Review

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Roles of innate lymphoid cells in metabolic and alcohol-associated liver diseases

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Summary

Innate lymphoid cells (ILCs) have been identified as potent regulators of inflammation, cell death and wound healing, which are the main biological processes involved in the progression of chronic liver disease. Obesity and chronic alcohol consumption are the leading contributors to chronic liver diseases in developed countries, due to inappropriate lifestyles. In particular, inflammation is a key factor in these liver abnormalities and promotes the development of more severe lesions such as fibrosis, cirrhosis and hepatocellular carcinoma. Opposite roles of ILC subsets have been described in the development of chronic liver disease, depending on the stage and aetiology of the disease. The heterogeneous family of ILCs encompasses cytotoxic natural killer cells, the cytokine-producing type 1, 2 and 3 ILCs and lymphoid tissue inducer cells. Dysfunction of these immune cells provokes uncontrolled inflammation and tissue damage, which are the basis for tumour development. In this review, we provide an overview of the recent and putative roles of ILC subsets in obesity and alcohol-associated liver diseases, which are currently the major contributors to end-stage liver complications such as fibrosis/cirrhosis and hepatocellular carcinoma.

Keywords: innate lymphoid cells; MASLD; ALD; fibrosis; hepatocellular carcinoma

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Introduction

The liver is a central organ that performs essential functions to maintain body homeostasis in response to metabolic and immunological cues. Hepatocytes account for 80% of total liver cells and the non-parenchymal fraction constitutes the remaining 20%.[1] The non-parenchymal fraction is mostly composed of sinusoidal endothelial cells and hepatic stellate cells (HSCs), which play a key role in the synthesis of extracellular matrix proteins and liver-resident immune cells. The functions of these immune cells are to maintain a state of tolerance as the liver is continuously exposed to food products, while defending against viruses, bacteria and toxins. Dysregulation of these functions may be the cause or the consequence of the development of chronic liver diseases which evolve from inflammation to fibrosis and towards more severe stages such as cirrhosis and cancer.[2]

Obesity is one of the main risk factors for developing the chronic liver diseases known as metabolic dysfunction-associated steatotic liver disease (MASLD).[3] The worldwide prevalence of MASLD is growing in parallel with obesity, reaching nearly 33% of the population in 2022.[4] MASLD has a wide pathological spectrum ranging from "simple" steatosis, characterised by lipid accumulation in hepatocytes, to metabolic dysfunction-associated steatohepatitis (MASH). MASH is a progressive and severe condition characterised by steatosis, a persistent inflammatory state, hepatocyte death and a predisposition to more severe liver abnormalities such as fibrosis/cirrhosis and hepatocellular carcinoma (HCC).[2,4–7] The chronic inflammation associated with MASH is associated with the recruitment of circulating immune cells and polarization of liver innate immune cells towards a more inflammatory phenotype.[2]

Gut and adipose tissue are key players in the development and progression of MASLD. Alteration of the gut barrier and dysbiosis lead to increased intestinal permeability and consequently to a release of bacterial-derived products (pathogen-associated molecular patterns) that reach the liver via the portal vein.[8–10] Pathogen-associated molecular patterns bind to pattern recognition receptors and accumulate in the liver, activating local immune cells and hepatocytes.[2,11] The adipose tissue also plays an important role in the progression of MASLD. When energy intake is excessive, adipocyte storage is rapidly saturated, causing a deregulation of lipid metabolism. In parallel, macrophages that produce T helper 1-type cytokines (tumour necrosis factor-α [TNF-α], interferon-γ [IFN-γ]) are recruited into the adipose tissue, leading to increased lipolysis and the development of insulin resistance associated with hyperlipidaemia.[12] All these alterations are responsible for a modification in the secretion of several mediators at the hepatocyte (hepatokines) and

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adipocyte (adipokines) level, activating the immune system and thus contributing to the development of MASLD.[13] These intra- and extrahepatic mechanisms promote the development of MASLD and increase the susceptibility of patients to infection and cancers.[14,15]

Alcohol-related liver disease (ALD) is the most common chronic liver disease in the world and causes more than 3.3 million deaths every year.[16] A chronic and large consumption of alcohol (more than 20 g/day of alcohol for females and 30 g/day of alcohol for males) leads to a build-up of fats in the liver (steatosis) and to alcohol-related steatohepatitis, which is characterised by steatosis, hepatocyte ballooning and infiltration of neutrophils. Steatosis and steatohepatitis are critical in the development and progression of ALD. Furthermore, alcoholic hepatitis (AH) is a severe syndrome (symptoms of jaundice, fever, tachycardia, tachypnoea, hepatomegaly, leucocytosis with neutrophilia) that can occur at any stage of ALD. Like MASLD, adipose tissue and gut dysfunction trigger ALD pathogenesis. Indeed, the spectrum of liver alterations in ALD and MASLD share common mechanisms but with their own characteristics (alcohol metabolism, specific dysbiosis, insulin resistance and inflammation of adipose tissue).[17–19] Histopathological characteristics of ALD include acute portal inflammation, high neutrophil infiltration, alcoholic foamy degeneration and cholestasis.[20]

HCC is the sixth most common cancer in the world but the third leading cause of cancer deaths. Its incidence has tripled in developed countries over the past three decades,[21,22] and is associated with the incidence of ALD and MASLD. Dysregulation of the local microenvironment and chronic inflammation associated with altered genetic/epigenetic modifications promote HCC development and progression.[23,24] Advanced HCC (with portal invasion and/or extrahepatic spread) accounts for around a third of all treated cases. Recently, immune checkpoint inhibitors (ICIs), administered alone or with anti-angiogenic agents, have been shown to significantly improve survival and quality of life compared with the multi-targeted kinase inhibitors previously used in patients with advanced HCC. In fact, based on their improved efficacy and acceptable tolerability, ICIs are currently being evaluated in patients with mid-stage or even early-stage HCC.[25,26] However although a significant increase has been observed in the median survival rate, not all patients respond to ICIs.[27] Therefore, it is critical to better characterise the hepatic microenvironment during HCC development and to better understand the interplay between local immune cells and neighbouring cells in order to find new anti-tumour approaches and improve existing strategies.

