Regulatory T cell: a double-edged sword from metabolic-dysfunction-associated steatohepatitis to hepatocellular carcinoma

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Summary

Metabolic-dysfunction-associated steatotic liver disease (MASLD) is becoming a leading cause of end-stage liver disease globally. Metabolic-dysfunction-associated steatohepatitis (MASH) represents a progressive inflammatory manifestation of MASLD. MASH underlies a versatile and dynamic inflammatory microenvironment, accompanied by aberrant metabolism and ongoing liver regeneration, establishing itself as a significant risk factor for hepatocellular carcinoma (HCC). The mechanisms underlying the escape and survival of malignant cells within the extensive inflammatory microenvironment of MASH remain elusive. Regulatory T cells (Tregs) play a crucial role in maintaining homeostasis and preventing excessive immune responses in the liver. Paradoxically, Tregs have been implicated in inhibiting tumour-promoting inflammation and facilitating the evasion of cancer cells. Recent studies have unveiled distinct behaviours of Tregs at different stages of MASLD, suggesting a dual role in the pathogenesis. In this review, we explore the fate of Tregs from MASLD to HCC, offering recent insights into potential targets for clinical intervention.

Keywords: Regulatory T cell; Metabolic-dysfunction-associated steatotic liver disease; Metabolic-dysfunction-associated steatohepatitis; Hepatocellular carcinoma; Immunotherapy

Introduction

Due to the dramatic modification in lifestyle over the past decades, the global prevalence of metabolic-dysfunction-associated steatotic liver disease (MASLD) has surged to 25% and continues to rise. MASLD, as with the previous term non-alcoholic fatty liver disease (NAFLD), encompasses a spectrum from metabolic-dysfunction-associated steatotic liver (MASL) with simple steatosis to metabolic-dysfunction-associated steatohepatitis (MASH) and cirrhosis. In the US, MASL and MASH affect 30% and 5% of the population, respectively.[1] Mounting evidence indicates that MASH, with or without fibrosis, has rapidly become a primary risk factor for hepatocellular carcinoma (HCC).[2] The pathogenesis of MASH-associated HCC (MASH-HCC) comprises a complex landscape involving immune responses, DNA damage, oxidative stress, and autophagy.[3] Effective therapies to prevent or reverse liver injury in MASLD and its subsequent complications remain limited.

The progression of MASLD entails a dynamic hepatic inflammatory microenvironment characterised by hepatocyte senescence, cell DNA damage, and continual liver regeneration.[4] Both innate and adaptive immune responses shape this fluctuating hepatic immune microenvironment, contributing to severe steatohepatitis, cirrhosis, or HCC.[5] The immune system is also recognised as crucial in monitoring aberrant hepatocytes before giving rise to HCC,[5] a phenomenon termed immune surveillance. The mechanisms of carcinogenesis in the MASLD liver remain unascertained, potentially involving the ever-changing types of immune cells, cytokines, and molecules within the hepatic microenvironment.

Regulatory T cells (Tregs), characterised by the expression of forkhead box protein P3 (Foxp3), constitute a specialised immunosuppressive lineage crucial for regulating immune responses in both healthy and pathological conditions.[6] Tregs also exert inhibitory effects on anti-tumour immune responses, hindering protective immunosurveillance against pre-cancerous cells. Significantly, an increased number of hepatic or peripheral Tregs is associated with poor prognosis in patients with MASH-HCC.[7,8] While the roles of Tregs have been extensively studied in chronic liver diseases such as viral hepatitis and autoimmune liver disease,[9,10] their precise contributions to the pathogenesis of

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MASLD and MASH-HCC remain contentious.[11–14] Recently, we elucidated the pro-tumourigenic role of Tregs in MASH liver.[15] In this context, we present a comprehensive summary of the latest advancements in our knowledge of Tregs in MASLD and related HCC, specifically focusing on the dynamic process from the hepatic immune microenvironment to the tumour microenvironment (TME). Additionally, we discuss the interplay between Tregs and other immune cell populations, as well as the potential therapeutic strategies targeting Tregs in the context of MASLD or MASH-HCC.

MASLD: a catalyst for HCC

Primary liver cancer has become the third most common cause of cancer-related deaths globally.[16] MASH has emerged as the fastest-rising etiology of HCC worldwide.[2] The number of patients with MASH-HCC on the liver transplantation waiting list in the US between 2002 and 2016 increased 7.7-fold (from 2.1% to 16.2%).[16] Surprisingly, patients with MASLD can develop HCC in the absence of liver fibrosis and cirrhosis. However, how MASLD leads to oncogenic mutations and creates the pro-tumourigenic microenvironment that facilitates HCC initiation is not completely known.

Innate immune responses from MASLD to HCC

The hepatic immune microenvironment in MASLD progression is regulated by a complex immune network, which undergoes constant changes over time (Fig. 1). Innate immune mechanisms are widely considered key elements in promoting pathological manifestations in MASLD progression. Kupffer cells (KCs), macrophages, neutrophils, dendritic cells (DCs), natural killer (NK) cells, mast cells (MCs), mucosal-associated invariant T (MAIT) cells, and other liver-resident lymphocytes (Innate Lymphoid Cells, ILCs) have proven to be crucial in the coordinated network of the innate immune system in MASLD.[17–19] Our recent study further demonstrated that neutrophils forming neutrophil extracellular traps (NETs) could promote HCC initiation in MASH liver.[19] Moreover, hepatic natural killer T (NKT) cells can induce liver damage and fibrogenesis in MASLD, but NKT cell dysfunction promotes HCC initiation in the later stage.[20] Additionally, γδT cells, a unique subset of innate lymphocytes within the $CD3^+$ T cell population defined by expressing γδ T cell receptor (TCR) rather than the conventional αβ TCR, can be categorised into at least two distinct subsets, specifically Interferon-γ (IFN-γ)- and IL-17-producing γδ T cells.[21] In MASH, γδT cells exhibited a noteworthy increase, exacerbating liver steatosis, damage, and cirrhosis in a MASH mouse model.[21] It is also worth noting that innate immune function can be exerted by hepatocytes and liver sinusoidal endothelial cells (LSECs), both of which modulate the progression and complications of MASLD.[22,23] Additionally, oxidative stress, a prevalent characteristic of MASLD, has the capacity to generate various byproducts, including reactive oxygen species, which have the potential to amplify innate immune responses.[3]

Adaptive immune responses from MASLD to HCC

Recently, adaptive immunity has also been recognised as contributing to liver inflammation in MASLD (Fig. 1). B cells play a dual role in MASLD by secreting pathogenic antibodies and the protective cytokine IL-10.[24] The recruitment of activated cytotoxic $CD8^+$ T cells in the liver of both mouse models and patients with MASH has been observed, promoting insulin resistance, immune-mediated liver damage, and fibrosis.[23,25] However, $CD8^+$ T-cell-derived perforin participates in the mechanism regulating liver inflammation, thus conferring a protective role in MASH.[26] It has been reported the suppression of anti-tumour cytotoxic $CD8^+$ T cells by liver-resident IgA^+ regulatory B cells contributes to carcinogenesis in MASH liver.[27] In the later stage, $CD8^+$ T cells kill malignant cells, and improving anti-tumour $CD8^+$ T cell responses rescues mice with MASH-HCC to immune checkpoint inhibitor therapy.[28]

Several studies have also supported an increase in Th2 cells or serum levels of Th2 cytokine in patients with MASLD.[29] Type 17 T helper (Th17) cells, a pro-inflammatory subtype of $CD4^+$ T cells mainly secreting interleukin 17A (IL-17A), have shown a pronounced accumulation in the liver of patients with MASH. IL-17A blockers may prevent insulin resistance, MASH, and HCC in high-risk patients.[30] It is noteworthy that oxidative phospholipids and reactive aldehydes produced during lipid peroxidation form antigenic adducts termed oxidative stress-driven epitopes (OSEs). These OSEs may serve as antigenic stimuli, eliciting adaptive immune responses mediated by B and T-lymphocytes in MASLD.[31] Our recent investigation unveiled that Tregs contribute to the progression of MASH-HCC,[15] and oxidative stress has the potential to modulate the suppressor activity of Tregs in MASLD.[32–34] $CD4^+$ T cells, especially the interferon-γ (IFN-γ)-producing T helper (Th1) cells, can serve their immune surveillance role by cooperating with macrophages to kill pre-malignant senescent hepatocytes.[35] However, selective loss of $CD4^+$ T cells has been reported to impair immune surveillance of pre-malignant hepatocytes and promote hepatocarcinogenesis in different MASH-HCC mouse models.[35,36]

