Liver Research 6 (2022) 1–9

Contents lists available at ScienceDirect

Liver Research

journal homepage: http://www.keaipublishing.com/en/journals/liver-research

Review Article

Immune cells in alcohol-related liver disease☆

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ARTICLE INFO

Article history:
Received 27 September 2021
Received in revised form
18 November 2021
Accepted 7 January 2022

Keywords:
Alcohol-related liver disease (ALD)
Resident immune cells
Circulating immune cells
Infiltrating immune cells
Inflammation
Cytokines

ABSTRACT

Alcohol-related liver disease (ALD), which is caused by excessive alcohol consumption, is one of the most common types of liver disease and a primary cause of hepatic injury, with a disease spectrum that includes steatosis, steatohepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma. Various lines of evidence have indicated that immune cells play a significant role in the inflammatory processes of ALD. On the one hand, the liver contains various resident immune cells that have been proven to perform different functions in ALD. For example, in the progression of the disease, Kupffer cells (KCs) are activated by lipopolysaccharide-Toll-like receptor 4 signaling and release various proinflammatory cytokines. Moreover, alcohol intake has been shown to depress the function of natural killer cells. Additionally, two types of unconventional T cells (natural killer T cells and mucosal-associated invariant T cells) are involved in the development of ALD. On the other hand, alcohol and many different cytokines stimulate the recruitment and infiltration of circulating immune cells (neutrophils, T cells, macrophages, and mast cells) into the liver. The neutrophils can produce proinflammatory mediators and cause the dysfunction of anti-infection processes. Additionally, alcohol intake can change the phenotype of T cells, resulting in their increased production of interleukin-17. Aside from KCs, infiltrating macrophages have also been observed in patients with ALD, but the roles of all of these cells in the progression of the disease have shown both similarities and differences. Additionally, the activated mast cells are also associated with the development of ALD. Herein, we review the diverse roles of the various immune cells in the progression of ALD.

1. Introduction

Alcohol overconsumption can lead to various health problems, including alcohol-related liver disease (ALD), immune system disorders, digestive problems, and heart and brain diseases.[1,2] From 2005 to 2010, global alcohol consumption per capita had increased from 5.5 liters to 6.4 liters. The prevalence of alcohol consumption has been increasing steadily in China and the USA for the past two decades, and this trend is expected to continue in the future.[3,4] If left untreated, excessive alcohol intake remains one of the leading causes of death, with three million people having died from alcohol-related causes in 2016 (5.3% of all deaths) worldwide.[5] Approximately 25% of deaths caused by alcohol were associated with cirrhosis and hepatocellular carcinoma (HCC).[6,7] ALD is one of the most common types of liver disease and a primary cause of hepatic injury, with a disease spectrum ranging from simple steatosis (the earliest response to alcohol consumption, characterized by fat accumulation in hepatocytes) to alcoholic steatohepatitis (characterized by immune cells infiltration and hepatocellular injury), fibrosis, cirrhosis, and HCC.[8–10] A number of mechanisms contribute to ALD, including the hepatotoxicity caused by ethanol and its catabolism (*e.g.*, acetaldehyde, acetate), oxidative stress, increased intestinal permeability, and decreased liver regeneration.

Aside from being the main site for protein production, nutrient metabolism, and toxin clearance, the liver is also an immunological organ that provides an ideal environment for immune cells-pathogen interactions.[11,12] The liver contains several types of resident immune cells, namely, Kupffer cells (KCs) and some innate lymphocytes, such as natural killer (NK) cells and unconventional T cells.[13,14] These immune cells are abundant in the liver, accounting

☆ Edited by Peiling Zhu and Genshu Wang.

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https://doi.org/10.1016/j.livres.2022.01.001

for 10–20% of the total cells of the organ. They process the blood that enters the liver, secrete large amounts of antigens or pathogens that may be present in the bloodstream, and maintain immune tolerance in the microenvironment.[15] In addition to its resident immune cells, the liver can rapidly recruit circulating immune cells, such as neutrophils, T cells, and macrophages.[16] In some morbid states of the liver, such as those resulting from excessive alcohol intake, drug-induced liver injury, and massive fat accumulation, immune cells also promote the occurrence and development of diseases.[17,18]

Although the exact details regarding the development and progression of ALD have yet to be fully elucidated, several lines of evidence have indicated that immune cells play a significant role in the various inflammatory processes of the disease, such as the initiation of the immune response, inflammation, and incomplete liver repair.[19,20] The activation of immune cells due to intestinal barrier dysfunction and the translocation of bacteria and their products have been proven to contribute significantly to the development of ALD.[21,22] Inflammation is an immune response to danger signals, including pathogen-associated molecular patterns (PAMPs, produced mainly by the gut microbiome) and damage-associated molecular patterns (DAMPs, released by damaged cells).[23–26] Many types of immune cells, including neutrophils, KCs, NK cells, and natural killer T (NKT) cells, can bind to these danger signals through their surface receptors and are involved in cellular activation and intracellular signal transduction process. During ALD progression, immune cells play an important role not only in recognizing and responding to PAMPs and DAMPs but also in activating the inflammatory cascade and contributing to the severity of the disease.[27,28] Additionally, the development of the proinflammatory immune responses starts with impaired endotoxin clearance in the liver and cross-talk among different organs. In this review, we summarize the roles of the hepatic resident immune cells and the circulating immune cells recruited to the liver in the pathogenesis of ALD (Fig. 1).