The family of innate lymphoid cells (ILCs) has recently received attention in the field of liver diseases, as these cells regulate inflammation and fibrosis and therefore the development of cancer. Since they were first identified in 2008-2009, ILC subsets have been extensively described and discussed in many reviews.[28–30] Briefly, ILCs are non-T and non-B lymphocytes that lack rearranged antigen receptors; they form a heterogeneous group and can be divided into five subsets: cytotoxic natural killer (NK) cells, type 1, 2 and 3 ILCs (ILC1, ILC2, ILC3) and lymphoid tissue inducer (LTi) cells.[28,31–33] These cells share similarities with T helper cells and cytotoxic T cells at both the transcriptional and functional levels and most of them are cytokine producers and tissue-resident cells (gut, skin, lung, liver, adipose tissue), with the exception of NK cells which are circulating cytotoxic cells.[34] ILCs regulate tissue homeostasis but also contribute to the onset of disease through their ability to produce proinflammatory and regulatory cytokines in response to local injury or infection[35,36] (Fig. 1). Dysregulation of ILC activity can contribute to inflammatory disorders, thus they play a role in tumour-associated inflammation.[37–42] LTi cells are essential for the formation of the secondary lymphoid organs during embryogenesis. In adulthood, bone marrow Lti cells replace these embryonic Lti cells and Lti-like subsets have been described in adult gut mucosa. However, their role in adult diseases is not yet well understood and needs to be studied in more detail.[43] There is growing interest in the contribution of ILC subsets to the regulation of liver disease. Whether they play a pathogenic or protective role in the development of disorders is a matter of debate, which may be due to the difficulty of accurately identifying each subset.

Here, we summarize current knowledge regarding the involvement of ILC subsets in regulating the development and progression of metabolic and alcohol-related liver disease in order to better understand the pathogenesis of these liver diseases and the therapeutic potential of targeting these cells.

Key points

- Metabolic dysfunction-associated steatotic liver disease (MASLD) and alcohol-related liver disease (ALD) are the leading types of chronic liver diseases in the developed world.
- Hepatocellular carcinoma is one of the most lethal cancers and patients with alcohol use disorder or obesity are at increased risk of developing it.
- Natural killer (NK) cells, and innate lymphoid cell (ILC)1 and ILC2 subsets have been found to play opposite roles (protective or pathogenic) in MASLD and ALD, depending on the stage and aetiology of the disease.
- NK cells and ILC1 have opposite roles in triggering steatohepatitis depending on the aetiology.
- ILC2 may be protective in MASLD but deleterious in ALD and ILC3 seem to be protective in MASLD and ALD.
- NK cells protect against liver fibrosis and HCC while ILC1, 2 and 3 may be deleterious.

NK cells and ILC1 in MASLD

The pathogenic or protective role of NK cells in MASH/MASLD has long been debated. This may be due to the difficulty of distinguishing conventional NK cells from tissue-resident hepatic ILC1 (also called liver-resident NK cells in some studies), which share some similarities, but which are functionally different. Conventional NK cells and ILC1 together account for approximately 30% and 10% of the total lymphocytes in the livers of humans and mice, respectively.[44] NK cells have more “killing” properties, with higher expression of granzyme B and perforin than ILC1, although recent studies indicate that under certain conditions, ILC1 also exhibit cytotoxicity.[45,46] However, the phenotypic and developmental properties that distinguish NK cells and ILC1 are often unclear and confusing, based on evidence from different organs and pathological conditions.[47,48] RNA sequencing approaches have therefore been increasingly used to better characterise and differentiate NK cells and ILC subsets.[49–51] The purpose of our review is not to resolve these discrepancies, which are widely discussed elsewhere, but rather to outline the pathogenic or protective roles of these cells in chronic liver diseases associated with obesity or alcohol consumption.

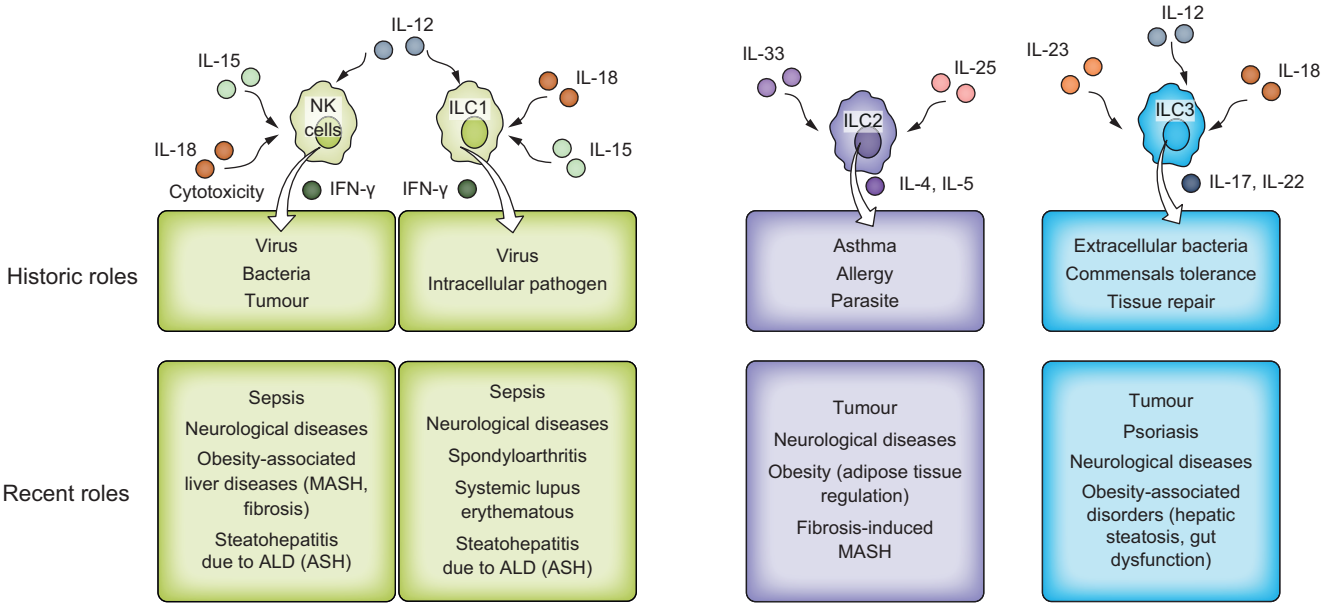

Fig. 1. Major contributions of ILC subsets to immunity. Historically described roles and the main recently discovered contributions of ILCs to immune responses are described. ILC(s), innate lymphoid cell(s); ILC1-3, type 1-3 ILC(s); MASH, metabolic dysfunction-associated steatohepatitis; MASLD, metabolic dysfunction-associated steatotic liver disease; NK, natural killer.