Treg: the regulator of immune tolerance in the liver

In general, $Foxp3^+$ Tregs are primarily derived from the thymus (tTreg), and can also be generated extrathymically from $Foxp3^-$ T-conventional cells (Tconv) at peripheral sites (pTreg).[37] Broadly, Tregs are classified into

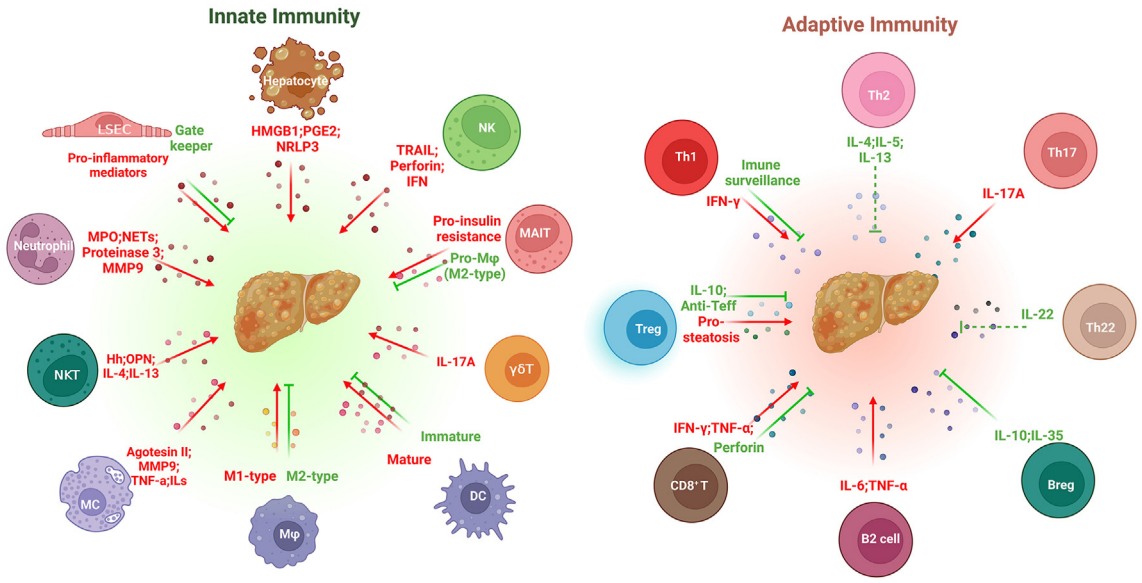

Fig. 1: **Innate and adaptive immune responses in MASLD**. Adaptive and innate immune cells collaborate to establish the hepatic microenvironment of MASLD before the initiation of HCC. In innate immune responses, NK cells, γδT cells, MCs, NKT cells, and neutrophils promote MASLD progression. MAIT cells, DC cells, macrophages, and LSECs exhibit dual roles in MASLD progression. Innate immune functions in MASLD are exerted by hepatocytes and LSECs. In the adaptive immune responses, effector T cells such as Th17 cells, Th1 cells, and cytotoxic $CD8^+$ T cells enhance immune-mediated liver injury, while Th2 cells, Th22 cells, and perforin produced by $CD8^+$ T cells impede MASLD progression. Th1 cells monitor abnormal hepatocytes, and Tregs prevent this surveillance while promoting steatosis. B cells play dual roles in MASLD. MASLD, Metabolic-dysfunction-associated steatotic liver disease; LSEC, Liver sinusoidal endothelial cell; NKT, Natural killer T cell; MC, Mast cell; Mφ, Macrophage; DC, Dendritic cell; MAIT, Mucosal-associated invariant T cell; NK, Natural killer cell; MPO, Myeloperoxidase; NET, Neutrophil extracellular trap; MMP9, metalloproteases 9; Hh; Hedgehog; OPN, Osteopontin; IL, Interleukin; TRAIL, Tumour necrosis factor-related apoptosis-inducing ligand; IFN, Interferon; TNF, Tumour necrosis factor; HMGB1, High mobility group protein 1; PEG2, Prostaglandin E2; NRLP3, NLR family pyrin domain containing 3; Teff, Effector T cell; Th, T helper cell; Treg, Regulatory T cell; Breg, Regulatory B cell.

two subsets: $CD44^{lo}CD62L^{+}$ central Tregs (cTregs) and $CD44^{hi}CD62L^{lo/-}$ effector Tregs (eTregs). cTregs maintain a quiescent state during circulation within secondary lymphoid tissues, while eTregs, characterised by high proliferative activity, represent the dominant Treg population in non-lymphoid tissues, demonstrating a relative tissue-resident phenotype.[37] The suppressive function of Tregs usually works through classical mechanisms, encompassing: (1) Competitive binding of IL-2 to other effector T (Teff) cells via the abundantly expressed IL-2 receptor, CD25.[6] (2) Direct binding of cytotoxic T-lymphocyte-associated protein 4 (CTLA4) on Tregs to CD80 and CD86 on antigen-presenting cells, results in the suppression of antigen-presenting cell (APC) function and preventing their activation of Teff cells.[38] (3) Secretion of immunosuppressive cytokines, including IL-10, IL-35, and transforming growth factor-β (TGF-β), which inhibits the activity of APC and Teff cells.[6] (4) Production of granzyme B and perforin for directly killing effector T cells. (5) Release and conversion of adenosine triphosphate (ATP) to adenosine, suppressing effector T cells through metabolic control.[34] Notably, the function of Tregs is dynamic and can be influenced by various internal and external factors.

The liver is a central, frontline immune tissue with an anti-inflammatory or immunotolerant default immune status. Tregs have been reported to participate in the pathogenesis of various liver diseases, including infectious hepatitis, sterile hepatitis, and liver cancer. Specifically, the protective roles of Tregs were confirmed in immune-mediated liver injuries, such as viral hepatitis and autoimmune liver diseases.[9,10] Adoptive transfer of Tregs has been considered a potential clinical therapy to control extensive inflammation in certain conditions.[10] Beyond their role in inflammation control, eTregs have been recognised for their role in tissue regeneration and repair across diverse non-lymphoid tissues, with a primary reliance on IL-33 and amphiregulin. Depletion of Tregs during postnatal week 2 in mice resulted in an upsurge in pro-inflammatory cytokines, and notably, liver tissue injury was more prominent compared with other non-lymphoid tissues.[39] In line with observations in other tissues, hepatic Tregs in mice with liver fibrosis exhibited an elevated expression of ST2, indicative of an augmented release of IL-33 following hepatocyte injury.[40] Furthermore, $ROR\gamma^{+}$ Tregs improve IL-17A-driven inflammation and liver regeneration in

MASH-fibrosis.[41] Different from other hepatitis, MASH stands out as a distinctive sterile hepatitis characterised by steatosis, hepatocyte damage, excessive lymphocyte infiltration, and a predisposition towards HCC. In our recent investigation, a notable discovery emerged as Tregs exhibited a selective increase in both murine models and patients with MASLD.[15] This elevation of hepatic Tregs prompts an intriguing prospect that these Tregs may impede immunosurveillance, potentially fostering carcinogenesis in MASH. Paradoxically, the adoptive transfer of Tregs to MASH mice aggravated liver steatosis and liver injury, indicating an additional role for Tregs beyond tissue regeneration. Further investigations are warranted to elucidate the precise contributions of hepatic Tregs in the context of MASLD and related HCC.

