitis.[37]

Patients with gastrointestinal haemorrhage and shock may have tissue damage, for example in the liver and kidneys, resulting in the release of pro-inflammatory DAMPs. These patients may also have intestinal ischaemia-reperfusion injury resulting in the translocation of bacterial PAMPs from the intestinal lumen to the systemic circulation, which in turn may induce inflammatory responses.[38]

Soluble mediators of inflammation in ACLF

Protein mediators

In 2016, Clària, Stauber *et al.* were the first to report the existence of a cytokine storm in a large series of patients (n = 237) who presented with ACLF.[6] The term "cytokine storm" refers to the

presence of elevated circulating cytokine levels that lead to secondary organ dysfunction (*e.g.*, renal, hepatic, pulmonary),[39] which is a collateral damage caused by an excessive inflammatory response (Box 1). In the study by Clària, Stauber *et al.*, patients with ACLF simultaneously had organ failures and elevated levels of 14 out of the 17 cytokines detected in plasma.[6] These included pro-inflammatory cytokines or chemokines, such as interleukin (IL)-6, tumour necrosis factor-α (TNF-α), C–C motif chemokine ligand 2 (CCL2, also known as MCP-1, chemotactic for monocytes), C-X-C motif chemokine ligand 8 (CXCL8, also known as IL-8, chemotactic for neutrophils); anti-inflammatory cytokines (IL-10, IL-1RA); the type-1 immune markers interferon-γ and CXCL10 (also known as IP-10, chemotactic for activated Th1 cells); the type 2 immune marker eotaxin; and the type 3 immune marker granulocyte-macrophage colony-stimulating factor, among others. In addition, this study showed that the circulating levels of molecules that comprised the cytokine storm were roughly similar whichever precipitants were present, with the exception of three cytokines (IL-6, IL-8 and TNFα), which had different levels according to the precipitants.[6] In particular, the levels of IL-6 and TNFα were higher among patients with bacterial infection alone or combined with alcohol-related hepatitis than among those with severe alcohol-related hepatitis alone or those without clinically apparent precipitants. In contrast, IL-8 levels were higher among patients with severe alcohol-related hepatitis alone or combined with bacterial infection than among those with bacterial infection alone or those without clinically apparent precipitants.

More recently, Weiss, de la Grange *et al.* measured 37 inflammatory markers in 16 patients with ACLF at presentation (of whom seven had severe sepsis) and six healthy individuals and found that these patients had elevated circulating levels not only of all inflammatory mediators already identified by Clària, Stauber *et al.*[6] but also of type 2 (IL-1α and IL-4) and type 3 (IL-17A) immune markers.[5] In addition, several inflammatory markers that were undetectable in healthy individuals were elevated in patients with ACLF, including the B-cell activator CD40 ligand, the pro-inflammatory chemokines CCL22 (also known as MDC; chemotactic for activated T cells) and CXCL1 (chemotactic for neutrophils), the markers of macrophage activation CD163 and CD206, the marker of fibrinolysis inhibition t-PAI-1 (for tissue plasminogen activator inhibitor; with extremely high levels in patients), and markers of endothelial dysfunction such as intercellular adhesion molecule 1 and vascular cell adhesion molecule 1 (which were both present at extremely high levels in patients). These authors also compared the blood levels of each inflammatory cytokine and chemokine with their expression at the mRNA level in whole blood and found a very weak correlation, a finding that suggests that elevated levels of circulating inflammatory mediators may be explained by their spillover from peripheral tissues (which are the sites of production) rather than increased production by circulating immune cells.

Together these findings show that patients with ACLF have deregulated tissue immunological responses indicated by a full-blown cytokine storm (in particular with simultaneous increases in circulating levels of immune markers for type 1, type 2 and type 3 immunity), which is associated with features of activation of macrophage function, inhibition of fibrinolysis (a prothrombotic effect) and endothelial dysfunction. Both alterations in fibrinolysis and endothelial function likely developed in the microvasculature of vital organs where they favoured the formation of microthrombi and leukocyte migration from blood to tissues, respectively.

Box 1. Cytokine storm.

Patients with a cytokine storm (also known as cytokine release syndrome) present with acute inflammatory symptoms (fever, elevated levels of CRP), elevated circulating cytokine levels, and secondary organ dysfunction (*e.g.* renal, hepatic, pulmonary), which is collateral damage caused by an excessive inflammatory response.[39] Cytokine storm is associated with poor clinical outcome. Acute infections are the most common causes of cytokine storm. Cytokine storm frequently refers to elevated levels of both pro-inflammatory cytokines such as IL-1α, IL-1β, IL-18, TNFα, IL-6 and the anti-inflammatory cytokines IL-10 and IL-1RA. However, the profile of elevated circulating cytokines is variable across studies, and very few have performed an extensive analysis of circulating cytokines in patients with acute infections. Of note, severe COVID-19 is associated with a maladapted immune response profile, in particular characterised by the simultaneous activation of type 1, type 2 and type 3 immune responses.[39]

CRP, C-reactive protein; IL-, interleukin-; TNFα, tumour necrosis factor-α.

Lipid mediators

Lipid mediators are bioactive lipids generated from structural lipid species (*i.e.* phospholipids containing polyunsaturated fatty acids), which compose the lipid bilayer of cell membranes.[40] Most lipid mediators are eicosanoids, which means that they are derived from the essential omega-6 fatty acid arachidonic acid.[41] The eicosanoid family consists of prostaglandins, thromboxane A_2, leukotrienes, lipoxins and epoxyeicosatrienoic acids. Except for lipoxins, eicosanoids have potent pro-inflammatory properties and, in fact, prostaglandins and thromboxane A_2 are the primary targets for non-steroidal anti-inflammatory drugs.[42,43] Like cytokines, eicosanoids are released in large quantities by immune cells in response to infections or tissue injury, initiating the so-called “eicosanoid storm”.[40] However, unlike cytokines, much less information is currently available on the role of lipid mediators in ACLF. The formation and actions of lipid mediators, mainly eicosanoids, in patients with acutely decompensated cirrhosis have been extensively studied in the past in the context of renal dysfunction,[44] but their role in the context of systemic inflammation and immunosuppression in ACLF has only been tackled more recently. Recent evidence has shown that increased circulating levels of prostaglandin E_2 (PGE_2) may drive immunosuppression and magnify the risk of infection in patients with acutely decompensated cirrhosis.[45] However, in a targeted lipidomic analysis of more than 100 lipid mediators in 200 patients with acutely decompensated cirrhosis with and without ACLF, López-Vicario *et al.* did not confirm increased plasma PGE_2 levels nor identify any association of this lipid mediator with the presence or risk of developing infections during hospitalisation.[46] Instead, these authors reported increased expression of the PGE_2-degrading enzyme 15-hydroxy-PG dehydrogenase in patients with acutely decompensated cirrhosis.[47]