Cytokines are known potent regulators of immune cell development, activation and functions.[52] In particular, interleukin (IL)-12, IL-15 and IL-18 together control the homeostasis of NK cells and the ILC1 subset.[53] *Il15*-knockout (KO) mice fed a high-fat diet (HFD) show a reduction of hepatic steatosis and liver expression of chemokines (CCL2, CCL5, CXCL10) known to attract NK cells. In turn, immune and NK cell infiltration into the liver are lower in diet-induced obese mice in comparison to control-diet mice.[54] Albeit only correlative, these results suggest that the absence of IL-15 could affect the biology of these cells as well as other immune cells such as NKT cells and/or subsets of CD8 T cells. Consistent with this, it was recently shown that IL-15 induces auto-aggressive hepatic CXCR6^{+} CD8 T cells by down-regulating FOXO1 (forkhead box O1) transcription factor expression during MASH, causing increased liver damage.[55] The study by Cepero *et al.* also hints that NK cells play a pathogenic role in the onset of MASH.[54] Therefore, although IL-15 is a key cytokine for NK cell development and biology at steady state, a local overproduction of IL-15 appears to be deleterious during MASH. A recent study supported this observation, showing that activated NK cells promote diet-induced MASH (methionine- and choline-deficient diet or choline-deficient HFD).[56] When challenged with a MASH diet, *Nfil3* (nuclear factor interleukin 3 regulated) KO mice, which lack NK cells but retain ILC1, showed reduced liver steatosis, inflammation and injury compared to control mice. This protective mechanism involves the reduction of JAK-STAT and NF-kB-p65 signalling in hepatocytes. In contrast, depletion of NKp46^{+} cells, which encompass NK cells and ILC1, aggravates MASH.[57] These data suggest that NK cells play a pathogenic role while ILC1 possibly play a protective role during MASH development. Further, the depletion of NK cells/ILC1 is not liver specific in *Nfil3*-KO mice. Thus, we cannot rule out that NK cells/ILC1 act via the regulation of gut and adipose tissue function, which are also important in the development and progression of MASLD. Further investigation is needed to explore the regulation of mechanisms controlling NK cell and ILC1 functions to determine the precise role of these subsets in MASH progression.

NK cells and ILC1 in ALD

The immunosuppressive effect of alcohol has clearly been established for several years, and patients with chronic alcohol consumption are more susceptible to infection.[58,59] Regarding NK cells, it has been reported that chronic alcohol consumption decreases the number and cytotoxicity of NK cells in human peripheral blood. The reduction in NK cell degranulation capacity has been related to decreased natural killer group 2, member D (NKG2D) receptor expression.[60–63] Primary pre-clinical studies have provided insights into the effect of alcohol on NK cells and their contribution to the development of ALD. As observed in circulating NK cells in humans, alcohol has an immunosuppressive effect on murine hepatic NK cells, with a decrease in their number and cytotoxic functions after chronic alcohol consumption.[64] Furthermore, chronic plus single-binge ethanol consumption suppressed NK cell activity, which is partly due to IL-10 secreted by NKT cells, leading to the aggravation of liver steatosis.[60] In addition, the removal of HSCs by hepatic NK cells is impaired in ethanol-fed mice which is mainly related to a reduction in the expression levels of NKG2D and IFN-γ. This decrease in NK cell activity has been associated with aggravation of liver fibrosis.[65] Alcohol consumption also compromises the development and maturation of NK cells due to IL-15 deficiency.[66,67] This reduction in liver IL-15 levels upon ethanol feeding has been related to a decrease in IL-15-producing cells (IL15^{+}CD11c^{hi} cells), and exogenous IL-15/IL-15 Rα supplementation is sufficient to normalise hepatic NK cell numbers.[68] However, all these studies defined NK cells as CD3^{-}NK1.1^{+} and did not distinguish NK cells from ILC1. The study of Zhang *et al.* addressed this point and demonstrated that 3 months of chronic

alcohol consumption significantly decreased Eomes-expressing NK cells without affecting ILC1.[66] Furthermore, the relative contributions of murine hepatic NK cells and ILC during alcoholic steatohepatitis have recently been investigated.[69] Chronic (10-days chronic) plus single-binge ethanol feeding in mice induced apoptosis of NK cells resulting in ILC1 dominance and a negative outcome. Restoration of NK cells by cell transfer protected mice from alcohol-induced steatohepatitis, revealing the protective role of NK cells. In contrast, hepatic ILC1 promoted the development and aggravation of steatohepatitis through the secretion of IL-17.[69] In parallel, NK-cell-derived IFN-γ, which is significantly downregulated after chronic alcohol consumption, inhibits IL-17 A production. This study highlighted the protective role of NK cells in ALD, whereas ILC1 appeared to be deleterious. It now remains to be determined whether this phenomenon also occurs in humans and thereby whether targeting the balance between NK cells and ILC1 holds therapeutic potential.

