Review

The importance of immune dysfunction in determining outcome in acute liver failure

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Acute liver failure (ALF) shares striking similarities with septic shock with regard to the features of systemic inflammation, progression to multiple organ dysfunction and functional immunoparesis. While the existence of opposing systemic pro- and anti-inflammatory profiles resulting in organ failure and immune dysfunction are well recognised in septic shock, characterization of these processes in ALF has only recently been described. This review explores the evolution of the systemic inflammation in acute liver failure, its relation to disease progression, exacerbation of liver injury and development of innate immune dysfunction and extra-hepatic organ failure as sequelae. Defects in innate immunity are described in hepatic and extra-hepatic compartments. Clinical studies measuring levels of pro- and anti-inflammatory cytokines and expression of the antigen presentation molecule HLA-DR on monocytes, in combination with \textit{ex-vivo} experiments, demonstrate that the persistence of a compensatory anti-inflammatory response syndrome, leading to functional monocyte deactivation, is a central event in the evolution of systemic immune dysfunction. Accurate immune profiling in ALF may permit the development of immunomodulatory strategies in order to improve outcome in this condition.

Keywords: Systemic inflammatory response; Multiple organ dysfunction syndrome; Acute liver failure; Compensatory anti-inflammatory response; Monocyte HLA-DR; Monocytes/macrophages

1. Introduction

Acute liver failure (ALF) is a clinical syndrome characterized by peripheral vasodilatation, encephalopathy and coagulopathy culminating in multiple organ dysfunction syndrome (MODS) and death. There is increasing evidence that activation of systemic immune responses plays a pivotal role in its pathogenesis and outcome. Large studies demonstrate that the presence of the systemic inflammatory response syndrome (SIRS) in ALF is associated with a worsening of encephalopathy and a poor prognosis [1,2]. Trewby et al. initially recognised that the haemodynamic profile in ALF mirrors that of septic shock [3]. Although these two conditions are distinct disease entities, their striking phenotypic similarity implies that they may also share pathogenic mechanisms such as uncontrolled activation of the systemic inflammatory cascade responsible for the transition from SIRS to refractory MODS. This review focuses on the immunological alterations identified in ALF with particular emphasis on the association between SIRS, progressive MODS, hepatocyte death, and systemic immune dysfunction.

2. The importance of SIRS, MODS and sepsis in acute liver failure

There is substantial evidence that SIRS contributes to morbidity and mortality in ALF, with MODS and complicating sepsis being significant sequelae. In a
large study, the King’s College group documented the association of infection with progression of encephalopathy through activation of SIRS, with attendant poor prognosis [1]. Subsequently, Vaquero et al. reporting the experience of the US ALF group [2], noted that in aceterminophen-induced ALF, the progression of encephalopathy was significantly more frequent in the presence of infection than in its absence. Of note, both studies went on to show that SIRS, independent of infection, is associated with worsening of the encephalopathy score and a poorer prognosis. A recent report by Schmidt and Larsen [4] confirmed that the presence of SIRS in ALF carries a poor prognosis and also identified correlations between composite organ failure scores (SOFA), disease severity and SIRS components. Rolando et al. identified the importance of infection in ALF in 1990, documenting frequencies between 50% and 90% [1,2,5]. Infection develops early in the course of ALF, with a median onset time of 2–5 days following admission to hospital [6], and is responsible for late death in at least a quarter of cases [5]. The relatively recent introduction of corticosteroids in the treatment of functional adrenal insufficiency associated with circulatory shock in ALF [7,8], may further increase susceptibility to infections. Despite improvement in medical care and routine use of prophylactic antibiotics, sepsis remains a major cause of mortality in ALF.

3. SIRS and evolution to MODS

SIRS is the clinical expression of an abnormal generalised inflammatory reaction in organs distant from the initiating insult. This term, originally coined by Bone et al., defines: “the systemic inflammatory response to a variety of severe clinical insults, manifested by two or more of the following conditions: (1) temperature >38 °C or <36 °C; (2) heart rate >90 beats/min; (3) respiratory rate >20 breaths/min or PaCO2 <32 mm Hg (<4.3 kPa); (4) WBC count >12,000/mm3, <4000/mm3, or >10% immature (band) forms” [9,10]. The definition is adopted to describe systemic inflammation as [9,10] consequence of infection, comprising a strictly linked sequence of pathological states from sepsis to septic shock. Other conditions associated with a systemic inflammatory response include polytrauma, major surgery, severe burns and pancreatitis [11–16]. There is considerable evidence showing that the severity of SIRS correlates with the severity of organ dysfunction and mortality rate [12,16,17]. Over the past decade, studies have shown that the inflammatory reaction constituting SIRS is characterized by the elevation of circulating levels of both pro- (TNF-α, IL-1, IL-6) [18–21] and anti-inflammatory cytokines (IL-10) [22–24]. The magnitude of increase of both of these functionally opposing sets of cytokines strongly correlates with severity of organ failure and mortality [18,22]. A sequence of events linking the initial inflammatory insult to subsequent MODS and immune dysfunction has been postulated.

