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Cell-to-cell and organ-to-organ crosstalk in the pathogenesis of alcohol-associated liver disease

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Abstract

Alcohol-associated liver disease (ALD) is a growing global health concern and its prevalence and severity are increasing steadily. While bacterial endotoxin translocation into the portal circulation is a well-established key factor, recent evidence highlights the critical role of sterile inflammation, triggered by diverse stimuli, in alcohol-induced liver injury. This review provides a comprehensive analysis of the complex interactions within the hepatic microenvironment in ALD. It examines the contributions of both parenchymal cells, like hepatocytes, and non-parenchymal cells, such as hepatic stellate cells, Kupffer cells, neutrophils, and liver sinusoidal endothelial cells, in driving the progression of the disease. Additionally, we explored the involvement of key mediators, including cytokines, chemokines and inflammasomes, which regulate inflammatory responses and promote liver injury and fibrosis. A particular focus has been placed on extracellular vesicles (EVs) as essential mediators of intercellular communication both within and beyond the liver. These vesicles facilitate the transfer of signalling molecules, such as microRNAs and proteins, which modulate immune responses, fibrogenesis and lipid metabolism, thereby influencing disease progression. Moreover, we underscore the importance of organ-to-organ crosstalk, particularly in the gut-liver axis, where dysbiosis and increased intestinal permeability lead to microbial translocation, exacerbating hepatic inflammation. The adipose-liver axis is also highlighted,

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particularly the impact of adipokines and free fatty acids from adipose tissue on hepatic steatosis and inflammation in the context of alcohol consumption.

INTRODUCTION

Alcohol-associated liver disease (ALD) presents a significant global public health challenge due to its strong link to excessive alcohol consumption.[1 2] ALD is the leading cause of liver-related mortality, encompassing a wide range of liver disorders, from simple hepatic steatosis to more severe conditions like alcohol-associated hepatitis (AH), cirrhosis, and eventually hepatocellular carcinoma (HCC).[2 3] The progression of ALD is influenced by a complex interplay of genetic, environmental and metabolic factors, highlighting the urgent need to deepen our understanding of its pathogenesis to develop effective prevention and treatment strategies.[4 5]

The pathophysiology of ALD is multifaceted, involving numerous interconnected mechanisms triggered by alcohol metabolism.[6] On ingestion, alcohol is primarily metabolised in hepatocytes by enzymes such as alcohol dehydrogenase (ADH) and cytochrome P450 2E1 (CYP2E1), producing acetaldehyde and reactive oxygen species (ROS).[6] These byproducts induce oxidative stress, lipid peroxidation and the formation of DNA and protein adducts, causing direct hepatocellular damage and impaired cellular functions.[6 7] Chronic alcohol consumption also disrupts the gut epithelial barrier, increasing intestinal permeability and allowing bacterial products like lipopolysaccharides (LPS) to enter the portal circulation.[8] This translocation exacerbates liver injury by activating macrophages and recruiting neutrophils, which release pro-inflammatory cytokines and chemokines that amplify the local inflammatory response.[7] Alcohol-induced protein adducts can also trigger an adaptive immune response by activating T cells, while chronic alcohol use impairs $CD4^+$ T cell immunometabolism, promoting their differentiation into a pro-inflammatory phenotype.[9]

Hepatic stellate cells (HSCs) play a pivotal role in the progression of ALD. When exposed to chronic alcohol-induced inflammation, HSCs become activated, transdifferentiating into myofibroblasts.[10] These myofibroblasts are responsible for the excessive production of extracellular matrix (ECM) components, which leads to liver fibrosis, a key feature in the transition from early stage ALD to cirrhosis.[10]

The intricate and multifaceted pathogenesis of ALD also involves various other mediators that contribute to inflammation and fibrosis. Cytokines, such as tumour necrosis factor-alpha (TNF-α), interleukins (ILs) and transforming growth factor-beta (TGF-β), play central roles in promoting inflammation and fibrosis.[7 10] Additionally, extracellular vesicles (EVs), which are released by damaged hepatocytes and other liver cells, carry signalling molecules like microRNAs (miRNAs) and proteins that modulate immune responses, fibroblast activation and lipid metabolism.[11] These EVs act as critical mediators of intercellular communication, spreading inflammatory and fibrogenic signals both locally within the liver and systemically, contributing to extrahepatic complications of ALD.

The systemic nature of ALD is further highlighted by the interplay between the liver and extrahepatic organs, particularly through the gut-liver and adipose-liver axes. This gut-derived inflammation fuels liver injury by triggering immune responses and enhancing HSC activation.[12] Similarly, the adipose-liver axis involves the release of adipokines and free fatty acids from adipose tissue, which aggravate hepatic steatosis and inflammation, further contributing to ALD progression.[13]

Given the rising prevalence and complexity of ALD, it is critical to synthesise existing knowledge and identify gaps in our understanding of its pathogenesis. This review aims to provide a comprehensive overview of the multifactorial mechanisms underpinning ALD, emphasising the roles of key cellular players, inflammatory mediators and the significance of intercellular communication pathways. By integrating these diverse aspects of ALD, we hope to inform future research directions and therapeutic strategies, ultimately leading to improved management of ALD.

Hepatic parenchymal and non-parenchymal cells, their roles and interactions in the pathogenesis of ALD

The liver's complex architecture is composed of both parenchymal and non-parenchymal cells, each contributing to its overall function and response to injury. Hepatocytes, the predominant parenchymal cells, are primarily responsible for the liver's metabolic, detoxifying and synthetic activities, including alcohol metabolism.[6] However, non-parenchymal cells, such as HSCs, Kupffer cells (KCs), infiltrating macrophages, neutrophils and liver sinusoidal endothelial cells (LSECs), are equally vital in maintaining liver homeostasis and orchestrating its response to alcohol-induced damage.[7] The intricate crosstalk between these parenchymal and non-parenchymal cells is key to understanding the progression of ALD (figure 1).

Hepatocytes—Alcohol metabolism predominantly occurs in the liver,[6] where key enzymes such as ADH1, CYP2E1, catalase and aldehyde dehydrogenase 2 (ALDH2) are responsible for converting alcohol into acetaldehyde and then into acetate.[6] This metabolic process generates ROS and shifts the cellular NAD+/NADH ratio by increasing NADH levels, leading to a reduction in NAD+, a crucial cofactor in numerous metabolic pathways.[6 14] This imbalance promotes hepatic steatosis, as the excess NADH favours fatty acid synthesis over oxidation.[14]

The increased metabolic activity in the liver during ethanol breakdown also results in higher oxygen consumption, especially in the pericentral zone of the liver lobule, where oxygen tension is already lower.[14] This localised hypoxia exacerbates liver damage by generating additional ROS, which further impairs mitochondrial fatty acid oxidation, while simultaneously upregulating genes involved in lipid synthesis.[14]

Acetaldehyde, a highly reactive intermediate produced during alcohol metabolism, compounds liver injury by forming adducts with proteins, DNA and lipids.[15] These acetaldehyde-protein and acetaldehyde-DNA adducts disrupt cellular functions by inhibiting DNA repair, protein synthesis and enzymatic activities, thereby triggering oxidative stress and inflammation.[15] Acetaldehyde also depletes glutathione, a critical antioxidant

in hepatocytes, rendering them more vulnerable to ROS-induced damage.[16] Moreover, acetaldehyde acts as a signalling molecule that activates HSCs, contributing to liver fibrosis.[17]

The oxidative stress induced by ROS causes direct hepatocellular damage and leads to the release of damage-associated molecular patterns (DAMPs) such as high mobility group box-1 (HMGB1) and mitochondrial DNA (mtDNA).[18] These DAMPs activate pattern recognition receptors (PRRs) such as toll-like receptors (TLRs) and NOD-like receptors, which initiate inflammatory responses and recruit immune cells to the site of injury. In particular, HMGB1 has been shown to play a critical role in the pathogenesis of ALD.[18] Studies have demonstrated that hepatocyte-specific knockout of HMGB1 ameliorates alcohol-induced liver injury, highlighting its importance in promoting liver inflammation.[19] Similarly, TLR4 signalling within hepatocytes has been implicated in ALD progression. Mice with hepatocyte-specific deletion of TLR4 exhibit reduced steatosis and inflammation following chronic or acute ethanol exposure.[20]

Emerging research has also focused on hepatocyte reprogramming, particularly in the context of AH. In severe cases of AH, hepatocytes undergo dedifferentiation, adopting a hepatobiliary phenotype, which is associated with poor clinical outcomes.[21] CXCR4, a chemokine receptor, has been implicated in driving this dedifferentiation process, and its inhibition has been shown to reverse these effects in experimental models.[21]

Additionally, transcription factors play a pivotal role in regulating hepatocyte responses to alcohol exposure. One such factor, forkhead box protein O1 (FoxO1), has been shown to downregulate the expression of miR-148a in hepatocytes in response to ethanol.[22] This suppression of miR-148a leads to overexpression of thioredoxin-interacting protein (TXNIP), which activates the NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome and promotes hepatocyte pyroptosis, a form of programmed cell death associated with inflammation.[22] Interestingly, restoring miR-148a levels in hepatocytes has been shown to reverse ethanol-induced steatosis and inflammation.[22]

This expanding body of research highlights the intricate interplay between alcohol metabolism, oxidative stress and inflammatory pathways in the liver. By focusing on mitigating oxidative damage, controlling inflammatory cascades and modulating hepatocyte reprogramming, we can advance the treatment of ALD.