The plasma lipid mediator landscape of patients with ACLF is also characterised by a deficit in anti-inflammatory/pro-resolving lipid mediators.[46,48] This is consistent with the presence in these patients of a higher omega-6 to omega-3 ratio, a surrogate marker of systemic inflammation and impaired resolution.[49] Indeed, a deficit in lipoxin A4 formation and reduced levels of the pro-resolving lipid mediator lipoxin A5 have been described in these

patients.[46,48] Analysis of lipid mediators bound to serum albumin (lipid mediators travel in the circulation attached to this protein) from patients with acutely decompensated cirrhosis at risk of developing ACLF has confirmed a lower content of anti-inflammatory/pro-resolving lipid mediators in this condition.[47] Likewise, a lower content of the pro-resolving lipid mediator resolvin E1 has recently been reported in extracellular vesicles from patients with ACLF.[50] Finally, it has been shown that the survival of patients with ACLF is associated with a shifted profile in the levels of pro-resolving lipid mediators.[51] Together, these findings suggest that systemic inflammation in ACLF can also be driven by a loss of anti-inflammatory and pro-resolving molecules involved in the control of acute inflammation.

Immune cells in ACLF

Characteristics of circulating immune cells

At presentation, patients with ACLF have marked changes in clinical blood counts characterised by leukocytosis and neutrophilia which contrast with lymphopenia[5] (Fig. 2). Of note, these patients have no apparent changes in the clinical monocyte count. The dichotomy between leukocytosis (made up of neutrophils) and lymphopenia is a hallmark of ACLF. The blood white cell count is a component of the CLIF-C ACLF score which assesses the probability of death at 1 month and 3 months in patients with ACLF; the higher the white cell count the greater the probability of death. These findings underline the association between increased biomass (leukocytosis) and outcomes in patients with ACLF. Although the landscape of circulating immune cells associated with ACLF has not yet been investigated in-depth in large series of patients, the results obtained from microarray analysis of RNA expression in whole blood and neutrophils, RNA sequencing in specific monocyte subsets, and flow cytometry in small series of patients have provided the first clues of perturbations in the blood immune cell compartment in ACLF.

Neutrophils

With the use of microarray analysis of whole blood RNA expression in patients with ACLF and healthy individuals, Weiss, de la Grange *et al.* identified a large number of genes that were upregulated in patients compared to healthy individuals.[5] Enrichment analyses revealed that genes coding for components of neutrophil