ILC2 in MASLD

Liver ILC2 represent less than 5% of all ILCs and have been little studied in the context of liver diseases. However, their role in white adipose tissue (WAT) dysfunction has been well described during obesity, which could influence the progression of associated liver diseases. In response to local IL-33, WAT ILC2 produce enkephalins and type 2 cytokines and are involved in the browning of adipose tissue via IL-4Rα signalling. Further, the activation of ILC2 by IL-33 is sufficient to promote the growth of functional beige fat.[70,71] Adipose tissue browning by increasing caloric expenditure could limit the development of obesity and improve insulin sensitivity, dyslipidaemia and MASLD.[72,73] It has been reported that elevated brown adipose tissue (BAT) activity is associated with improvement of metabolic disorders, and patients with MASLD displayed lower BAT activity, which could be due to defective ILC2. Specifically, it has been suggested that increased BAT ILC2 activity could ameliorate chronic liver diseases associated with obesity.[74,75] However, the role of hepatic ILC2 in MASLD remains to be determined.

ILC2 in ALD

Currently there are no reports concerning the potential role of ILC2 in ALD. However, as for other ILCs, it is possible that alcohol may cause a deregulation of ILC2. In a cohort of 12 patients with cirrhosis of different aetiologies, those with alcohol-related cirrhosis (n = 4) showed the highest expression of IL-33.[76] IL-33 is a potent activator of ILC2, which may facilitate the progression of fibrosis towards cirrhosis through the subsequent release of IL-13 by activated ILC2 (discussed below). Further studies are needed to evaluate the contribution of ILC2 to ALD more precisely.

ILC3 in MASLD

ILC3 are relatively rare in the liver but are present in large numbers in the intestine where they actively protect against infections. Depending on the expression of the activator receptor NKp46, ILC3 express RAR-related orphan receptor-gamma t (RORγt) and are classified into three subpopulations: NKp46^{+} ILC3 (NCR^{+} ILC3), NKp46^{-} ILC3 (NCR^{-} ILC3) and LTi cells. It has been shown that NCR^{+} ILC3 are the main source of hepatic IL-22, unlike NCR^{-} ILC3 which produce significant quantities of IL-17 A.[77,78] The role of ILC3 and IL-22 in metabolic diseases has also been addressed. It has been shown that IL-22 secreted by ILC3 plays a protective role in HFD-induced hepatic steatosis via its regulation of hepatic lipid metabolism.[79] Further, in genetically obese leptin receptor-deficient (db/db) mice and HFD-fed mice, administration of exogenous IL-22 reverses many metabolic symptoms, such as insulin resistance and hyperglycaemia.[80] Recently, a study described the protective role of ILC3 in mice with high-fat diet-induced steatohepatitis.[81] ILC3 deficient mice (RORγt$^{KI/KI}$) fed an HFD display significant fatty liver and liver fibrosis, as well as elevated hepatic and circulating palmitic acid levels, a key activator of proinflammatory macrophages. These activated macrophages are an important source of IL-23 which enhances the proliferation/activation of IL-22-producing ILC3. The secreted IL-22 in the liver then regulates lipid metabolism, decreases inflammation and has anti-apoptotic activity. Further, exogenous IL-22 administration ameliorates liver injury, inflammation, and fibrosis in diet-induced MASH mice, by correcting liver oxidative stress and attenuating inflammatory functions of hepatocyte-derived, mitochondrial DNA-enriched extracellular vesicles.[82]

ILC3 in ALD

To date, no study has directly described the role of hepatic ILC3 in ALD in humans and mice. However, it has been shown in a mouse model that gut ILC3 secrete decreased levels of IL-22 after chronic plus binge ethanol feeding. This reduction in IL-22 secretion was reported to be the result of ethanol-induced intestinal dysbiosis and decreased levels of indole-3-acetic acid, a microbiota-derived aryl hydrocarbon receptor ligand, which regulates IL-22 expression. This deregulation leads to reduced expression of the antimicrobial peptide REG3 (regenerating islet-derived protein 3 gamma), which promotes bacterial translocation to the liver and leads to the development of alcohol-related steatohepatitis. This study also showed that in mice fed with *Lactobacillus reuteri* engineered to produce IL-22, along with a chronic-binge alcohol diet, liver inflammation and injury were reduced and intestinal expression of IL-22 was upregulated.[83] Thus, even if the role of hepatic ILC3 is not clearly described in ALD, intestinal ILC3 seem to play a protective role in the development of alcohol-induced hepatic complications through the gut/liver axis.

ILCs in fibrosis

Fibrosis and its advanced stage, cirrhosis, are caused by the repeated death of a critical fraction of hepatocytes due to persistent inflammation or infection, resulting in a loss of liver tissue organisation and function. HSCs, located between hepatocytes and the endothelial cells of the sinusoids in the space of Disse, are key actors in the development of fibrosis. During chronic inflammation, HSCs are activated by cytokines and transdifferentiate into myofibroblasts, resulting in the production of huge amounts of fibrous proteins (collagen, laminin, fibronectin). The balance between the production of this extracellular matrix and its degradation influences the evolution of fibrosis.[84]

Because ILCs have only recently been identified, few studies have investigated their role in the pathogenesis of fibrosis induced by a metabolic and/or alcoholic insult and such studies have mainly used mouse models of liver damage induced by administration of hepatotoxic compounds, such as carbon tetrachloride (CCl4). NK cells are the most well-studied ILC subset

