The quest for new therapies in patients with cirrhosis and sepsis

Jean-Jacques QUIOC (1), Khalid A. TAZI (1), Didier LEBREC (1, 2), Richard MOREAU (1, 2)
(1) INSERM, U 773, Centre de Recherche Biomédicale Bichat-Beaujon CRB 3, (2) Service d’Hépatologie, Hôpital Beaujon, Clichy.

In Europe, the prevalence of cirrhosis varies from 1.1 to 4.5% [1, 2] making the prevalence of cirrhosis in France about 600,000 with at least 100,000 symptomatic patients [3]. Fifteen thousand patients with cirrhosis die each year, a substantial number from bacterial infections, mainly due to Gram-negative bacteria. Thirty to 50% of patients with cirrhosis have bacterial infections at admission to hospital or develop them during hospitalization. Moreover, 15 to 35% of patients with cirrhosis admitted to hospital develop infections, which is twice or four times higher than in the general hospitalized population. The mortality rate in cirrhotic patients with severe bacterial infection still remains high. For example, 20% of patients with spontaneous bacterial peritonitis die while in hospital [4]. Thus, many of these patients cannot benefit from the only curative treatment of cirrhosis, i.e., liver transplantation.

Sepsis is defined as a systemic inflammatory response to a suspected or proven infection. This condition associated with acute organ dysfunction characterizes severe sepsis. In the general population, the mortality rate from severe sepsis ranges from 30% to 50%. In the United States, approximately 750,000 cases of sepsis occur each year, at least 225,000 of which are fatal [5]. As a result substantial efforts have been made to improve our knowledge of the immunopathogenesis of sepsis. This complex clinical syndrome is mainly due to overactivation of the innate immune system, resulting in a generalized inflammatory and procoagulant response to infection (figure 1). Indeed, the first line of host defence against infection can, under some circumstances, be dysregulated and cause cell and tissue damage, the clinical hallmark of sepsis (coagulopathy, vasodilatation and impaired tissue perfusion) [6]. Advances in the understanding of the pathophysiology of sepsis have led to the identification of many promising new therapeutic targets. Unfortunately, the impact of these novel therapies has been modest, particularly in cirrhosis.

Immunopathogenesis of sepsis

The pathogen responsible for sepsis is identified in about half the cases, and Gram-negative bacteria account for about 60% of cases in the general population [6]. Studies on host-microbe interactions led to the discovery of bacterial motifs called pathogen-associated molecular patterns (PAMPs) and their cognate binding partners on the phagocytes called pattern recognition receptors (PRR) [7]. In Gram-negative bacteria, a molecule embedded in the bacterial cell wall, the lipopolysaccharide (LPS, also known as endotoxin) binds on LPS-binding protein (LBP) and on a soluble or plasma cell opsonic receptor cluster of differentiation 14 (CD14). This LPS-LBP-CD14 complex is then recognized by a phagocyte specific receptor called the Toll-like receptor 4 (TLR4) [8]. So, TLR4 is an LPS receptor (figure 2).

Recently, rapid advances have improved our understanding of the molecular mechanisms that mediate TLR4 signalling. Stimulation of TLR4 and its cytosolic domain, Toll interleukin-1 receptor (TIR), triggers the association of the myeloid differentiation primary-response protein 88 (MyD88), an adaptor molecule which in turn recruits the interleukin-1 receptor-associated kinase-4 (IRAk-4) allowing the association of IRAK-1. IRAK-4 then induces phosphorylation of IRAK-1. Tumor necrosis factor (TNF) receptor-associated factor (TRAF)-6 (TRAF-6) is also recruited to the receptor complex, by associated with phosphorylated IRAK-1. Then, TRAF-6 induces the activation of the transforming growth factor β-activated kinase-1 (TAK-1) which in turn phosphorylates both the mitogen-activated protein kinases (MAPK) and the IKK complex (Inhibitor of nuclear factor-κB (IκB) kinase). The IKK complex phosphorylates IκBα, which leads to its ubiquitylation and degradation. The degradation of IκBα, bound on nuclear factor-κB (NF-κB) in basal conditions, results in the release of NF-κB, a dimer composed of 2 sub-units called p55 and p65, which then become free and can translocate into the nucleus, and induce the expression of its target genes [9].