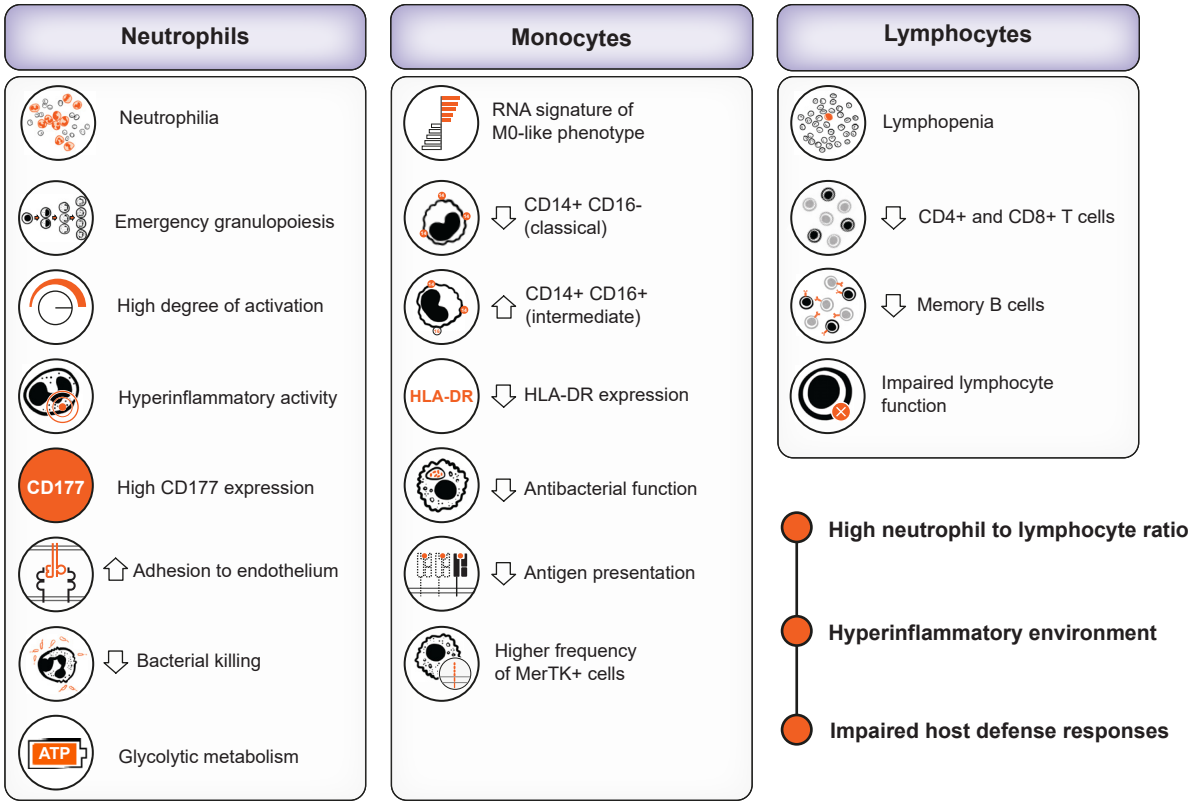

Fig. 2. The immunopathological landscape in peripheral blood of patients with acutely decompensated cirrhosis and ACLF is characterised by neutrophilia and severe lymphopenia. The monocyte population can be slightly increased or within the normal range. In addition to increased neutrophil counts, patients with acute-on-chronic liver failure (ACLF) exhibit an augmented number of immature neutrophil precursors, indicating enhanced emergency granulopoiesis. Blood neutrophils from patients with ACLF also show signs of hyperactivation, increased CD177 expression and adhesion to endothelium. Paradoxically, in this hyperinflammatory environment, the bactericidal activities of neutrophils are impaired. All these neutrophil activities are nurtured through a very intense glycolytic metabolism. The blood monocytes circulating in patients with ACLF also present reduced antigen presentation, HLA-DR expression and antibacterial function. Despite the number of monocytes not being decreased, patients with ACLF exhibit reduced numbers of CD14+CD16- (classical) and CD14+CD16+ (intermediate) monocytes. Also, a higher number of MerTK+ (immunosuppressed) monocytes is observed in this condition. On the other hand, in addition to decreased lymphocyte counts, mainly affecting CD4+ and CD8+ T lymphocytes, and memory B cells, patients with ACLF exhibit impaired lymphocyte function. The increased neutrophil to lymphocyte ratio and the above-described features set the ground for the presence of a concomitant hyperinflammatory and defective host defences in the peripheral blood of patients with acutely decompensated cirrhosis and ACLF.

granules were overrepresented among genes that were upregulated in ACLF, features indicating that ACLF-associated neutrophilia was made up of activated neutrophils. The increased transcription of granule genes was confirmed by bulk transcriptome analysis in circulating neutrophils from patients with ACLF *vs.* healthy individuals.[5] In addition, genes coding for key enzymes in glycolysis, another feature of neutrophil activation, were upregulated in neutrophils from the blood of patients with ACLF. Alterations in the neutrophil transcriptome observed in patients with ACLF were not found in patients with acutely decompensated cirrhosis without ACLF, a finding highlighting the specificity of transcriptional changes in ACLF. Together, these findings are consistent with dramatic increases in *de novo* production of neutrophils by bone marrow, a process called emergency granulopoiesis, in patients with ACLF; this results from enhanced myeloid precursor cell proliferation in response to PAMPs (*e.g.* lipopolysaccharide [LPS]) or cytokine storm (that includes elevated blood levels of granulocyte colony-stimulating factor [G-CSF]).[52] Indeed, myeloid precursor cells express pattern recognition receptors that sense PAMPs (*e.g.* Toll-like receptor [TLR]4 that senses LPS) and cytokine receptors (*e.g.*, receptors for G-CSF).[52]

Weiss, de la Grange *et al.* also showed that activated blood neutrophils from patients with ACLF overexpressed the protein CD177 and that CD177-overexpressing neutrophils firmly adhered to endothelial cells.[5] This increased adhesion of neutrophils to the endothelium may be the first step in the migration of neutrophils to tissues, which is otherwise favoured by endothelial dysfunction combined with the overproduction of the master neutrophil-attracting chemokine IL-8 by inflamed tissues.[5]

Of note, additional functional studies indicated that, despite being activated at the transcriptional level, the production of reactive oxygen species (ROS) stimulated by N-formylmethionyl-leucyl-phenylalanine was markedly reduced in neutrophils from patients with ACLF.[5] ROS release plays a crucial role in neutrophil-mediated bacterial killing and thus a defect in ROS production by neutrophils may contribute to the elevated prevalence of infections in patients with ACLF, at their presentation and during follow-up (secondary infections).

Monocytes

Weiss, de la Grange *et al.* showed that gene signatures related to monocytes were overrepresented among genes that were upregulated in blood from patients with ACLF, indicating that transcription was also increased in monocytes from these patients.[5] Using the CIBERSORT software package to deconvolute the results of blood RNA expression, they found that increased RNA features were related to monocytes with a "macrophage M0-like phenotype", whereas signatures related to other monocyte subsets (*i.e.*, signatures for M1-like and M2-like phenotypes) were not changed. Thus, these findings suggest that alterations of the monocyte compartment may not be homogeneous in ACLF. The results of some studies are consistent with this hypothesis. A recent study which used flow cytometry in blood from patients with ACLF showed that these patients had decreases in the frequency of classical monocytes ($CD14^+CD16^-$), increases in intermediate monocytes ($CD14^+CD16^+$), and no changes in non-classical monocytes ($CD14^{dim}CD16^+$).[53] Moreover, classical monocytes, but not the other monocyte subsets, exhibited reduced expression of the HLA-DR isotype. In addition, elevated frequencies of cells producing the anti-inflammatory cytokine IL-10 were found among the classical and intermediate monocyte populations. Finally, transcriptional profiling of isolated classical monocytes in ACLF revealed upregulation of an array of immunosuppressive parameters and compromised antibacterial and antigen presentation machinery.[53] Other studies mainly based on flow cytometry have shown an increased frequency of circulating $CD14^+$ monocytes expressing the receptor tyrosine kinase MerTK[54] and $CD14^+CD15^-HLADR^-$ myeloid-derived suppressor cells[55] in patients with ACLF. Both subsets of myeloid mononuclear cells had suppressed innate responses to bacterial PAMPs, suggesting that some mechanisms induced a tolerance to PAMP-induced activation of circulating mononuclear cells from patients with ACLF. Of note, MerTK is induced by tolerogenic stimuli such as glucocorticoids[56] and is known to inhibit the innate immune response.[57] The role for PGE_2 in the suppression of innate immune responses in acutely decompensated cirrhosis is still controversial (see above).[45,46] It has also been suggested that the tolerant phenotype of peripheral blood immune cells from patients with decompensated cirrhosis may be explained by prolonged exposure to LPS from gut bacteria.[58] Therefore, tolerance of mononuclear cells, in addition to functional defects in neutrophils, may contribute to the elevated prevalence of infections reported in patients with ACLF.