Liver sinusoidal endothelial cells—LSECs, the most abundant non-parenchymal cells in the liver, form the vascular bed of the liver and play a critical role in maintaining hepatic homeostasis. Their unique structural features, including the presence of fenestrae and the absence of a basement membrane, facilitate the selective filtration of blood components and ensure efficient exchange between the bloodstream and liver parenchymal cells. These fenestrae are essential for maintaining liver function, as they allow macromolecules to pass freely between the blood and hepatocytes, facilitating nutrient and waste exchange.

Beyond their physical filtration role, LSECs serve several functional roles, including scavenging bloodborne molecules, regulating immune responses and maintaining HSC

quiescence, which prevents excessive liver fibrosis. These functions are critical for overall liver health and are essential for preventing liver diseases, such as fibrosis and cirrhosis, particularly in conditions like metabolic dysfunction-associated steatotic liver disease (MASLD).[23] LSECs are also involved in the metabolism of various substances, including ethanol, through the expression of key enzymes such as ADH1 and CYP2E1.[24] While much research has focused on the role of LSECs in MASLD, their involvement in ALD is less well understood and requires further exploration. In ALD, LSECs metabolise ethanol primarily through CYP2E1, which is upregulated on ethanol exposure. This ethanol metabolism in LSECs leads to a decrease in nitric oxide (NO) production, largely due to alterations in the acetylation of heat shock protein 90 (Hsp90) and its interaction with endothelial nitric oxide synthase.[24] Importantly, studies suggest that blocking Hsp90 acetylation may restore NO production and improve LSEC function.[24]

In addition to ethanol metabolism, LSECs are implicated in the progression of fibrosis and inflammation in liver diseases. For instance, capillarisation of LSECs, characterised by the loss of fenestrae and the development of a basement membrane, has been observed in liver diseases such as metabolic-associated steatohepatitis and AH.[25] Inducing apoptosis in capillarised LSECs has been shown to reduce fibrosis and inflammation in murine models, highlighting the significance of maintaining LSEC integrity to prevent disease progression.[25]

One of the key regulators of redox homeostasis and inflammation in LSECs is TXNIP, which is significantly elevated in both patients and murine models of ALD.[26] TXNIP modulates the inflammatory response and plays a pivotal role in ethanol-induced liver injury. Specific knockout of TXNIP in LSECs leads to sinusoidal capillarisation, reduced NO production and increased inflammation, thereby aggravating liver injury, fibrosis and HCC development in ALD.[26] Conversely, overexpression of TXNIP reverses these effects.[26]

The loss of fenestrae and the capillarisation of LSECs are also associated with disrupted crosstalk between LSECs and other liver cells, including hepatocytes, HSCs and macrophages, further contributing to the development of liver fibrosis.[27] Moreover, elevated plasma markers such as vascular cell adhesion molecule-1, intercellular adhesion molecule-1, E-selectin and von Willebrand factor are observed in patients with severe AH and are associated with disease severity and prognosis.[28] These endothelial dysfunction markers suggest that LSECs play a critical role in the pathogenesis of ALD and may serve as predictors of patient outcomes in AH.

Furthermore, LSECs have been shown to recruit bone marrow progenitor cells in response to liver injury, such as partial hepatectomy or toxin exposure, facilitating liver regeneration.[29] However, their role in the regenerative response in ALD remains unclear and is an area of ongoing research. Understanding how LSECs contribute to liver repair mechanisms in ALD could unlock new therapeutic strategies to enhance liver regeneration in these patients.

In summary, LSECs are integral to various aspects of ALD pathogenesis, including ethanol metabolism, oxidative stress regulation, inflammation, fibrosis and potentially regeneration. Given their central role in liver function and disease progression, targeting LSECs and

their associated molecular pathways represents a promising therapeutic approach for treating ALD and improving patient outcomes.

Macrophages—The liver contains two distinct populations of macrophages: resident KCs and infiltrating macrophages derived from circulating monocytes. KCs are specialised macrophages that maintain liver homeostasis by clearing pathogens and cellular debris under normal conditions.[30] However, during liver injury, such as chronic ethanol exposure, the liver's inflammatory environment triggers the recruitment of circulating monocytes.[31] These monocytes differentiate into infiltrating macrophages, which play a crucial role in tissue homeostasis, particularly in response to the heightened demands of pathogen clearance, tissue repair and immune modulation.[30]

In mouse models, infiltrating macrophages arise from two major subsets of circulating monocytes: classical Ly-6C^{hi} and non-classical Ly-6C^{low} cells.[32] These are analogous to the human monocyte subsets CD14^{hi}CD16^{-} and CD14^{dim}CD16^{+} monocytes, respectively.[32] Ly-6C^{low} macrophages are characterised by their anti-inflammatory and tissue-protective properties.[30] They promote tissue repair and help resolve inflammation, thus contributing to liver recovery following injury.[30] In contrast, Ly-6C^{hi} macrophages adopt a pro-inflammatory phenotype, releasing cytokines that exacerbate liver damage and inflammation.[30] Interestingly, Ly-6C^{hi} macrophages can transition to a Ly-6C^{low} phenotype after phagocytosing apoptotic hepatocytes, indicating a possible regulatory mechanism that aids in resolving inflammation and promoting tissue repair.[30]

In ALD, both infiltrating macrophages and neutrophils are key players in the immune response.[33] These cells collaborate to clear necrotic cellular debris, which is essential for maintaining tissue integrity.[33] However, macrophage activation plays a central role in modulating the inflammatory response, as inhibiting macrophage activation reduces neutrophil recruitment to the liver, potentially decreasing inflammation and injury.[34] This suggests that macrophages are involved in direct pathogen clearance and regulate neutrophil-mediated responses, further illustrating their complex role in ALD pathogenesis.

The pivotal role of KCs in ALD has been demonstrated through the use of gadolinium chloride, a compound that selectively depletes KCs.[35] Studies have shown that blocking KC activity can alleviate liver injury, underscoring their importance in the progression of ALD.[35] Activated KCs contribute to liver fibrosis by secreting profibrogenic factors, such as TGF-β and platelet-derived growth factor (PDGF), which promote the activation of HSCs, the main effector cells in liver fibrosis.[35]

One of the key mechanisms driving macrophage and KC activation in ALD is the upregulation of TLRs, particularly TLR4, in response to ROS.[36] Chronic alcohol consumption leads to increased ROS production, which sensitises KCs and macrophages to bacterial endotoxins, such as LPS.[37] This sensitisation amplifies the inflammatory response in the liver.[37] ROS-mediated activation of the CD14/TLR4 receptor complex on macrophages perpetuates the inflammatory cascade, contributing to ongoing liver injury.[31 37]

Macrophages, including KCs, exhibit significant plasticity, enabling them to transition between pro-inflammatory (M1) and anti-inflammatory (M2) phenotypes in response to environmental stimuli.[32] This dynamic polarisation allows macrophages to adapt to different stages of liver injury and recovery, playing distinct roles in ALD.[32] M1 macrophages are typically activated by pathogen-associated molecular patterns (PAMPs) like LPS, primarily via TLRs. On activation, M1 macrophages release a host of pro-inflammatory cytokines, such as TNF-α, IL-6, IL-1β and ROS, which are crucial in initiating and perpetuating inflammatory responses.[38] In addition to cytokines, M1 macrophages secrete chemokines like C-X-C motif chemokine ligand (CXCL) 9 and CXCL10, which further recruit T helper 1 (Th1) cells and propagate inflammation in the liver.[39]

In contrast, M2 macrophages are associated with the resolution of inflammation and tissue repair.[38] These macrophages are activated by signals indicating that the tissue injury or infection is subsiding, and they release anti-inflammatory mediators like TGF-β, IL-10 and chemokines such as C-C motif chemokine ligand (CCL)17, CCL18, CCL22 and CCL24.[38] These signalling molecules help attract regulatory T cells (Tregs), which contribute to suppressing immune responses and promoting tissue regeneration.[35 40] The balance between M1 and M2 macrophages is crucial for maintaining liver homeostasis, as prolonged M1 activation can exacerbate liver injury, whereas M2 macrophages aid in repairing damaged tissue and resolving inflammation.[34 41]

In ALD, there is a notable increase in both circulating monocytes and liver macrophages, particularly in severe forms like AH and experimental models of chronic alcohol consumption.[30 42] TLR4 signalling, along with its adaptor protein myeloid differentiation primary response gene 88 (MyD88), plays a central role in alcohol-induced inflammation.[43] The increased intestinal permeability seen in chronic alcohol use leads to the translocation of bacterial endotoxins such as LPS into the bloodstream.[43] These endotoxins activate the TLR4/MyD88 signalling pathway in KCs and infiltrating macrophages, resulting in the production of pro-inflammatory cytokines and chemokines that drive liver inflammation.[43]

A particularly interesting mechanism in ALD involves the complement receptor of the immunoglobulin superfamily (CRIg), which is predominantly expressed on macrophages, including KCs.[44] CRIg plays a protective role in liver health by aiding the clearance of translocated pathobionts, potentially pathogenic organisms that migrate from the intestine to the liver during alcohol-induced gut permeability.[44] This protective mechanism helps reduce the inflammatory burden in the liver by preventing the accumulation of harmful bacterial products.[44] However, in both murine models and patients with ALD, CRIg expression is significantly reduced, leading to impaired clearance of pathobionts and sustained liver inflammation.[44] The reduction in CRIg function exacerbates the inflammatory response and contributes to continued liver damage, as the inability to clear intestinal bacteria leads to persistent immune activation.[44]

Furthermore, strategies aimed at promoting M2 macrophage polarisation have shown promise in mitigating alcohol-induced liver injury. For instance, overexpression of prostacyclin synthase or inhibition of miRNA-34a has been demonstrated to shift macrophage populations towards an M2 phenotype, reducing inflammation and enhancing

tissue repair in experimental models of ALD.[34 41] These findings highlight the therapeutic potential of targeting macrophage polarisation to balance pro-inflammatory and anti-inflammatory responses, offering a strategy to mitigate the liver damage caused by chronic alcohol consumption.

