

Interleukin-22 in acute-on-chronic liver failure: A matter of ineffective levels, receptor dysregulation or defective signalling?

To the Editor:

Xiang *et al.* published interesting insights about the role of interleukin (IL)-22 in acute-on-chronic liver failure (ACLF).¹ The authors elegantly analysed the effects of IL-22 in a mouse model of ACLF after chronic carbon tetrachloride (CCl₄)-treatment (0.2 ml/kg, 2×/week, i.p.) and acute precipitating injury using 2 injections of 0.4 ml/kg CCl₄ in combination with an i.p. challenge with *Klebsiella pneumoniae* (K.P.), mimicking a bacterial infection. The model seems to induce a shift in the hepatic IL-22 signalling from the pro-regenerative IL-6/STAT3 towards the anti-regenerative IFN γ /STAT1 pathway paralleled by suppression of liver regeneration. Interestingly, treatment with IL-22Fc fusion protein reduces bacterial load, improves microcirculation (hepatic/renal) and survival rate in mice with ACLF. The authors suggest that an impaired production of IL-6 from “tolerant” Kupffer cells in the fibrotic liver might be responsible for reduced liver regeneration in ACLF. They demonstrated that high-dose treatment with IL-22Fc restores downstream STAT1/STAT3 signalling imbalances. Using IL-22Fc to ameliorate ACLF is an elegant approach that can be translated into clinical trials using the IL-22 agonist F-652.^{2,3}

However, there are significant discrepancies between the data reported by Xiang *et al.* and data reported earlier in human ACLF. We recently demonstrated that circulating IL-22 levels are significantly elevated and strongly associated with mortality in these patients.⁴ Circulating levels of the endogenous IL-22, however, were not significantly elevated in the study by Xiang *et al.* unlike the human experience. To further assess the role of IL-22 in ACLF, we have developed different animal models. In a C57BL/6J mouse model of ACLF, we combined chronic liver disease (CLD) (CCl₄/ethanol⁵ or CYP2D6-linked adenovirus (ADV)-induced autoimmune hepatitis⁶) with different precipitating events (2 binges of ethanol or i.p. polymicrobial infection) (Fig. S1A+D). After 7 weeks CCl₄/ethanol we induced ACLF with 2 binges of alcohol (alcohol gavage with 31.5% Vol.) with an interval of 3 days in between the binges. Mice showed mortality (Fig. S1B), as well as systemic inflammation (Fig. 1D) and significant elevation of serum alanine aminotransferase (ALT) levels (Fig. S1C) alongside other ACLF features. In mice with chronic autoimmune hepatitis we induced polymicrobial infection according to an adapted protocol by Wynn *et al.*⁷ to achieve dose-dependent bacterial infection. Cecal slurry of a naïve donor C57BL/6J mouse was used to prepare a stool suspension (concentration 50 mg cecal slurry/1 ml sodium chloride solution) and 1 mg cecal slurry per g of bodyweight was injected.⁸ After injection of cecal slurry mice develop a serious systemic infection (Fig. 1D), mortality (Fig. S1E) and elevation of ALT serum levels (Fig. S1F). In our opinion polymicrobial sepsis is closer to human infection-triggered ACLF than the K.P. injection used in the study