in the development of fibrosis. Several studies using mouse models of diet and/or chemical induced-liver fibrosis have reported that NK cells work against fibrosis by eliminating HSCs.[57,85–88] HSCs can be "killed" by NK cells through the interaction of activating/inhibitory NK cell receptors and ligands differentially expressed by the activated HSCs during their differentiation, for example, RAE-1 (ribonucleic acid export-1) recognised by the NKG2D receptor, or a lack of MHC I expression, which triggers NK cell activation.[89–91] Other mechanisms involve the anti-fibrotic activity of IFN-γ, which inhibits HSC proliferation and attenuates pro-fibrogenic transforming growth factor-β (TGF-β) signalling.[87] The source of IFN-γ might be hepatic NK cells but could also be ILC1, making both cell types important in the resolution or prevention of fibrosis in chronic liver diseases. Using mouse models of MASLD-induced hepatic fibrosis, it has been shown that the regulation of fibrotic MASH depends on the interaction between liver NK cells and macrophages. The depletion of NK cells and/or ILC1 favours the polarization of alternatively activated macrophages at the expense of the proinflammatory macrophages that lead to MASLD progression.[57,92] These data provide insight into the contributions of NK cells and ILC1 during the development of fibrotic MASH in a metabolic context.[93] Furthermore, ILC2 could also be involved in fibrosis associated with MASLD in an IL-33-dependent manner, as reported in a mouse model of diet-induced MASH.[94] The results showed an increase in the expression of IL-33 and its receptor ST2 in the livers of mice fed a methionine- and choline-deficient diet or a HFD. While IL-33 treatment attenuated hepatic steatosis, reduced serum alanine aminotransferase activity, and improved systemic insulin resistance and glucose intolerance, liver fibrosis was aggravated in an ST2-dependent manner. Although this study does not clearly indicate the involvement of ILC2, the results are in line with previous data showing the involvement of ILC2 in liver fibrosis.[95] In a mouse model of liver fibrosis induced by CCl_4 treatment, IL-33 was released in response to chronic hepatocellular stress, leading to the accumulation and activation of hepatic ILC2 in a ST2 signalling-dependent manner. In turn, activated ILC2 produce IL-13, promoting the activation of HSCs. Human studies confirmed that ILC2 activation is strongly correlated with fibrosis severity.[76] Since patients with fibrotic livers are at increased risk of developing cancer, this could suggest ILC2 plays a pathogenic role in HCC development. It has recently emerged that IL-22- and IL-17-producing ILC3 subsets are also important in liver fibrosis. The role of IL-22 in fibrosis development has mainly been studied in mouse models of CCl_4-induced fibrosis. A decade ago, it was reported that IL-22 ameliorates hepatic fibrosis by activating the STAT2 pathway, thereby inducing HSC senescence and inhibiting HSC activation.[96] However, a more recent study refutes this hypothesis and highlights the pathogenic role of ILC3 in hepatic fibrosis.[97] An increase in ILC3 ($Lin^-CD127^+ROR\gamma t^+$) was found in mouse fibrotic livers, which contained more subpopulations of ILC3 IL-17 A^+ and ILC3 IL-22^+ than ILC3 populations from control mice. Furthermore, the adoptive transfer of ILC3 into *Rag1*$^{-/-}$ ILC-depleted mice resulted in a significant increase in HSC activation. These apparently contradictory results might be explained by differences in technical approaches, such as the sorting and transfer of the very rare population of ILC3 *in vivo*, and thus further investigation is needed to ascertain the role of ILC3 in liver fibrosis. Recently, in human fibrotic liver, an "unconventional" ILC3-like cell producing the ILC2-related cytokine IL-13 was identified. The frequency of this subset was higher in fibrotic livers than in healthy livers, and IL-13^+ ILC3-like cells can modulate HSC activation by upregulating inflammatory genes/proteins such as the monocyte-chemoattractant chemokine CXCL8.[98]

Together, these data strongly support that ILC subsets play a complex but important role in the pathogenesis of fibrosis and must be considered in future studies and therapeutic strategies.

ILCs in HCC

Studies examining the role of ILCs in cancer, including HCC, have yielded contradictory results, with some studies suggesting a role in tumour progression while others suggest that ILCs confer antitumor properties.[99] However, the results are more consistent for the NK cell subset. NK cells represent a key weapon against tumours, as they have the unique ability to detect and eliminate malignant cells without any sensitization.[91,100] It has been shown that the number of $CD56^+$ NK cells infiltrating the liver in patients with HCC (aetiology not provided) positively correlated with survival and the elimination of cancer cells, confirming that NK cell activity protects against cancer.[101] In addition, decreased IFN-γ production, upregulation of the expression of NK cell inhibitory receptors (NKG2A, TIM3, CD96) and downregulation of the expression of NK cell activating receptors (NKp46, NKG2D, TIGIT, Siglec7, CD160 and NKp30) were reported in patients with HCC.[101] Furthermore, in patients with advanced HCC, NKp30-positive NK cells show reduced expression of immunostimulatory splice variants of NKp30 (NKp30a and NKp30 b) and increased expression of the inhibitory variant NKp30c.[102] These studies indicate that tumour-infiltrating NK cells are deregulated in HCC and have a reduced capacity to eliminate cancer cells. This reduction in NK cell activity could be mediated by different cells and mechanisms, for example by tumour-derived monocytes/macrophages, which boost the CD48/2B4 inhibitory axis, or by myeloid-derived suppressor cells, which reduce the NKp30 receptor activating signalling pathway.[103,104] Co-culture of NK cells with cancer-associated fibroblasts (HCC-associated fibroblasts from tumour tissues) also leads to inhibition of NK cell functions with a reduction in the expression of activating receptors and the production of granzyme B, perforin, IFN-γ and TNF-α.[105] Zhang *et al.*, uncovered the infiltration of double-negative $CD11b^-CD27^-$ NK cells into the tumour tissue of patients with HCC. This population displays an immature phenotype with impaired cytotoxic capacity and IFN-γ production. These infiltrating double-negative NK cells could explain the NK cell dysfunction observed in patients with HBV-related HCC and their tumour progression.[106]

Currently, most findings regarding the involvement of NK cells in HCC have been observed in humans while few preclinical studies are available. This may be explained by the difficulty in generating mouse models that reach the HCC stage exclusively through diet-induced MASLD. Mouse models of HCC are most often transplanted tumour models where cells from human cancer cell lines are injected subcutaneously into mice. In the study of Yu *et al.*, the Hep3B cell line, representative of the invasive and oncogenic nature of HCC, was used to investigate the role of NK cells. The NK cells infiltrating these tumours showed a non-functional phenotype associated with high expression of the transcription factor NR4A1, a regulator of antitumor immunity.[107] The results thus appear to be consistent with human data, where dysfunctional NK cells in HCC are believed to promote tumour progression.

Table 1. Pathogenic and protective roles of ILCs in metabolic, alcohol-related.