In summary, the plasticity of macrophages in ALD, particularly their ability to switch between M1 and M2 phenotypes, plays a crucial role in both liver injury and repair.[32] While M1 macrophages drive the inflammatory response during alcohol-induced liver damage,[32] M2 macrophages facilitate tissue healing and inflammation resolution.[45] Disruptions in this balance, as seen with reduced CRIg expression, exacerbate liver injury by allowing sustained inflammation.[44] Therefore, therapeutic strategies that promote M2 polarisation or enhance CRIg function could hold promise for treating ALD and reducing its progression to more severe forms, such as fibrosis and cirrhosis.

Neutrophils—Neutrophils, the most abundant type of white blood cells derived from the bone marrow, play a crucial role in the innate immune response.[46] They circulate in the bloodstream, constantly patrolling for pathogens and potential threats.[46] Neutrophils are equipped with a variety of defence mechanisms, including the production of ROS, phagocytosis of pathogens, cytokine release, degranulation of antimicrobial proteins and the formation of neutrophil extracellular traps (NETs).[46] These mechanisms are essential for neutralising and eliminating pathogens during infections.[46] In the context of ALD, neutrophils are central players in both liver injury and repair.

In ALD, multiple liver cells including HSCs, KCs, Th17 cells, LSECs and hepatocytes, along with DAMPs and PAMPs, collaborate to prime, activate and recruit neutrophils to the liver.[47 48] Alcohol-induced liver damage triggers the release of these molecular signals, which initiate an inflammatory cascade, leading to neutrophil infiltration.[47 48] This process is particularly pronounced in patients with AH, where large numbers of neutrophils accumulate in the liver and contribute to liver injury, largely through excessive ROS production.[48] ROS, while essential for pathogen elimination, can also cause significant oxidative stress, damaging hepatocytes and contributing to the progression of liver inflammation and injury in ALD.[48]

However, recent research has highlighted the dual role of neutrophils in ALD, showing that while they contribute to liver injury, they are also involved in resolving inflammation and promoting tissue repair.[49–51] Neutrophils destroy pathogens and clear cellular debris through phagocytosis[48] and play a critical role in shaping the immune environment by influencing macrophage polarisation.[50] Specifically, neutrophils can promote the transition of macrophages from a pro-inflammatory M1 phenotype to an anti-inflammatory M2 phenotype, thereby aiding in the resolution of liver inflammation.[50] This functional plasticity suggests that neutrophils may help to fine-tune the immune response in ALD, balancing pro-inflammatory and tissue-repairing activities. Additionally, neutrophils contribute to liver regeneration by producing hepatocyte growth factor, which is essential for hepatocyte proliferation and tissue repair in patients with AH.[52]

One key regulator of neutrophil function in ALD is miR-223, which is highly expressed in neutrophils and acts as a critical modulator of inflammation and liver injury.[51 53] miR-223 limits alcohol-induced liver damage by inhibiting the IL-6-phagocytic oxidase 47 ($p47^{phox}$) pathway, which is involved in ROS production and neutrophil activation.[51] In mouse models, the deletion of miR-223 exacerbates liver injury, indicating its protective role in ALD.[51] By dampening excessive neutrophil activation and reducing ROS-mediated hepatocyte damage, miR-223 represents a potential therapeutic target for mitigating neutrophil-driven liver injury in ALD.[51]

It is well-known that IL-8 and neutrophils are elevated in patients with AH. Recently, Guan *et al* found that patients with severe AH exhibited significantly higher IL-8-positive neutrophils in the liver compared with patients with alcohol-associated liver cirrhosis, contributing to persistent inflammation. This indicates that targeting IL-8-positive neutrophils could be a potential therapy for AH.[54]

A newly identified subset of neutrophils, known as low-density neutrophils (LDNs), has been linked to alcohol exposure and shows distinctive characteristics that differentiate them from traditional neutrophil populations.[49] LDNs exhibit functional exhaustion, meaning they have diminished capacity to perform typical neutrophil functions such as pathogen elimination and inflammation resolution.[49] Additionally, they display resistance to macrophage efferocytosis, the process by which dying or dead cells are engulfed and cleared by macrophages.[49] This impaired clearance exacerbates tissue damage and inflammation in the liver, further contributing to the progression of ALD.[49]

One of the hallmark defence mechanisms of neutrophils is the formation of NETs.[46] NETs consist of DNA and cationic proteins, which serve to capture and neutralise pathogens.[49 55 56] However, in the context of ALD, alcohol exposure disrupts the formation of NETs, leading to decreased pathogen clearance and exacerbation of liver inflammation.[49 55 56] This impairment in NET formation reduces the ability of neutrophils to contain infections, contributing to the pathogenesis of liver injury in ALD. Furthermore, the clearance of NETs by macrophages is also impaired in ALD, which exacerbates hepatic inflammation and injury.[49 55 56] Accumulation of uncleared NETs can trigger additional immune responses, amplifying the cycle of inflammation and tissue damage in the liver.[49 55 56]

A key biomarker that has gained attention in ALD is the neutrophil-to-lymphocyte ratio (NLR), which has emerged as a strong predictor of mortality and acute kidney injury in patients with ALD.[57 58] The NLR reflects the balance between the innate and adaptive immune responses, with elevated levels indicating an increased inflammatory state driven by neutrophils.[57 58] Higher NLR values have been associated with worse clinical outcomes, making it a useful tool for risk stratification and guiding treatment decisions in patients with ALD.[57 58]

In summary, despite their well-established role in promoting liver injury through ROS production and inflammatory signalling, neutrophils also play a protective role by facilitating liver repair and regeneration. This duality highlights the complex nature of neutrophil involvement in ALD and suggests that therapeutic strategies aimed at modulating

neutrophil activity, such as enhancing their tissue-protective functions while limiting their pro-inflammatory effects, may hold promise for treating ALD. Additionally, LDNs and impaired NET formation represent emerging mechanisms by which alcohol-induced neutrophil dysfunction exacerbates liver injury in ALD. Further research is needed to fully elucidate the molecular mechanisms underlying neutrophil plasticity in ALD and to identify potential therapeutic targets for minimising liver injury while promoting recovery.

T cells—T cells are vital players in the adaptive immune system, orchestrating responses to pathogens and damaged cells, including in the liver. They are primarily categorised into two main groups: $CD8^+$ cytotoxic T cells and $CD4^+$ Th cells. These cells are central to the immune response against liver damage, including that caused by alcohol exposure, yet chronic alcohol use compromises their function, exacerbating liver injury and contributing to the progression of ALD.

$CD8^+$ cytotoxic T cells are primarily responsible for directly attacking and killing virus-infected or damaged cells.[59] In ALD, these T cells can recognise and respond to liver cells that have been modified by alcohol metabolites, such as protein adducts. Alcohol metabolism, particularly through CYP2E1, generates ROS and other toxic byproducts that form neoantigens.[6 40] However, chronic alcohol exposure compromises the cytotoxic function of $CD8^+$ T cells,[60 61] reducing their ability to effectively clear damaged hepatocytes.[62 63] This impairment is linked to both intrinsic T cell dysfunction and an altered liver microenvironment, which becomes more immunosuppressive under the influence of chronic alcohol intake.