3.1. Local inflammatory response

Local tissue injury or invading microbial agents trigger local mediators of innate immunity to release an array of pro-inflammatory mediators through the activation of macrophages, polymorphonuclear phagocytes, endothelial cells and the complement system. This activation leads to elimination of pathogens, with attendant damage to tissue, but also promotes tissue regeneration [11,25].

3.2. Systemic pro-inflammatory response

Chemotactic mediator release and endothelial cell activation serve to recruit circulating immune effector cells (monocytes, lymphocytes), augmenting the local immune response to injury/infection [11,13]. Systemic inflammation occurs following intravascular “spill over” of local inflammatory mediators generated at the site of injury/infection. Pro-inflammatory cytokines recruit further circulating neutrophils, B and T cells and increase vascular permeability by activating vasoactive mediators, platelets and the coagulation cascade.

3.3. Compensatory anti-inflammatory response syndrome (CARS)

A rise in the circulating levels of anti-inflammatory cytokine and mediators in response to systemic pro-inflammatory cytokine release represents a counter-regulatory homeostatic mechanism aimed at preventing overwhelming inflammation. This is termed the compensatory anti-inflammatory response syndrome (CARS) and is defined by persistently elevated circulating levels of anti-inflammatory cytokines (IL-4, -10, transforming growth factor [TGF]-β), and by impairment in cellular immune function. This is demonstrable in phenotypic and functional changes that occur in monocytes, resulting in monocyte deactivation, an event central to the development and evolution of CARS. Here a decrease in pro-inflammatory cytokine secretion is accompanied by loss of antigen presenting capability, while production of IL-10 is not only preserved but increased. The point of transition from a homeostatic response to the pathological state of CARS, characterized by “excessive” immunosuppression and increased predisposition to infection, has not been clearly defined.

3.4. Immunological dissonance

This phase represents the persistence of an “unbalanced” inflammatory response that results in refractory
multiple organ failure and increased risk of death. While an excessive SIRS accounts for the early mortality in patients with septic shock [26,27], the late mortality in refractory septic shock is most likely due to persistent CARS [28]. Here abrogation of pro-inflammatory cytokine production and monocyte deactivation prevent clearance of infection and recovery from organ failure (Fig. 1). The clinical sequelae of monocyte deactivation will be discussed in more detail.

4. The central role of monocytes in SIRS and CARS

Monocytes/macrophages are central to both innate and adaptive arms of the immune responses (Fig. 2). Following the binding of microbial constituents (such as lipopolysaccharide (LPS) and cell wall constituents of Gram-positive bacteria) to complementary receptors on the cell surface of monocytes/macrophages, large amounts of both pro- and anti-inflammatory cytokines are produced. In addition to being activated after exposure to non-specific microbial stimuli, monocytes have, in turn, the ability to trigger adaptive immune responses. After enzymatic digestion and processing, they express [12–18] amino acid long peptides from these digested antigens on their cell surface within the groove of an HLA class II molecule, typically HLA-DR. This peptide/HLA molecule complex present on the monocytes can activate T lymphocytes in a very specific manner, through ligation to the complementary T cell receptor molecule and, in the presence of appropriate co-stimuli, T cells become activated and set in motion an adaptive immune response.

Endotoxin tolerance has been described in experimental animal models where repeated exposure to endotoxin (LPS) [29] induces qualitative and quantitative alterations in monocyte function, particularly in inflammatory cytokine production and surface expression of HLA class II antigens (Fig. 3). Following repetitive LPS challenge, there is an alteration in inflammatory mediator secretion with an attenuated pro-inflammatory [30–32] and enhanced anti-inflammatory cytokine [33–35] secretion profile. Endotoxin tolerance induces reduced surface expression of the antigen presenting HLA class II molecules, impairing the ability of these cells to induce T cell responses following antigenic stimulation [35]. Exposure to a wide range of mediators such as lipoteichoic acid, peptidoglycan, bacterial lipoprotein, catecholamines [36,37], glucocorticoids [38–43] and IL-10 [29] can also induce a state comparable to endotoxin tolerance.