Together, these findings suggest that the clinical blood monocyte count is apparently "normal" in patients with ACLF because a significant fraction of circulating monocytes might have left the circulation toward tissues in response to monocyte-attracting chemokines produced by these tissues. The case has been made that MerTK-overexpressing blood monocytes from patients with ACLF migrate to different tissues, including the liver, where they may contribute to tissue homeostasis through clearance of apoptotic cells.[56]

Dendritic cells

The ACLF-associated profile of circulating DCs is unclear. In their study of the whole-blood transcriptome, Weiss, de la Grange *et al.* found that genes assigned to DCs were enriched among genes that were upregulated in patients with ACLF compared to healthy individuals.[5] There was no overrepresentation of DC genes among genes that were downregulated in patients with ACLF *vs.* healthy individuals. In contrast, another study found decreased frequencies of DCs and plasmacytoid DCs in patients with ACLF related to severe alcohol-related hepatitis.[59]

Lymphocytes

In their microarray analysis of whole-blood RNA expression in patients with ACLF and healthy individuals, Weiss, de la Grange *et al.* identified a large number of genes that were downregulated in patients.[5] Genes assigned to T cells, NK cells and B cells were overrepresented among genes that were downregulated in ACLF, consistent with the presence of peripheral lymphopenia associated with ACLF. In addition, they performed deconvolution of whole blood transcriptome data using the CIBERSORT software and found that patients with ACLF had decreases in RNA signatures related to resting memory CD4 T cells, CD8 T cells, resting NK cells, and memory B cells. Together, these findings indicate that blood from patients with ACLF is characterised by a decrease in lymphocytes of both the innate (NK cells) and adaptive (T and B cells) immune systems.

Of note, all the alterations of the lymphocyte compartment observed in ACLF were absent or much less marked in patients without ACLF. Importantly, these lymphocyte alterations were found at the presentation of ACLF, findings in sharp contrast with those observed in the general population of patients with sepsis in whom lymphopenia is delayed, *i.e.* observed only in patients with protracted sepsis. Future studies should determine whether peripheral blood lymphopenia associated with ACLF is the result of one or more of the following mechanisms: i) generalised lymphocyte death (a mechanism that has been proposed to explain lymphopenia associated with protracted sepsis[60]); (ii) the activation of the tryptophan-kynurenine pathway seen in ACLF[61] (some kynurenine metabolites known to inhibit T-cell proliferation[62] may cause defective renewal of a depleted lymphocyte compartment); iii) emergency granulopoiesis (the expansion of bone marrow granulopoiesis paralleled by a decrease in bone marrow lymphopoiesis[52]); iv) impaired lymphocyte egress from lymph organs[63] and v) migration of lymphocytes from blood to lymphoid and non-lymphoid tissues in response to several lymphocyte-attractant chemokines identified in ACLF. Elucidating the mechanisms of peripheral lymphopenia associated with ACLF is of importance given the crucial role played by lymphocytes in the immune responses against pathogens (Fig. 1).

Metabolic changes in ACLF

Inflammatory responses, which are highly energy-demanding, represent a priority at the organismal level.[19] In immune cells, inflammatory signals modulate cell-specific metabolic programmes, a process known as immunometabolism. In non-immune cells, inflammatory signals induce a reallocation of metabolic resources through different mechanisms, including a reduction in the utilisation of nutrients by peripheral organs and the induction of a dormancy programme (hypometabolism).[22] Below, we describe the major metabolic alterations present in patients with ACLF.

Anabolic reprograming of innate myeloid cells

Metabolites have been identified with the use of untargeted metabolomics by liquid chromatography coupled to high-resolution mass spectrometry in the blood of a large series of patients prospectively enrolled in the CANONIC study (including patients with and without ACLF) and healthy individuals.[64,65] Compared to the other study groups, patients with ACLF

Box 2. High metabolic demand of activated innate myeloid cells and adaptation of non-immune organs to immune metabolic demand.

This Box summarises the current knowledge on metabolic changes resulting from acute inflammation in the general population. During acute inflammation (*e.g.* sepsis), exposure to PAMPs and cytokines results in activation, *i.e.* reprograming, of innate myeloid cells (*e.g.* macrophages, neutrophils) to produce nucleotides, proteins and lipids that support leukocyte proliferation (increased biomass) and the biosynthesis of a myriad of biomolecules involved in host defence, including, for example, soluble proteins such as cytokines and chemokines, or bioactive lipids, among others.[21, 22] Reprograming of innate myeloid cells involves the orchestrated integration of intracellular glucose metabolism, non-essential amino acids, and the one-carbon metabolism network (folate and methionine cycles) toward an anabolic goal. Thus, in activated/reprogrammed innate myeloid cells, glucose extracted from blood is channelled through aerobic glycolysis to produce pyruvate, lactate and ATP (two ATP per glucose molecule). Glucose is also used in the pentose phosphate pathway (a branch of glycolysis) to produce precursors for the synthesis of purine and pyrimidine nucleotides. The oxidative pentose phosphate pathway generates NADPH which is a crucial cofactor for the production of antimicrobial reactive oxygen species. In addition, glucose can give rise to the non-essential amino acid serine, which together with serine extracted from blood form a serine pool used by the folate cycle to produce a one-carbon atom and the amino acid glycine, both used for nucleotide synthesis. Intermediates are extracted from the Krebs cycle (a process called cataplerosis) for anabolic purposes. For example, the intermediates α-ketoglutarate and oxaloacetate are used to give rise to the non-essential amino acid aspartate which is a major contributor to de novo nucleotide production. Two anaplerotic reactions are used to replenish the Krebs cycle; i) the conversion of pyruvate to oxaloacetate, and ii) the extraction of glutamine from blood to produce glutamate and then α-ketoglutarate. Through the methionine cycle, the essential amino acid methionine contributes to nucleotide production, the synthesis of the methyl donor S-adenosylmethionine (involved in epigenetic regulation), polyamines (that stimulate autophagy), and cystathionine (which fuels the transsulfuration pathway that gives rise to the antioxidant glutathione).