$CD4^+$ Th cells play a regulatory role in liver inflammation during the pathogenesis of ALD by differentiating into subsets like Th1, Th2, Th17 and Tregs. Th1 cells produce pro-inflammatory cytokines, promoting chronic liver inflammation by activating $CD8^+$ T cells and KCs. In contrast, Th2 cells release anti-inflammatory cytokines, although an imbalance favouring Th1 often worsens inflammation. Alcohol exposure significantly impairs T cell cytotoxicity and activation, contributing to liver damage in ALD.[61] A marked decrease in C-C chemokine receptor type 5 (CCR5) expression on $CD4^+$ T cells has been observed in patients with AH, which may disrupt the balance between pro-inflammatory Th1 cells and anti-inflammatory Th2 cells, leading to a more severe inflammatory response.[64] Th17 cells, which produce IL-17, drive inflammation by recruiting neutrophils to the liver,[65] further exacerbating inflammation and injury.[66] Elevated IL-17 levels are commonly seen in patients with ALD, particularly in those with advanced diseases such as AH.[66 67] Studies suggest that targeting and blocking the IL-17 pathway can reduce alcohol consumption and liver damage, highlighting IL-17 as a therapeutic target.[67] Additionally, the imbalance between Tregs and Th17 cells is critical in ALD progression. While Th17 cells drive inflammation, Tregs are responsible for suppressing immune activation[68] and promoting tissue repair. Alcohol exposure often leads to a decrease in Tregs, further exacerbating liver injury.[69 70] Restoring the balance between these two cell types, by reducing Th17-mediated inflammation and enhancing Treg activity, may offer a therapeutic strategy to mitigate alcohol-induced liver damage and promote liver recovery.[71]

In addition to conventional $CD8^+$ and $CD4^+$ T cells, several populations of innate-like T cells contribute to liver immunity and the pathogenesis of ALD. These cells include natural killer T (NKT) cells and mucosa-associated invariant T (MAIT) cells. NKT cells are a unique subset of T cells that share characteristics of both T cells and natural killer (NK) cells.[72] They recognise lipid antigens presented by CD1d molecules and can rapidly produce large amounts of cytokines in response to liver injury.[72 73] In ALD, NKT cells play a complex role in modulating liver inflammation. Type I NKT cells, in particular, have been shown to promote liver damage by producing pro-inflammatory cytokines such as TNF-α and interferon (IFN)-γ.[72] In contrast, type II NKT cells tend to exert anti-inflammatory effects, helping to protect the liver from excessive immune-mediated damage.[72] The balance between type I and type II NKT cells is critical in determining the outcome of liver injury in ALD, with type I NKT cells generally contributing to disease progression. Mice that are deficient in or have inhibited type I NKT cells show reduced liver injury.[73] Cytokines and chemokines, including osteopontin and CXCL2, were significantly reduced in type I NKT cell-deficient mice with ethanol feeding, further highlighting their central role in liver inflammation.[73] Moreover, NKT cells interact with other immune cells, such as KCs and neutrophils, to exacerbate liver injury in ALD, emphasising the complex immune crosstalk involved in alcohol-related liver damage.[74]

MAIT cells are a specialised population of T cells that recognise microbial metabolites presented by MHC-related protein, MR1 molecules.[75] These cells are abundant in the liver and play a vital role in controlling gut microbiota, responding to bacterial infections, and regulating inflammatory diseases.[76 77] In ALD, exposure to bacterial products can result in the depletion and dysfunction of MAIT cells, further exacerbating the disease.[78] This highlights the intricate interplay between immune cell populations in the pathogenesis of ALD, where the disruption of one immune component can intensify liver inflammation and injury.

Hepatic stellate cells—HSCs are mesenchymal cells located in the space of Disse, positioned between the sinusoidal endothelial cell layer and hepatocytes. They play a central role in liver fibrosis and tissue repair by producing fibrogenic components, such as collagen types I and III, and ECM proteins like fibronectin. Under normal conditions, HSCs remain in a ‘quiescent’ state, storing vitamin A (retinol) in lipid droplets and balancing ECM production and degradation to prevent fibrosis. However, exposure to alcohol triggers the activation of HSCs, leading to the loss of retinol storage and their trans-differentiation into myofibroblasts, which secrete ECM.[10] This activation is driven by factors such as TGF-β1, ROS, lipid peroxides, PDGF and epidermal growth factor.[79 80]

Recent studies have expanded the understanding of HSC functions beyond ECM production. Alcohol-induced glutamate release from hepatocytes stimulates HSCs to produce 2-arachidonoylglycerol, which binds to cannabinoid receptor 1 on hepatocytes, promoting lipogenesis and triglyceride synthesis, thereby contributing to alcohol-induced steatosis.[81] Additionally, HSCs regulate steatohepatitis through neuropilin-1 (NRP-1) signaling.[82] Selective deletion of NRP-1 in HSCs has been shown to ameliorate hepatic steatosis, inflammation and fibrosis in alcohol-fed mice.[82] These findings emphasise the complex crosstalk between HSCs and hepatocytes in alcohol-induced liver injury.

In summary, the interaction between hepatic parenchymal and non-parenchymal cells is central to the pathogenesis of ALD. Hepatocytes metabolise alcohol, leading to the production of ROS and acetaldehyde. LSECs also play a key role in alcohol metabolism. However, alcohol exposure leads to their dysfunction and capillarisation, which contributes to the development of liver fibrosis by impairing nutrient and oxygen exchange and promoting fibrogenic signalling. Macrophages, both KCs and infiltrating monocyte-derived macrophages, play dual roles in ALD, mediating inflammation and tissue repair. Their plasticity allows them to either exacerbate or mitigate liver injury depending on their activation state, switching between pro-inflammatory (M1) and anti-inflammatory (M2) phenotypes. Neutrophils are rapidly recruited to the liver during ALD, driven by cytokines and chemokines released from various liver cells. These immune cells can both promote liver injury through oxidative burst and protease release and contribute to tissue repair by clearing dead cells and resolving inflammation. T cells, particularly Th17 cells, are key players in the inflammatory response. Th17 cells produce IL-17, which recruits neutrophils to the liver, exacerbating inflammation and tissue damage. Conversely, Tregs attempt to limit excessive inflammation, although their numbers are often reduced in ALD. NKT and MAIT cells are also involved in the immune response, with NKT cells contributing to liver inflammation and MAIT cells, although typically protective against bacterial infections, becoming depleted and dysfunctional in ALD. HSCs become activated in response to alcohol-induced damage. Once activated, they transform into myofibroblasts, producing excess collagen and ECM, leading to fibrosis. Their interaction with hepatocytes and other immune cells further drives inflammation, steatosis and the fibrotic processes that characterise advanced stages of ALD. The complex crosstalk between these diverse cell populations accelerates the progression of liver damage in ALD.

Signalling mediators and modulators in ALD

Signalling mediators and modulators are critical in the pathogenesis of ALD, orchestrating the complex interplay between hepatocytes and non-parenchymal cells. These cells communicate through a variety of mediators, including cytokines,[83] EVs[84] and inflammasomes,[85] all of which contribute to liver damage.

Cytokines—Cytokines play a central role in the pathogenesis of ALD by driving the inflammatory response and promoting liver injury.[83] Chronic alcohol consumption induces hepatic stress and inflammation, leading to the release of various cytokines, including TNF-α, IL-6, IL-1β and TGF-β, each contributing to different aspects of ALD pathophysiology.[86]

TNF-α is a key pro-inflammatory cytokine that exacerbates liver damage by promoting inflammation, oxidative stress and cellular necrosis. TNF-α amplifies the inflammatory cascade by recruiting immune cells such as neutrophils and monocytes to the liver, further aggravating inflammation.[86] Despite its role in driving ALD progression, early attempts to block TNF-α as a therapeutic approach were met with disappointing results.[87] Clinical trials revealed that TNF-α blockade, while initially promising, was associated with severe adverse events, including a higher risk of infections and increased mortality rates in patients with advanced ALD.[87] This outcome highlights the critical need for caution when targeting

inflammatory pathways in ALD, as immune suppression can lead to life-threatening complications in an already vulnerable population.

TGF-β is another pivotal cytokine in ALD, primarily involved in liver fibrosis, one of the hallmarks of chronic liver injury.[88] Ethanol and its metabolite, acetaldehyde, increase the expression of TGF-β1 in HSCs, activating them to produce ECM proteins such as collagen, which leads to the accumulation of fibrotic tissue in the liver.[89] In addition to its fibrogenic effects, TGF-β inhibits matrix-degrading proteolytic enzymes, further contributing to fibrosis by preventing the breakdown of the ECM.[89] TGF-β also exerts direct effects on hepatocytes, inducing apoptosis and inhibiting proliferation, which exacerbates liver injury and impairs the liver's ability to regenerate following damage.[90 91] Furthermore, TGF-β influences immune cell function by polarising macrophages towards an M2 phenotype, which is associated with tissue remodelling and fibrosis.[92] Despite its central role in fibrosis, TGF-β exhibits complex and sometimes contradictory effects.[93 94] While it promotes fibrogenesis, it can also have anti-inflammatory and tissue repair functions.[93] This multifunctional nature presents a significant challenge in targeting TGF-β as a therapeutic strategy for ALD. Inhibiting TGF-β could reduce fibrosis but may interfere with its protective roles in resolving inflammation and promoting tissue repair, leading to unintended consequences.[93 94] Therefore, therapeutic approaches targeting TGF-β must be carefully considered and finely tuned to balance its profibrotic and anti-inflammatory effects.

ILs play diverse and critical roles in the immune response within the liver, with significant implications for ALD. IL-6, in particular, exhibits a dual role in ALD.[95 96] While deletion of IL-6 has been shown to exacerbate liver injury, suggesting a protective function, IL-6 also contributes to mitigating alcohol-induced liver damage and inflammation through the activation of the Signal Transducer and Activator of Transcription 3 (STAT3) pathway.[97] This protective effect of IL-6 is crucial for maintaining liver homeostasis during chronic alcohol consumption. However, IL-6 is also involved in the differentiation of Th17 cells, indicating its role in promoting inflammation.[66 98] The multifaceted nature of IL-6 highlights its complex contribution to both liver protection and inflammation in ALD.

