HHS Public Access

Author manuscript

eGastroenterology. Author manuscript; available in PMC 2024 December 31.

Published in final edited form as:

eGastroenterology. 2024 October ; 2(4): . doi:10.1136/egastro-2024-100166.

New therapeutic target for alcohol-associated hepatitis (AH): AH-associated IL-8+ neutrophils

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Alcohol-associated liver disease (ALD) is one of the leading chronic liver diseases worldwide, encompassing a spectrum of conditions from simple steatosis to steatohepatitis, cirrhosis, and hepatocellular carcinoma.[1] Histologically, alcohol-associated steatohepatitis (ASH) is characterised by lipid accumulation in hepatocytes, hepatocellular damage, ballooning of hepatocytes, formation of Mallory-Denk bodies, inflammation and pericellular fibrosis.[1] Over the past several decades, research has elucidated key molecular mechanisms underlying ALD, such as ethanol-induced hepatotoxicity, oxidative stress, endoplasmic reticulum stress and organ–organ crosstalk pathways like the gut-liver and adipose tissue-liver axes.[2] Patients with underlying chronic ALD, particularly those with recent episodes of excessive drinking, are at risk of developing acute alcohol-associated hepatitis (AH), a condition characterised by severe clinical symptoms, including jaundice.[3] Severe AH (sAH) is commonly linked to alcohol-associated cirrhosis (AC) and carries a high short-term mortality rate. Due to the limited availability of effective pharmacological therapies, early liver transplantation is frequently required as a life-saving intervention.[3] Identifying molecular mechanisms driving AH progression and liver failure is thus essential for developing targeted therapies for this life-threatening disease. AH, first described in 1961, is recognised as an acute inflammatory liver disease characterised by extensive inflammatory cell infiltration.[4] Currently, AH is recognised as a form of acute-on-chronic liver injury involving various types of immune cells, including macrophages, neutrophils, T cells and B cells, as evidenced by immunohistochemical analysis.[5] Recent RNA-sequencing analyses

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Contributors YG and DF wrote the manuscript. LM and YW edited the manuscript. BG supervised the whole project and edited the manuscript.

Competing interests BG is an Associate Editor for *eGastroenterology*.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Provenance and peer review Commissioned; internally peer reviewed.

have highlighted that AH is associated with the upregulation of numerous inflammatory mediators, including pro-inflammatory cytokines, chemokines and adhesion molecules.[5–7] However, the key mechanisms that drive the transition from ALD to sAH remain unclear.

Neutrophil infiltration, a hallmark of AH, plays a critical role in ALD progression.[8] In AH, multiple CXC chemokines that mediate neutrophil migration, such as interleukin (IL)-8 (CXCL8), CXCL1, CXCL5, CXCL6 and CXCL10, are significantly elevated.[9] Although these chemokines likely work in concert to promote neutrophil infiltration and activation, further research is needed to elucidate the specific cell types that produce these chemokines, the mechanisms driving their induction and precise functions in AH. Notably, compared with humans, mice have lower neutrophil counts (~1×10^9/L in mice vs ~4×10^9/L in humans) and lack key neutrophil chemoattractants like human IL-8 and CXCL6,[10] making it challenging to study IL-8 and CXCL6 functions in AH through mouse models. To overcome these limitations of mouse models, we recently analysed human sAH samples by performing single-cell RNA sequencing (scRNA-seq) analysis and identified AH-associated IL-8^+ neutrophils in sAH livers but not in the blood.[11] IL-8, the most important chemokine that is involved in the recruitment and activation of neutrophils,[12] is significantly elevated in patients with AH, correlating with AH severity.[9] While previous studies reported rat hepatocytes and Kupffer cells produced IL-8 analogue CINC-1 (cytokine-induced neutrophil chemoattractant-1) in the setting of ethanol,[13 14] and human hepatocytes produced IL-8 in response to fatty acid stimulation,[15] our recent studies provided several lines of evidence suggesting that neutrophils are the dominant source of IL-8 in sAH. First, scRNA-seq analysis revealed a distinct IL-8^+ neutrophil population enriched in sAH livers but not in AC livers. Second, multiplex immunofluorescence staining analyses of liver tissues revealed that the majority of hepatic neutrophils in sAH are IL-8 positive, whereas circulating neutrophils in the same patients have minimal IL-8 expression. Third, immunofluorescence staining analyses of isolated neutrophils also confirmed neutrophils from sAH livers expressed much higher levels of IL-8 than those from the blood. In addition to IL-8, several other neutrophil chemokines such as CXCL1, CXCL5 and CXCL6, also upregulated in sAH livers, as shown by RNA sequencing and ELISA.[11] Notably, scRNA-seq analyses of sAH livers demonstrated that hepatocytes express CXCL1, hepatic stellate cells produce CXCL5, neutrophils predominately express IL-8, while macrophages and hepatocytes express low levels of IL-8.[11] Thus, an autocrine IL-8 loop may drive recruitment and activation of neutrophils in sAH, and IL-8 expression in neutrophils is sustained by elevated IL-1β and tumour necrosis factor (TNF)-α levels in the liver via the activation of p38 mitogen-activated protein kinase (MAPK) (figure 1).