Treg: a double-edged sword in MASLD

Quantity of Tregs in MASLD

Studies have presented conflicting data of Tregs in the pathogenesis of MASLD over the past decade. Tregs with markedly elevated transcript levels of molecules involved in migration, such as chemokine receptors (CCRs) and chemokine (C-X-C motif) ligands (CXCLs), have been noted as highly enriched in the abdominal fat of normal mice.[42] However, these distinctive Tregs were specifically diminished in obesity models, potentially allowing fat inflammation to escape from immune modulation.[42] Another study reported that in the fatty liver, increased oxidative stress led to the apoptosis of Tregs, resulting in a reduction of Tregs in the livers of mice fed a high-fat diet (HFD).[33] In contrast, our findings revealed a significant increase in the number of hepatic Tregs of mice fed with a Western diet (WD).[15] Another group additionally reported that the frequency of ST2-positive Tregs and total Tregs significantly increased in the livers of HFD-fed mice.[43] Consistently, an increased number of Tregs were observed in the liver tissue of 31 patients with MASH.[44] A study based on liver specimens from patients with MASLD elucidated the spatial distribution of Tregs in both the portal/periportal and intralobular areas, revealing distinct patterns in children and adults. Severe inflammation was reported to be associated with more intralobular Tregs in children, and lower portal/periportal Tregs and higher intralobular Th17 cells in adults,[45] denoting a Treg/Th17 balance in the pathogenesis. Patients with significant MASH-fibrosis exhibit lower portal/periportal Tregs regardless of age. Table 1 presents an overview of the fluctuations in Treg abundance observed in MASLD, encompassing both human and murine investigations. The disparities observed may arise from variations in murine models and limitations in sample size and heterogeneity in human specimens. Nevertheless, overall, there is a general trend of increased Treg numbers as MASLD progresses.

Tregs prevent liver inflammation in MASLD

Although different numerical situations of Tregs have been observed in MASLD, the protective role of Tregs in immune-mediated liver injury in MASLD has been recognised.[60] In the Methionine-choline deficient diet (MCD)-MASH model, depletion of Tregs led to more aggravated inflammation.[12] The double deficiency of B7.1/B7.2 molecules aggravated inflammation in diet-induced obesity in mice, which is associated with a reduced number of Tregs.[56] Consistently, they also found that inhibition of B7 co-stimulation with Tregs effectively alleviated MASH, providing evidence for the protective role of Tregs in MASLD. The unique visceral adipose tissue-resident Tregs are regulated by peroxisome proliferator-activated receptor (PPAR)-γ,[42] and can be upregulated by treating PPAR-γ agonist to attenuate MASLD in an HFD mouse model.[50] The suppressive function of Tregs on effector T cells plays a critical role in MASH,[47,48,52,54,55] and the equilibrium between Tregs and Th17 cells has long been regarded as an indicator to assess the degree of inflammation in the MASH liver.[46,53] Evidence has substantiated the role of Tregs in mitigating insulin resistance in mice and patients with MASLD,[49–51,57] thereby constraining the subsequent metabolic inflammation. In line with previous reports highlighting the anti-inflammatory properties of Tregs in viral hepatitis and autoimmune liver disorders, evidence has demonstrated the protective function of Tregs in ameliorating immune-mediated liver injury in MASLD (Table 1). However, the role of Tregs in MASH is multifaceted due to the intricate microenvironment associated with MASH, characterised by steatosis, hepatocyte damage, and liver inflammation.

Tregs promote steatosis in MASLD

We and others recently found that Tregs may aggravate hepatic steatosis in mice,[11,13,15] but the mechanisms behind this process remain to be elucidated. In a MASH-HCC mouse model (stelic animal model, STAM[19]) and a MASH mouse model (WD), we found that depletion of Tregs alleviated steatosis and hepatocyte injury in the mouse liver at the early stage.[15] Adoptive transfer of Tregs has been considered an emerging therapy for immune-mediated liver injury, with a phase I clinical trial being initiated in autoimmune hepatitis.[61] However, a recent study reported that adoptive Treg transfer unexpectedly increased steatosis and liver injury, as indicated by increased alanine aminotransferase level.[13] Another group has also provided similar results by confirming that the adoptive transfer of Tregs exacerbates hepatic steatosis in mice fed a high-fat, high-fructose diet.[11] Likewise, either the adoptive Treg transfer or anti-cluster of differentiation

Disease stages	Role of Tregs		Factors regulating Tregs	Mouse/Human	Treg quantity		Type of Treg study	Potential targets/ Clinical insights	Year/ Ref.	Cells interacting
					Liver	Periphery				
Steatosis	Preventing immune-mediated liver injury	Anti-effector T cells	–	H	→	↓ (Blood)	In vivo	–	2016[29]	Hepatocytes
		Anti-Th17 cells	Ferroptosis	M (SAM)	↑	↑	In vivo	Porphyromonas gingivalis	2023[46]	Neutrophils
		Anti-Th1 cells	Gut microbiota	M (HFD)	–	↓(MLN)	In vivo	Fecal microbiota transplantation	2023[47]	γδT cells Th1 cells Th17 cells
		Anti-effector T cells	Gut microbiota	M (HFD)	↓	↓ (MLN)	In vivo	Administration of Lactobacillus	2019[48]	
		Preventing inflammation	Oxidative stress	M (HFD)	↓	→ (Spleen)	In vivo & vitro	Antioxidant	2007[33]	
	Preventing insulin resistance	Improving insulin tolerance	Adiponectin	M (HFD)	–	–	In vivo	Adiponectin-expressing Treg precursors	2021[49]	
		Anti-insulin resistance	PPAR-γ	M (HFD)	↓	↓ (Spleen)	In vivo & vitro	Pioglitazone	2017[50]	
MASH	Preventing insulin resistance	Anti-insulin resistance	TGF-β	H	–	–	In vivo	Oral AdmiOKT3 MAb	2015[51]	Hepatocytes Neutrophils
	Preventing immune-mediated liver injury	Anti-Th17 cells	Hyperlipidemia	H	–	↓	In vivo	Apolipoprotein B-100	2022[52]	Macrophages HSCs
		Anti-effector T cells	–	H (MASH vs steatosis)	↑	–	In vivo	–	2011[44]	γδT cells CTLs
		Anti-Th17 cells	–	H (Adult vs pediatric)	↓/↑[a]	–	In vivo	Age is a key point when treatment	2021[45]	Th1 cells Th17 cells
		Anti-Th17 cells	Gut microbiota	M (HFD)	–	↓ (Blood)	In vivo	A.muciniphola or B. bifidum	2023[53]	
		Anti-effector T cells	–	M (HFD)	↓	–	In vivo & vitro	Clostridium butyricum B1	2017[54]	
		Anti-effector T cells	–	M (HFD)	↓	→ (Spleen)	In vivo	PPC	2017[55]	
		Preventing inflammation	B7 costimulation	M (HFD)	↓	↓ (Blood)	In vivo	Inhibition of B7 costimulation	2014[56]	
		Anti-effector T cells	TLR7-TNF-α/IFN-γ	M (MCD)	↓	–	In vivo	Manipulating the TLR7-Treg axis	2018[12]	
	Limiting metabolic dysfunction	Preventing metabolic inflammation	Aging	M (HFD/CD) (Aged vs young)	–	↑ (Spleen)	In vivo	IL-10 production	2021[57]	
	Promoting Steatosis	Enhancing metabolic inflammation	–	M (HF-HC)	↑	↑ (Spleen)	In vivo	Tregs and immunomodulatory therapies: reverse effect	2022[13]	
		Exacerbating steatosis	–	M (HFHFD)	→	↑ (SAT)	In vivo	ADT can exacerbate MASH	2020[11]	
MASH-fibrosis	Inhibiting fibrosis	Anti-IL-17A	Gut microbiota	M (CDAHFD)	↓/↑[b]	–	In vivo	Microbiota-dependent RORγ$^+$ Tregs	2023[41]	HSCs NK cells
		Ant-Th17 cells	HIF1α	M (HFD)	–	↓ (Blood)	In vivo & vitro	Improving hypoxia condition	2018[58]	NKT cells γδT cells Th17 cell
		Immunosuppressive function	ALR protein	M (HF-HC)	↓	–	In vivo	ALR upregulation promoting Tregs	2020[59]	
MASH-HCC	Promoting carcinogenesis and MASH-HCC development	Suppressing antitumour immunity	Bacterial extract	H	–	↑ (Blood)	In vivo & vitro	Leaky gut and dysbosis of micobiota in MASH-HCC	2021[8]	HSCs Neutrophils
		Antitumour immune responses	Special TME in the MASH liver	H (MASH-HCC vs HBV/HCV-HCC)	–	↑ (Blood)	In vivo	MASH-Tregs contribute to the resistance to immunotherapy	2019[7]	Macrophages B cells DCs
		1. Exacerbate steatosis; 2. Impairing immune surveillance	Neutrophils/NETs	M (STAM/WD)	↑	–	In vivo & vitro	Depleting Tregs prevents HCC initiation in MASH	2021[15]	NK cells NKT cells γδ T cells CTLs
		Inducing CD8+ T cell exhaustion	Il-33 release from HSCs	M (HFD)	↑	–	In vivo	IL-33 enhances the activities of ST2$^+$ Tregs	2022[43]	

H, Human; M, Mouse; MASH, Metabolic-dysfunction-associated steatohepatitis; HCC, Hepatocellular carcinoma; PPAR-γ, Peroxisome proliferator-activated receptor; TLR, Toll-like receptor; ALR, Augmenter of liver regeneration; TME, Tumour microenvironment; HSC, Hepatic stellate cell; SAM, Spontaneous animal model; HFD, High-fat diet; CD, Choline-deficient; HFHFD, High-fat high-fructose diet; HF-HC, High-fat/high-carbohydrate; STAM, Stelic animal model; WD, Western diet; CDAHFD, Choline deficient, amino-acid-defined, and high-fat diet; MLN, Mesenteric lymph nodes; SAT, Subcutaneous adipose tissue; PPC, Polyene phosphatidylcholine capsules; ADT, Adoptive transfer; NK cell, Natural killer cell; NKT cell, Natural killer T cell; DC, Dendritic cell; CTL, Cytotoxic T lymphocyte. [a]Treg quantity changed depending on the liver zones, age, and disease stages. [b]An increase in total liver Tregs but a substantially reduced population of RORg$^+$ Tregs.