2. Resident immune cells of the liver

2.1. KCs

KCs represent approximately 20% of the non-parenchymal cells in the liver and are the largest population of the resident macrophage.[29] These cells are much larger than the other cells in the liver and are typically found in the sinusoidal vascular system and adjacent to hepatocytes. KCs scan immediately for and then phagocytose all foreign bodies passing through the hepatic sinuses, including endotoxins and intestinal bacteria and their expressed antigens and metabolites, such as lipopolysaccharide (LPS).[30] The destruction of KCs by gadolinium chloride was shown to prevent alcohol-induced hepatocyte damage, indicating how important this cell type is in the development of ALD.[31]

Studies have indicated that alcohol consumption disrupts the gut microbiota and increases intestinal permeability, which allows more gut microorganisms and their metabolites (*e.g.*, LPS) to enter the liver through the portal system, thereby activating the KCs.[32] The reduction of the gut microbiota by antibiotics or their direct clearance by KCs could relieve ALD.[33,34] On the basis of their phenotypic properties, KCs can be divided into classical (M1) and alternative (M2) types, which interchange in response to environmental signals. The M1 phenotype releases reactive oxygen species (ROS) and cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and monocyte chemoattractant protein-1 (MCP-1), and is therefore characterized as being proinflammatory.[35] By contrast, the M2 phenotype specifically produces IL-10, which is considered as an anti-inflammatory mediator.[36] The balance between M1 and M2 plays an important role in the progression of ALD. M2 promotes M1 apoptosis through the release of IL-10, whereas ethanol inhibits the M2 production of IL-10.[37] Under excessive exposure to alcohol, the LPS molecules produced by pathogenic gut microorganisms bind to Toll-like receptor (TLR) 4 on the KCs, activating the cells. The activated KCs then trigger the release of various proinflammatory cytokines and chemokines, resulting in the inflammatory cascade, which is characterized by infiltrating immune cells and hepatocytes damage.

TNF-α is an important signal transducer in the inflammatory cascade, regulating the activation, proliferation, cytotoxicity, and apoptosis of various immune cells, which are considered important processes in the development of ALD. Studies have shown that anti-TNF-α therapy could attenuate alcohol-induced liver damage and alcoholic steatosis in mice.[34,38] According to a study of Nagy,[39] LPS was in fact helpful in maintaining the stabilization of TNF-α mRNA in KCs, which led to more TNF-α production by these macrophages after ethanol exposure. Using mouse models, Kono *et al.*[40] found that excessive alcohol intake easily resulted in the generation of ROS with the help of NADPH oxidase, and the oxidative stress triggered the activation of the transcription factor nuclear factor-kappa B (NF-κB) and contributed to the KC production of TNF-α. The fact that NF-κB inhibition protects the liver from alcohol-induced damage, even under oxidative stress, was corroborated in the study by Uesugi *et al.*[41] In a recent study that focused on microRNAs (miRs) of KCs, miR-181b-3p was found to be sensitive to ethanol and could inhibit TNF-α expression in these macrophages.[42] By contrast, there was a decrease in miR-181b-3p levels in the liver of mice with ALD. Additionally, Bala *et al.*[43] had determined that the levels of miR-155 expression were high in both KCs and hepatocytes of mice with ethanol-induced steatosis, whereas there was a significant reduction in fat accumulation and immune cells infiltration in miR-155 knockout mice.

It was previously demonstrated that the increase in KCs-mediated IL-1β secretion occurred in a paracrine manner, having a significant pathogenic effect on alcohol-induced inflammation, steatosis, liver damage, and fibrosis.[44] Another study showed that activated KCs could produce more IL-1β through the inflammasome, resulting in the activation of NKT cells and the promotion of ALD.[45] In our recent study on the functions of TLRs in the development of ALD, we found that contemporaneous TLR2 inhibition and TLR3 agonism could promote M1 to M2 polarization and IL-10 production through signal transducer and activator of transcription 3 (STAT3) activation, which was effective in protecting the liver against ALD-associated injury.[46]

The involvement of MCP-1 in ALD was first recognized through its conspicuously higher levels in the liver and mononuclear cells of patients with alcoholic hepatitis.[47] Similarly, the excessive KC expression of MCP-1 was confirmed in rodent models of alcohol-induced liver damage.[48] Further research revealed that MCP-1 could exacerbate alcohol-induced liver injury by promoting the production of proinflammatory cytokines, such as IL-1β and TNF-α, and inducing the expression of genes related to fatty acid oxidation.[49] Notably, KCs can produce high amounts of profibrogenic factors, such as produce transforming growth factor-beta (TGF-β) and platelet-derived growth factor (PDGF) and contribute to the activation of hepatic stellate cells (HSCs) and the promotion of liver fibrosis during ALD.[50]