In addition to sAH, IL-8^+ neutrophils have been detected in other diseases such as severe SARS-CoV-2 infection,[16] but have not been carefully investigated in other types of liver diseases other than sAH. Previous studies highlight a key distinction between sAH and other liver diseases such as metabolic dysfunction associated steatohepatitis and viral hepatitis, IL-8^+ neutrophils most likely exist in these diseases but probably at much lower frequencies compared with sAH patients.[17–19] Interestingly, low abundant IL-8^+ neutrophils have been identified in hepatocellular carcinoma, where they are linked to tumour progression and immunosuppression.[20 21]

As discussed above, IL-8$^+$ neutrophils amplify neutrophil recruitment and activation and subsequently intensify liver inflammation specifically in sAH patients. This notion makes IL-8$^+$ neutrophils a promising therapeutic target, with the potential to selectively attenuate the inflammatory profile of sAH without affecting other hepatic conditions. Several strategies have been developed for targeting IL-8 signalling.[12] For example, anti-IL-8 antibodies, such as ABX-IL-8 and HuMax-IL-8 (BMS-986253), are currently being investigated in Phase I/II clinical trials for various cancers and inflammatory diseases.[12] Another approach involves inhibiting IL-8 receptors CXCR1 and CXCR2 using short lipopeptides (pepducins), which have been shown to ameliorate mild ASH in mouse models.[22] Multiple CXCR1/CXCR2 antagonists are currently being tested in clinical trials for cancer, diabetes and inflammation,[12] making them potential candidates for sAH therapy. Targeting inflammatory stimuli and signals such as p38 MAPK that drive IL-8 expression is also an alternative option to block IL-8. Given the association between bacterial infection and sAH, monitoring for infections is crucial when implementing anti-IL-8 or anti-CXCR1/2 therapies. Combination therapy with antibiotics or IL-22, a cytokine with potent hepatoprotective and antibacterial effects, could help mitigate infection risk and promote liver repair and regeneration.[23 24] In summary, we believe that IL-8 signalling represents a novel, specific, and promising target for the treatment of sAH, with several drug candidates currently available for evaluation in clinical trials.

Acknowledgements

The authors thank Dr Suthat Liangpunsakul (Indiana University) for editing the manuscript.

Funding

The work was supported by the intramural programme of NIAAA and NIH (AA000369 and AA000368).

Data availability statement

No data are available.