Table 1: **The role of Treg across various stages of MASLD.**

(CD)3 therapy has been shown to increase steatosis and the alanine aminotransferase level.[13] These pieces of evidence collectively suggest the detrimental role of Tregs in promoting hepatic steatosis, thereby aggravating hepatocyte damage and subsequent liver inflammation in MASLD. Further, the extensive hepatocyte damage contributes to lymphocyte infiltration and Treg recruitment. However, the underlying mechanisms through which Tregs exacerbate hepatocyte steatosis remain to be fully elucidated.

Tregs play dual roles in MASLD-fibrosis

To date, the majority of available evidence supports that Tregs possess antifibrotic properties, primarily attributed to their immunosuppressive effects.[58,60] Augmenter of liver regeneration (ALR) protein was significantly reduced in patients with MASH-cirrhosis.[59] Knocking out ALR contributed to liver fibrosis in mice, resulting in the reduction of Tregs. In another study, the number of Tregs was observed to change with the severity of hepatic fibrosis in MASH.[62] This evidence also suggests a protective role of Tregs in MASH-fibrosis. Nevertheless, TGF-β, which can be released by Tregs, is an important profibrotic factor and activates hepatic stellate cells (HSCs) in the presence of MASLD or MASH.[63] Additionally, in a MASH-HCC mouse model with fibrosis, IL-33 release from senescent HSCs promoted HCC development via the activation of ST2-positive Tregs.[43] Consequently, Tregs exhibit dual functionality in the progression of MASLD-associated fibrosis, as their distinct temporal dynamics throughout the disease process can lead to contrasting outcomes.

We have summarised the multifaceted roles that Tregs might assume during the pre-cancerous stages of MASLD, accompanied by the corresponding clinical implications inferred from diverse preclinical data in Table 1. In the pre-cancerous milieu of MASLD, Tregs exhibit dual effects, at times fostering disease progression and at other times impeding it. More specifically, at the initiation of the disease, hepatic Tregs may contribute to steatosis and hepatocyte injury. Following inflammatory cell infiltration in the liver at the latter stage of MASH, Tregs increase and start conferring their traditional mission, which inhibits the intense immune response. Finally, the progressively infiltrating Tregs will continue aggravating steatosis and form a suppressive immune microenvironment, which leads to the survival of abnormal pre-malignant hepatocytes and their escape from immune surveillance (Fig. 2). While the adoptive transfer of Tregs may be efficacious in other hepatic conditions such as autoimmune hepatitis, it holds the potential to exacerbate the severity of MASLD. In summary, we propose that Tregs function as initiators, promoting hepatocyte steatosis and necrosis, as well as instigating subsequent inflammatory responses, despite their potential to mitigate liver damage induced by infiltrating inflammatory cells.

Tregs in the initiation of MASH-HCC

When the MASH liver begins progressing to HCC, Tregs become more detrimental. Increased infiltrated Tregs have been well documented in the blood and of patients with MASH-HCC,[7,8] representing both a potential prognostic marker and a therapeutic target. However, the cell fate of Tregs in the process from MASH to HCC remains unclear. As inferred, Tregs may play a dual role in the different stages from MASLD to HCC. Various activated immune cells, different cytokine profiles, and other stimuli during the disease process form a fine-tuned cellular network in the liver to modulate Treg development directly or indirectly. Tregs may subsequently exert further primed physiological functions, which can also affect other immune cells (Fig. 3).

Interaction of Treg with adaptive immune cells

The suppressive function of Tregs on effector T cells has been implicated in autoimmune disorders and cancer, while their role in carcinogenesis is less well understood. Cytotoxic $CD8^+$ and $CD4^+$ T cells mainly contribute to cancer immunosurveillance in the liver, while Tregs promote tumour cell escape by inhibiting their function.[64] However, how Tregs modulate effector T cells in the pre-malignant stage in the liver of MASLD needs to be further studied. The increase of $CD4^+$ T helper cells (Th1 cells and Th17 cells) and $CD8^+$ cytotoxic T cells in the liver has been observed in mouse models and patients with MASH.[23,25] However, HCC still develops at the later stage of MASH with abundant Teff cells in the liver, indicating the impaired monitor function of the Teff cells. $ST2^+$ Tregs play a crucial role in the liver TME in promoting obesity-associated HCC development by inducing $CD8^+$ T cell exhaustion.[43] Th1 cells, the main subtype of effector $CD4^+$ T cells, have been confirmed to have the ability to recognise and clear senescence pre-malignant hepatocytes.[4,5,65] Tregs inhibit the function of these anti-malignant Th1 cells, thus impairing immunosurveillance in the MASH liver. Th17 cells have been recently shown to correlate positively with disease progress in MASH-HCC.[30] Moreover, Th17/Treg balance is described as a risk factor for HCC.[66] In general, Tregs may relieve liver inflammation but also impair premalignant or cancer immunosurveillance of T cells in the later stages of MASLD.

Interaction of Treg with innate immune cells

Given the critical role of the innate immune system in MASLD, multiple infiltrated innate immune cells in the disease process may affect the fate of Tregs in MASH-HCC development. Neutrophils aggregating in the MASH liver and promoting carcinogenesis,[19] can recruit Tregs into the hepatic microenvironment by secreting chemokine (C–C motif) ligand (CCL) 2 and CCL17.[67] Our recent study confirmed that neutrophil extracellular traps further promoted Tregs differentiation and