2.2. NK cells

NK cells are innate lymphocytes that are present in various organ tissues, particularly in the liver where it represents approximately 33–50% of all lymphocytes.[51] Unlike T and B lymphocytes, NK cells can attack infected cells and tumorous cells directly and

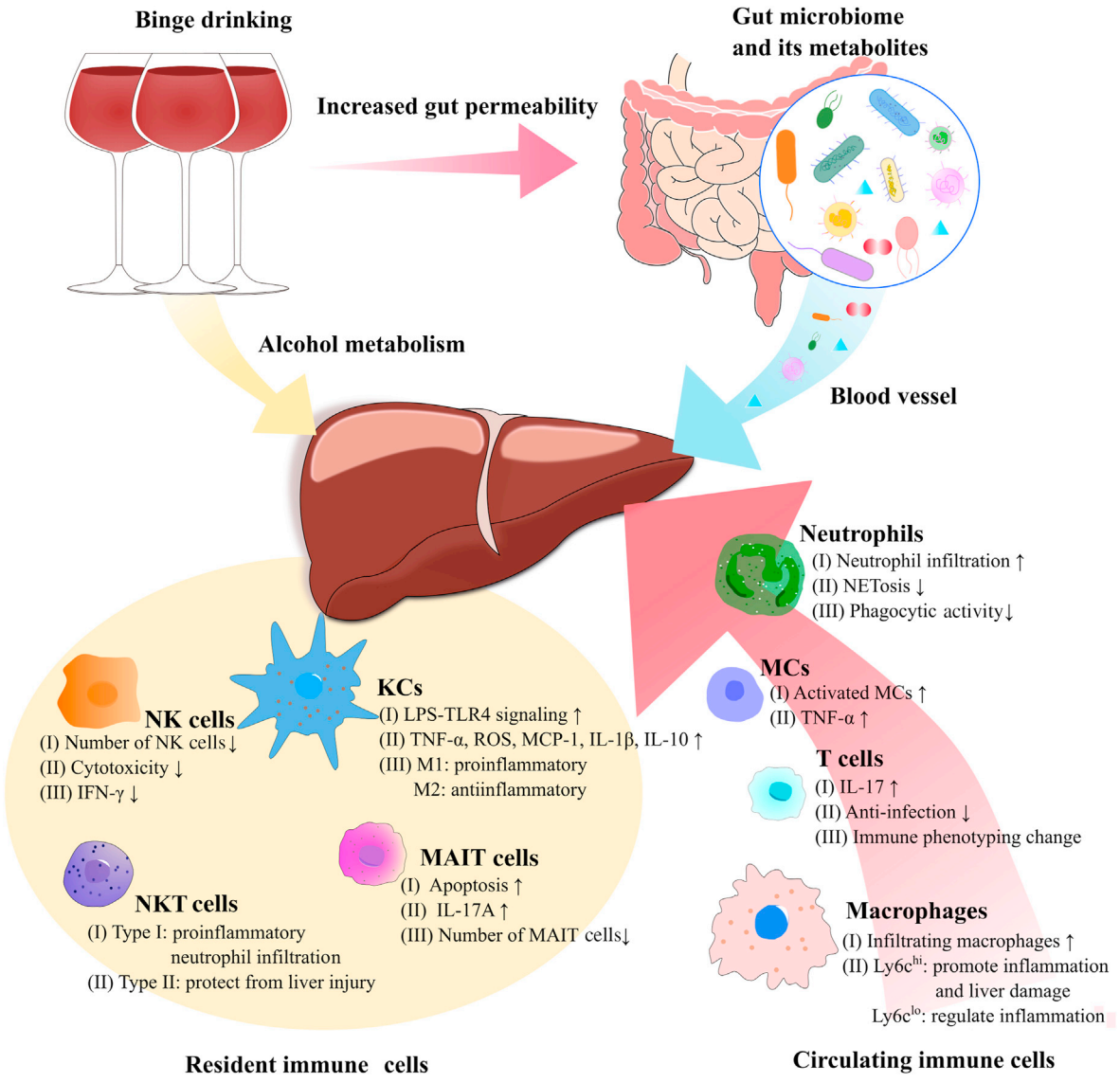

Fig. 1. Immune cells in ALD. The pathogenesis of ALD involves many immune cells, which can be divided into resident immune cells and recruited circulating immune cells. In the progression of ALD, KCs could be activated by LPS-TLR4 signaling, whereupon various proinflammatory cytokines are released. Alcohol intake has been shown to decrease the function of NK cells. Two types of unconventional T cells (NKT and MAIT cells) have also been reported to be involved in the development of ALD. By contrast, alcohol and many cytokines stimulate the infiltration of circulating immune cells (neutrophils, T cells, and macrophages) into the liver. Infiltrating neutrophils could produce proinflammatory mediators and cause dysfunction in anti-infection processes. Moreover, alcohol intake could change the phenotype of T cells and result in their increased production of IL-17. Aside from KCs, infiltrating macrophages were observed in patients with ALD. However, the KCs and infiltrating macrophages have displayed both similarities and differences in their roles in the progression of the disease. Additionally, the activated MCs are also associated with the development of ALD. Abbreviations: ALD, alcohol-related liver disease; IFN-γ, interferon-gamma; IL, interleukin; KCs, Kupffer cells; LPS, lipopolysaccharide; Ly6c, lymphocyte antigen 6 complex; MAIT, mucosal-associated invariant T; MCP-1, monocyte chemoattractant protein-1; MCs, mast cells; NK, natural killer; NKT, natural killer T; ROS, reactive oxygen species; TLR4, Toll-like receptor 4; TNF-α, tumor necrosis factor-alpha.

rapidly without the assistance of antigen-specific receptors. NK cells possess a wide range of receptors for the various cytokines that play a key role in the inflammatory responses. On the one hand, these cytokines promote the immediate recruitment of NK cells to the infected tissue. On the other hand, they activate NK cells and enhance their anti-infection activities and antitumor capabilities.[52] Furthermore, NK cells are postulated to play an antifibrotic role by directly killing HSCs and promoting their apoptosis through the production of cytokines such as interferon-gamma (IFN-γ).[53,54] It has been revealed that there are various subsets of hepatic NK cells with distinct and diverse phenotypes and functions that promote or inhibit liver diseases.[55,56] The activation of NK cells by hepatitis viruses not only promotes recovery from the infection but also contributes significantly to liver destruction through the cytolytic activity of the lymphocytes against the hepatocytes.[57–59] Similar evidence has been shown in non-alcoholic fatty liver disease, in which NK cells cause liver injury by directly attacking hepatocytes, which express a high level of NK cell-activating ligands.[60]