	Metabolic dysfunction-associated steatotic liver disease	Alcohol-related liver disease	Liver fibrosis	HCC
NK cells	**Pathogenic role:** Responsible for inflammatory macrophage polarization[93] JAK-STAT and NF-kB pathway activation in hepatocytes resulting in oxidative stress and hepatocyte damage[56]	**Protective role:** IFN-γ produced by NK cells inhibits IL-17 A production and consequently also liver inflammation[69]	**Protective role**: Leads to HSC apoptosis[57,85,86,89–91] IFN-γ inhibits HSC proliferation and pro-fibrogenic TGF-β signalling[87]	**Protective role:** Anti-tumoural activity[101]
ILC1	**Pathogenic or protective role:** Not yet defined	**Pathogenic role:** Drives liver inflammation by producing IL-17 A[69]	**Potential protective role:** IFN-γ inhibits HSC proliferation and pro-fibrogenic TGF-β signalling[87]	**Potential pathogenic role:** NK cell conversion into ILC1-like cells by tumour microenvironment[112,113]
ILC2	**Potential protective role:** IL-33 treatment attenuated hepatic steatosis and liver injury[94]	**Pathogenic or protective role:** Not yet defined	**Pathogenic role:** Activated ILC2 promote HSC activation through IL-13 production[76,94,95]	**Potential pathogenic role:** Could contribute to an immunosuppressive microenvironment which favours tumour development[114] **Potential protective role:** High ILC2/ILC1 ratio is associated with better survival in patients with HCC[113]
ILC3	**Potential protective role:** IL-22, produced by ILC3, decreases metabolic syndrome[79,80]	**Potential protective role:** Gut ILC3 indirectly promote the development of hepatic complications through gut/liver axis[83]	**Potential pathogenic role:** Lead to an increase in HSC activation[97]	**Potential pathogenic role:** IL-22 inhibits apoptosis and promotes tumour growth via STAT3[117,118]

HCC, hepatocellular carcinoma; ILC(s), innate lymphoid cell(s); ILC1-3, type 1-3 ILC(s); NK, natural killer.

The contribution of ILC1 to the development of HCC is yet to be well documented. The tumour microenvironment could play an essential role in the orientation of the ILC1 phenotype, which may lead these cells to a variety of different fates.[40,108] For example, in a mouse mammary tumour model, the abundance of IL-15 within the microenvironment leads to an increase in a specific subset of ILC1 that lacks expression of some ILC1-related proteins (CD127, TNF-α) but expresses granzyme B.[109] The inflammatory environment in a mouse model of cutaneous squamous cell carcinoma also favours the increase of a peculiar subset of ILC1 that produce less IFN-γ but more of the proinflammatory cytokine IL-6 in pre-cancerous lesions, which could contribute to tumour progression.[40] Furthermore, studies in other cancers (fibrosarcoma, colon carcinoma, lung carcinoma) have shown that NK cells can be converted into "ILC1-like" cells, mainly mediated by TGF-β released by the tumour microenvironment.[110,111] Consistent with this, elevated concentrations of TGF-β were reported in the supernatant derived from human HCC.[112] The conversion of NK cells into "ILC1-like" cells could occur in HCC and could explain the decreased NK cell numbers discussed above. Heinrich *et al.* showed that the cytokine profile in the tumour microenvironment shapes ILCs in patients with HCC. Single-cell RNA sequencing and flow cytometry analysis of biopsies from patients with HCC also demonstrated the presence of NK-like cells in the non-tumour tissue. These cells lose their cytotoxic capacity as they evolve towards an ILC1-like profile.[113] It would be of interest to evaluate whether HCC is infiltrated by the recently described cytotoxic ILC1 subset and, if so, how the HCC environment controls the fate of these cells.[45,46] Taken together, these studies suggest that the reduced ability of NK cells to clear cancer cells or their reduced frequency through possible conversion into ILC1-like cells, as well as the increase in ILC1, could lead to HCC development.

As discussed above, recent studies have demonstrated that ILC2 play a pro-fibrogenic role in the liver in mice and in humans. Therefore, they may also contribute to HCC onset in the long term. In line with this, a significant correlation has been reported between hepatic ILC2 number and poor prognosis in patients with HCC.[114] The same study showed decreased expression levels of ILC2 activators (IL-33, IL-25) in HCC samples, and the loss of expression of KLRG1, a marker of ILC2 maturation. This suggests the existence of an HCC-derived ILC2 subpopulation that is functionally divergent from canonical ILC2. This population also produced elevated amounts of IL-13 and chemokines (such as CXCL2 and CXCL8) involved in neutrophil recruitment. The transfer of ILC2 into tumour-bearing mice confirmed the observation that these cells are associated with neutrophil recruitment, increased arginase expression and tumour burden.[114] These hepatic ILC2 identified in mouse and human HCC could contribute to an immunosuppressive microenvironment that favours tumour development. However, the study published by Heinrich *et al.* reports contradictory results, suggesting that ILC2 play a protective role in human HCC. Specifically, the authors show that a high ratio of ILC2/ILC1 within patients' tumours is associated with better survival. The high ratio corresponds to an increase in tumour-associated ILC2 that could be dependent on cytokine levels, in particular IL-33, which may promote ILC2 activation.[113] This discrepancy may be due to different origins of ILC2. ILC2 derived from ILC1 plasticity may not have the same phenotype as the ILC2 described by Xu *et al.* Furthermore, in human studies, the stages of HCC are not clearly specified and may potentially influence the type of ILC2 and their role in the tumour. The composition of the tumour microenvironment also seems to play an essential role in the behaviour of these cells and may potentially explain the divergent roles observed by the two studies.