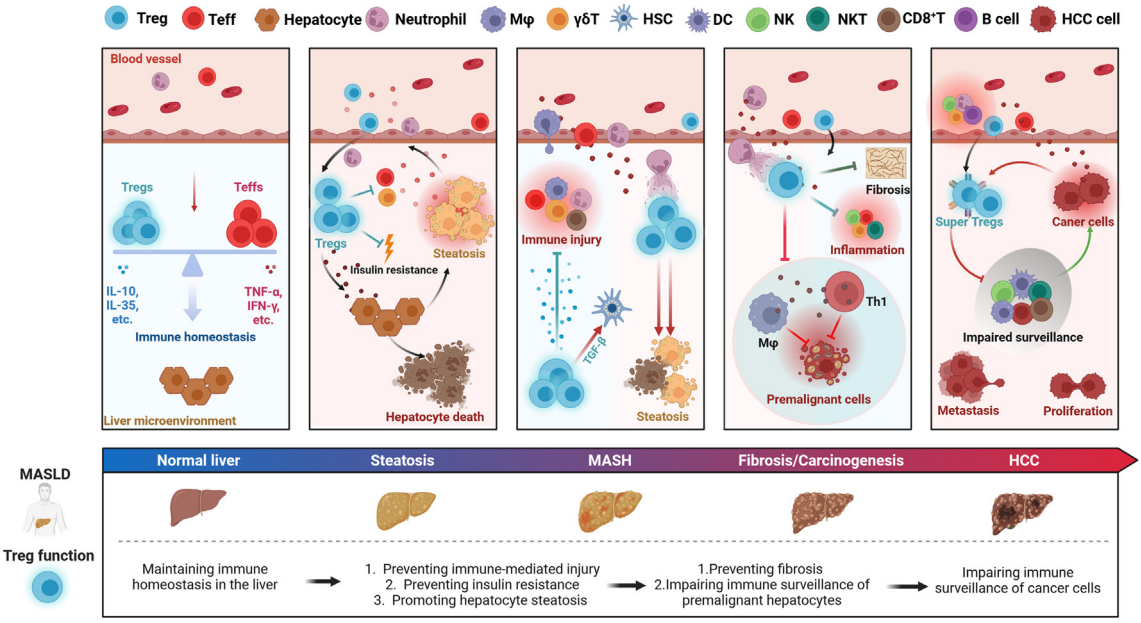

Fig. 2: **The fate of Tregs from MASLD to HCC**. Tregs perform different roles in various disease stages, from early steatosis to MASH-HCC. Tregs maintain immune tolerance in the normal liver. In the steatosis stage, Tregs are recruited into the liver, promoting steatosis and hepatocyte injury. As steatosis progresses to MASH, extensive immune cell aggregation occurs in the liver. Tregs play anti-inflammatory roles by inhibiting effector cells, yet further promote steatosis in the MASH liver. Th1 cells recognise pre-malignant hepatocytes and collaborate with macrophages for their elimination; Tregs inhibit this immune surveillance, creating an immune-suppressive microenvironment for pre-malignant cells. After HCC development, cancer cells recruit more Tregs into the TME, enhancing their suppressive function. Super Tregs in the TME impair immune surveillance by effector T cells and promote HCC development. MASLD, Metabolic-dysfunction-associated steatotic liver disease; MASH, Metabolic-dysfunction-associated steatohepatitis; HCC, Hepatocellular carcinoma; Treg, Regulatory T cell; Teff, Effector T cell; Th, T helper cell; Mφ, Macrophage; HSC, Hepatic stellate cell; DC, Dendritic cell; NK, Natural killer cell; NKT, Natural killer T cell; IL, Interleukin; TNF, Tumour necrosis factor; IFN, Interferon.

function in the MASH liver, promoting HCC initiation and development,[15] Evidence indicates that macrophages, aggregating in MASH liver, can contribute to the aggregation of Tregs by secreting IL-10 or CCL20/CCL22, which are associated with the Treg differentiation and recruitment mechanism, respectively.[68,69] Moreover, human and mouse MASH-HCC KCs displayed a decreased ratio of pro-inflammatory phenotype compared with MASH KCs.[70] Nevertheless, Treg activities in the MASH liver were reported to be related to the increased anti-inflammatory macrophages.[13] On the other hand, macrophages can kill pre-malignant senescent hepatocytes (abundant in the MASH liver[4]) after recognizing the signal from Th1 cells.[65] Tregs can suppress the function of the macrophages in a contact-independent manner in obesity,[71] which may promote abnormal cell survival in the liver. These studies indicate dynamic crosstalk between macrophages and Tregs in the progression of MASH-HCC.

NK cells limit MASH from developing fibrosis and HCC by polarizing infiltrating monocytes towards the anti-inflammatory phenotype.[72] However, Tregs bearing TGF-β on their membrane can present it directly to NK cells, leading to a reduction of their functions.[73] Hepatic NKT cells are activated early during inflammation and accumulate in MASH,[74] which can modulate Treg activity quantitatively and qualitatively. Tregs, in turn, suppress the proliferation, cytokine release, and cytotoxic activity of NKT cells by direct cell-contact mechanisms,[75] contributing to dysfunctional NKT cell-related surveillance of carcinogenesis. As the main providers of IL-17A, γδ T cells can exacerbate steatohepatitis but inhibit HCC progression.[21,76] The impaired effector function of γδ T cells was also partially mediated by Tregs in a TGF-β and IL-10-dependent manner in patients with HCC.[77]

The local tumour-related factors may favor the generation of Tregs through the inhibition of DC maturation, as evidenced by the *in vitro* induction of Tregs through HCC cell culture supernatants.[5] DC cells, the main source of CCL22, can recruit Tregs to the liver, which is substantiated through *in vitro* Treg migration assays.[78] On the contrary, macrophages and DCs isolated from HCC livers can upregulate the expression of leptin receptor (LEPR) in Tregs,[79] which can combine with leptin (an adipokine usually expressed in fat tissue) and lead to the inhibition

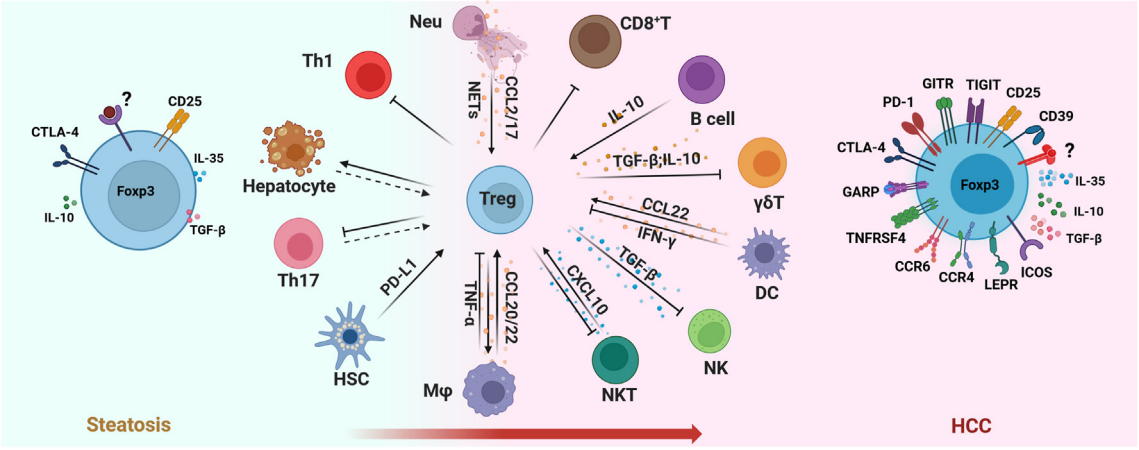

***Fig. 3:* The interaction of Tregs and other immune cells from MASLD to HCC**. In MASLD, Tregs ameliorate liver inflammation by inhibiting effector T cells and may influence malignant transformation by suppressing Th1 cells. Activated HSC expand Tregs through up-regulating PD-L1. Neutrophils enhance Treg activity through NETs and secreted chemokines, recruiting Tregs in the MASH liver. Once HCC develops, Tregs suppress anti-tumour immune responses. Multiple tumour-associated innate immune cells (macrophages, γδT cells, neutrophils, NK cells, NKT cells, and DC cells) contribute to Treg recruitment or their suppressive function in HCC by secreting chemokines and cytokines. Tregs aggregate with higher functionality in HCC. High-level expression of CC chemokine receptors contributes to the recruitment of Tregs into the tumour area in HCC. Increased inhibitory cytokines such as IL-10, IL-35, and TGF-β are secreted by intratumoural Tregs. LEPR expressed on intratumoural Tregs allows leptin, an adipokine usually expressed in fat tissue, to inhibit Treg activation and function. $ST2^+$ Tregs are active in MASLD. $CD39^+Foxp3^+$ Treg infiltration is correlated with high expression of IL-35. MASLD, Metabolic-dysfunction-associated steatotic liver disease; HCC, Hepatocellular carcinoma; Treg: Regulatory T cell; Foxp3, Forkhead box protein P3; Th, T helper cell; Neu, Neutrophil; HSC, Hepatic stellate cell; Mφ, Macrophage; NKT, Natural killer T cell; NK, Natural killer cell; DC, Dendritic cell; IL, Interleukin; Tumour necrosis factor; IFN, Interferon; TGF, Transforming growth factor; NETs, Neutrophil extracellular traps; CCL, Chemokine (C–C motif) ligand; CXCL, Chemokine (C-X-C motif) ligand; PD-L1, Programmed cell death protein 1; CTLA-4, Cytotoxic T-lymphocyte-associated protein 4; GARP, Glycoprotein A repetitions predominant; TNFRSF4, Tumour necrosis factor receptor superfamily member 4; CCR, Chemokine receptor; LEPR, Leptin receptor; ICOS, Inducible T cell co-stimulator; TIGIT, T cell immunoreceptor with immunoglobulin and ITIM domain; GITR, Glucocorticoid-induced tumour necrosis factor receptor.

of Treg activity. Activated HSCs are not formally innate immune cells, but they take on strong immune–modulatory activities in promoting MASH-HCC development,[80] which is partially through the induction of Treg expansion.[81] In summary, during the process from MASLD to HCC, Tregs expand and obtain an enhanced suppressive function to modulate other immune cells in the hepatic microenvironment (Fig. 3). Tregs interact with diverse cells to form a dynamic immune network, together providing a suppressive immune microenvironment for abnormal hepatocytes to escape the immune surveillance in the liver.

