Editorial

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Regeneration in acute-on-chronic liver failure – the phantom lost its camouflage

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Current aspects of ACLF pathogenesis: inflammation and regeneration

Acute-on-chronic liver failure (ACLF) develops in patients with cirrhosis as the consequence of precipitating events leading to acute decompensation and multi-organ failure.[1,2] Extensive work has focussed on describing the co-existence of an overwhelming systemic inflammatory response (that leads to tissue injury) and immune paralysis (that predisposes patients to infectious complications, which are the main determinants of adverse prognosis).[3] However, patients who survived the initial phase of ACLF frequently showed a prolonged state of convalescence and recovered with sequelae, notably with persisting organ dysfunction, and thus poor prognosis.[4] A general lack of regeneration was considered responsible for the prolonged recovery.[4] However, profound scientific evidence validating this hypothesis as well as an exploration of the underlying molecular mechanisms were lacking. Two recent studies in patients with acutely decompensated liver cirrhosis[5] and ACLF[6] showed that hepatocyte proliferation (Ki67), which is the major regenerative response after mild to moderate injury, was abrogated in end-stage liver disease.[6] However, these descriptive studies could not provide information about influencing factors or mechanisms. In line, pre-clinical studies on modulating regeneration in ACLF are scarce. Therefore, our current knowledge about regeneration in ACLF has been limited to the awareness of its existence, but the *how* and *why* required more clarification. The study presented by Xiang *et al.*[7] in the current issue of the *Journal of Hepatology* represents a major step forward in understanding and potentially targeting regeneration in ACLF.

IL22-STAT3 pathway promotes tissue regeneration in ACLF

The first novel aspect of the paper is the most obvious and most profound finding. The IL22-STAT3 pathway is essential in mediating regenerative responses (Fig. 1). The authors showed that in a newly established mouse model of ACLF, which combined parenchymal injury (by repetitive as well as intensified carbon tetrachloride [CCl_4] exposure) with *Klebsiella pneumonia* infection, there was a lack of hepatocyte proliferation.[7] The STAT3 pathway is assumed to be pro-regenerative and can be activated by IL6 leading to upregulation of the cell cycle regulator Cyclin D1 and antiapoptotic BCL2.[7] STAT1 is involved in mediating anti-regenerative capacities via overexpression of p21 in response to IFNγ stimulation.[7] Whereas high-dose CCl_4 injection in animals without pre-existing chronic liver injury led to high levels of IL6 and STAT3 activation, the ACLF model was characterised by IL6 suppression and IFNγ-mediated STAT1 activation.[7] As a conclusion, pre-existing liver fibrosis initiated a switch from a pro-regenerative to anti-regenerative pathway(s) activation in response to acute tissue injury. The authors also showed that the administration of IL22Fc, which is a dimer of IL22 with longer half life and therefore prolonged stimulation of STAT3, was associated with higher levels of BCL2 and thus reduced liver injury as well as increased expression of pro-proliferative markers such as Cyclin D1.[7]

This study is a prime example of a translational approach, describing the path from a clinical observation to the unravelling of a mechanism and discovery of a therapeutic intervention. To target the IL22 pathway is not only interesting because it stimulates regeneration in general, the fact that the IL22 receptor is only expressed on epithelial cells and tissue but not immune cells makes it an ideal mediator of tissue repair in ACLF, a disease in which further stimulation of the immune system should be avoided.[7]

The complex interplay of IL22 and its binding protein IL22BP

These data are strongly supportive of a strategy to translate IL22Fc into man, and are underpinned by other pre-clinical studies demonstrating improved liver regeneration.[8] However, the situation in man is likely to be more complex, partly due to