There is considerable evidence to indicate a role for monocyte “dysregulation” as part of CARS in conditions previously identified as uniquely due to SIRS. Ex-vivo studies of monocytes from patients with septic shock, show a profound reduction in HLA-DR expression associated with reduced production of pro-inflammatory cytokines (TNF-α, IL-1, IL-6) [28,34] and augmented production of IL-10 [29] following stimulation with LPS. This finding has been linked with a high incidence of superimposed infections and poor clinical outcome. Numerous clinical studies have assessed monocyte HLA-DR expression as a phenotypic marker of functional monocyte deactivation. A profound reduction in monocyte HLA-DR expression is a consistent finding, present in septic shock [41,44–48], trauma and post operative patients [48–52]. Secondly, HLA-DR levels are consistently lower in non-survivors than in survivors [48,50]; survival being associated with recovery to
normal levels. Collectively, these studies show that monocyte deactivation is a dynamic event, varying through the evolution of systemic inflammatory responses and directly influencing outcome in these conditions.

5. Evolution of pro- and anti-inflammatory responses and profiles in systemic inflammation

The temporal evolution of pro- and anti-inflammatory responses has been delineated in experimental mod-

![Diagram](image_url)

**Fig. 2.** Schematic representation of the role of monocytes/macrophages in the activation of inflammatory responses following the encounter with microbes and microbial products. Lipopolysaccharide (LPS) is released from Gram-negative bacteria and forms a complex with LPS binding protein. Interaction with cell surface receptor, CD14, on monocytes/macrophages (mono/mφ) and toll-like receptors (TLR-2/4) activates the production of NF-κB inducible inflammatory cytokines. Monocyte and neutrophil (nu) engagement results in the activation of the systemic inflammatory cascade, and this, if uncontrolled, leads to multiple organ dysfunction syndrome (MODS). Monocyte activation also triggers adaptive immune responses via its antigen presentation capabilities (MHC class II expression) to CD4+ helper T cells whose engagement leads to an orchestrated immune response capable of eliminating the microbial organism.

![Diagram](image_url)

**Fig. 3.** Schematic representation of the in vitro model of functional monocyte deactivation – the endotoxin tolerant monocyte (ET monocyte). Following repeated lipopolysaccharide (LPS) stimulation an ET monocyte phenotype emerges and evolves. This in vitro phenomenon closely mirrors functional monocyte deactivation encountered in vivo. [This figure appears in colour on the web.]
 els of sepsis [53,54] and human studies [55,56]. Contrary to the concept of an initial pro-inflammatory followed by an anti-inflammatory response, these functionally opposing processes occur simultaneously, though over time the balance may be tilted towards an anti-inflammatory response. In murine polymicrobial sepsis, elevated levels of pro-inflammatory (TNF-α, IL-1β), IL-6, chemokines and anti-inflammatory (TNF soluble receptors, IL-1 receptor antagonists, IL-10) mediators are detected within six hours of onset of sepsis [53], confirming earlier reports that following endotoxin exposure an early TNF-α secretion is soon matched by IL-10 release [54,55].

A considerable amount of interest in the anti-inflammatory role of IL-10 in systemic inflammatory responses has stemmed from its ability to induce monocyte deactivation in in vitro and in vivo studies [35,57]. Experimental models of endotoxaemia and human studies of sepsis have documented a biphasic response to IL-10 secretion, where an early rise is paralleled by release of pro-inflammatory mediators [53,54,58]. The second peak in IL-10 occurs in the later stages of endotoxaemia and is associated with monocyte deactivation and poor outcome [56,58,59]. Hepatic macrophages have been implicated as the main source of the late IL-10 peak [54]. Elevations in circulating TGF-β levels have also been described in patients with SIRS [58,60]. There is no evidence, however, indicating a pathogenic role of TGF-β in CARS. The observation that TGF-β can suppress monocyte function warrants further investigation [61,62].

The anti-inflammatory properties of cyclopentenone prostaglandins (cyPGs) have also been described in animal models of endotoxaemia and sepsis [62–65]. These studies reveal that the anti-inflammatory effects are mediated through inhibition of IKK-β/NF-κB dependent pro-inflammatory gene transcription [66], induction of anti-inflammatory proteins (heat shock protein 70, haeme oxygenase-1) [64] and apoptosis [63]. Recent work supports the role of these anti-inflammatory mediators in the resolution of inflammation through inhibition of pro-inflammatory mediator release and promotion of apoptosis of activated macrophages [67,68]. Further work may better define the role of cyPGs in systemic inflammatory responses.