Several studies using orthotopic models or hepatotoxic agents highlight the potential pro-tumoural role of liver ILC3 subsets in HCC development. The pro-tumorigenic role of IL-17 A, the cytokine noted above that is secreted by NCR$^-$ ILC3, T helper 17 cells and γδ T cells, is clearly described in the literature.[115] It has also been reported that liver NCR$^-$ ILC3 promote the

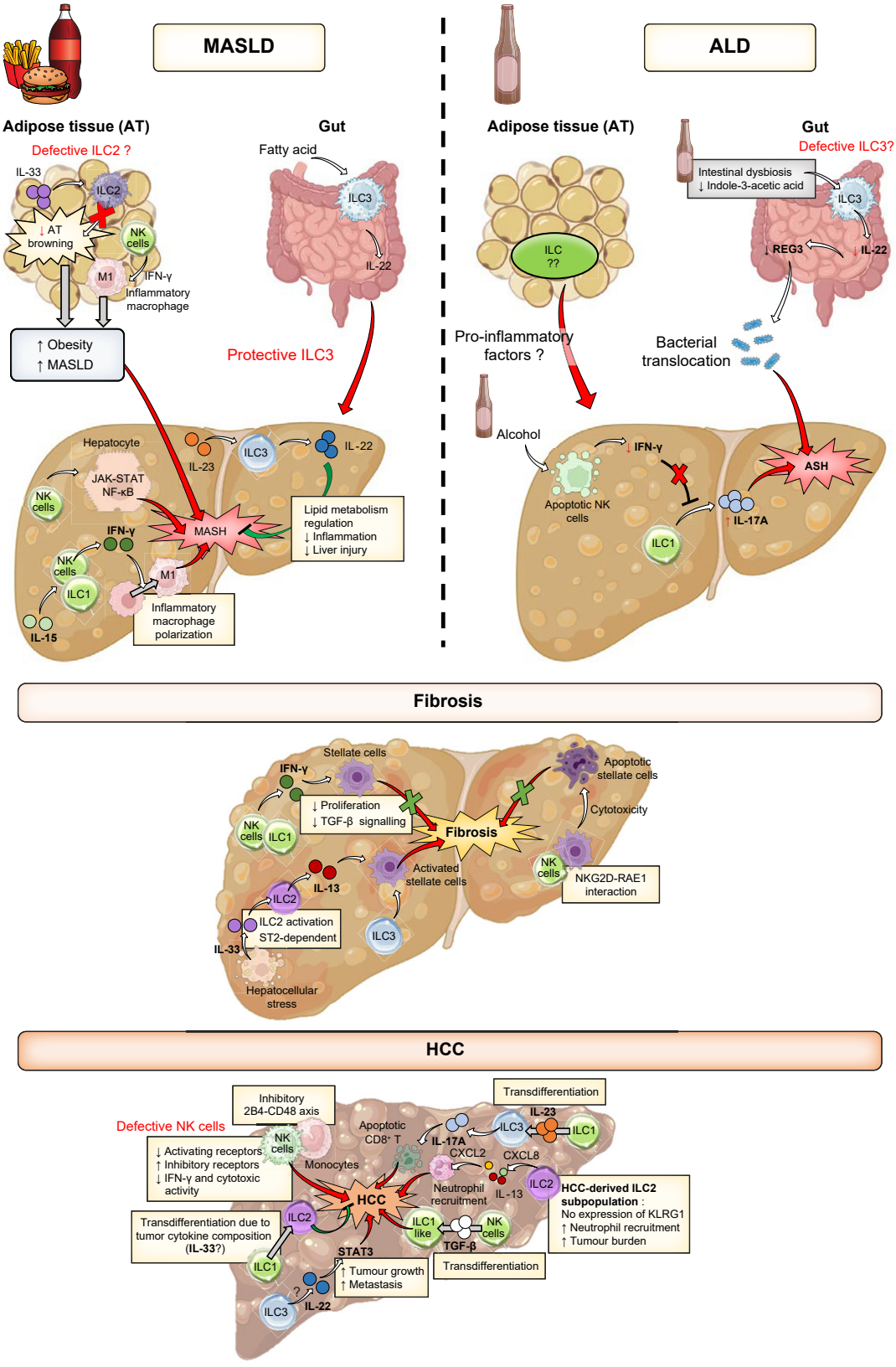

Fig. 2. Roles of ILC subsets in chronic liver diseases. ILC involvement in the development of: liver abnormalities after high-caloric (top left) or excessive/chronic alcohol consumption (top right); fibrosis (middle); and HCC (bottom). ALD, alcohol-related liver disease; ASH, alcohol-related steatohepatitis; AT, adipose tissue; HCC, hepatocellular carcinoma; ILC(s), innate lymphoid cell(s); ILC1-3, type 1-3 ILC(s); MASH, metabolic dysfunction-associated steatohepatitis; MASLD, metabolic dysfunction-associated steatotic liver disease; NK, natural killer.

development of HCC in response to IL-23 in an orthotopic mouse model (injection of hepa1.6 cell line into the liver) and a model of diethylnitrosamine-induced HCC.[116] IL-23 promotes the differentiation of ILC1 into ILC3 in the tumour microenvironment which in turn produces IL-17 A but not IL-22. IL-17 A-producing NCR^- ILC3 respond to IL-23 present in the tumour microenvironment and directly promote $CD8^+$ T apoptosis and in turn HCC progression.[116] The role of NCR^+ ILC3 in HCC progression has not yet been described. However, one study showed that patients with HCC had significant amounts of IL-22 in the tumour microenvironment. IL-22 inhibits apoptosis and promotes tumour growth and metastasis via STAT3 activation.[117] Furthermore, a liver-specific IL-22 transgenic mouse model highlighted the involvement of IL-22 in liver carcinogenesis. IL-22 acts as a local paracrine factor capable of stimulating the proliferation of liver cancer cells.[118] Thus, this study hints that IL-22-producing NCR^+ ILC3 could play a pathogenic role during the development of HCC. Taken together, these studies support a potential pro-tumoural role for liver ILC3 subsets in HCC development. Further work is needed to investigate whether this is also true for metabolic and alcohol-induced HCC.