Elevated levels of IL-17 have been observed in ALD, correlating with the severity of hepatic inflammation and liver damage.[66] IL-17 is heavily implicated in the recruitment of neutrophils to the liver, where it promotes alcohol-induced HCC by synergising with other inflammatory mediators.[99] IL-17 amplifies the production of pro-inflammatory cytokines, including TNF-α and IL-6, further driving the progression of liver injury.[66 100] In patients with ALD, particularly those with AH and cirrhosis, IL-17-secreting cells, such as T lymphocytes and neutrophils, are abundant and significantly contribute to the inflammatory response.[66] Studies have shown that HSCs expressing the IL-17 receptor can recruit neutrophils on IL-17 stimulation,[66] promoting liver inflammation through the secretion of inflammatory mediators such as IL-8 and growth-related oncogene alpha.[66] Blocking IL-17 has demonstrated protective effects in mouse models of alcohol-induced liver injury,[67] including a reduction in voluntary alcohol consumption in alcohol-dependent mice,[67] suggesting that targeting IL-17 could be a promising therapeutic strategy to attenuate liver inflammation and injury in ALD.

IL-1, particularly IL-1β, is another key pro-inflammatory cytokine in ALD.[101] Chronic alcohol exposure activates the NLRP3-caspase inflammasome, leading to elevated levels of IL-1β,[102] which in turn promotes liver inflammation by triggering the activation of invariant NKT cells and the infiltration of polymorphonuclear neutrophils.[103] Targeting IL-1β through the use of IL-1 receptor antagonists has been explored as a potential therapeutic approach for inflammasome-dependent AH.[104]

IL-22 plays a predominantly protective role in ALD.[105] It is essential for maintaining epithelial cell integrity, hepatocyte survival and liver regeneration.[105] IL-22 activates STAT3-mediated signalling pathways, which promote the expression of anti-apoptotic and mitogenic genes, fostering tissue repair and regeneration in the liver.[105] By enhancing hepatocyte survival and supporting liver regeneration, IL-22 mitigates alcohol-induced liver injury, making it a potential therapeutic target for protecting the liver in ALD.[106]

IL-10 is a pivotal anti-inflammatory cytokine that plays a key role in counteracting the actions of pro-inflammatory cytokines, helping to regulate the balance between inflammatory and anti-inflammatory responses in the liver. It is central to downregulating inflammation, and its deficiency has been associated with heightened inflammatory responses to alcohol consumption.[107] Interestingly, studies in IL-10 knockout mice fed alcohol have shown increased inflammation but improved steatosis, potentially due to elevated IL-6/STAT3 activation.[107] This activation downregulates lipogenic genes while upregulating genes involved in fatty acid oxidation.[107]

IFN-γ, primarily produced by T cells and NK cells, is another important cytokine in ALD. It modulates immune responses and has been shown to exert antifibrotic effects.[108 109] By attenuating fibrotic processes in the liver, IFN-γ offers a promising approach for limiting fibrosis in alcohol-induced liver disease.

Chemokines, a subset of cytokines that includes CCL2, CXCL5, CXCL8 and monocyte chemoattractant protein 2, are crucial in the recruitment of immune cells, particularly neutrophils, to sites of inflammation.[110] Elevated levels of these chemokines have been strongly associated with increased neutrophil infiltration in patients with AH.[111] This excessive neutrophil activity is linked to worse outcomes and a poorer prognosis in AH, underscoring the significant role of chemokines in the pathogenesis and progression of ALD.[111]

CCL20 is significantly upregulated in the livers of patients with AH.[112] Both macrophages and HSCs are identified as major producers of CCL20 within the liver.[112] Elevated CCL20 levels are associated with the severity of liver fibrosis, portal hypertension and endotoxemia,[112] suggesting its involvement in multiple aspects of AH pathogenesis. In mouse models, CCL20 knockdown in LPS-treated mice has been shown to reduce liver damage.[112] This suggests that targeting CCL20 may offer therapeutic potential by mitigating the inflammatory response and attenuating liver injury in AH. A summary of the roles and functions of various cytokines in the pathogenesis of ALD is provided in table 1.

In summary, cytokines play critical roles in mediating liver injury, inflammation and fibrosis in ALD. Pro-inflammatory cytokines such as TNF-α, IL-6 and IL-1β drive the inflammatory

cascade, while IL-17 promotes neutrophil recruitment and contributes to the severity of liver damage. Anti-inflammatory cytokines like IL-10 counterbalance this response but can have complex effects on lipid metabolism. Chemokines, including CCL20, exacerbate liver damage by promoting immune cell infiltration and are closely linked with disease severity in AH.

Extracellular vesicles—EVs represent a diverse population of small, non-replicating, membrane-encapsulated particles released by nearly all cell types.[113 114] Based on their biogenesis and size, EVs are categorised into three main groups: exosomes (50–150 nm), microvesicles (100–1000 nm) and apoptotic bodies (500–2000 nm).[113 114] These nanosized particles carry a distinct molecular signature composed of proteins, RNA (including miRNAs and long non-coding RNAs), lipids and even DNA fragments, which reflect the cell type of origin and the specific homeostatic or pathological stimuli that trigger their release.[115] EVs are emerging as sophisticated mediators of intercellular communication. They play a pivotal role in transmitting responses to both physiological and pathological stimuli, thereby influencing a wide range of cellular processes in neighbouring and distant cells.[115] This intercellular crosstalk is particularly relevant in the context of ALD, where EVs can mediate the propagation of inflammatory signals, fibrotic pathways and hepatocellular injury.

Studies have revealed elevated levels and altered contents of circulating EVs in individuals with ALD and in mice exposed to alcohol.[116 117] These EVs play a critical role in orchestrating intercellular communication within the liver microenvironment and between distant organs.[84 118] By transmitting molecular information encapsulated in their cargo, EVs modulate and propagate pathological processes associated with ALD,[119] ultimately influencing disease progression and clinical outcomes.

One notable finding from animal studies is the unique protein signature observed in EVs from alcohol-fed mice, which reflects inflammatory responses, cellular development and cell migration compared with control EVs.[120] When EVs from alcohol-exposed mice were transferred to hepatocytes from mice without alcohol exposure, there was an increased expression of monocyte chemoattractant protein 1 mRNA and protein, suggesting that these EVs play a direct role in promoting liver inflammation and injury.[120] Additionally, mice receiving EVs from alcohol-fed counterparts displayed significant alterations in the populations of KCs and infiltrating monocytes.[120] Specifically, there was an increase in pro-inflammatory F4/80^{hi} CD11b^{lo} KCs, which are typically associated with an inflammatory phenotype.[120] This shift was further characterised by an elevated percentage of TNF-α^{+}/IL12/23^{+} KCs and monocytes, indicative of a pro-inflammatory (M1) state, while the percentage of anti-inflammatory (M2) KCs decreased.[120] This study illustrated how alcohol-derived EVs skew the immune balance towards a more inflammatory environment in the liver.

Hepatocytes subjected to chronic-plus-binge ethanol feeding release microvesicles enriched with mtDNA.[121] These mtDNA-enriched EVs promote neutrophil recruitment, thereby exacerbating liver injury.[121] In murine models of AH, elevated levels of circulating EVs were observed, and hepatocyte-derived EVs were enriched in mtDNA.[12] These mtDNA-

enriched EVs activated TLR9 in macrophages, leading to increased production of pro-inflammatory cytokines such as IL-1β and IL-17.[12] Furthermore, the EVs promoted HSCs activation, contributing to both liver fibrosis and inflammation.[12]

Additionally, hepatocytes treated with ethanol also release EVs enriched with CD40 ligand (CD40L), which induce M1 phenotypic switch in macrophages, characterised by elevated expression of pro-inflammatory cytokines.[122] Increased levels of CD40L-expressing EVs have also been detected in the serum of patients with AH, suggesting their potential clinical relevance.[122] In a murine model of AH, specific miRNA cargos within EVs derived from hepatocytes, such as miR-27a and miR-181, were found to contribute to HSC activation by repressing Nlrp2 expression, a marker of quiescent HSCs.[12]

Moreover, hepatocytes exposed to alcohol secrete miR-122-containing EVs that sensitise liver macrophages to LPS, leading to increased levels of pro-inflammatory cytokines through the inhibition of the heme oxygenase-1 pathway.[123] Additionally, EVs released from intestinal epithelial cells exposed to alcohol have been shown to decrease hepatocyte viability and increase lipid deposition,[124] highlighting the potential crosstalk between the gut and liver in the context of ALD pathogenesis. Furthermore, IL-1β-containing EVs derived from hepatic macrophages have been implicated in alcohol-induced liver injury and steatosis, mediated by macrophage-inducible C-type lectin and dependent on gasdermin D (GSDMD).[125]

Understanding the intricate interactions and molecular changes mediated by EVs provides valuable insights into the detailed mechanisms underlying ALD. These findings present opportunities for innovative therapeutic strategies targeting EV-mediated pathways and hold promise for identifying novel biomarkers for ALD management. A summary of the different cell types releasing EVs and their functions is shown in table 2.