6. "Systemic" immune defects in ALF

6.1. Monocyte dysfunction

In light of the alterations of monocyte phenotype and function in septic shock, monocyte studies have been conducted in ALF. Despite a marked SIRS, implying a major role for monocytes, only a few studies have investigated relationships between the functional state of circulating monocytes with the profile and degree of inflammatory mediator production in ALF [69–71]. In patients with acetaminophen-induced ALF, circulating monocytes have reduced ability to secrete TNF-α following endotoxin challenge when compared to healthy controls [69,70]. This impaired secretion, possibly reflecting monocyte exhaustion, is associated with poor outcome.

The King’s College group evaluated monocyte TNF-α expression in a cohort of ALF patients, finding high levels of monocyte TNF-α expression in comparison to pathological and healthy controls [72]. Furthermore, monocyte TNF-α expression strongly correlated with circulating levels of the cytokine, suggesting that monocytes are an important source of its enhanced production. However this work does not provide dynamic information on monocyte function during the evolution of ALF. By quantification of cytokine production following LPS challenge, we have recently demonstrated endotoxin tolerance in monocytes from patients with ALF. Thus, while there is no significant increase in TNF-α secretion upon endotoxin stimulation, a significant rise in IL-10 secretion is seen [73]. This finding, novel in ALF, supports the concept of a shift towards a monocyte anti-inflammatory profile in the progression of ALF.

IL-10 is a pleiotropic cytokine with well characterized anti-inflammatory and immunosuppressive properties. A subset of IL-10 producing monocytes, described in patients with ovarian carcinoma, inhibits T cell proliferation and Th1 type cytokine production [74]. In addition to producing IL-10, these cells have low HLA-DR expression and consequently reduced antigen presentation capabilities. In a recent publication we highlight the value of assessing monocyte deactivation in monitoring the severity of acute hepatic injury and predicting adverse outcome in ALF. The main finding is a marked reduction in HLA-DR expression on the surface of circulating monocytes – a sign of functional monocyte deactivation in parallel with increased serum levels of IL-10 [71]. This observation is reminiscent of that in septic shock where it was first described. Reduced monocyte HLA-DR expression and the inability to recover its expression are shown to be predictors of poor prognosis and also parallel disease severity. Collectively, these studies provide evidence that a systemic compensatory anti-inflammatory response, accounting for a grossly impaired HLA-DR expression on monocytes, is present in ALF.

Given the strong relationship that has been demonstrated between high levels of circulating IL-10, low monocyte HLA-DR expression and poor clinical outcome in ALF [71], we suggest a role for a phenotypically similar monocyte population. Thus we found a 7- to 16-fold expansion in IL-10 producing monocytes in ALF patients compared to pathological and healthy controls [72]. Furthermore, ex vivo experiments show a marked reduction in IFN-γ evoked responses to a panel of common recall antigens, indicating an impairment in antigen
presentation capabilities [73]. A possible mechanism for progression to functional monocyte deactivation during systemic inflammatory responses in ALF is illustrated in Fig. 4. This shows the critical steps from acute liver injury to systemic immune dysfunction mediated by IL-10 and its effect on monocyte function, thus rendering the patient vulnerable to sepsis, MODS and death.

6.2. Complement

Several defects of innate immunity in ALF affecting the complement system, neutrophil and Kupffer cell function have been demonstrated. The liver is the major site of complement synthesis, and reductions in serum complement levels and functional activity were identified over 20 years ago [75]. ALF patients have qualitative and quantitative changes in both classical and alternative complement pathways; well documented are the defects of C3, C5 and CH50 [75–78]. In vitro studies have shown that serum from ALF patients deficient in C3 and C5, has reduced opsonic capacity against Escherichia coli and yeasts [75], and poor chemoattractant ability for neutrophils [76].

6.3. Neutrophils

Through a series of ex-vivo studies, Rolando et al. demonstrated that neutrophils are also functionally impaired in ALF. Reductions in superoxide/hydrogen peroxide production [78,79], phagocytosis/intracellular killing capacity and complement receptor expression [79] were identified. Exposure to exogenous granulocyte colony-stimulating factor (G-CSF) improved neutrophil phagocytic and killing capacities in both in vivo and in vitro experiments [79,80]. Given its ability to reverse defective neutrophil function, the administration of G-CSF has been considered for restoring immune responses in an effort to reduce the incidence of infection in ALF. Large prospective studies are required to evaluate this possibility.