Contrary to what has been described above, it is increasing to note that chronic alcohol ingestion can decrease both the proportion and the number of NK cells in mice and humans.[61,62] Additionally, the status of NK cells in ALD is usually described as being repressed rather than excited, indicating that ethanol and its metabolites can interfere with the functions of these lymphocytes, such as prevention of infections and tumor proliferation.[63] Various mechanisms have been proposed to explain how ethanol and its metabolites depress NK cells. For example, Jeong *et al.*[64]

demonstrated that alcohol consumption could compromise the anti-fibrotic effect of NK cells in three ways. First, ethanol changed the expression of NK cell surface receptors, including TNF-related apoptosis-inducing ligands, natural killer group 2 member D (NKG2D), and IFN-γ, which reduced the cytotoxicity of the lymphocytes toward HSCs. Second, in mice administered excess amounts of alcohol, the activated HSCs were able to supply more TGF-β molecules, which inhibited the NK cells, indicating that the animals were more resistant to the cytolytic activity of the lymphocytes. Third, the activated HSCs could also express an inhibitor of IFN-γ that protected them from the apoptotic effect associated with IFN-γ stimulation.[60] Additionally, high alcohol ingestion was shown to increase the apoptosis of splenic NK cells and blocked the release of these lymphocytes from the bone marrow.[65]

2.3. Unconventional T cells

T cells are categorized into alpha beta (αβ) and gamma delta (γδ) subtypes according to their surface expression of T-cell receptors (TCRs). The αβ subtype, also known as conventional T cells, recognizes the molecular complexes (*i.e.*, antigen peptide-major histocompatibility complex) presented by antigen-presenting cells (APCs). These conventional T cells can be further classified into $CD4^+$ T cells, $CD8^+$ T cells, and regulatory T (Treg) cells. In addition, some unconventional T cells can identify antigens such as lipids, proteins, and heat shock proteins (HSPs) without the help of APCs.[66] The most common unconventional T cells in the liver are the NKT cells and mucosal-associated invariant T (MAIT) cells, which have been confirmed to be related to the progression of ALD.