Because acute inflammatory responses are a priority at the organismal level[19], these responses benefit from prioritisation and reallocation of nutrients (*i.e.*, glucose, amino acids, fatty acids). However, because pro-inflammatory signals such as TNFα, IL-6, and prostaglandins act on the hypothalamus to induce the sickness behaviour of anorexia[17], mobilisation of stored fuels is critical for host immunity. This mobilisation is orchestrated directly or indirectly by acute inflammatory signals (*e.g.* GDF15)[62] that inform the brain stem which in turn induces an “alarm response”. For example, acute inflammation activates the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system resulting in intense catabolic metabolism including, in particular, liver glycogenolysis (stimulated by adrenaline), and skeletal muscle proteolysis (stimulated by glucocorticoids), providing immunity with glucose and amino acids, respectively.

During inflammation-induced anorexia there is a substrate shift from ingested glucose to ketone bodies[22], a situation close to physiological fasting. However, an intriguing effect of acute inflammation is the reprograming of hepatic β-oxidation of fatty acids, resulting in the downregulation of this pathway downstream of the intracellular formation of acylcarnitines (that are a combination of fatty acids with carnitine).[22] This downregulation has two remarkable consequences: first, the inhibition of hepatic β-oxidation contributes to both elevated blood levels of fatty acids (in concert with stimulated lipolysis) and blood accumulation of acylcarnitines.[22] Second, the inhibition of hepatic β-oxidation results in decreased production of acetyl-CoA, and therefore of ketone bodies, from fatty acids. As a result, catabolism of ketogenic amino acids is used as an alternative source of acetyl-CoA to produce ketone bodies[22], which are unique sources of energy in the context of nutrient scarcity.

Because the immune responses are energetically expensive, immune cells and non-immune tissues likely compete for energy, a competition that therefore requires trade-offs. At the organismal level, engagement of immunity is a vital priority; in this context, the brain stem, which integrates the energy demand from each of the major maintenance programmes, may “decide” to engage a physiological energetic trade-off which comprises a dormancy programme of hypometabolism (characterised by dramatic decreases in mitochondrial oxygen consumption (*i.e.* mitochondrial respiration and ATP production) in peripheral organs.[22] The mechanisms by which the brain stem induces a dormancy programme are still poorly understood.

GDF15, growth and differentiation factor 15; IL-, interleukin-; PAMPs, pathogen-associated molecular patterns; TNFα, tumour necrosis factor-α.

showed a generalised accumulation of metabolites in peripheral blood. The most distinctive features that characterise the metabolomic signature of patients with ACLF are as follows: First, in patients with ACLF, there is evidence of channelling of intracellular glucose into cytosolic aerobic glycolysis, as indicated by: i) increased blood levels of lactate (pyruvate, the end-product of glycolysis, being preferentially used to produce lactate rather than to fuel the mitochondrial Krebs cycle), and ii) increased blood levels of metabolites of the pentose phosphate pathway (a branch of glycolysis that produces NADPH and fuels *de novo* purine and pyrimidine synthesis through ribose-5-phosphate). All these features suggest a preferential cellular use of glucose for anabolism rather than for energy production (ATP) in mitochondria, a preference known as the Warburg effect. Second, a metabolite signature of activated serine-glycine one-carbon metabolism (folate cycle, which is also involved in nucleotide synthesis) has been observed in ACLF. Third, a metabolic network comprising the amino acids aspartate and glutamate and the methionine cycle is activated, contributing to increased nucleotide synthesis. In addition, activation of the methionine cycle is associated with engagement of the transsulfuration pathway, which is an antioxidant process. Together, these findings are consistent with the capture of the anabolic reprogramming of innate myeloid cells by the blood metabolome, as described in Box 2.

A limitation of blood metabolomic studies in cirrhosis is that it is difficult to discern whether the metabolic changes are due to liver failure *per se* or to changes in the metabolic programmes within the circulating immune cells. However, we propose that the metabolic changes observed in the blood of patients with ACLF are likely related to granulopoiesis, metabolic alterations in circulating innate myeloid cells, or both. This view is supported by recent work by Zhang *et al.*, which demonstrated similar changes in the blood metabolome and in immune cells freshly isolated from patients with ACLF.[66] Indeed, using metabolic flux and gene expression analyses, these authors showed that leukocytes from patients with ACLF preferentially use intracellular glucose through aerobic glycolysis and the pentose phosphate pathway.[66] They also confirmed that glutamine-derived glutamate fuelled reactions of the Krebs cycle, giving rise to aspartate which is involved in *de novo* nucleotide synthesis. They also found evidence of decreased mitochondrial fatty acid β-oxidation in these cells, suggesting that fatty acids were skewed to molecular synthesis instead of ATP production.[66] Importantly, with the use of transmission electron microscopy, Zhang *et al.* demonstrated that the number of mitochondria per leukocyte was higher in patients with ACLF, accompanied by a reduction in their size and changes in mitochondrial ultrastructure distinguished by cristae rarefication and swelling (Fig. 3),[66] findings that require further studies given the central role of mitochondria in immunometabolism. To better summarise all this information and integrate it with systemic inflammation and organ failure in peripheral organs, a simplified schematic representation of the major abnormalities in the metabolism of glucose, fatty acids and amino acids in patients with ACLF is provided (Fig. 4).