6.4. Fibronectin

Plasma fibronectin is a hepatically synthesised glycoprotein that aids the clearance of circulating microbes and microbial products through their opsonisation and subsequent microbial uptake and clearance by Kupffer and other cells of the reticuloendothelial system (RES).

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Fig. 4. Proposed mechanism of functional monocyte deactivation during the evolution of acute liver failure. Acute liver injury stimulates TNF-α production by Kupffer cells, followed by inflammatory cell recruitment, which exacerbates the initial liver injury. Systemic “spill-over” of inflammatory cytokines triggers the systemic inflammatory response including monocyte activation. Following initial activation, monocytes secrete large amounts of both pro-(TNF-α, IL-1, IL-6) and anti-(IL-10) inflammatory cytokines. The predominant cytokine secretion profile is pro-inflammatory at an early stage of the inflammatory response (SIRS). However, the systemic spill-over resulting in high circulating levels of IL-10, initially secreted to counteract the damaging effect of pro-inflammatory cytokines, leads to severe inhibition of HLA-DR expression on monocytes and functional monocyte deactivation (CARS – compensatory anti-inflammatory response syndrome). This results in inability of CD4+ T cells to orchestrate cellular and humoral immune responses. If the hepatic inflammatory insult remains uncontrolled, persistent functional monocyte deactivation ensues with attendant manifestations of immunoparesis, namely recurrent infections and ultimately refractory multiple organ dysfunction syndrome (MODS).
The circulating levels of this opsonic molecule are markedly reduced in ALF. Studies in man have shown a correlation between low serum fibronectin concentration and impaired Kupffer cell function, as documented by the low systemic clearance of microaggregated albumin [81], and by an increased predisposition to infection and a higher mortality rate [82,83].

7. The inflammatory cytokine cascade following acute liver injury

Human studies have provided insights into the deleterious effects of cytokines with regard to the severity of circulatory dysfunction and organ failure. However, most data supporting a pathogenic role for inflammatory cytokines are derived from animal models. It is helpful to consider these cytokines as part of a compartmental model.

7.1. Hepatic compartment

TNF-α plays a critical role in the pathogenesis of hepatic injury through activation of pro-apoptotic pathways (see below). Although the monokine IL-6 is likened to TNF-α in systemic inflammatory processes, acting synergistically in generating the acute phase response, their respective roles diverge in acute liver injury. IL-6 prevents liver injury in murine models of ALF through its ability to down-regulate TNF-α production [84]. Numerous studies have also shown the pivotal role of this cytokine in hepatic regenerative responses following partial hepatectomy and in experimental ALF models [85,86].

In the experimental animal, there is good evidence that the anti-inflammatory response, characterized by IL-10 production, initially ameliorates acute liver injury. This has been reported in numerous immune [87–94] and non-immune [95] mediated ALF models through the ability of the cytokine to reduce the levels of pathogenic pro-inflammatory cytokines, in particular TNF-α [91,92].

7.2. Systemic compartment

Table 1 summarises human studies evaluating circulating inflammatory cytokine levels in ALF. Irrespective of ALF aetiology, most studies report increased levels of pro-inflammatory cytokines including TNF-α [70,71,94,96–99], IL-1 [96,99,100], IL-6 [70,71,94,100,101] and IL-8 [100]. Recent work from our unit has demonstrated a strong relationship between circulating levels of pro-inflammatory cytokines and the degree of vasodilatory shock [100], vasopressor requirement and severity of acute hepatic injury [71]. Given the parallel between levels of circulating pro-inflammatory cytokines and degree of systemic inflammation in ALF, a few studies suggest that measuring pro-inflammatory cytokines may be of value in discriminating survivors from non-survivors. Early studies, based on small numbers of patients, have shown that the levels of TNF-α and IL-6 are higher in non-surviving than in surviving ALF patients [94,98,100,101]. A large study from our institution, of predominantly acetaminophen- induced ALF cases, identified markedly higher circulating levels of TNF-α and IL-6 in non-surviving or transplant-requiring ALF patients compared to those who survived spontaneously [71]. This indicates the value of soluble effectors of immune response as predictors of outcome. Their inclusion into prognostic scoring systems should be evaluated.

Raised circulating levels of IL-10 have been recently reported in ALF with viral and non-viral aetiology [94,97]. Our group has found that levels of IL-10 are not only increased in ALF but strongly correlate with levels of pro-inflammatory cytokines and markers of severity of acute hepatic injury [71]. These findings, novel in the ALF setting, indicate that activation of the systemic inflammatory process shares similarities with other pathological states where a profound SIRS is counterbalanced by a strong and persistent CARS leading to systemic immune dysregulation and poor outcome. Our data, those in the experimental animal and in other clinical conditions, all support the role of CARS in augmenting a predisposition to sepsis and refractory MODS, a characteristic of end-stage ALF.