by Xiang *et al.* This may be the reason why the authors tried to induce ACLF with cecal ligation and puncture surgery. Yet, this method may have drawbacks, since it is not easy to control the amount of cecal contents spilled into the peritoneal cavity, additionally the mice are exposed to a surgical procedure, which influences outcome as well. In our polymicrobial infection model, we could find a method to control the severity of infection with a specific amount of cecal slurry (Fig. S1). For both precipitating events, IL-22 serum concentrations in ACLF were elevated when compared to CLD alone. Of note, in infections, IL-22 levels are elevated regardless of the presence of CLD (Fig. 1A), suggesting that the mouse model used in the study by Xiang and co-workers is potentially not a suitable model to study mechanistic effects of IL-22, specifically in ACLF. Based on the paper by Xiang and co-workers, one may speculate that the increased IL-22 levels might be an endogenous attempt to improve organ function. Yet, the clinical course of disease shows no benefit from elevated IL-22 levels, which cannot be explained by the data published. A possible explanation is that IL-22 might be scavenged by the IL-22 binding protein (IL-22BP), which is highly upregulated in this condition as we recently showed in patients with ACLF⁴ and Xiang *et al.* also show in their mouse model. Correspondingly, using a rat model of ACLF, IL-22BP levels are elevated in peripheral blood mononuclear cells in ACLF (lipopolysaccharide 6.25 μ g day 21 and day 25, n = 4) compared to acute decompensation (AD, lipopolysaccharide 6.25 μ g day 21, n = 5) (Fig. 1B). The novel experimental data are in line with our previous findings on ACLF and IL-22 in humans, showing that a significant elevation of IL-22BP is strongly associated with mortality in patients with ACLF and *in vitro* inhibition of hepatocellular IL-22 signalling in human hepatoma cells.⁴ Hence, the lack of a hepatoprotective effect of IL-22 in ACLF could potentially be due to reduced circulating levels of this cytokine in its free/unbound form. Alternatively, based on differential expression levels of the receptor subunits IL-22R1 and IL-10R2, we infer that IL-22 signalling could be modified at the receptor level, possibly by impaired receptor assembly (Fig. 1C). Such a mechanism could cause ineffective signalling or potentially modulate the downstream signalling cascade as suggested by Xiang *et al.* It seems likely that both mechanisms, IL-22BP scavenging and modified IL-22 receptor assembly, are not mutually exclusive but rather could exist in parallel. However, mechanisms on the IL-22 receptor level remain largely unexplained. In addition, Xiang and co-workers suggest that an increase in hepatic IL-6 might be beneficial in ACLF and could lead to enhanced liver regeneration. This conclusion seems puzzling given that in human ACLF high IL-6 levels were associated with worse outcomes in several independent studies.^{9,10} Systemic inflammation including an increase in IL-6 precedes ACLF in humans and is, even in the absence of infection, strongly associated with the development of ACLF and death.^{9,10} In our mouse models, IL-6 is elevated in ethanol- as well as in infection-triggered ACLF (Fig. 1D). One may

Received 20 April 2020; received in revised form 12 May 2020; accepted 12 May 2020
<https://doi.org/10.1016/j.jhep.2020.05.012>