"Alarm response" and intense peripheral catabolism

Patients with ACLF exhibit metabolic features related to the "alarm response" mediated by the central nervous system. For example, metabolomic studies found that ACLF was associated with elevated blood levels of 4-hydroxy-3-methoxyphenylglycol sulphate, a marker of increased sympathetic nervous activity.[64,65] Also, patients with ACLF have elevated levels of the mitokine GDF15 (growth and differentiation factor 15),[66] which is known to be produced by immune and non-immune cells during acute inflammation and to act on the brain stem to stimulate sympathetic outflow to the liver and the hepatic release of triglycerides.[67] In addition, the blood metabolome of patients with ACLF exhibits elevated levels of most proteinogenic amino acids released secondarily to skeletal muscle catabolism, which is a consequence of the "alarm response".[64,65] Furthermore, an untargeted lipidomics study showed that the circulating lipid landscape of patients with ACLF was characterised by marked increases in fatty acids, reflecting inflammation-enhanced lipolysis.[68] Together, these findings are consistent with the view that signals related to acute inflammation stimulate the brain stem to elicit an "alarm response" which controls peripheral metabolism (see Box 2 for a summary of the most relevant features of the "alarm response"). Although more data need to be collected, we suggest that ACLF-associated acute inflammation is associated with an intense peripheral catabolism resulting in the release of nutrients (amino acids, fatty acids) from peripheral storage sites (skeletal muscles, adipose tissue) that likely fuel the vigorous anabolic demand of activated innate myeloid cells.

Another metabolic characteristic of patients with ACLF is blood accumulation of products of the catabolism of amino acids which are known to be neurotoxic, including quinolinic acid (a metabolite of the kynurenine pathway), pipecolate (a product of lysine degradation) and N-acetyl-L-aspartic acid (derived from aspartate).[61,64,65] These metabolites may, therefore, be involved in the development of brain failure in patients with ACLF.

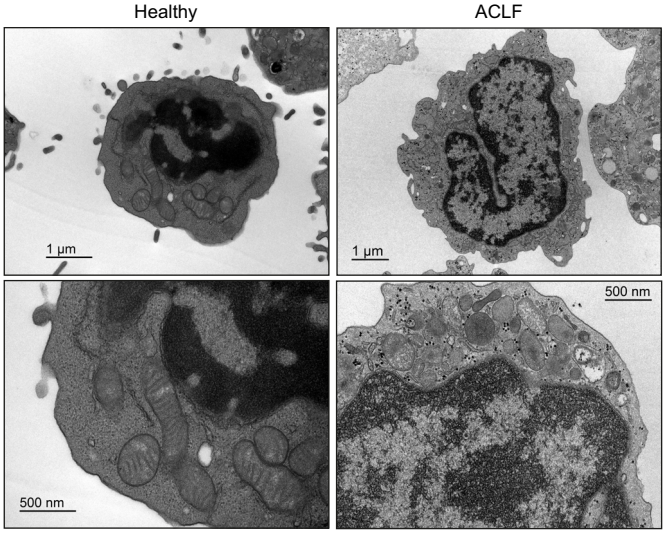

Fig. 3. Representative electron microscopy images at different magnifications of leukocytes from healthy individuals and from patients with ACLF. Leukocytes from patients with acute-on-chronic liver failure (ACLF) present higher numbers of mitochondria per cell, but of smaller size. These leukocytes also present altered mitochondrial morphology characterized by more apparent cristae rarefication, swelling and lack of connection. These alterations in mitochondrial ultrastructure indicate severely disorganized mitochondria and extensive degradation of these organelles in blood leukocytes from patients with ACLF. Images reproduced from Zhang *et al. J Hepatol.* 2022;76:93-106.[66]