8. Innate immunity and development of acute liver injury

The innate immune system is based on broadly specific recognition and effector processes that do not require the more specialised antigen recognition pathways of adaptive immune responses. The liver is a unique organ that is rich in cellular effectors of the innate immunity, and studies have shown that these are central to the process of hepatocellular injury.

8.1. Kupffer cells and macrophages

Kupffer cells (KC) exert a pivotal “filtering” role over microbes and microbial products carried via the portal circulation. Early studies documented high circulating levels of endotoxin in ALF suggesting impaired RES activity [102,103]; defective KC function has been indirectly demonstrated by reduced [125] I-labelled microaggregated albumin and galactose clearance [77,104]. The degree of KC dysfunction reflects severity of liver injury, and a return to normal function is observed in patients who recover spontaneously.

KC and macrophages contribute to liver injury in a wide spectrum of experimental models of acute liver failure [105–109]. In the non-inflamed liver, KC secrete anti-inflammatory mediators, such as IL-10 [110,111],...
endogenous prostanoids [112] and TGF-β [113], following exposure to pro-inflammatory mediators (LPS, bacterial DNA) derived from the portal circulation. The net effect of this exposure is a reduction in liver sinusoidal endothelial (LSEC) adhesion molecule expression, antigen uptake and presentation, and, consequently, reduction in T cell activation [114,115]. In the acutely inflamed liver, the hepatic insult activates KC/macrophages to secrete copious amounts of pro-inflammatory mediators. The contributory role of KC/macrophage-derived cytokines (TNF-α [116–120], IFN-γ [117,121–123]), chemokines (MCP-1, IL-8 [124,125]), reactive oxygen/nitrogen species [106] and expression of death ligands in the process culminating in hepatocyte death is well documented.

The LPS/ranitidine model of hepatotoxicity illustrates how KC may initiate and sustain acute liver injury [126]. Liver injury is induced through co-treatment of KC with low-dose LPS and ranitidine. In isolation, neither of these agents induces hepatotoxicity – while in combination they do – supporting the notion of a critical “threshold” of KC activation above which the release of inflammatory mediators causes liver injury. TNF-α is the key mediator of hepatocyte death acting through both direct and indirect mechanisms (Fig. 5). In ALF, however, these cells, have also been shown to secrete cytokines (IL-6, -10, -18) that compensate the deleterious effects of the pro-inflammatory response [95,127,128]. A homeostatic counter-regulatory process is therefore operating in acute liver injury aimed at offsetting the damaging effects of unhindered pro-inflammatory KC/macrophage activation.

8.2. Natural killer (NK) and natural killer T cells (NKT)

NK and NKT cells are another major cellular component of innate immunity, much more abundant in the liver than in lymphoid organs and peripheral blood [114]. The roles of NK/NKT cells in the progression of liver injury are described in acetaminophen [129] and Con-A ALF [130] models. Upon stimulation, NK/NKT cells release large amounts of IL-4, IL-5 and especially IFN-γ, which participate in liver injury through induction of hepatocyte apoptosis [121,123], chemokine production [122,129], LSEC adhesion molecule expression [122], activation/recruitment of other immune effector cells [129,131]. NK and NKT cells also directly
Fig. 5. Role of Kupffer cells and infiltrating macrophages in the initiation and propagation of acute liver injury. Following Kupffer cell (KC) and macrophage (m) activation by a noxious stimulus (e.g. lipopolysaccharide [LPS], unmethylated CpG motifs of bacterial DNA [CpG]) hepatocyte death is induced by both direct and indirect mechanisms. Hepatocyte apoptosis can occur through the direct interaction of TNF-α and Fas ligand (FasL) with their respective ligands on hepatocytes. Indirect mechanisms of hepatocyte death occur through TNF-α induced activation of liver sinusoidal endothelial cell (LSEC). Platelet activation, sinusoidal fibrin deposition, reduced fibrinolysis (increased concentrations of plasminogen activator inhibitor [PAI]-1) and widening of the LSEC fenestrations lead to hepatic microvascular dysfunction/ischaemia and hepatocyte death. Increased expression of LSEC adhesion molecules and chemokine production (e.g. macrophage inflammatory protein [MIP]-2, macrophage chemotactic protein [MCP]-1) promotes influx of further immune effector cells to mediate hepatocyte death. Neutrophils (N) recruitment and activation occurs through TNF-α mediated chemokine production (e.g. macrophage inflammatory protein [MIP]-2, macrophage chemotactic protein [MCP]-1). The release of reactive oxygen species (ROS), peroxynitrite and proteases from these activated neutrophils induce intracellular hepatocyte oxidative stress and oncotic necrosis. [This figure appears in colour on the web.]

induce hepatocyte death, through surface expression of death ligands and perforin release [129,132–134] (Fig. 6). The role of NK/NKT cells in the development of ALF in man needs to be explored.