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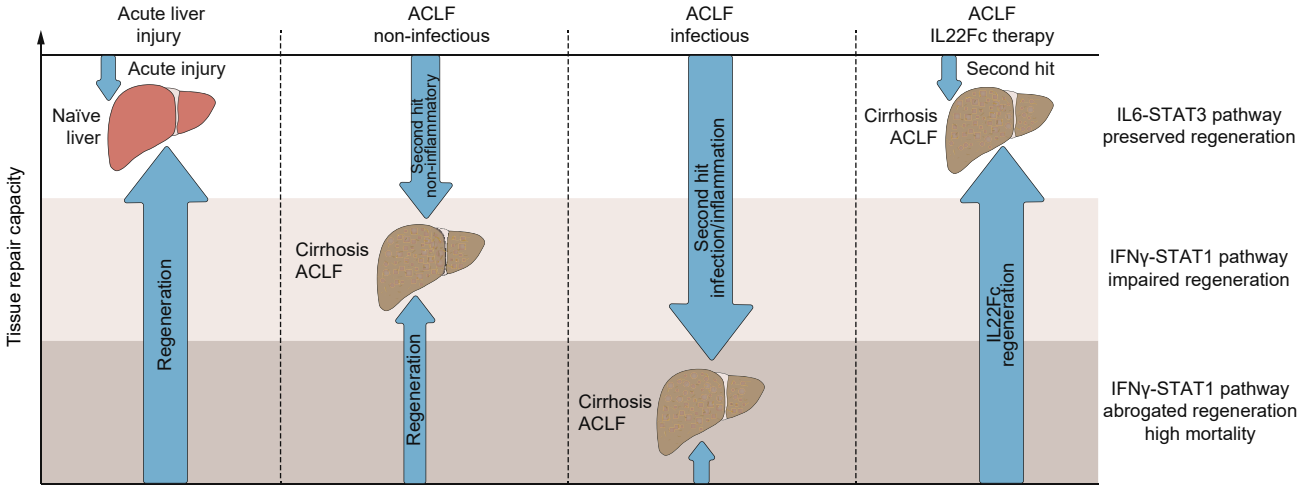

Fig. 1. Regenerative capacities in acute-on-chronic liver failure. The naïve liver has a high regenerative capacity allowing full recovery after acute liver injury. Once cirrhosis is present the ability of hepatocytes to proliferate is impaired due to a switch from STAT3 to STAT1 pathway activation in response to injury. Additional infections in the context of ACLF abrogate regenerative capacities and lead to insufficient recovery with high mortality. Stimulation of the STAT3 pathway by IL22Fc has the potential to restore the liver's ability to regenerate in ACLF. ACLF, acute-on-chronic liver failure.

the action of regulatory proteins such as the IL-22 binding protein (IL-22BP). Binding of IL-22 to its transmembrane receptor is prevented by IL-22BP, which acts as a 'decoy' receptor with higher affinity for IL-22. Thus, higher circulating levels of IL-22BP lead to less free IL-22, and vice versa.

Mice deficient in IL-22BP are more susceptible to acute liver injury, and have delayed regeneration.[9] In humans it was demonstrated that lower IL-22BP levels in alcoholic hepatitis were associated with increased mortality.[10] More recently, these results were replicated in a larger cohort of patients with cirrhosis and ACLF, where low IL-22BP/IL-22 ratio and high IL-22 levels were associated with adverse outcome,[11] as IL-22 signalling may not only be pro-regenerative but also induce hepatocellular production of acute-phase proteins, thus augmenting pro-inflammatory responses.[12] The interaction with IL-22BP will need to be carefully monitored in any prospective human studies.

The new link between disease phenotype and regenerative mechanisms

Another novelty of this paper is less obvious but provides a major contribution to understanding the clinical phenotype of ACLF. Our current knowledge emphasizes the special role of bacterial infections which, among all other confounding factors, have the worst impact on the disease course. Infections are among the most frequent (>30% patients) precipitating events[13] of ACLF and more than 50% of patients with ACLF develop an infection during their hospital stay, increasing their death rate from 34% to 71%.[14,15] Infections have a sustained negative effect on patients' outcome even if successfully treated, as the 1-year mortality increases by the factor 3.75 compared to non-infected patients with cirrhosis.[16] Up to now, responsibility was assigned specifically to a paralysed immune system and a subsequent lack of bacterial clearance.[3] Although this explains the high prevalence and relevance of infections in ACLF it does not necessary explain the negative impact on the long-term disease course, as true mechanistic explanations have been lacking.