TRAF-6 is involved in the activation of the other signalling pathways, i.e., MAPK, through the activation of extracellular signal-regulated kinase 1/2 (ERK1/2), p38 kinase and c-Jun N-terminal kinase (JNK). These stress kinases lead to activation of the activated-proteins-1 (AP-1) transcription family members Jun and Fos. These molecular mechanisms result in the overproduction of early pro-inflammatory cytokines such as TNFα, interleukin-6 (IL-6) and interleukin-1β (IL-1β) among others [10, 11]. Thus, severe sepsis is characterized as an excessive innate immune host response to microbial products (for example, LPS).

Advances in therapies in the general population

Recent therapeutic advances have been shown to improve survival in patients with sepsis. Optimization of tissue perfusion and intensive insulin therapy could substantially reduce mortality [12, 13]. Indeed, it has been suggested that in patients with severe sepsis, tissue perfusion may be improved by early goal-directed therapy. This strategy is based on early hemodynamic
Monitoring (central venous pressure, mean arterial pressure, central venous oxygen saturation) to match oxygen delivery with oxygen demand [early use of crystalloid, colloid, vasoactive and inotropic agents, transfusion of red cells] before admission to intensive care units. Moreover, low doses of hydrocortisone and hydrocortisone can also reduce mortality in patients with septic shock [14]. A better control of the inflammatory host response in these patients, with relative adrenal insufficiency induced by sepsis, could explain these results. But, there is no information on the efficacy of hydrocortisone replacement therapy in patients with cirrhosis and severe sepsis.

However, despite major breakthroughs in the fundamental biology of the host response to infection, there have been relatively few therapeutic advances based on the immunopathogenesis of sepsis. Indeed, progress in our understanding of the innate immunity and its role in sepsis had opened the door to "a treasure trove for drug development" [15]: the possibility of altering the clinical course of sepsis by targeting its molecular mediators, and in this way the possibility to improve survival. Unfortunately, despite apparently promising results in animal models, the translation of this possibility into clinical reality has been modest. Potential sepsis therapies have included many pharmacological agents targeting micro-organisms and their products, early host inflammatory cytokines (especially with anti-TNFα antibodies), bioactive lipid mediators, nitric oxide, immuno-stimulatory molecules and coagulation disorders. Despite the dozens of randomized clinical trials, these attempts have been largely disappointing. There are many reasons for these failures but in particular the complex and redundant characteristics of the innate immune response might not be completely under control [16].

Fortunately, one new agent has encouraged research on mediator-directed therapies for sepsis. Bernard et al. [17] have studied the effects of recombinant human activated protein C (aPC), a molecule with anti-coagulant, pro-fibrinolytic and anti-inflammatory properties (inhibition of TNFα) on the course of sepsis. This randomized controlled trial of aPC therapy resulted in a significant survival benefit in treated patients. However, in this study, patients with cirrhosis were not included because they were considered at high risk of bleeding with this anti-coagulant therapy. On the other hand, it should be noted that anti-coagulant therapy is used in certain patients with cirrhosis and portal thrombosis. Thus, additional information is needed on the safety and efficacy of aPC in patients with cirrhosis. This example further emphasizes the importance of looking for new therapeutic targets since the liver is an immunologic filter and a major source of cytokines and bacterial infections, particularly Gram-negative bacteria infections, are frequent and severe in liver diseases [18]. Moreover, in cirrhotic patients with sepsis, coagulation disorders are significantly related to mortality, independent of the severity of cirrhosis [19].

Cirrhosis and sepsis

There is an individual diversity in TLR4-mediated responses [20]. Genetic factors are known to have a significant impact on adverse outcome following infection. A polymorphism in the promoter region of the TNFα gene is associated with higher TNFα levels and increased mortality in sepsis [21]. These polymorphisms also exist in genes for TLRs, CD14, IL-1 and IL-10. Predisposing factors are not limited to genetic variability, but may also include pre-morbidity illnesses [22]. Despite advances in critical care, the prognosis of severe sepsis is worse in patients with cirrhosis than in patients without. Indeed, the rate of severe sepsis is 80% in cirrhosis, whereas it ranges from 30 to 50% in non-cirrhotic patients [23].