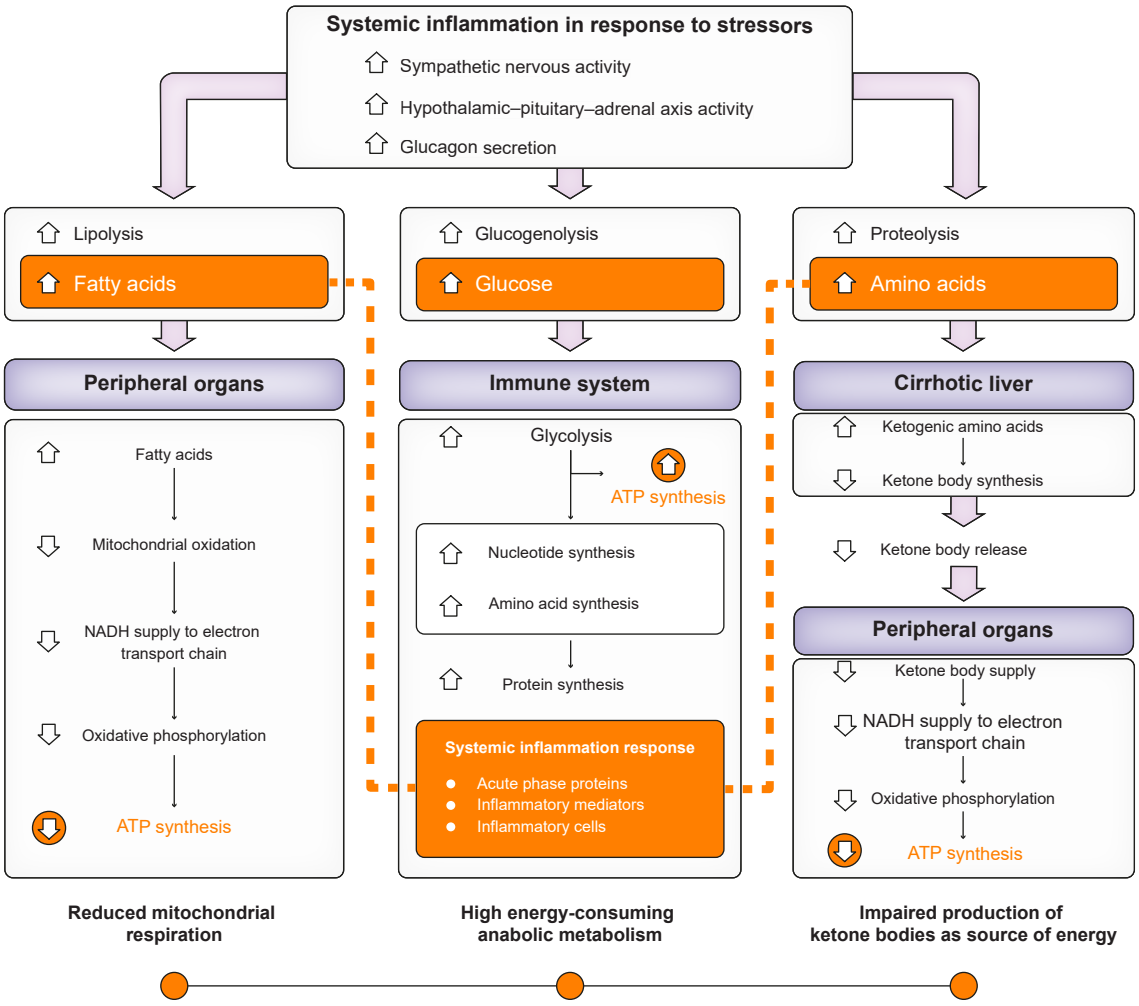

Fig. 4. This figure shows the three major disorders of energetic metabolism in patients with ACLF. i) Increased systemic catabolic metabolism in response to systemic inflammation (upper part of the panel), which leads to intense lipolysis, glycogenolysis and proteolysis and release of fatty acids, glucose and amino acids to the immune system and peripheral organs; ii) anabolic and increased energetic metabolism (ATP synthesis) by innate immune cells (central box); iii) reduced mitochondrial respiration and energy production by peripheral (non-immune) cells (lateral boxes). Figure adapted from Arroyo *et al. J Hepatol.* 2021;74:670-685.

Reprograming of hepatic fatty acid β-oxidation

Ketone bodies become an important source of energy in ACLF because glucose is prioritised to fuel inflammatory responses and because of glucose scarcity (related to the sickness behaviour of anorexia). As expected in the context of acute systemic inflammation,[22] patients with ACLF exhibit increased blood levels of acylcarnitines, reflecting the inhibition of hepatic β-oxidation and, therefore, suppression of this source of ketone bodies.[64] These patients also exhibit features of increased catabolism of ketogenic amino acids, a logical mechanism to compensate for the inhibition of β-oxidation. However, blood metabolomics did not detect any increase in ketone bodies in blood from patients with ACLF,[64] suggesting that the activation of ketogenic amino acid catabolism failed to give rise to its end-products, *i.e.* ketone bodies. These findings suggest that ACLF is associated with a dramatic decrease in the production of ketone bodies and, therefore, deprivation of an important source of energy for peripheral tissues.

Prognostic value of metabolic changes

Several blood metabolites have been shown to be associated with short-term death (by 28 and 90 days after presentation) in a large series of patients with acutely decompensated cirrhosis who had not received early liver transplantation.[69] Moreover, with the use of multivariable analyses, a prognostic score was developed which included, age, the international normalised ratio, bilirubin, 4-hydroxy-3-methoxyphenylglycol sulphate and the acylcarnitine, hexanoylcarnitine; this score was more accurate for assessing short-term death than other prognostic scores, including the MELD-Na score, the CLIF-C ACLF score (for patients with ACLF) and the CLIF-C AD score (for patients with acutely decompensated cirrhosis without ACLF).[69] Together these

findings suggest that metabolites may be of prognostic value because they are markers of metabolic alterations that underlie poor outcomes in patients with acutely decompensated cirrhosis.

Unifying hypothesis for organ failure development in ACLF

Portal hypertension and liver failure are determinants of hallmark complications of cirrhosis, including ascites, hyperammonaemia-related hepatic encephalopathy, and variceal haemorrhage. In addition, splanchnic and systemic vasodilation, both components of the hyperdynamic circulation in patients with advanced cirrhosis, stimulate endogenous anti-natriuretic systems that promote avid sodium retention by renal tubules and subsequent fluid accumulation (ascites).[70] We suggest that, on top of these mechanisms, intense acute inflammation is a driving mechanism that explains the development of organ failure in patients with acutely decompensated cirrhosis. Our hypothesis is as follows (Fig. 5). Inflammation starts in a tissue because of infection, tissue damage (*e.g.* liver injury), or both (*e.g.* tissue damage caused by microbial pathogens). In the context of infection, the inducers of inflammation are PAMPs and virulence factors, whereas DAMPs are inducers of inflammation in the context of tissue damage. When local inflammatory responses (that involve various myeloid and lymphoid cells and a variety of cytokines and lipid mediators) fail to eliminate the source of perturbation (*i.e.* pathogens, tissue damage, or both), local inflammation becomes more intense (with a spillover of locally produced cytokines into the blood), and the immune system engages in intense systemic inflammation, which can cause organ failure through five different mechanisms that are not mutually exclusive. First, PAMPs, cytokines (*e.g.*, TNFα) and lipid mediators (*e.g.* leukotriene B4) stimulate the production of the vasorelaxant nitric oxide in the walls of the splanchnic arterioles. This effect leads to enhanced splanchnic and systemic vasodilation, which induces enhanced activity of endogenous vasoconstrictor systems that cause renal vasoconstriction and acute kidney injury. Second, PAMPs, cytokines (*e.g.* G-CSF) and lipid mediators (*e.g.* PGE_2) stimulate emergency granulopoiesis, which explains the neutrophilia seen in patients with ACLF. Neutrophils that reach the systemic circulation are rewired to adhere to the vascular endothelium (*e.g.* through the surface CD177 protein). Cytokines/chemokines (*e.g.* IL-8) and lipid mediators (*e.g.* leukotriene B4) activate the endothelium in microvessels of vital organs, an effect that favours the migration of neutrophils (and probably other leukocytes) in surrounding tissues where neutrophils can cause tissue damage (a process called immunopathology) and thereby contribute to organ failure. Third, cytokines and lipid mediators (*e.g.* thromboxane A_2) promote the formation of microthrombi in microvessels, an effect that impairs tissue oxygenation and therefore function. Fourth, acute inflammation stimulates intense peripheral catabolism of amino acids, the products of which may be metabotoxins affecting central nervous system activity. These toxic metabolites may, therefore, contribute to brain failure in ACLF. Fifth, acute inflammatory responses, which include the production of a broad variety of biomolecules (protein and lipids), respiratory burst, acute-phase response, and increases in biomass (*i.e.*, granulopoiesis requiring *de novo* nucleotide synthesis) are energetically expensive processes that require large amounts of