8.3. Neutrophils

During acute liver injury, neutrophils are recruited to hepatic sinusoids and also primed by an array of pro-inflammatory mediators released by innate immune cells (TNF-α, IL-1, platelet activating factor [PAF], IL-8 [135–139]) and by dying/dead hepatocytes (high mobility group box protein 1 [HMGB1, lipid peroxidation products [140,141]). Mediators released from dying or dead hepatocytes and CXC chemokines are very potent at promoting neutrophil extravasation into the hepatic parenchyma. Once within the hepatic parenchyma, neutrophils are fully activated and induce intracellular hepatocyte stress and oncocytic necrosis through the release of ROIs and proteases [142]. Neutrophil-induced hepatocyte injury has been implicated in ischaemia–reperfusion [143,144], endotoxic shock [145,146], Con-A [147] models of ALF.

8.4. Liver sinusoidal endothelial cells

The LSEC do not solely constitute a physical barrier between the sinusoidal lumen and parenchyma, but actively participate in acute liver injury in both hepatic and systemic inflammatory conditions. A distinct function of such cells is their ability to clear pro-inflammatory substances, such as LPS, from sinusoidal blood without inducing widespread inflammation. This unique ability is mediated through an enhanced production of anti-inflammatory mediators (endogenous prostanooids [148], TGF-β [113], IL-10 [111]), reduction in the expression of adhesion (ICAM/VCAM) and antigen presentation/co-stimulator molecules (MHC class II, CD80/86 [149]) during exposure to pro-inflammatory mediators such as LPS.
Although the LSEC is not directly responsible for hepatic neutrophil recruitment in LPS/galactosamine-induced ALF, the increased expression of the adhesion molecule E-selectin, promotes neutrophil activation and increases the expression of the adhesion glycoprotein Mac-1 (CD11b/CD18), that favours hepatic transmigration and neutrophil-dependent cytotoxicity [138,145,150].

In this ALF model, TNF-α widens LSEC fenestrations permitting direct contact of neutrophils with the hepatic parenchyma, thereby promoting hepatocyte injury. Platelet and red blood cell extravasation through these fenestrations also contributes to hepatocyte death through the induction of microcirculatory disturbances and hepatic ischaemia [151].

Of note, activated LSEC participate in hepatocyte injury during systemic endotoxaemia/sepsis. Circulating TNF-α activates LSEC to produce chemoattractants, such as IL-8 and PAF that lead to the increased expression of β-integrins (LFA-1 and Mac-1) on leucocytes, initiating leucocyte transmigration into the hepatic parenchyma and ensuing hepatocellular injury [152]. Early in polymicrobial sepsis, hepatic microvascular dysfunction occurs as a consequence of platelet activation, recruitment and adhesion to the hepatic sinusoidal endothelium. This triggers a sequence of events that culminate in leucocyte adhesion, sinusoidal fibrin deposition and hepatocyte hypoxia/death [153–155].

9. Hepatocyte apoptosis and immune dysregulation

Hepatocyte apoptosis has been documented in viral, ischaemia/reperfusion, cholestatic and drug/toxin-induced liver injury. In this section, we briefly discuss the engagement of signalling pathways that results in hepatocyte apoptosis.

9.1. TNF- receptor mediated hepatocyte death

The pathogenic role of TNF-α has been extensively documented, in experimental and human models of acute liver failure, largely operating through hepatocyte apoptosis [94,116–120,143,156–159]. TNF-α can also stimulate hepatocyte proliferation and promote regeneration following partial hepatectomy [85]. The pleiotro-
The pro-apoptotic effects of TNF-α remain to be elucidated. Hepatocyte sensitisation to the balance towards a pro- or anti-apoptotic outcome is necessary to render the hepatocyte sensitive to the pro-apoptotic effects of TNF-α [116,118,158]. Other experimental ALF models support the concept that the degree of oxidative/intracellular stress determines whether or not the hepatocyte will be sensitive to apoptosis following TNF-α exposure [119].