Super Treg in the TME of HCC

Consistent with other types of HCC, such as chronic viral hepatitis-related HCC, intratumoural Tregs in MASH-HCC exhibited higher frequencies and more suppressive phenotypic functions compared with those in peritumour and periphery.[7] The interaction between tumour cells and Tregs in the TME of HCC contributes to the activity of Tregs (Fig. 4). Several potential mechanisms have been explored for the expansion of Tregs in the TME of progressive HCC: (1) Increased CCLs secreted by tumour cells or immune cells in the TME recruit Tregs from periphery to the tumour area.[82] (2) Elevated TGF-β levels or some functional proteins in patients with HCC may contribute to Treg differentiation in the TME.[83] (3) The change of crucial receptors on the surface of Tregs in the TME may facilitate Treg expansion or recruitment.[38] (4) Epigenetic control in $CD4^+$T cells contributes to the high percentage of Tregs in HCC.[84]

The elevated presence of Tregs within the TME exerts direct and indirect effects on tumour cells. Tregs within the TME secrete abundant levels of TGF-β, promoting malignancy and metastasis in HCC.[83] Meanwhile, Tregs exert their suppressive function within the TME of patients with HCC by upregulating the expression of various receptors, such as CTLA-4, programmed cell death protein 1 (PD-1), tumour necrosis factor receptor superfamily member 4 (TNFRSF4), T cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT), and glucocorticoid-induced tumour necrosis factor receptor (GITR).[83,85] The upregulated expression of these functional molecules closely correlates with suppressive cytokines produced by Tregs, including IL-10 and IL-35,[83] which impair cancer immunosurveillance. Overall, as HCC progresses, Tregs can effectively promote immune evasion, allowing cancer cells to escape immunosurveillance.

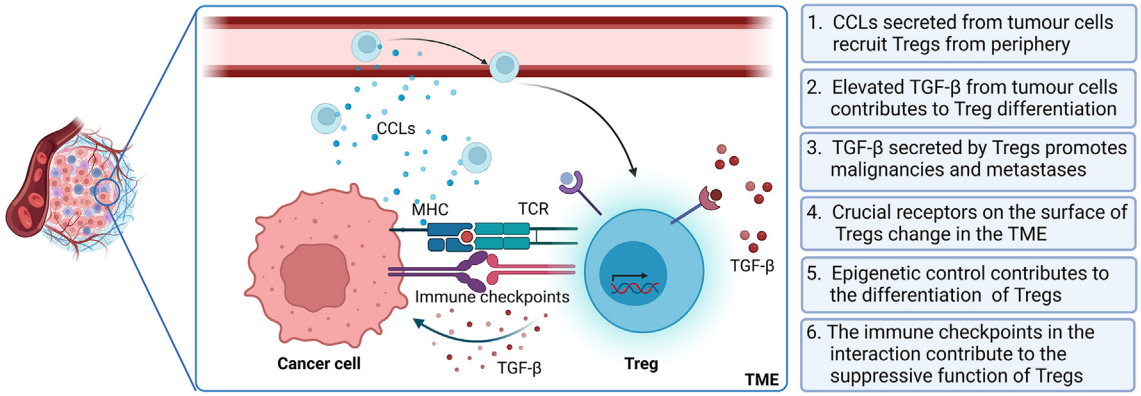

Fig. 4: **The crosstalk between cancer cells and Tregs in HCC**. In HCC, tumour cells secret CCLs to recruit Tregs to TME. TGF-β from tumour cells promotes Treg differentiation while abundant Tregs secret TGF-β, affecting tumour cells reciprocally. Crucial receptors such as CCRs contribute to Treg expansion or recruitment. Tumour cells activate immune checkpoints (PD-1 and CTLA-4) on Tregs to enhance their suppressive function. Epigenetic control promotes Treg activity. HCC, Hepatocellular carcinoma; Treg, Regulatory T cell; CCL, Chemokine (C–C motif) ligand; MHC, Major histocompatibility complex; TCR, T-cell receptor; TME, Tumour microenvironment; TGF, Transforming growth factor.

Additionally, anti-tumour immune responses may differ between HCC caused by MASH and HCC associated with hepatic viral infection. Tregs were studied in patients with HCC separated by causes.[7] The anti-tumour immune responses in the MASH-HCC group were weaker than the responses in the hepatitis B and C-related HCC, while the frequencies of eTregs strongly expressing cytotoxic CTLA-4 were high. In the HFD-induced HCC mouse model, ST2$^+$ Tregs increased and the molecules important for their function such as CTLA-4 and killer cell lectin-like receptor G1 (KLRG1) were strongly up-regulated.[43] These alterations were mediated by lipoteichoic acid and gasdermin D amino terminus, which were detected in HSCs both in murine and human MASH-HCC, suggesting the capacity of the unique MASH microenvironment to prime hepatic Tregs. However, further studies need to be conducted to elucidate the "special" Tregs in patients with HCC that develop from MASLD.

Immunotherapy targeting Treg in cancer

Recent decades have witnessed the transformative therapeutic potential of T cell-based immunotherapy in cancer. There is increasing evidence supporting that the removal of Tregs can evoke an anti-tumour immune response. However, systemic depletion of Tregs may also elicit autoimmunity. Specifically targeting differentiated eTregs rather than all foxp3$^+$ Tregs is one therapeutic strategy for arousing effective tumour immunity without autoimmune destruction. One of the breakthroughs in cancer immunotherapy is the clinical use of checkpoint blockade antibodies, which may have possible anti-Treg effects. Significant associations have been documented between the therapeutic efficacy of ipilimumab and the decreased presence of Tregs within tumour tissues in patients with cancer.[38] Moreover, the blockade of neuropilin-1 (Nrp-1) on Tregs in mouse models restores antitumour immunity and optimises tumour growth control by existing immunotherapies without sacrificing peripheral tolerance.[86] Other molecules predominantly expressed by Tregs, such as PD-1, TIGIT, GITR, and TNFRSF4[83] can also serve as potential targets for functional modulation.

Immune checkpoint blockade has shown strong anti-tumour efficacy in patients with HCC, but not all patients can benefit from it. Patients with liver metastases demonstrate significantly worse outcomes when treated with anti-PD-1 immunotherapy, which can be improved when Tregs were depleted (anti-CTLA4) or destabilised (EZH2 inhibitor).[87] This evidence suggests that the hepatic immune microenvironment may feed Tregs to become more immunosuppressive. Depleting ST2 on Tregs in MASH-HCC leads to reduced expression levels of functional Treg markers, such as CTLA-4 and KLRG1, which rescues the exhausted cytotoxic CD8$^+$ T cells in the liver TME.[43] Another recent study confirmed that MASH-HCC might be less responsive to immunotherapy,[88] due to MASH-related aberrant Treg activities.[7,8] The free fatty acids (FFAs), cholesterol deposition, and the continuously dying fatty hepatocytes may continually feed the hepatic Tregs to promote HCC survival and progression, but further studies are needed to confirm this hypothesis. Based on the studies about the enhancement of Treg activity in MASLD, we can, therefore, anticipate that Tregs may become more "powerful" in HCC developed from MASH due to the special hepatic microenvironment based on severe steatohepatitis.

Conclusions

MASLD emerges as a prominent etiological factor for HCC on a global scale. Throughout the continuum from

Search strategy and selection criteria

Data for this Review were identified by searches of PubMed between 2007 and 2023 and cross-references from relevant articles, using the search terms "Treg", "Steatohepatitis", "MASLD", "MASH", "NAFLD", "NASH", "Fibrosis", "HCC", "Liver cancer", "NAFLD-HCC", "MASLD-HCC", "NASH-HCC" and "MASH-HCC". Only articles published in English were included. With a preference for those published after 2019. The final reference list was generated after screening the abstracts and reviewing in-depth the relevance to this Review.