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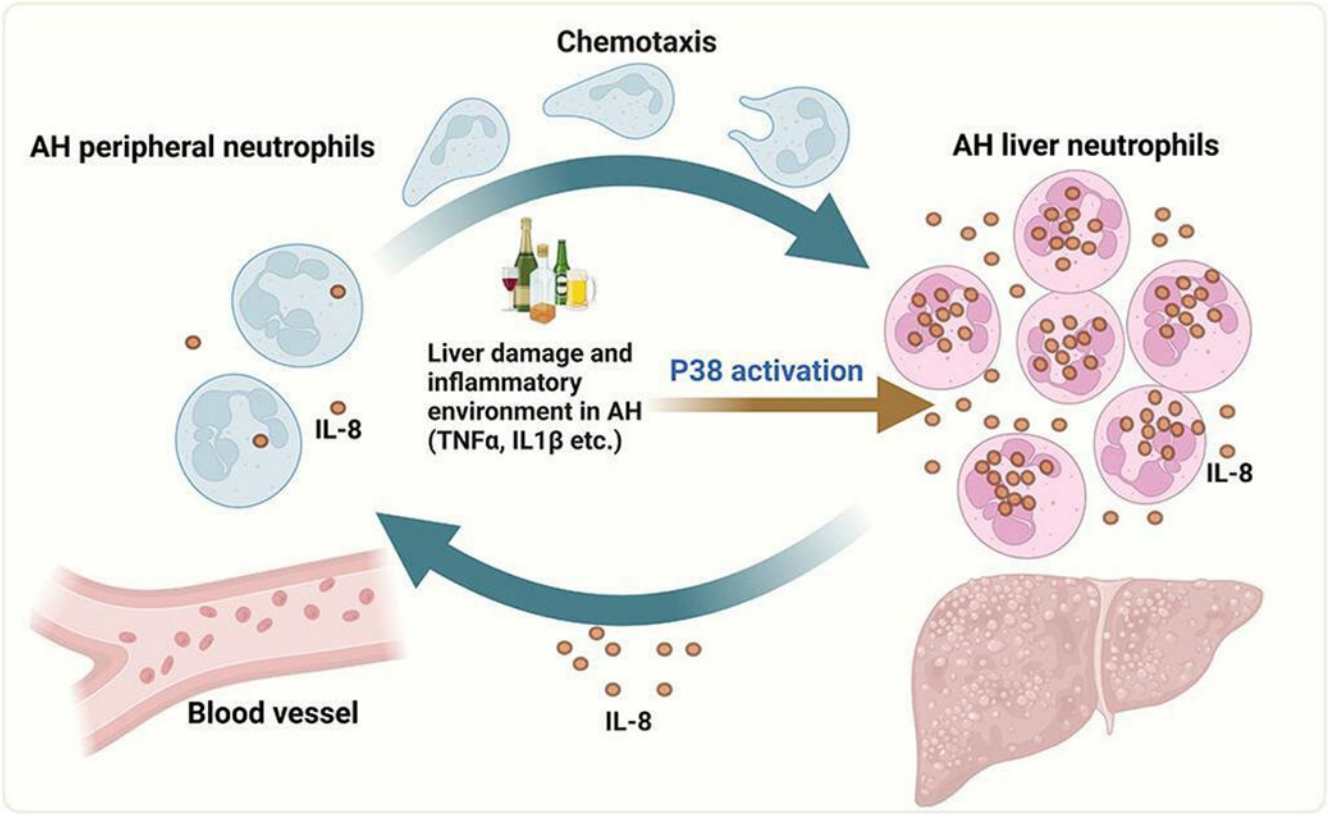

Figure 1.

IL-8^{+} neutrophils induce a vicious cycle that drives unstoppable inflammation in AH. Peripheral neutrophils from sAH patients initially express low levels of IL-8. However, on infiltrating the sAH liver, these neutrophils are stimulated by the inflammatory environment, including mediators such as TNF-α and IL-1β, to produce high levels of IL-8. This IL-8 production amplifies systemic IL-8 levels, further recruiting neutrophils to the liver and perpetuating a vicious cycle of inflammation. As central drivers of this inflammatory loop, IL-8^{+} neutrophils represent promising therapeutic targets for managing sAH. Diagram was created with BioRender.com. AH, alcohol-associated hepatitis; IL, interleukin; sAH, severe AH; TNF, tumour necrosis factor.