Inflammasomes—Inflammasomes play a pivotal role in the pathogenesis of ALD.[85] These intracellular multiprotein complexes are responsible for recognising danger signals, such as PAMPs and DAMPs, which trigger the activation, maturation and release of pro-inflammatory cytokines, including IL-1β and IL-18.[126 127] Additionally, inflammasomes can initiate pyroptosis, a highly inflammatory form of programmed cell death that further contributes to liver damage.[128]

Among the various inflammasomes studied in ALD, the NLRP3 inflammasome is the most extensively investigated.[129] The NLRP3 complex comprises the NLRP3 sensor, the adaptor protein apoptosis speck protein and pro-caspase-1.[130] Activation of the NLRP3 inflammasome is a two-step process.[131] First, PAMPs or DAMPs engage TLRs, leading to the activation of nuclear factor Kappa-light-chain-enhancer of activated B cells.[131] This transcription factor induces the expression of NLRP3 and pro-inflammatory cytokines like pro-IL-1β and pro-IL-18.[131] In the second step, NLRP3 oligomerises, forming a complex that activates caspase-1, which in turn cleaves pro-IL-1β and pro-IL-18 into their active forms. Caspase-1 also cleaves GSDMD, facilitating pyroptosis and contributing to the release of inflammatory cytokines.[131] Patients with AH show increased serum IL-1β levels.[132] Experimental studies in alcohol-fed mice also confirm higher hepatic NLRP3,

IL-1β and caspase-1 activity.[104] Notably, mice deficient in the NLRP3 inflammasome pathway experience reduced liver damage when chronically exposed to ethanol compared with controls.[104] IL-1β exacerbates liver injury by promoting immune cell recruitment and impairing liver regeneration in ALD models,[103 133] while IL-18 has been associated with increased gut permeability in alcohol-fed mice.[134 135] In both mice and patients with AH, GSDMD, a key driver of pyroptosis activated downstream of caspase-11/caspase-4, shows increased activation. In mouse models, caspase-11 deficiency reduces GSDMD activation, bacterial load in the liver and the severity of AH.[136]

In addition to the canonical NLRP3 pathway, a non-canonical inflammasome pathway has been identified. In humans, this pathway involves caspase-4 and caspase-5, while in mice, it involves caspase-11.[131] Unlike the canonical pathway, the non-canonical pathway directly activates the NLRP3 inflammasome, leading to the release of IL-1β and IL-18 without requiring their direct cleavage by caspases.[136] Non-canonical pyroptosis, triggered by these caspases, exacerbates the inflammatory response in ALD.[129 136]

In conclusion, inflammasomes, particularly the NLRP3 inflammasome, play a central role in the pathogenesis of ALD by mediating inflammation and liver damage. These complexes detect danger signals such as PAMPs and DAMPs, leading to the activation of caspase-1 and the maturation of pro-inflammatory cytokines IL-1β and IL-18. This activation drives the inflammatory response and triggers pyroptosis, a form of programmed cell death that further exacerbates liver injury. The involvement of both canonical and non-canonical inflammasome pathways highlights the complexity of the inflammatory processes in ALD. Elevated inflammasome activity in both patients and experimental models underscores their contribution to the disease's progression.

Organ-organ crosstalk in ALD

ALD is a multifaceted condition that extends beyond the liver, with systemic effects involving multiple organs and tissues. The intricate relationships between the liver and other organs contribute to both the progression and severity of ALD, highlighting the importance of organ-organ crosstalk in this disease. Two key organs that are particularly vulnerable to the harmful effects of excessive alcohol consumption are the gut and adipose tissue. These organs play critical roles in metabolism and immunity and maintain close functional and biochemical interactions with the liver (figure 2).

The gut-liver axis—The gut-liver axis is a complex and dynamic interplay between the gut and liver that is essential for maintaining homeostasis and overall health.[137] Alcohol disrupts the integrity of the intestinal barrier, leading to increased permeability. This allows the translocation of microbial products, such as LPS, into the portal circulation, which subsequently triggers hepatic inflammation and promotes liver injury.[8] It is recently reported that gut and liver work together to clear systemic acetaldehyde and drive alcohol intake.[138] In a healthy gut, commensal bacteria play a vital role in maintaining barrier integrity. They promote mucus production and stimulate the immune system, reinforcing both innate and adaptive immune defences. Additionally, they produce short-chain fatty acids (SCFAs) such as butyrate, which protect the gut lining by promoting epithelial integrity and

reducing inflammation.[139] However, individuals with ALD show a reduction in SCFAs, which correlates with impaired gut barrier function.[137 139] Alcohol-induced dysbiosis, characterised by an imbalance in the microbial community, also results in a decrease in microbial metabolites called indoles.[140] This decrease contributes to lower levels of IL-22, an essential cytokine for maintaining gut integrity.[140] The reduction in IL-22 weakens the gut barrier, facilitating bacterial translocation into the liver and contributing to the progression of ALD.[141] Alcohol consumption has been shown to reduce bacterial diversity and alter the abundance of various bacterial taxa in mice.[142–144] Studies involving alcohol administration in animal models have demonstrated a decrease in the abundance of the Lactobacillus, Bacteroidetes and Firmicutes phyla.[142–144] Conversely, there is an observed increase in the Proteobacteria and Actinobacteria phyla, as well as in Verrucomicrobia, Enterobacteria and Enterococcus.[142–144] Similar dysbiotic patterns have been noted in human studies. Patients with alcohol-related cirrhosis exhibit significant reductions in beneficial bacterial families such as Ruminococcaceae, Lachnospiraceae and Clostridiales Family XIV Incertae Sedis.[145–147] Notably, *A. muciniphila*, a bacterium important for maintaining gut barrier integrity, is decreased in both ALD mouse models and patients with AH.[148] Additionally, patients with AH show elevated levels of cytolytic-positive faecal enterococci, which correlate with the severity of liver disease and mortality.[149] These findings highlight the pivotal role of dysbiosis in driving the progression of liver disease through the gut-liver axis. Table 3 summarises key studies documenting changes in gut microbiota associated with ALD, particularly focusing on bacterial species reported in more than two studies.[145 146 149–172]

Immune cells within the gut, including dendritic cells, innate lymphoid cells, MAIT cells and γδ T cells, form the immunological barrier.[8] These cells secrete antimicrobial peptides and IgA, which play crucial roles in maintaining tolerance to commensal microbes and defending against pathogenic organisms.[173] Chronic alcohol consumption impairs this immune function. It alters the distribution and activity of dendritic cells, leading to dysfunctional immune responses and an increased risk of systemic inflammation.[174] Recent research has shown that intestinal conventional type 1 dendritic cells (cDC1s) play a key role in regulating the gut microbiota, particularly *A. muciniphila*, a bacterium that helps maintain the gut barrier.[175] In alcohol-fed mice, the number of cDC1s in the intestine decreases significantly. This reduction worsens the downregulation of antimicrobial peptides, which are crucial for supporting *A. muciniphila*.[175] As a result, the weakened gut barrier becomes more permeable, allowing bacterial toxins like LPS to enter the bloodstream, increasing inflammation and liver damage.[175] Ethanol-fed mice and patients with alcohol use disorder (AUD) exhibit reduced numbers of macrophages in the duodenum and display dysregulated cytokine secretion and impaired phagocytosis.[176] Moreover, reduced intestinal IgA secretion has been observed in patients with decompensated cirrhosis compared with those with compensated cirrhosis.[177] Additionally, patients with AUD, even at precirrhotic stages, exhibit reduced intestinal T cell numbers and immune dysfunction, further exacerbating the risk of gut-derived liver inflammation.[150]

The interplay between bile acid (BA) metabolism and the gut microbiota is another critical factor in ALD. Primary BAs are synthesised in hepatocytes and delivered to the intestine via bile ducts, where a large portion (approximately 95%) is reabsorbed back into the liver

through the portal vein, in a process known as enterohepatic circulation.[178] The remaining BAs undergo biotransformation by gut microbiota, converting primary BAs into secondary bile acids.[179]

Chronic alcohol consumption disrupts both BA levels and composition,[180 181] negatively affecting the gut microbiota and its ability to metabolise BAs. This dysregulation sets up a feedback loop where intestinal dysbiosis and altered BA metabolism mutually reinforce each other, driving the progression of ALD.[179] For instance, reduced BA secretion during ALD diminishes their bactericidal effect, leading to bacterial overgrowth in the gut.[179] Additionally, BA metabolites produced by gut microbiota influence the balance of Th17 cells and Tregs, key immune players in the progression of liver disease.[182]

Farnesoid X receptor (FXR) is a nuclear receptor that regulates BA synthesis and modulates gut-liver communication.[183] When BAs bind to FXR, they inhibit the expression of Cyp7a1/CYP7A1 (cholesterol 7α-hydroxylase), a key enzyme in the BA synthesis pathway.[180] FXR activation protects the liver from alcohol-induced injury by maintaining gut barrier integrity and limiting gut-derived inflammation.[184] However, alcohol impairs FXR function,[184] leading to gut barrier dysfunction, dysbiosis and an exacerbation of ALD progression.[185] Studies have demonstrated that FXR deficiency, particularly in the intestine rather than in hepatocytes, worsens alcohol-induced liver damage.[185 186] This suggests that enhancing FXR activity, especially within the gut, could be a therapeutic strategy for mitigating the harmful effects of alcohol on the liver.