MASLD to HCC, Tregs manifest a dualistic function, characterised by dynamic quantitative and functional shifts over time. Enhancing Treg activity shows potential in alleviating immune-mediated liver injury in the MASLD liver; nevertheless, it may concurrently foster steatosis and expedite the malignant procession by impeding immunosurveillance in later stages. Consequently, Treg depletion during the early stages of steatosis holds potential as a therapeutic approach to hinder the progression from MASLD to HCC. Moreover, existing research predominantly concentrate on anti-Treg therapies within the TME of HCC to augment clinical responses to immune checkpoint therapy. The resistance of MASH-patients with HCC to immunotherapy might be attributed to the MASH-affected Tregs in the TME.

Outstanding questions

Specific investigations regarding Tregs in HCC derived from MASLD remain scarce. Tracking and delineating Treg fate in the hepatic microenvironment from MASLD to HCC represents an enticing avenue for future exploration direction. The comprehensive elucidation of the mechanisms through which Treg fosters hepatocyte steatosis requires further investigation. The optimal temporal parameters for Treg-based intervention within the contexts of MASLD and MASH-HCC necessitate additional experimental scrutiny. Further research is warranted to unveil the enigmatic aspects of Tregs at different stages in MASLD and related HCC, ultimately contributing to a more comprehensive clinical understanding.

Contributors

H.H, designed the study and revised the manuscript. H.W. collected materials, drew the figures, and wrote the manuscript. A.T. and L.M. reviewed the manuscript. All authors read and approved the final version of the manuscript.

Declaration of interests

The authors have no conflict of interest to declare.

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References

1 Ioannou GN. Epidemiology and risk-stratification of NAFLD-associated HCC. *J Hepatol*. 2021;75(6):1476–1484.
2 Huang DQ, Singal AG, Kanwal F, et al. Hepatocellular carcinoma surveillance - utilization, barriers and the impact of changing aetiology. *Nat Rev Gastroenterol Hepatol*. 2023;20(12):797–809.
3 Peiseler M, Schwabe R, Hampe J, Kubes P, Heikenwalder M, Tacke F. Immune mechanisms linking metabolic injury to inflammation and fibrosis in fatty liver disease - novel insights into cellular communication circuits. *J Hepatol*. 2022;77(4):1136–1160.
4 Papatheodoridi AM, Chrysavgis L, Koutsilieris M, Chatzigeorgiou A. The role of senescence in the development of nonalcoholic fatty liver disease and progression to nonalcoholic steatohepatitis. *Hepatology*. 2020;71(1):363–374.
5 Ringelhan M, Pfister D, O'Connor T, Pikarsky E, Heikenwalder M. The immunology of hepatocellular carcinoma. *Nat Immunol*. 2018;19(3):222–232.
6 Dikiy S, Rudensky AY. Principles of regulatory T cell function. *Immunity*. 2023;56(2):240–255.
7 Inada Y, Mizukoshi E, Seike T, et al. Characteristics of immune response to tumor-associated antigens and immune cell profile in patients with hepatocellular carcinoma. *Hepatology*. 2019;69(2):653–665.
8 Behary J, Amorim N, Jiang XT, et al. Gut microbiota impact on the peripheral immune response in non-alcoholic fatty liver disease related hepatocellular carcinoma. *Nat Commun*. 2021;12(1):187.
9 Tseng CW, Wu SF, Chen CY, Ho YC, He YT, Tseng KC. Characteristics of regulatory T-cell function in patients with chronic hepatitis B and C coinfection. *J Viral Hepat*. 2020;27(8):800–809.
10 Richardson N, Wootton GE, Bozward AG, Oo YH. Challenges and opportunities in achieving effective regulatory T cell therapy in autoimmune liver disease. *Semin Immunopathol*. 2022;44(4):461–474.
11 Van Herck MA, Vonghia L, Kwanten WJ, et al. Adoptive cell transfer of regulatory T cells exacerbates hepatic steatosis in high-fat high-fructose diet-fed mice. *Front Immunol*. 2020;11:1711.
12 Roh YS, Kim JW, Park S, et al. Toll-like receptor-7 signaling promotes nonalcoholic steatohepatitis by inhibiting regulatory T cells in mice. *Am J Pathol*. 2018;188(11):2574–2588.
13 Dywicki J, Buitrago-Molina LE, Noyan F, et al. The detrimental role of regulatory T cells in nonalcoholic steatohepatitis. *Hepatol Commun*. 2022;6(2):320–333.
14 Riaz F, Wei P, Pan F. Fine-tuning of regulatory T cells is indispensable for the metabolic steatosis-related hepatocellular carcinoma: a review. *Front Cell Dev Biol*. 2022;10:949603.
15 Wang H, Zhang H, Wang Y, et al. Regulatory T-cell and neutrophil extracellular trap interaction contributes to carcinogenesis in non-alcoholic steatohepatitis. *J Hepatol*. 2021;75(6):1271–1283.
16 Younossi Z, Stepanova M, Ong JP, et al. Nonalcoholic steatohepatitis is the fastest growing cause of hepatocellular carcinoma in liver transplant candidates. *Clin Gastroenterol Hepatol*. 2019;17(4):748–755.e3.
17 Lombardo J, Broadwater D, Collins R, Cebe K, Brady R, Harrison S. Hepatic mast cell concentration directly correlates to stage of fibrosis in NASH. *Hum Pathol*. 2019;86:129–135.
18 Hegde P, Weiss E, Paradis V, et al. Mucosal-associated invariant T cells are a profibrogenic immune cell population in the liver. *Nat Commun*. 2018;9(1):2146.
19 van der Windt DJ, Sud V, Zhang H, et al. Neutrophil extracellular traps promote inflammation and development of hepatocellular carcinoma in nonalcoholic steatohepatitis. *Hepatology*. 2018;68(4):1347–1360.
20 Syn WK, Agboola KM, Swiderska M, et al. NKT-associated hedgehog and osteopontin drive fibrogenesis in non-alcoholic fatty liver disease. *Gut*. 2012;61(9):1323–1329.
21 Torres-Hernandez A, Wang W, Nikiforov Y, et al. Gammadelta T cells promote steatohepatitis by orchestrating innate and adaptive immune programming. *Hepatology*. 2020;71(2):477–494.
22 Hammoutene A, Rautou PE. Role of liver sinusoidal endothelial cells in non-alcoholic fatty liver disease. *J Hepatol*. 2019;70(6):1278–1291.