9.2. Fas–Fas ligand (Fas–FasL) mediated hepatocyte death

One mechanism by which hepatic macrophages and NKT cells induce hepatocyte apoptosis is via the Fas–FasL signalling pathway [160,161]. Fas is constitutively expressed on hepatocyte membranes and is overexpressed at the time of liver damage [162]. Both murine and human studies of acute liver failure describe the triggering of intracellular caspase-dependent signalling pathways through Fas–FasL interaction, resulting in hepatocyte apoptosis [160,162–164]. The pathogenic role of this pathway has been confirmed by reports that depletion or silencing of Fas/FasL protects against murine acetaminophen-induced ALF [129,165].

10. Potential immunomodulatory strategies in ALF

Given the complexity of innate immune responses and cytokine networks in acute liver injury, several factors need to be evaluated when considering immunomodulating strategies.

10.1. Modulation of innate immune effector cells

Inhibition of KC [106,107,127,166,167], NK/NKT cell [129,130] and neutrophil [145,168,169] function can attenuate the severity of acute liver injury in experimental ALF. A degree of redundancy exists whereby a single hepatic insult can activate numerous effector cells which perform overlapping effector functions resulting in hepatocyte death. This is evident in acetaminophen-induced ALF, where the inhibition of either KC/macrophage [106,170] or NK/NKT [129] cell function can ameliorate, though not fully prevent, liver injury. Thus, inhibition of a single effector cell type is unlikely to provide the “magic bullet” to arrest completely liver injury.

10.2. Modulation of cytokine networks

10.2.1. “Early” hepatotoxic stage

The cytokine milieu generated following a specific liver insult is important when considering immunomodulatory strategies. For example, immunodepletion of IFN-γ, but not TNF-α, ameliorates liver injury in acetaminophen-induced ALF [123,171,172], whereas inhibition of TNF-α is protective in both LPS/galactosamine [116,118] and concanavalin-A induced ALF [120].

ALF models have shown that the early response to liver injury involves simultaneous activation of both pro- and anti-inflammatory mediators, the balance of which determines the degree of liver injury incurred. Studies have shown that IL-6 [173], IL-10 [91,95], IL-15 [174], and prostaglandin E1 [175–177] are protective at the onset of hepatic injury through modulation of pro-inflammatory mediator release. However, the persistence of an anti-inflammatory activity mediated by IL-10 and TGF-β, may in fact impair hepatic recovery at the later stages of acute hepatic injury [89].

10.2.2. “Late” regenerative stage

Following peak hepatotoxicity, the cytokine milieu will alter in order to favour the resolution of acute inflammation and promote regeneration. Numerous cytokines (TNF-α [178,179], IL-6 [180,181]) and growth factors (hepatocyte growth factor, epidermal growth factor [85]) are implicated in hepatic regeneration. In the partial hepatectomy model, the administration of immunosuppressants (calcineurin inhibitors) in conjunction with an exogenous growth factor (e.g. G-CSF) may “tip” the balance towards the resolution of inflammation and promote hepatocyte regeneration [182–184].

10.2.3. Intracellular signalling pathways

Given the degree of pleiotropy of cytokine signalling and immune effector functions, a promising approach to promote hepatocyte survival in acute liver injury may be represented by the inhibition of intracellular signalling pathways (c-jun terminal kinase [185]) and genes (Fas, caspase 8) responsible for the induction of apoptosis. This approach has been recently highlighted in murine ALF where small interfering RNA ameliorated liver injury through inhibition of Fas and caspase 8 expression [186,187].

10.2.4. Modulation of systemic inflammatory responses

A homeostatic response following acute hepatic injury should comprise balanced and opposing pro- and anti-inflammatory components, serving to limit the extent of parenchymal damage and simultaneously promoting tissue recovery, without lasting adverse effects to the host. However, this is not the case in ALF, where a massive and uncontrolled activation of the systemic inflammatory response, paralleled by an
equally uncontrolled anti-inflammatory response, results in progressive MODS. Recent work in ALF and non-ALF conditions provides insights into the immunological profile prevailing in systemic inflammation. While in the past only the pro-inflammatory component was considered, recent evidence, as discussed above, indicates that the development and persistence of CARS, with attendant functional monocyte deactivation, is inextricably linked with enhanced susceptibility to sepsis and death.

Further work is needed to identify the individual immune components involved in the process, their relative contribution, functional relationship and evolution over time in patients who survive and in those who do not. Staging of the immune status during evolution of disease and recognition of pro- or anti-inflammatory predominance may provide experimental grounds on which to base intervention with immunomodulatory agents.

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References


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