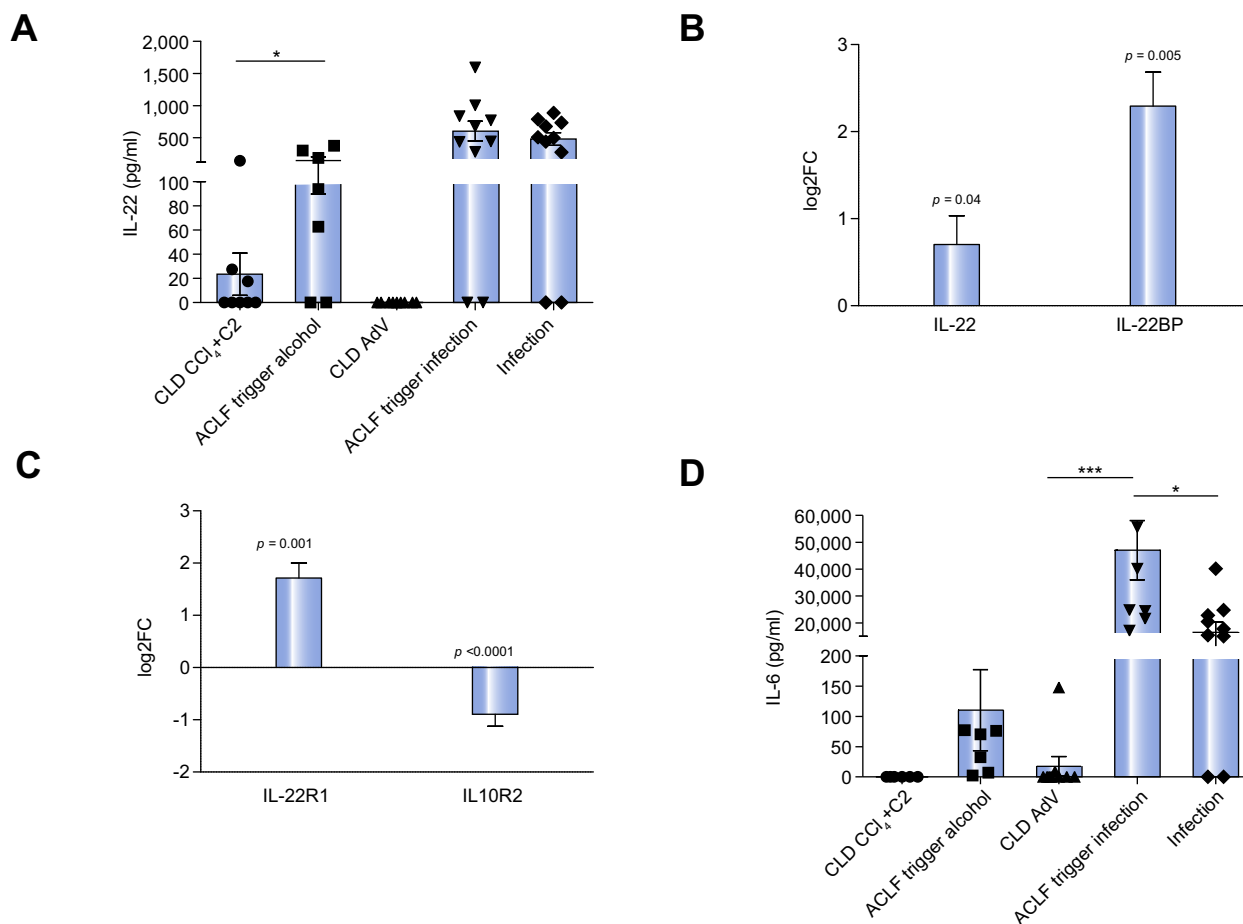


Fig. 1. IL-22 and IL-6 expression in ACLF models. (A) Increase of IL-22 serum concentration in ACLF mice with alcohol-triggered ACLF and infection-triggered ACLF in comparison to mice with chronic alcoholic liver cirrhosis (CLD CCl₄+C2) or chronic autoimmune hepatitis (CLD ADV) [n = 7–10]. (B) Transcriptome data of PBMCs: upregulation of IL-22 (ACLF vs. AD; $p = 0.04$) and IL-22BP (ACLF vs. AD $p = 0.005$) in ACLF [n = 4–5]. (C) IL-22R1 subunit of the IL-22 receptor is significantly upregulated in ACLF (ACLF vs. AD; $p = 0.001$) while the IL-10R2 subunit of the IL-22 receptor was downregulated (ACLF vs. AD; $p < 0.0001$) [n = 4–5]. (D) IL-6 serum concentration in alcohol-triggered ACLF and infection-triggered ACLF in comparison to mice with chronic alcoholic liver cirrhosis (CLD CCl₄+C2), chronic autoimmune hepatitis (CLD ADV) or infection only (Infection) [n = 9–10]. A and D measured by ELISA, statistical evaluation with unpaired 2-tailed t test. B and C transcriptome data of PBMCs, statistical evaluation with Welch's t test. ACLF, acute-on-chronic liver failure; AD, acute decompensation; ADV, adenovirus; CCl₄, carbon tetrachloride; CLD, chronic liver disease; IL-, interleukin; PBMCs, peripheral blood mononuclear cells.

speculate that high levels of IL-6 reflect dysfunction of the immune system and lead to failure in the regeneration process.¹¹ Given the different lines of evidence and lack of mechanistic detail, caution is required in the interpretation of the results and further studies on IL-22 are needed to fully understand its impact on the course of liver disease and ACLF, as well as its therapeutic or preventive potential.

Financial support

Dr. Katharina Maria Schwarzkopf was funded by Nachwuchsförderung of Goethe University, Frankfurt, Germany.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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

Authors' contributions

KMS designed and conducted the experimental studies and wrote the paper; LE, FEU, SK, RS, MMM conducted some experimental studies, performed data analysis and edited the paper; LS, JC, SZ, EH, UC, CML helped with experimental design and edited the paper; JT and CW designed the experimental studies, supervised the whole project and wrote the paper.

Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jhep.2020.05.012>.

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