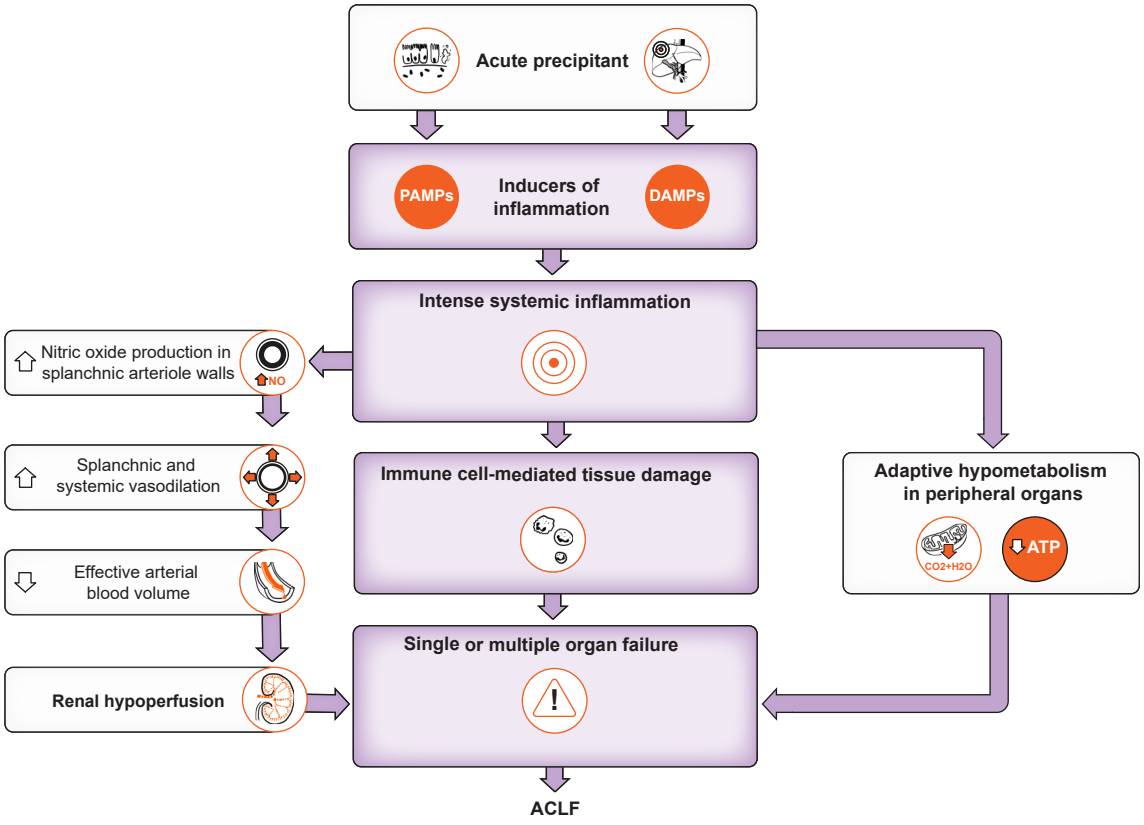

Fig. 5. Unifying hypothesis for organ failure development in ACLF.

nutrients. Therefore, immunity competes with other maintenance programmes for energy. In patients with ACLF, activated innate immune responses compete with energy-consuming processes such as an elevated resting cardiac output, active sodium reabsorption by renal tubules, and active transport systems within the liver, among others. In the liver, the induction of the hepatic acute-phase response (*i.e.*, *de novo* synthesis of a broad variety of secretory proteins such as CRP, all involved in the host immune response)[71] by IL-6 and TNFα competes for energy with several ATP-consuming transport systems. The brain stem integrates the energy demand of each organ system, with immunity considered a top priority. We hypothesise that the brain stem "makes the decision" of a trade-off, which is the induction of a dormancy programme (similar to torpor) allowing for the shutdown of mitochondrial oxygen consumption (*i.e.* respiration: the reduction of oxygen into water) and oxidative phosphorylation (*i.e.* ATP production) in peripheral organs. In the context of acutely decompensated cirrhosis, the consequence of a shutdown of mitochondrial respiration and ATP production is a dramatic reduction in organ function (*e.g.* decreases in kidney function or a decrease in cardiac output as described in ref.[72]) In our hypothesis, liver failure would indicate the engagement of an energy trade-off; in other words, liver failure would result in large part from the arrest of ATP-consuming transport systems as an adaptation to the increased energy demand related to *de novo* synthesis of a broad variety of acute-phase proteins which are prioritised because of their major role in host defence.[71]

The proposed hypothesis is a "snapshot" taken when ACLF has developed. Further studies should investigate patients longitudinally, before the development of ACLF, to better understand the dynamics of immune and metabolic changes that precede the development of organ failures.

Abbreviations

ACLF, acute-on-chronic liver failure; CCL, C–C motif chemokine ligand; CXCL, C-X-C motif chemokine ligand; CRP, C-reactive protein; DAMPs, damage-associated molecular patterns; DC, dendritic cells; G-CSF, granulocyte colony-stimulating factor; IL-, interleukin-; LPS, lipopolysaccharide; NK, natural killer; PAMPs, pathogen-associated molecular patterns; ROS, reactive oxygen species; TLR, Toll-like receptor; TNFα, tumour necrosis factor-α.

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Conflict of interest

None of the authors have competing financial interests to declare.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

All authors participated in drafting and writing of the manuscript and were involved in its critical revision for intellectual content.

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Supplementary data

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Author names in bold designate shared co-first authorship

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