Targeting ILCs in liver diseases

Understanding the exact role of ILCs in inflammatory diseases or cancers is still the subject of intense research and the specific targeting of these cells for therapy is still in its infancy. Chronic inflammation is a key driver of MASLD and many efforts have been made recently to target inflammatory immune cells. Strategies to limit immune cell recruitment into the liver (CCR2/CCR5, CXCR2 antagonists) and/or their activation (inhibition of TLR4, NLRP3 inflammasome) in MASH have led to limited results, and they mainly target myeloid cells.[119] NK cells/ILCs can also express these markers, at lower levels, thus it is possible that their functions will also be impacted by these treatments. In any case, since immune cells interact continuously and influence each other, NK cells/ILCs could also be affected indirectly by such approaches. The therapeutic manipulation of NK cells is much more advanced for oncological indications than for liver diseases. To date, over 200 clinical trials (clinicaltrials.gov) have been conducted to exploit their anti-tumoural properties. NK cell-based therapeutic approaches leading to promising results include infusion of activated and/or engineered NK cells, NK cell stimulation with cytokines and the use of agonistic and blocking antibodies that target NK cells.[120] Regarding the treatment of HCC, it has been shown that injecting allogenic NK cells into patients increased progression-free survival.[121,122] The use of chimeric antigen receptor (CAR)-modified NK cells also represents a promising approach in HCC treatment. CAR-NK cells have been engineered against glypican-3, c-MET oncoproteins, CD147 glycoprotein (immune cell activator) and TGF-β (inhibitor of NK cell cytotoxicity). These modified NK cells are fully functional, with increased IFN-γ production and cytotoxicity against HCC cell lines, and are able to reduce tumour growth in xenograft models.[123–126] Cytokines targeting activation and expansion of NK cells (and/or other ILC subsets) are also being tested in cancer therapies, for example IL-2, IL-15 and IL-12. The strongest anti-tumoural response is induced by infusion of IL-2 but the use of this cytokine is limited by major adverse effects, such as an increase in regulatory T cells and tissue inflammation.[127] Mutated IL-2, engineered to limit toxicity, represents an attractive approach to boost NK cell anti-tumoural properties in HCC.[128] IL-15 is known to activate NK cell proliferation and constitutes an alternative to IL-2, as it has the capacity to recover antitumor functionality in NK cells inhibited by in vitro exposure to HCC cell lines or extracted directly from HCC.[129] However, as mentioned above, the use of IL-15 could be deleterious in treating MASH as it favours the expansion of aggressive CD8 T cells and inflammatory NK cells, highlighting that the nature of the microenvironment constrains the use of this type of approach. Finally, strategies that target NK cell or ILC receptors and ICIs are also promising against chronic liver diseases. T cells were the first immune cells targeted by immunotherapy. However, NK cells and other ILC subsets have also been shown to express immune checkpoints (PD-1, TIGIT, LAG3, *etc.*) and thus studies focusing solely on T cells have underestimated the potential of targeting these cells as well.[100] Multispecific engagers, which target one or more activating NK cell receptors (NKp46, NKG2D, etc.) and/or cytokine receptors (IL-2 receptor) designed to stimulate NK cell function, represent a new class of therapeutic molecules being developed against cancer.[130] It would be of interest to evaluate the use of such molecules in chronic liver diseases and HCC.

A better characterisation of liver NK cells and ILC subsets at different stages of chronic liver diseases could lead to a better understanding of the mechanisms that regulate the local immune response and could help in the development of therapeutic strategies tailored to the liver microenvironment.

Conclusions

The involvement of ILCs in obesity- and alcohol-related liver disease is undeniable (Table 1). However, the exact nature of the protective or pathogenic roles these cells play remains difficult to elucidate. In this review we highlighted that their roles can rapidly change depending on the stage of progression of the disease due to changes in the microenvironment (Fig. 2). Currently, NK cells, due to their predominance among ILC populations in the liver, are the ILCs whose roles are best described. However, their close resemblance to ILC1 and their phenotypic heterogeneity often make their role difficult to assess. ILC2 and ILC3 are poorly represented in the liver and their involvement in this organ is not yet very well described, unlike their respective roles in adipose tissue and the gut. However, existing studies have allowed us to put forward several hypotheses that point to a pathogenic role for these cells that leads to HCC. New approaches combining single-cell RNA sequencing, flow cytometry and spatial transcriptomic technologies would help to better characterise ILC subsets in order to better understand their role within the tissue. The study of ILC metabolism could provide additional indicators of the role of these cells in different stages of obesity- and alcohol-related liver diseases, by giving insights into the functional activity of ILCs. Finally, targeting the immune system holds great promise in the fight against diseases, and in particular in cancer. Immunotherapy strategies that target ICIs and their effects on the host have mostly focused on T cells. ILCs also express inhibitory receptors and could thus be targeted by similar strategies. Recently developed cell therapy approaches that target NK cells (CAR-NK cells, multispecific NK cell engagers) to enhance their activities represent promising weapons in the fight against cancer. The development of ILC-based cell therapies that target these cells at different stages of MASLD and ALD could similarly represent a new therapeutic option that deserves further investigation.

Abbreviations

AH, alcoholic hepatitis; ALD, alcohol-related liver disease; BAT, brown adipose tissue; CAR, chimeric antigen receptor; CCl_4, carbon tetrachloride; HCC, hepatocellular carcinoma; HFD, high-fat diet; HSCs, hepatic stellate cells; ICIs, immune checkpoint inhibitors; IFN-γ, interferon-γ; IL-, interleukin-; ILC(s), innate lymphoid cell(s); ILC1-3, type 1-3 ILC(s); KO, knockout; MASLD, metabolic dysfunction-associated steatotic liver disease; NK, natural killer; NKG2D, natural killer group 2, member D; TGF-β, transforming growth factor-β; TNF-α, tumour necrosis factor-α; WAT, white adipose tissue.

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Conflict of interest

The authors of this study declare that they do not have any conflict of interest.

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

Authors' contributions

Manon Bourinet and Carmelo Luci designed and wrote the manuscript. Rodolphe Anty contributed to the clinical section of the introduction. Philippe Gual gave guidance on the outline and revised the manuscript. All authors contributed to the article and approved the submitted version.

Supplementary data

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