Strategies to mitigate liver injury associated with alcohol-induced intestinal dysbiosis focus on restoring a healthy gut microbiome and strengthening the gut-liver axis. Key approaches include the use of antibiotics and probiotics, which have shown promise in alleviating liver damage.[187 188] Antibiotics can selectively target harmful bacterial populations, reducing endotoxin production and bacterial translocation that drive liver inflammation.[188] However, long-term use of antibiotics carries the risk of resistance and further gut microbiota disruption, so their application must be carefully managed. Probiotics, on the other hand, aim to restore a healthy balance of gut microbes by introducing beneficial bacterial strains, such as *Lactobacillus* and Bifidobacterium.[187] These probiotics support gut barrier function by stimulating the production of protective mucus, strengthening tight junctions between epithelial cells, and producing SCFAs like butyrate, which have anti-inflammatory effects.[187 189] Additionally, probiotics can modulate immune responses within the gut by promoting the secretion of IgA and enhancing the activity of immune cells, such as macrophages and dendritic cells.[190] Therapies may also include faecal microbiota transplantation (FMT) and prebiotics.[187 191] FMT involves transplanting healthy microbiota from a donor to restore gut balance,[191] while prebiotics encourage the growth of beneficial microbes.[187]

Adipose tissue-liver axis—Adipose tissue, especially white adipose tissue (WAT), plays a key role in regulating lipid and energy balance in the body. WAT stores excess energy as triglyceride-rich lipid droplets within its cells. During times of energy deficit, like fasting, these triglycerides are broken down into fatty acids and glycerol through a process called lipolysis. Two enzymes, adipose triglyceride lipase (ATGL) and hormone-sensitive lipase,

are responsible for breaking down triglycerides, with ATGL being the rate-limiting enzyme in this process.[192]

Persistent alcohol exposure disrupts the coupling between lipolysis and thermogenesis in WAT, leading to increased lipolysis, which contributes to ALD.[193] Fasting in ethanol-fed rats has been shown to reduce hepatic steatosis by preventing further influx of adipose-derived fatty acids to the liver and promoting increased fatty acid oxidation.[194] Studies using models deficient in adipose lipolysis have demonstrated that inhibiting this breakdown can protect against alcohol-induced liver fat accumulation and lipid peroxidation.[195]

Adipokines, proteins secreted by adipose tissue, play a significant role in ALD.[196] Two notable adipokines are adiponectin and leptin. Adiponectin helps protect against alcoholic liver steatosis by activating various signalling pathways involving, silent mating type information regulation 2 homolog 1 (Sirtuin, SIRT1), AMP-activated protein kinase, peroxisome proliferator-activated receptor-gamma coactivator 1 alpha, peroxisome proliferator-activated receptor alpha and sterol regulatory element binding protein 1.[197] These pathways collectively reduce lipogenesis, enhance fat oxidation and prevent lipid accumulation in the liver.[197] However, chronic alcohol consumption leads to a decrease in adiponectin levels.[197] Leptin is another important adipokine that regulates energy balance by suppressing energy intake and increasing energy expenditure.[198] Alcohol exposure lowers leptin levels, which can worsen alcohol-related diseases.[198] Administration of leptin has been shown to mitigate alcohol-induced hepatic steatosis.[198] Additionally, fibroblast growth factor 21, an adipokine secreted by WAT, can be induced by alcohol consumption, further promoting lipolysis.[199]

Alcohol exposure also causes significant changes in cytokine levels and leads to an influx of immune cells, such as macrophages, $CD4^+$ T cells and dendritic cells, into adipose tissue, contributing to inflammation.[13] For example, TNF-α secreted by adipose tissue can induce apoptosis in hepatocytes and activate KCs, further exacerbating the inflammatory response associated with ALD.[13 200]

In summary, the gut-liver axis plays a critical role in maintaining homeostasis, but alcohol disrupts intestinal barrier integrity, leading to increased permeability and the translocation of microbial products like LPS into the portal circulation. A healthy gut microbiome, rich in beneficial bacteria, supports barrier integrity and produces protective metabolites such as SCFAs, which are diminished in individuals with ALD. Alcohol-induced dysbiosis results in the loss of beneficial bacteria and an increase in harmful species, contributing to inflammation and liver disease progression. The immune system in the gut, including various immune cells, is also impaired by chronic alcohol consumption, leading to a dysfunctional immune response and heightened systemic inflammation. Furthermore, alcohol disrupts bile acid metabolism, negatively impacting gut microbiota and exacerbating ALD. Strategies to mitigate these effects focus on restoring a healthy gut microbiome and enhancing gut-liver communication through interventions like probiotics, antibiotics, faecal microbiota transplantation and prebiotics. Additionally, adipose tissue plays a significant role in lipid regulation and energy balance; alcohol alters lipolysis and the secretion of adipokines, leading to inflammation and exacerbation of liver injury.

CONCLUSION

In conclusion, the rising prevalence and severity of ALD underscore its significance as a global health challenge. This review highlights the multifaceted nature of ALD, emphasising the interplay between parenchymal and non-parenchymal liver cells in mediating liver injury and fibrosis through intercellular communication. Key mediators, including cytokines, chemokines and EVs, play pivotal roles in regulating inflammatory responses and influencing disease progression. Furthermore, the dysregulation of the gut-liver and adipose-liver axes reveals how alterations in microbiota and adipokine signalling exacerbate hepatic inflammation and steatosis. Understanding these complex interactions is crucial for developing targeted therapeutic strategies to mitigate ALD and improve patient outcomes.

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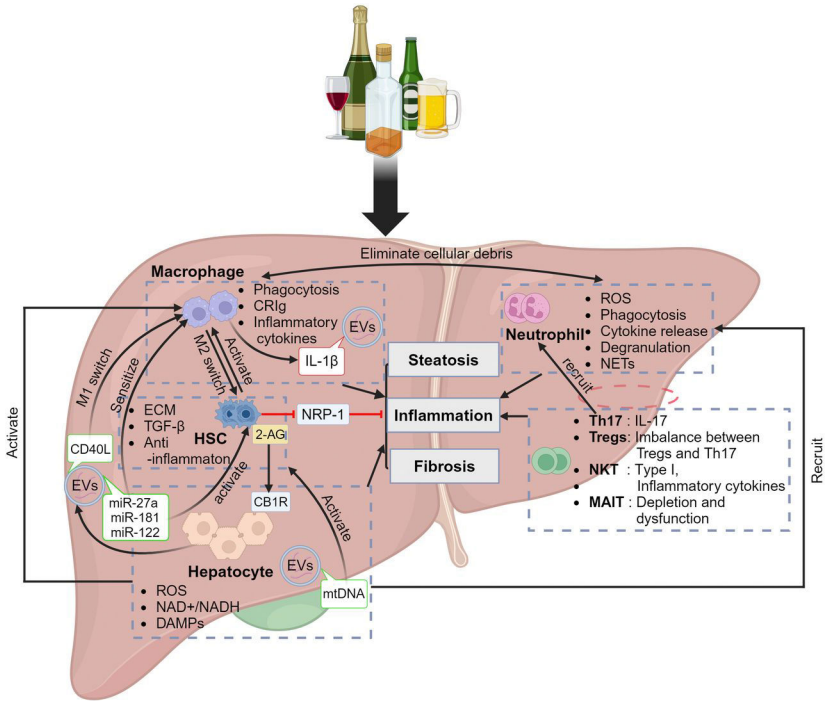

Figure 1.

Intercellular crosstalk in the pathogenesis of alcohol-associated liver disease (ALD). In response to alcohol, various cellular and molecular changes occur in the liver, contributing to hepatic steatosis, inflammation and fibrosis. Hepatocytes exhibit increased production of reactive oxygen species (ROS) and release damage-associated molecular patterns (DAMPs) along with extracellular vesicles (EVs) containing mitochondrial DNA (mtDNA), microRNA (miRNA) and CD40 ligand (CD40L). This, combined with a reduced NAD+/NADH ratio, promotes the development of hepatic steatosis, inflammation and fibrosis. Macrophages work alongside neutrophils to clear cellular debris; however, continuous alcohol consumption activates macrophages, leading to hepatic inflammation through cytokine secretion and phagocytosis. Kupffer cells (KCs) also contribute to this inflammation by activating hepatic stellate cells (HSCs). A reduced expression of complement receptor of the immunoglobulin superfamily (CRIg) on macrophages has been linked to the progression of ALD. Moreover, interleukin (IL)-1β-containing EVs derived from hepatic macrophages have been implicated in alcohol-induced liver injury and steatosis. Neutrophils contribute to inflammation in ALD through mechanisms such as ROS production, phagocytosis, cytokine release, degranulation and the formation of neutrophil extracellular traps (NETs). Various T cells in the liver, including T helper (Th) 17 cells, regulatory T cells (Tregs), natural killer T (NKT) cells and mucosal-associated invariant T (MAIT) cells, also play important roles in liver injury in response to alcohol exposure. HSCs respond to alcohol by producing increased amounts of extracellular matrix (ECM) and transforming growth factor-beta (TGF-β), leading to liver fibrosis. Alcohol stimulates HSCs to produce 2-arachidonoylglycerol (2-AG), which binds to cannabinoid receptor 1 (CB1R) on hepatocytes, promoting steatosis. Interestingly, HSCs deficient in neuropilin-1 (NRP-1) have been shown to reduce hepatic steatosis, inflammation and fibrosis in murine models of

ALD. Additionally, TGF-β produced by activated HSCs can polarise macrophages towards an M2 phenotype, which exhibits anti-inflammatory functions.