Experimental and clinical research has been performed to explain this difference. TNFα and IL-6 plasma levels are higher in infected cirrhotic patients than in infected patients without cirrhosis [24]. In rats with cirrhosis, endotoxin induces an amplified pro-inflammatory response in vivo [25]. Moreover, ex vivo experimental studies have demonstrated excessive bacterial LPS-induced production of cytokines in mononuclear cells of patients with cirrhosis compared with controls [26]. Since cytokines (like TNFα and IL-6) with inflammatory and pro-coagulant properties can lead to multi-organ failure, excessive production of these cytokines could play a key role in the high mortality rate of sepsis in patients with cirrhosis. However, the mechanisms responsible for the overproduction of early inflammatory cytokines in cirrhosis have not yet been identified.

Furthermore, experimental studies performed in rats with cirrhosis have shown promising approaches for the treatment of sepsis. In rats with secondary biliary cirrhosis, it has been shown that tezosantan, a non-selective endothelin receptor antagonist, prevents LPS-induced liver injury, decreases plasma TNFα levels and increases survival [27]. In addition, another study demonstrated that terlipressin, a vasopressin analog, inhibits LPS-induced aortic...
inducible nitric oxide synthase (iNOS) expression [28]. Thus, it could be a novel approach for the treatment of hemodynamic disorders in patients with cirrhosis and severe sepsis.

**Toward new therapeutic approaches**

Many of the components of the innate immune response that are normally involved in host defences against infections can, in cirrhosis, cause cell damage and hence multigorgan failure. In 1972, Lewis Thomas wrote about sepsis that “our arsenals for fighting off bacteria are so powerful… that we are more in danger from them than the invaders. It is our response to their presence that makes the disease” [29]. We hypothesize that an imbalance develops between pro-inflammatory (enhanced) and anti-inflammatory (inhibited) signalling pathways. But, in this excessive immune response to LPS, we still do not know which is the preferential transducing pathway: NF-xB or MAPK. Indeed, there are no studies comparing LPS, NF-xB, ERK1/2, p38 or JNK levels in monocytes of patients with and without cirrhosis. It would be interesting to clearly identify the key molecules involved in the “cytokine storm” in response to LPS in chronic liver diseases. Key molecules may become the targets of novel therapeutic approaches aimed at modifying both the innate immune response and inflammatory cascade [30]. There are numerous unsuccessful trials of anti-inflammatory agents in patients with sepsis. But, we can hypothesize that the success of these drugs depends on the nature and the stage of sepsis, and on the host immune status. Indeed, inflammatory responses are not identical in young people and in the elderly, in meningococcemia and in nephritis. Measurements of circulating cytokine concentrations in evaluating the stage of sepsis could be useful for tailoring the administration of pro- or anti-inflammatory drugs [31]. Although sepsis is associated with an amplified production of early inflammatory cytokines in patients with cirrhosis, it would not be surprising that these cytokine levels collapse in the later stages of sepsis. The initial hyper-inflammatory immune response could rapidly progress to hypo-inflammatory as in old patients with malnutrition. Other studies are necessary.

**Pentoxifylline: why not?**

Studies have demonstrated that pentoxifylline can reduce, in vivo and in vitro, the production of inflammatory cytokines (TNFα, IL-1β and IL-6), IL-8 chemokine and inter-cellular adhesion molecule-1 (ICAM-1) [32-34]. Pentoxifylline can inhibit, in a dose-dependent manner, LPS-induced monocyte secretion of IL-12, and stimulate IL-10 and PGE2 production. This suggests that pentoxifylline could correct an imbalance between pro- and anti-inflammatory cytokines [35].

The anti-inflammatory properties (in particular anti-TNFα) of pentoxifylline have motivated many experimental studies. Promising results have already been obtained for various conditions such as septic shock, inflammatory bowel diseases, non alcoholic steato-hepatitis or sarcoidosis [36-39]. In practice, pentoxifylline is already used to treat severe acute alcoholic hepatitis. Indeed, pentoxifylline improves short term survival in severe acute alcoholic hepatitis. Indeed, pentoxifylline could correct an imbalance between pro- and anti-inflammatory cytokines [35].

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**Perspectives**

Future studies on sepsis and cirrhosis should have at least two aims. First, to understand the molecular mechanisms of early cytokine overproduction in patients with cirrhosis and sepsis. Second, to improve our knowledge of mechanisms involved in the later stages of the immune response to micro-organisms. All these studies should help identify “À la carte” therapies which could be used according to stage of sepsis. These novel therapies should reduce the mortality rate in sepsis, and could become a "bridging treatment" toward liver transplantation.

**REFERENCES**