2.3.1. NKT cells

NKT cells are innate-like lymphocytes that are abundant in the liver sinusoids. CD56 and TCRs, which are the typical receptors of NK cells and conventional T cells, are expressed concurrently on the surface of NKT cells.[67] In general, NKT cells are divided into types I and II according to their expression of TCRs. Type I NKT cells are usually known as invariant NKT (iNKT) cells.[68] Injury to the liver triggers a very rapid response from the NKT cells, with the two subtypes playing different roles in the process. iNKT cells are proinflammatory and can promote liver damage, whereas type II NKT cells influence iNKT cells activity and protect the liver from injury.[69] On the basis of the finding that alcohol consumption could activate the release of more IL-1β from the KCs, Cui *et al.*[45] confirmed that the release of this proinflammatory cytokine promoted hepatic iNKT cells activation, further driving liver inflammation and neutrophil infiltration. Using a mouse model of chronic-plus-binge ethanol-induced liver injury, Mathews *et al.*[70] observed that iNKT cells became activated and released mediators that would encourage neutrophil infiltration into the liver. Maricic *et al.*[71] confirmed that alcohol consumption activated iNKT cells, which promoted inflammation and neutrophil recruitment in the liver, whereas type II NKT cells protected against ALD damage. Additionally, the researchers of another study proposed the migration of intestinal iNKT cells to the liver as being one of the important reasons for the apoptosis of hepatocytes during ALD.[72] Although iNKT cells make up more than 90% of all liver NKT cells, the direct influence of type II NKT cells on the development of ALD should not be overlooked, even though they are difficult to identify and we have a limited understanding of their functions. Thus, additional studies to confirm these findings are required.

2.3.2. MAIT cells

Among the unconventional immune cells residing in the liver, MAIT cells are the most prevalent, making up almost 50% of the lymphocytes in the organ. Although these cells usually take part in the antibacterial defenses of the host, they are significantly depleted and hyperactive in ALD, showing a defective response to antibacterial cytokines.[73] It has been proposed that poor intestinal integrity and increased amounts of gut microbiome and bacterial products could be contributing factors to these disturbances in the MAIT cells.[73] Marrero *et al.*[74] focused on the activation of three unconventional T cells in patients with severe alcoholic hepatitis and in chronic alcoholics, whereupon they noticed that the number of MAIT cells was significantly low in all patients with ALD. The results of our recent studies are consistent with these findings, in that we observed significantly reduced levels of MAIT cells in patients with ALD.[75] Moreover, the MAIT cells underwent apoptosis more easily, a phenomenon that was caused by the increased production of IL-12, IL-18, and IL-8.[75] Additionally, there was a change in MAIT cell function in patients with ALD cirrhosis, in that the cells produced more IL-17A and perforin and less TNF-α, which contributed to the pathogenesis of the disease.[75]

3. Recruitment of circulating immune cells

3.1. Neutrophils

Neutrophils are the most abundant type of white blood cells in the human body, making up 50−70% of all circulating leukocytes, and are always at the forefront of immune responses.[76] These cells kill infectious agents through three different and typical ways: phagocytosis, degranulation, and neutrophil extracellular traps, the last of which are defined as fibrous networks that protrude from the surface of activated neutrophils.[77] Furthermore, neutrophils release numerous types of inflammatory cytokines. The most common cell surface receptors of neutrophils are pattern recognition receptors, including TLRs, C-type lectin receptors, and nucleotide-binding oligomerization domain-containing protein-like receptors.[78] Typically, the increased release of LPS, a relatively common PAMP produced by the gut microbiota in response to alcohol consumption, can be detected by TLRs on neutrophils, which then activate the neutrophils directly and lead to liver inflammation.[79] Moreover, the endotoxemia caused by the impaired phagocytosis of KCs could result in the infiltration of the activated neutrophils into the liver sinusoids.[80] The expression levels of the typical CXC chemokines are positively associated with the prognosis of alcoholic hepatitis.[81] C-X-C motif chemokine receptor (CXCR) 1 and CXCR2 are protein-coupled receptors of CXC chemokines that can bind with IL-8, which has a vital role in activating and recruiting neutrophils in severe alcoholic hepatitis.[82] Another study showed that the IL-17 pathway could also recruit neutrophils in patients with ALD.[83] There has been some evidence of the involvement of the TLR4 pathways in alcohol-caused liver damage.[84] Additionally, in a mouse model of chronic-binge ethanol intake, the TLR2 and TLR9 pathways were shown to mediate the production of C-X-C motif chemokine ligand (CXCL) 1, which in turn acted through its receptor CXCR2 to promote neutrophil infiltration into the liver, leading to ethanol-induced liver damage.[85] Das *et al.*[86] examined 90 patients with severe alcoholic hepatitis and observed that oxidized albumin could activate neutrophils to promote inflammation and oxidative stress.

Neutrophil clusters have been found in liver biopsies from patients with ALD since at least 40 years ago.[87] Interestingly, although the majority of heavy drinkers develop fatty liver, only approximately 35% develop alcoholic hepatitis with neutrophil infiltration, raising the question of the exact role of these white blood cells in the pathogenesis of ALD.[60] The migration of neutrophils was observed in patients with ALD in one study,[88] and the removal of neutrophils in mice before their treatment with a binge dose of alcohol was shown to decrease liver damage and inflammation in

another study.[89] Owing to the extensive neutrophil infiltration observed in ALD, which is one of the pathological features of the disease, the relationship between neutrophils and ALD has been the focus of considerable attention in the last few decades.[86,90–92] A multicenter study by Altamirano *et al.*[93] revealed that the degree of neutrophil infiltration was independently associated with the 90-day mortality of patients with alcoholic hepatitis. Research conducted by Aguilar-Bravo *et al.*[92] showed that ductular reaction cells have proinflammatory characteristics and express proinflammatory mediators, which are associated with neutrophil infiltration and systemic inflammation in ALD. Our previous studies had indicated that neutrophil infiltration and necroptosis were significantly associated with a poor prognosis in patients with end-stage ALD cirrhosis.[94] Iracheta-Vellve *et al.*[95] demonstrated that the inhibition of IL-1 reduced liver inflammation and neutrophil infiltration.