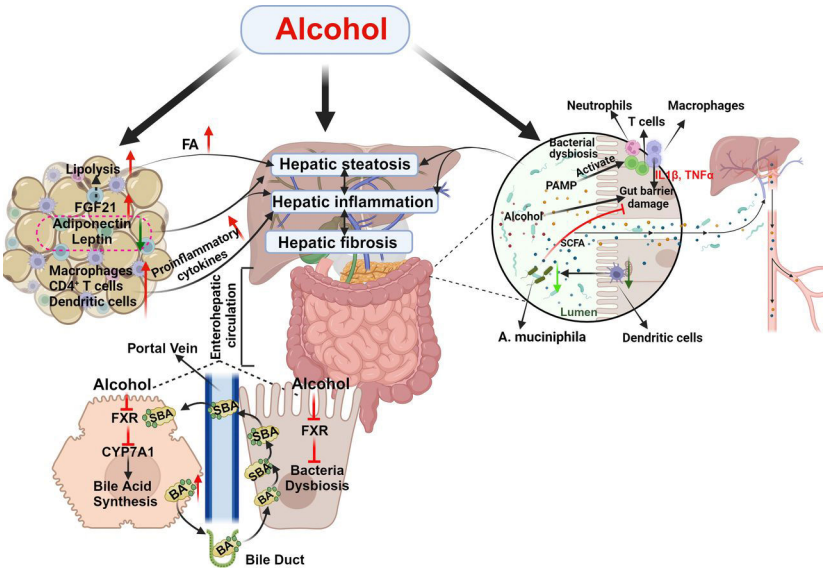

Figure 2.

Interorgan crosstalk in the pathogenesis of alcohol-related liver disease (ALD). Alcohol and its metabolites disrupt the gut barrier, leading to increased levels of pathogen-associated molecular patterns (PAMPs). These PAMPs trigger the recruitment and activation of immune cells, resulting in the production of pro-inflammatory cytokines and chemokines. In individuals with ALD, levels of short-chain fatty acids (SCFAs), typically produced by commensal microbes, are reduced, exacerbating gut permeability. Alcohol consumption is also associated with intestinal bacterial dysbiosis, marked by a decrease in beneficial bacteria such as *Akkermansia muciniphila*. In mice fed alcohol, there is a significant reduction in conventional dendritic cells in the intestine. The production of bile acids (BAs) is regulated by the farnesoid X receptor (FXR); however, alcohol diminishes the expression and function of FXR. Normally, the binding of BAs to FXR inhibits the expression of cholesterol 7-alpha hydroxylase (CYP7A1), an enzyme involved in BA synthesis. Alcohol exposure induces lipolysis in white adipose tissue (WAT), releasing fatty acids (FAs) that are transported to the liver and deposited as triglycerides, contributing to hepatic steatosis. Dysregulated adipokines further promote steatosis. Additionally, alcohol exposure leads to significant changes in cytokine levels and an increased influx of immune cells, which contribute to inflammation in the liver. IL, interleukin; SBA, secondary bile acid; TNF-α, tumour necrosis factor-alpha.

Table 1

Cytokines released by various cell types and their functions

Cytokines	Functions in ALD	Reference
TNF-α	A pro-inflammatory cytokine; mainly produced by monocyte-macrophage lineage; upregulates inflammatory mediators; activates and recruits immune cells to stimulate inflammation	86
TGF-β	A crucial profibrogenic cytokine; contributes to fibrosis by increasing ECM production and inhibiting ECM degradation; exhibits anti-inflammatory and tissue repair functions	88 89 93
IL-6	Mitigates alcoholic liver injury and inflammation by activating STAT3; contributes to liver inflammation and by increasing IL-17	66 97 98
IL-17	Predominantly produced by Th17 cells; recruits neutrophils; amplifies the production of pro-inflammatory cytokines such as TNF-α	65 66 100
IL-1	A key pro-inflammatory cytokine; closely linked to inflammasomes	102–104
IL-22	Exhibits predominantly protective roles	105
IL-10	An anti-inflammatory cytokine; implicated in promoting steatosis	107
IFN-γ	Produced primarily by T cells and NK cells; modulates immune responses; antifibrotic effects	108 109
CCL20	Mainly produced by macrophages and HSCs; highly upregulated in patients with AH; correlated with disease severity; promotes inflammation and fibrosis	112

AH, alcohol-associated hepatitis; ALD, alcohol-associated liver disease; CCL, C-C motif chemokine ligand; ECM, extracellular matrix; HSC, hepatic stellate cell; IFN, interferon; IL, interleukin; NK, natural killer; STAT3, signal transducer and activator of transcription 3; TGF-β, transforming growth factor-beta; Th, T helper; TNF-α, tumour necrosis factor-alpha.

Table 2

EVs released by different cell types and their functions

Model	EVs origin	EV contents	Functions of EVs	References
Gao-binge mouse model	Hepatocytes	mtDNA	Promote the recruitment of neutrophil and liver injury	[121]
Murine model of AH	Hepatocytes	mtDNA, miR-27a, miR-181	Activate TLR9 in macrophages, resulting in upregulated pro-inflammatory cytokine production, including IL-1β and IL-17; contribute to HSC activation via repressing *Nrld2* expression	[12]
HepG2 cells treated with ethanol	Hepatocytes	CD40-ligand	Induce macrophage M1 phenotypic switch, characterised by elevated expression of pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6	[122]
Huh7.5 cells treated with ethanol	Hepatocytes	miR122	Sensitise liver macrophages to the effects of LPS and increased levels of pro-inflammatory cytokines via inhibiting HO-1 pathway	[123]
Caco-2 cells exposed to ethanol	Intestinal epithelial cell	Not detected	Contribute to lower hepatocyte viability and lipid deposition	[124]
Gao-binge mouse model	Hepatic macrophages	IL-1β	Contribute to alcohol-induced liver injury and steatosis via macrophage-inducible C-type lectin and dependent on GSDMD	[125]

AH, alcohol-associated hepatitis; EV, extracellular vesicle; GSDMD, gasdermin D; HO-1, heme oxygenase-1; HSC, hepatic stellate cell; IL, interleukin; LPS, lipopolysaccharide; miR, microRNA; mtDNA, mitochondrial DNA; TLR, toll-like receptor; TNF-α, tumour necrosis factor-alpha.

Table 3

Gut microbiota changes in patients with ALD

Comparison objects	Changes of microbiota	Reference
Alcoholics or patients with ALD versus controls	Anaerobic bacteria ↑, aerobic bacteria ↑, Bifidobacteria ↓↑, Lactobacilli ↓↑, Enterococci ↓, *Enterococcus faecalis* ↑, *Candida* ↑, *Pichia* ↑, *Kluyveromyces* ↑, *Issatchenkia* ↑, *Propionibacterium* ↑, *Leuconostoc* phages ↓, Bacteroidetes ↓, Firmicutes ↓↑, *Streptococcus* ↑, *Rothia* ↑, Proteobacteria ↑, *Holdemania* ↓↑, Prevotellaceae ↓↑, Proteobacteria ↑, Fusobacteria ↑, *Faecalibacterium* ↓, Enterobacteriaceae ↑, Lachnospiraceae ↓, Ruminococcaceae ↓, Clostridiales ↓, *Lactococcus* ↑, *Akkermansia* ↓, *Ruminococcus* ↓↑, *Gemmiger* ↓, *Veillonella* ↑	145 146 149–167
Patients with cirrhosis with severe AH versus ALC	Actinobacteria ↑, Bacteroidetes ↓, *Haemophilus* ↑, Enterobacteriaceae ↑, *Bilophila* ↓, *Lactobacillus* ↑, *Lactococcus* ↑	168
Patients without cirrhosis and with AH versus patients without cirrhosis and without AH	*Dorea* ↑, *Wolbachia* ↑, *Rothia* ↑	168
Patients with AH with more severe disease versus non-severe AH	*Akkermansia* ↓, *Veillonella* ↑	169
Patients with severe AH versus patients without AH	*Bifidobacteria* ↑, Streptococci ↑, Enterobacteria ↑, *Atopobium* ↓	170
ALC versus patients with alcoholic fatty liver disease and healthy controls	*Streptococcus* ↑	163
ALC with active alcohol misuse versus abstinent ALC and healthy controls	Proteobacteria (Enterobacteriaceae) ↑, Lachnospiraceae ↓, Prevotellaceae ↓	171
Patients with alcohol dependence syndrome	*Ruminococcus gnavus* ↑, torques ↑, *Faecalibacterium* ↓, *Akkermansia* ↓	172
Patients with AUD with progressive liver disease versus patients with AUD with non-progressive liver disease	Phages targeting Enterobacteria ↑, *Lactococcus* species phages↑	154
AUD with impaired cognition and AUD with normal cognition	*Faecalibacterium* ↓, *Gemmiger* ↓, *Escherichia* ↓, *Fusobacterium* ↓	165
Patients with AUD after 2 weeks of alcohol abstinence	*Candida* ↓, *Malassezia* ↓, *Pichia* ↓, *Kluyveromyces* ↓, *Issatchenkia* ↓, *Candida albicans* species ↓ and *Candida* zeylanoides ↓, *Propionibacterium* ↑, *Lactobacillus* ↑, *Leuconostoc* phages↑	153 154

↓, decreased; ↑, increased; ↓↑, reported to increase in some studies and decrease in others; AH, alcohol-associated hepatitis; ALC, alcohol-associated liver cirrhosis; AUD, alcohol use disorder.