Authors’ reply to: G-CSF exacerbates liver injury in a mouse model of autoimmune hepatitis

Cornelius Engelmann\textsuperscript{1,2,3,4}, Rajiv Jalan\textsuperscript{2}

\begin{itemize}
  \item \textsuperscript{1} Department of Hepatology and Gastroenterology, Charité Universitätsmedizin Berlin, Berlin, Germany
  \item \textsuperscript{2} University College London, Institute for Liver and Digestive Health, London, United Kingdom
  \item \textsuperscript{3} Berlin Institute of Health (BIH), Berlin, Germany
  \item \textsuperscript{4} Division of Hepatology, Department of Medicine II, Leipzig University Medical Center, Leipzig, Germany
\end{itemize}

Corresponding author

Rajiv Jalan, Institute for Liver and Digestive Health, Division of Medicine, University College London, Rowland Hill Street, NW32PF London, United Kingdom

Tel.: +442074332795

Email: r.jalan@ucl.ac.uk

Funding: None

Table: 0

Figures: 0

Word count – 786 (main text excluding references)

Conflict of interests: Rajiv Jalan is the inventor of OPA, which has been patented by UCL and licensed to Mallinckrodt Pharma. He is also the founder of Yaqrit Discovery, a spin out company from University College London, Hepyx Limited and Cyberliver. He had research collaborations with Yaqrit Discovery. Cornelius Engelmann has received advisory fees from Novartis, CSL Behring and Albireo. He is shareholder of Hepyx Ltd. Rajiv Jalan and Cornelius Engelmann...
Engelmann are named inventors on the patents surrounding the use of G-TAK in ACLF, which have been filed as a priority application. This patent has been licensed to Hepyx Ltd.

Authors’ contribution. Both authors drafted and critically revised the manuscript.
Reply:

Inflammation and a lack of regeneration are pathomechanistic hallmarks of acute-on-chronic liver failure (ACLF) and its treatment is an unmet need (1). Granulocyte-colony stimulating factor (G-CSF), was considered to be the first disease modifying therapy in ACLF (2) but it failed to show efficacy in the first multicenter trials (GRAFT study) (3). Our recently presented data in the Journal of Hepatology emphasized that in endotoxin driven ACLF mouse models the administration of G-CSF causes an exaggerated inflammatory response, tissue injury and high rate of fatalities (4).

Yi Shen and colleagues presented data to support the finding that G-CSF acts deleterious in the context of severe liver disease (5). In a mouse model of concanavalin A (ConA) induced acute autoimmune liver disease they injected two different doses of G-CSF, 12.5\(\mu\)g/kg and 25\(\mu\)g/kg subcutaneously. G-CSF led to a more severe liver injury, infiltration of neutrophils (Ly6G+) and high mortality in a dose dependent manner (5). These results are in line with our recent data and other reports. It was shown that G-CSF therapy sensitizes the liver to endotoxin. Lipopolysaccharide (LPS) injection after G-CSF treatment led to an enhanced LPS uptake into the liver, high grade liver injury and increased tissue expression of TNF-\(\alpha\), IL-6 and IL1\(\beta\) (6). Likewise, G-CSF pre-treatment prior to 70% partial hepatectomy in mice and subsequent LPS injection led to pronounced erythrocyte congestion in the liver, microcirculatory disorders, neutrophil infiltration and 100% mortality. This G-CSF related sensitization of the liver was associated with an increased expression of toll-like receptor 4 (TLR4) (7) which provided the rational for combining G-CSF with a TLR4 inhibitor in our recently published study. TLR4 is also one of the central mediators of inflammation and organ failure in ACLF (6-9). Its inhibition was shown to reduce tissue injury in ACLF models (8). Therefore, the combination of liver disease and G-CSF seems to act as a dual sensitizing mechanism through TLR4 upregulation.

Accordingly, combining G-CSF with the TLR4 inhibitor TAK-242 prevented inflammation and enhanced regeneration in mouse models of ACLF.(4) This is now being evaluated in (A-TANGO) in patients with ACLF (https://a-tango.eu/). Whilst this approach is based on the observation that disease progression from cirrhosis to ACLF (8, 9) and/or the G-CSF driven excess inflammation is mediated by TLR4 (7). Yi Shen and colleagues were not able to link their G-CSF driven excess inflammation to TLR4 (5). In fact, they showed decreased TLR4 expression in the liver with G-CSF treatment, whilst the induction of autoimmune liver disease was associated with unchanged TLR4 levels. These results are intriguing as TLR4 signaling was shown to be an important mechanisms in both, acute and chronic liver disease (8). One explanation for this discordance may be may be that the ConA model has distinct effects on the receptor expression of immune cells, which are the main carrier of TLR4. However,
enhanced liver expression and activated receptor signaling after ConA injection was shown in several previous studies (10, 11). Inhibition of the TLR4 using JKB-122 reduced the severity of necrotic lesions and cytokinemia (12).

Another potential explanation may be that the overstimulated immune system leads to immune cell exhaustion and cell fate of TLR4 expressing cells. As already suggested by the authors, neutrophils may be the cells of interest in that context. High-density neutrophils are known to be activated in alcoholic hepatitis and LPS stimulation results in an exaggerated oxidative stress and neutrophil extracellular traps (NET). These, in turn increase the number of defective low-density neutrophils which cannot phagocytose bacteria (13). Whether the transition between both cellular phenotypes is associated with a loss if TLR4 expression remains speculative but additional immunofluorescence analyses by Shin et al showed that while there was a strong neutrophil recruitment into the liver the NET formation and hence a change of neutrophil phenotype was not relevant in their model.

The effect of immune cell mobilization by colony stimulating factors on TLR4 expression is controversial. G-CSF regulates the activity of hematopoietic stem cells through TLR4, hence requiring this pathway to maintain the activity in some cell subsets (14). Therefore, there is no plausible evidence in the literature that G-CSF may actually down-regulate TLR4. Furthermore, in vivo loss of TLR4 expression at one time point in a disease model does not preclude the overexpression and stimulation at an earlier time point and previous results from other pre-clinical experiments strongly indicate that TLR4 plays an overarching pathophysiological role across different types of liver disease.

Therefore, it seems obvious that the combination of G-CSF and TLR4 inhibition should be tested not only for ACLF and alcoholic hepatitis but also for other types of severe liver disease. Understanding the disease phenotype as well as similarities and differences across patients’ clusters will be key to select patient for treatments, which are tailored for the individual needs.

Bibliography