In recent years, much attention has been given to neutrophil extracellular traps, which are formed during the progression of ALD. Dysfunctional neutrophil cell death was detected in mice administered a binge dose of alcohol, which impaired the ability of the neutrophils to preclude infection, resulting in liver damage during ALD.[89] Another reason that patients with ALD are more prone to infections is the weakened antibacterial activity of neutrophils that is caused by their impaired ability to produce ROS. A seminal study by Rolas *et al.*[91] reported that the neutrophils of patients with advanced ALD cirrhosis were severely deficient in their expression of the NADPH oxidase catalytic core flavocytochrome b558 ($gp91^{phox}$), which correlated with impaired ROS production. Those authors proposed a novel treatment strategy of reversing the downregulation of $gp91^{phox}$ using TLR7/8 receptor agonists.[91]

Takeuchi *et al.*[96] revealed that cell adhesion molecules on neutrophils interacted with integrin beta-1 on cholangiocytes and decreased their level of type 3 inositol 1,4,5-trisphosphate receptor (ITPR3) expression, which resulted in cholestasis in patients with alcoholic hepatitis. Artru *et al.*[97] investigated the cellular pathways in patients with severe alcoholic hepatitis and found that the regulatory activity of the IL-33/ST2 pathway was decreased in the neutrophils, which could lead to a higher risk of infection in those patients. Patients with acute alcoholic hepatitis have weakened innate host defenses, and a variety of their immune cells (*e.g.*, neutrophils, NK cells, monocytes, and T cells) have defective antibacterial responses.[98] Lipocalin-2 (LCN2), a siderophore-binding peptide in neutrophils, has been confirmed to have anti-infective effects in several disease models. By contrast, Wieser *et al.*[99] found that LCN2 was involved in the development of ALD and promoted neutrophilic inflammation following ethanol induction. Furthermore, neutrophils with dysfunctional phagocytic activity were observed in 108 patients with stable liver cirrhosis, but the etiology of the disease was not discussed in that study.[100] Given that there is a dearth of reports on neutrophils with phagocytic dysfunction in patients with ALD, further studies are needed to investigate the phenomenon more clearly.

Since neutrophils are known to play an important role in ALD, some researchers have sought to predict the prognosis of the disease using neutrophil-related indicators, such as the neutrophil-to-lymphocyte ratio (NLR). Although a higher concentration of neutrophils suggests a more severe inflammatory response, and a high NLR indicates an altered immune system more clearly, the prognostic value of the NLR alone has been proven by several studies to be mediocre.[101,102] In contrast to these results, Forrest *et al.*[103] confirmed that the NLR of patients with severe alcoholic hepatitis was associated with the occurrence of infection and acute kidney injury. Furthermore, they noted that patients with acute alcoholic hepatitis who had an NLR of 5–8 responded better to corticosteroids.

3.2. $CD4^+$ and $CD8^+$ T cells

Mature T cells express the cell surface proteins CD4 or CD8, their primary function of which is to help the TCRs recognize antigens and activate the T cells; therefore, mature T cells are divided into $CD4^+$ and $CD8^+$ T-cell subtypes. The fact that patients with alcoholic hepatitis develop immune dysregulation indicates that the contribution of T cells to this phenomenon cannot be ignored. In 1993, Chedid *et al.*[104] reported that frozen liver biopsy specimens from 144 patients with ALD showed a higher prevalence and persistence of $CD4^+$ and $CD8^+$ T cells and that the interaction between T cells and hepatocytes contributed to the progression of the disease. Furthermore, researchers found that chronic alcohol consumption led to T-cell activation in both humans and mice.[105] Various ethanol adducts are produced in the liver of patients with ALD, which could then be presented to $CD4^+$ T cells by APCs, thereby stimulating clonal T-cell proliferation.[19] Ambade *et al.*[106] showed that C-C chemokine receptor types 2 and 5 (CCR2/5) signaling was increased in mice after alcohol feeding, leading to the hepatic recruitment of circulating immune cells, including T cells and macrophages, which caused hepatocyte damage, hepatic steatosis, and the upregulated expression of several cytokines. Moreover, cenicriviroc, an inhibitor of CCR2/5 signaling, could play a vital role in the treatment of alcohol-induced liver injury or steatohepatitis. Increasing lines of evidence suggest that various T-cell phenotypes are involved in the progression of ALD, and the functions of T cells are diverse.