Importantly, Xiang *et al.*[7] showed an important link between inflammation/infection and an impaired regenerative capacity in ACLF. Chronic liver injury reduced the proliferative response of hepatocytes due to a predominant STAT1 activation, whereas control animals showed an IL6-STAT3-mediated proliferative effect. That is not entirely surprising as the aforementioned human studies,[5,6] as well as pre-clinical studies focussing on the effect of TGFβ1, have already proposed that cirrhosis negatively modulates liver tissues' regenerative capacities.[17] Importantly, the acute hepatotoxic injury with CCl_4, despite limiting regeneration and inducing a severe liver injury, did not impair survival or long-term recovery.[7]

However, the additional induction of an infection by *Klebsiella pneumoniae* injection abrogated the regenerative liver capacity irrespective of the presence of a chronic liver injury. STAT1 and p21 overexpression inhibited hepatocyte proliferation and reduced survival. One might argue that it might be the consequence of an increasing severity of liver injury, as this has been shown to limit hepatocyte proliferation in a TGFβ1-dependent manner in models of acute liver injury[18] and severe hepatocyte injury is a typical feature of ACLF.[19] However, alanine aminotransferase levels were insignificantly affected by adding *Klebsiella* infections to the CCl_4 acute insult and lower if given as the only secondary hit in comparison to CCl_4. That observation strongly suggests that a sufficient degree of liver regenerative capacity is maintained even in the presence of chronic liver injury, however, once infections/inflammation develop tissue repair is insufficient.

This finding has significant implications as it improves our understanding of the role of inflammation and infections in ACLF and it re-directs the scientific focus at least in part towards anti-inflammatory and anti-infectious strategies.

The need for animal models in ACLF

ACLF is a multifactorial disease with multiple precipitators and complications and therefore varying disease phenotypes and organ failures. Its multifaceted character makes it almost impossible to develop a single pre-clinical animal model to mimic the entire disease spectrum, thus several different models which can cover the most important clinical features are required.

To date, well-described models included a rat model with bile duct ligation and injection of lipopolysaccharide (LPS) and a mouse model consisting of low dose CCl_4 or thioacetamide for 6-10 weeks followed by LPS injection.[20–22] The principle of all these models is to imitate the bi-factorial disease character consisting of a chronic liver injury, which leads to the development of progressive liver fibrosis, and a precipitating event inducing further organ injury. However, the clinical situation is often more complex, and different modulating factors might occur concurrently or sequentially. Typically, 50% of patients develop a bacterial infection as a complication of ACLF, although the (initial) precipitating event was non-inflammatory.[23] The authors of the current manuscript developed a new sequential tri-factorial model consisting of 8 weeks CCl_4 (0.2 ml/kg, twice a week, i.p.) followed by a double dose of CCl_4 (0.4 ml/kg) to induce an acute liver injury and subsequent injection of *Klebsiella pneumonia* (1,000 colony-forming units per mouse, i.p.) to mimic a systemic bacterial infection.[7] This model led to substantial liver and kidney injury and a high mortality rate of 87.5% after 7 days.[7] Therefore, this model not only imitates several features of ACLF, but it also enables the testing of new findings and pathways in different disease-related settings at the same time. However, it is unclear whether CCl_4 ± Klebsiella had any impact on the function of other organs, such as the brain or lungs, and using a double dose of CCl_4 as an acute liver toxin is certainly not comparable with the human situation, in which the "acute hit" might be caused by alcohol intake.

Taken together, the study presented by Xiang *et al.* provided us with a substantial piece of evidence to further understand the link between regeneration and disease phenotype in ACLF. Moreover, data about the therapeutic effect of IL22Fc might be strong enough to test this novel and original concept in human patients.

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Conflict of interest

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

Authors' contribution

All authors drafted and revised the manuscript critically for important intellectual content.

Supplementary data

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Author names in bold designate shared co-first authorship

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