First, T cells may contribute to the progression of ALD by releasing inflammatory mediators or by attacking hepatocytes directly. Lee *et al.*[107] found that in mice administered a binge dose of alcohol, the level of IL-17A expression in γδ T cells was increased in the early stage of the disease, whereas it was accelerated in $CD4^+$ T cells in the later stage only. Consistent with these results, Lin *et al.*[108] found that patients with ALD expressed significantly higher levels of IFN-γ and IL-17, and the degree of T helper (Th)1 cell response was directly related to the disease severity. Moreover, alcohol consumption significantly increased the number of Th17 cells, which are thought to contribute to the development of ALD.[109] Furthermore, because the elimination of IL-17A in KCs has been shown to result in the amelioration of HCC development, IL-17A (produced mainly by $CD4^+$ Th17 cells) could be a key regulator of the inflammatory responses in KCs, thereby promoting alcohol-induced liver injury and increasing the development of HCC.[110] Researchers found that the numbers of $CD4^+CD57^+$ and $CD8^+CD57^+$ T cells were increased in the circulating blood of patients with ALD. Additionally, the percentage of $CD44^{hi}$ T cells in mice fed chronic levels of alcohol was significantly higher than that in mice that were not fed alcohol.[105] By contrast, Gurung *et al.*[111] demonstrated the number of $CD8^+$ T cells and antigen-specific responses were significantly reduced in mice fed chronic levels of alcohol, suggesting that this subset of T cells was associated with the immunodeficiency caused by chronic alcohol consumption. Additionally, researchers discovered that excessive alcohol consumption could reduce the number of antitumor $CD8^+$ T cells, which resulted in a faster progression of HCC in mice.[112]

Excessive ethanol exposure over time causes the immune phenotype of T cells to change, which weakens the defenses of the body against injury, making it more susceptible to infections.[113] Kasztelan-Szczerbinska *et al.*[114] observed a higher expression level of programmed cell death protein 1/programmed cell death ligand 1 (PD-1/PD-L1)-positive T cells in female patients with ALD. On the basis of the theory that the PD-1/PD-L1 pathway is a

negative regulator for immune responses, they inferred that the above phenomenon might explain why immune activity is enhanced and inflammatory responses are induced, but antibacterial activity is decreased, in patients with ALD.

Furthermore, T cells can promote liver regeneration and play a positive role in ALD. It is noteworthy that in some surveys that compared immune response differences between patients who drank actively and those who abstained for a long period, the results showed that the individuals who were abstinent had a stronger IL-4 immune response, which was negatively related to the severity of the liver injury. This indicated that the IL-4 molecules secreted by hepatic mononuclear cells could inhibit cytotoxic $CD8^+$ T cells and IFN-γ-producing T cells, thereby playing a protective role in patients with ALD.[108] Similar effects were observed with IL-22; Radaeva *et al.*[115] observed for the first time the protective role that IL-22 played against T-cell-mediated hepatitis.

There are several other interesting findings related to circulating immune cells. For example, besides $CD4^+$ and $CD8^+$ T cells, Treg cells have been recognized as contributing to the progression of ALD. In the study by Almeida *et al.*,[116] circulating Treg cells were shown to be significantly low in number in patients with alcoholic hepatitis. Liu *et al.*[117] demonstrated that chronic alcohol consumption could boost the development of chronic liver disease in hepatitis B virus (HBV)-infected mice by activating Treg cells and stimulating HBV-induced abnormal lipid metabolism.

3.3. Infiltrating macrophages

When inflammation occurs in response to an infection or products from hepatic metabolism or sterile injury, monocytes, and neutrophils from the blood are recruited into the tissues, whereupon the monocytes eventually mature into macrophages. Despite the large amounts of KCs in the liver, infiltrating macrophages, mostly those derived from monocytes, play a crucial role in the pathogenesis of ALD.[118] Moreover, there is evidence that hepatic-recruited macrophages are derived not only from monocytes circulating in the blood but also from multiple compartments, such as the peritoneal cavity, from which the macrophages can be recruited quickly across the mesothelium into the afflicted tissue as a reaction to liver damage.[119]

Generally, infiltrating macrophages and KCs are considered to be hepatic macrophages and their roles in ALD have not been separately discussed. However, some studies have focused on recruited macrophages in ALD individually. Mouse blood monocytes, which are the precursors of infiltrating macrophages, can be categorized into two types depending on their lymphocyte antigen 6 complex (Ly6c) levels: $Ly6c^{hi}$ and $Ly6c^{lo}$. Acute and chronic carbon tetrachloride-induced liver damage resulted in $Ly6c^{hi}$ monocyte recruitment into the injured liver, promoting liver fibrosis.[120] Wang *et al.*[121] focused on whether alcohol intake caused a similar phenomenon. First, they differentiated between infiltrating macrophages and KCs by comparing their differences in levels of CD11b and F4/80 expression. The infiltrating macrophages were subsequently divided into two subsets according to their differential expression of Ly6c. The authors reported that chronic alcohol ingestion in mice could induce the accumulation of the two types of infiltrating macrophages mentioned above within the liver, but they governed inflammation and liver regeneration in opposite ways. Infiltrating macrophages with the $Ly6c^{hi}$ phenotype caused inflammation and tissue damage, whereas those with the $Ly6c^{lo}$ phenotype regulated inflammation and protected the tissues. Furthermore, the $Ly6c^{hi}/Ly6c^{lo}$ ratio was increased in mice fed chronic doses of ethanol, which contributed to liver injury.[121]

However, the influence of hepatic macrophages on liver diseases in humans remains unclear. Several animal experiments have demonstrated that hepatic macrophages are remarkably versatile and complex in their functions.[122] However, some of the functions of macrophages differ between humans and mice, whereas others are similar. For example, the phenotypic features of human $CD14^+CD16^+$ macrophages are similar to those of murine $Ly6c^{lo}$ hepatic macrophages, and both cell types share some similar functional characteristics, such as high phagocytic activities. By contrast, the human $CD14^+CD16^+$ macrophages are also characterized by their release of multiple proinflammatory mediators, which is close to the function of murine $Ly6c^{hi}$ macrophages.[123] In light of these facts, further investigations of the mechanisms underlying liver macrophage heterogeneity are essential.

3.4. Mast cells (MCs)

MCs are one of the most widely studied cells of immune cells, which exist in connective tissues and usually reside in low numbers within the liver.[124] However, the number of MCs in the liver is increased significantly during liver injury, demonstrating the important role of MCs during the pathophysiology of liver diseases.[125–127] When stimulated by antigens, the most eye-catching property of MCs is that they release multiple proinflammation mediators which have been stored performed, such as histamine, proteases, and proteoglycans.[128] Furthermore, MCs can produce and secrete numerous cytokines, chemokines, and growth factors that promote the recruitment of several immune cells, including neutrophils, macrophages, and T cells, as well as promoting liver inflammation.[129] Besides, evidence suggests that MCs are also involved in liver fibrogenesis when the liver is damaged by toxins, viral infections, and cholestasis.[130] On the one hand, it has been proposed that the MCs activated by chronic alcohol consumption accelerate the progression of ALD by impairing the intestinal barrier.[128] On the other hand, there is a view that elevated tryptase- and chymase-positive MCs, as well as MC-derived TNF-α, are the points by which MCs promote ALD.[131] The knowledge of MC function in ALD is still very limited and further fundamental studies are urgently required.

4. Treatment

The most studied therapeutic of ALD is how to intervene in inflammation. Corticosteroids are one of the most commonly considered options for reducing liver inflammation. Despite this, the use of corticosteroids is controversial since they are ineffective for most patients and remain a risk for sepsis, infections, and gastrointestinal bleeding.[132] In addition, the N-acetylcysteine, pentoxifylline, and anti-TNF agents had also been considered as treatments for ALD, but subsequent studies have confirmed that they have low efficiency and high risk that makes them unsuitable for clinical use.[133–135] However, different from anti-TNF, anakinra, is another monoclonal antibody therapy that against IL-1 has been tested in clinical trials and positive results are been expected (NCT04072822).[136] Since chemokines released by immune cells also play important role in the progression of ALD, it had been considered as a therapeutic key for interrupting inflammation. For example, researchers focused on cenicriviroc, a CCR2/5 antagonist, have reported some exciting results.[106] Additionally, macrophage migration inhibitory factor, a pluripotent immune regulator, has also been shown to protect ethanol-induced liver injury in mice.[137] Besides, there are multiple treatments of ALD that are being actively tried, including nutrition-based therapies, anti-infection, promoting gut microbiome (fecal microbiota transplantation and antibiotics), and liver regenerative methods, and so on. The treatments for ALD are advanced, but more research is needed to tackle it.

5. Conclusion

Although ALD is one of the major causes of chronic liver diseases worldwide, its pathogenesis remains unclear. However, what is certain is that various immune cells are involved in the pathogenesis of this disease (Fig. 1). On the one hand, the liver contains some resident immune cells, such as KCs, NK cells, NKT cells, and MAIT cells, all of which have been proven to play different roles in ALD. On the other hand, the liver also recruits circulating immune cells following stimulation by alcohol and many cytokines. Notably, many research studies focused on immune cells in ALD, have been conducted and some of the mechanisms of those immune cells in ALD have been elucidated. Additionally, several clinical trials have been conducted to verify the effectiveness of inflammatory therapeutic targets. Nonetheless, further research on the mechanisms of immune cells and more clinical trials of inflammatory moderators are needed to identify novel treatment strategies for ALD.

Authors' contributions

H. Wang conceived the idea and revised the manuscript critically, H. Xu and H. Wang wrote the manuscript, and H. Xu drew the figure. All authors approved the final version for publication.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (81900554) and the Major Program for Supporting Outstanding Talents in Colleges of Ministry of Human Resources and Social Security of the People's Republic of Anhui (gxyqZD2020013).

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