23 Wolf MJ, Adili A, Piotrowitz K, et al. Metabolic activation of intrahepatic CD8+ T cells and NKT cells causes nonalcoholic steatohepatitis and liver cancer via cross-talk with hepatocytes. *Cancer Cell*. 2014;26(4):549–564.
24 Karl M, Hasselwander S, Zhou Y, et al. Dual roles of B lymphocytes in mouse models of diet-induced nonalcoholic fatty liver disease. *Hepatology*. 2022;76(4):1135–1149.
25 Grohmann M, Wiede F, Dodd GT, et al. Obesity drives STAT-1-dependent NASH and STAT-3-dependent HCC. *Cell*. 2018;175(5):1289–1306.e20.
26 Wang T, Sun G, Wang Y, et al. The immunoregulatory effects of CD8 T-cell-derived perforin on diet-induced nonalcoholic steatohepatitis. *FASEB J*. 2019;33(7):8490–8503.
27 Shalapour S, Lin XJ, Bastian IN, et al. Inflammation-induced IgA+ cells dismantle anti-liver cancer immunity. *Nature*. 2017;551(7680):340–345.
28 Wabitsch S, McCallen JD, Kamenyeva O, et al. Metformin treatment rescues CD8(+) T-cell response to immune checkpoint inhibitor therapy in mice with NAFLD. *J Hepatol*. 2022;77(3):748–760.
29 Rau M, Schilling AK, Meertens J, et al. Progression from nonalcoholic fatty liver to nonalcoholic steatohepatitis is marked by a higher frequency of Th17 cells in the liver and an increased Th17/resting regulatory T cell ratio in peripheral blood and in the liver. *J Immunol*. 2016;196(1):97–105.
30 Gomes AL, Teijeiro A, Buren S, et al. Metabolic inflammation-associated IL-17A causes non-alcoholic steatohepatitis and hepatocellular carcinoma. *Cancer Cell*. 2016;30(1):161–175.
31 Sutti S, Albano E. Adaptive immunity: an emerging player in the progression of NAFLD. *Nat Rev Gastroenterol Hepatol*. 2020;17(2):81–92.
32 Vaikunthanathan T, Landmann E, Correa DM, et al. Dysregulated anti-oxidant signalling and compromised mitochondrial integrity negatively influence regulatory T cell function and viability in liver disease. *eBioMedicine*. 2023;95:104778.
33 Ma X, Hua J, Mohamood AR, Hamad AR, Ravi R, Li Z. A high-fat diet and regulatory T cells influence susceptibility to endotoxin-induced liver injury. *Hepatology*. 2007;46(5):1519–1529.
34 Maj T, Wang W, Crespo J, et al. Oxidative stress controls regulatory T cell apoptosis and suppressor activity and PD-L1-blockade resistance in tumor. *Nat Immunol*. 2017;18(12):1332–1341.
35 Weiskirchen R, Tacke F. Immune surveillance of liver cancer in non-alcoholic fatty liver disease: excess lipids cause CD4 T-cells loss and promote hepatocellular carcinoma development. *Hepatobiliary Surg Nutr*. 2016;5(5):433–437.
36 Ma C, Kesarwala AH, Eggert T, et al. NAFLD causes selective CD4(+) T lymphocyte loss and promotes hepatocarcinogenesis. *Nature*. 2016;531(7593):253–257.
37 Koizumi SI, Ishikawa H. Transcriptional regulation of differentiation and functions of effector T regulatory cells. *Cells*. 2019;8(8):939.
38 Gan X, Shan Q, Li H, et al. An anti-CTLA-4 heavy chain-only antibody with enhanced T(reg) depletion shows excellent preclinical efficacy and safety profile. *Proc Natl Acad Sci U S A*. 2022;119(32):e2200879119.
39 Li M, Zhao W, Wang Y, et al. A wave of Foxp3(+) regulatory T cell accumulation in the neonatal liver plays unique roles in maintaining self-tolerance. *Cell Mol Immunol*. 2020;17(5):507–518.
40 Ikeno Y, Ohara D, Takeuchi Y, et al. Foxp3+ regulatory T cells inhibit CCl(4)-induced liver inflammation and fibrosis by regulating tissue cellular immunity. *Front Immunol*. 2020;11:584048.
41 Hanna BS, Wang G, Galvan-Pena S, et al. The gut microbiota promotes distal tissue regeneration via RORgamma(+) regulatory T cell emissaries. *Immunity*. 2023;56(4):829–846.e8.
42 Feuerer M, Herrero L, Cipolletta D, et al. Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. *Nat Med*. 2009;15(8):930–939.
43 Yamagishi R, Kamachi F, Nakamura M, et al. Gasdermin D-mediated release of IL-33 from senescent hepatic stellate cells promotes obesity-associated hepatocellular carcinoma. *Sci Immunol*. 2022;7(72):eabl7209.
44 Soderberg C, Marmur J, Eckes K, et al. Microvesicular fat, inter cellular adhesion molecule-1 and regulatory T-lymphocytes are of importance for the inflammatory process in livers with non-alcoholic steatohepatitis. *APMIS*. 2011;119(7):412–420.
45 Cairoli V, De Matteo E, Rios D, et al. Hepatic lymphocytes involved in the pathogenesis of pediatric and adult non-alcoholic fatty liver disease. *Sci Rep*. 2021;11(1):5129.
46 Yao C, Lan D, Li X, Wang Y, Qi S, Liu Y. Porphyromonas gingivalis is a risk factor for the development of nonalcoholic fatty liver disease via ferroptosis. *Microbes Infect*. 2023;25(1-2):105040.
47 Zhou X, Zhang X, Niu D, et al. Gut microbiota induces hepatic steatosis by modulating the T cells balance in high fructose diet mice. *Sci Rep*. 2023;13(1):6701.
48 Su L, Wu Z, Chi Y, et al. Mesenteric lymph node CD4(+) T lymphocytes migrate to liver and contribute to non-alcoholic fatty liver disease. *Cell Immunol*. 2019;337:33–41.
49 Zhang Y, Cao H, Chen J, Li Y, Xu A, Wang Y. Adiponectin-expressing Treg facilitate T lymphocyte development in thymic nurse cell complexes. *Commun Biol*. 2021;4(1):344.
50 Xu Z, Wang G, Zhu Y, et al. PPAR-gamma agonist ameliorates liver pathology accompanied by increasing regulatory B and T cells in high-fat-diet mice. *Obesity*. 2017;25(3):581–590.
51 Lalazar G, Mizrahi M, Turgeman I, et al. Oral administration of OKT3 MAb to patients with NASH, promotes regulatory T-cell induction, and alleviates insulin resistance: results of a phase IIa blinded placebo-controlled trial. *J Clin Immunol*. 2015;35(4):399–407.
52 Plochg BFJ, Englert H, Rangaswamy C, et al. Liver damage promotes pro-inflammatory T-cell responses against apolipoprotein B-100. *J Intern Med*. 2022;291(5):648–664.
53 Nian F, Wu L, Xia Q, Tian P, Ding C, Lu X. Akkermansia muciniphila and bifidobacterium bifidum prevent NAFLD by regulating FXR expression and gut microbiota. *J Clin Transl Hepatol*. 2023;11(4):763–776.
54 Zhou D, Pan Q, Liu XL, et al. Clostridium butyricum B1 alleviates high-fat diet-induced steatohepatitis in mice via enterohepatic immunoregulation. *J Gastroenterol Hepatol*. 2017;32(9):1640–1648.
55 He B, Wu L, Xie W, et al. The imbalance of Th17/Treg cells is involved in the progression of nonalcoholic fatty liver disease in mice. *BMC Immunol*. 2017;18(1):33.
56 Chatzigeorgiou A, Chung KJ, Garcia-Martin R, et al. Dual role of B7 costimulation in obesity-related nonalcoholic steatohepatitis and metabolic dysregulation. *Hepatology*. 2014;60(4):1196–1210.
57 Moreno-Fernandez ME, Sharma V, Stankiewicz TE, et al. Aging mitigates the severity of obesity-associated metabolic sequelae in a gender independent manner. *Nutr Diabetes*. 2021;11(1):15.
58 Liu J, Li W, Zhu W, et al. Chronic intermittent hypoxia promotes the development of experimental non-alcoholic steatohepatitis by modulating Treg/Th17 differentiation. *Acta Biochim Biophys Sin (Shanghai)*. 2018;50(12):1200–1210.
59 Kumar S, Verma AK, Rani R, et al. Hepatic deficiency of augmenter of liver regeneration predisposes to nonalcoholic steatohepatitis and fibrosis. *Hepatology*. 2020;72(5):1586–1604.
60 Zhou Y, Zhang H, Yao Y, Zhang X, Guan Y, Zheng F. CD4(+) T cell activation and inflammation in NASH-related fibrosis. *Front Immunol*. 2022;13:967410.
61 Raffin C, Vo LT, Bluestone JA. Treg cell-based therapies: challenges and perspectives. *Nat Rev Immunol*. 2020;20(3):158–172.
62 Drescher HK, Schippers A, Clahsen T, et al. beta7-Integrin and MAdCAM-1 play opposing roles during the development of non-alcoholic steatohepatitis. *J Hepatol*. 2017;66(6):1251–1264.
63 Gough NR, Xiang X, Mishra L. TGF-Beta signaling in liver, pancreas, and gastrointestinal diseases and cancer. *Gastroenterology*. 2021;161(2):434–452.e15.
64 Gonzalez H, Hagerling C, Werb Z. Roles of the immune system in cancer: from tumor initiation to metastatic progression. *Genes Dev*. 2018;32(19-20):1267–1284.
65 Kang TW, Yevsa T, Woller N, et al. Senescence surveillance of pre-malignant hepatocytes limits liver cancer development. *Nature*. 2011;479(7374):547–551.
66 Li K, Liu H, Guo T. Th17/Treg imbalance is an indicator of liver cirrhosis process and a risk factor for HCC occurrence in HBV patients. *Clin Res Hepatol Gastroenterol*. 2017;41(4):399–407.
67 Zhou SL, Zhou ZJ, Hu ZQ, et al. Tumor-associated neutrophils recruit macrophages and T-regulatory cells to promote progression of hepatocellular carcinoma and resistance to sorafenib. *Gastroenterology*. 2016;150(7):1646–1658.e17.
68 Wu Q, Zhou W, Yin S, et al. Blocking triggering receptor expressed on myeloid cells-1-positive tumor-associated macrophages induced by hypoxia reverses immunosuppression and anti-programmed cell death ligand 1 resistance in liver cancer. *Hepatology*. 2019;70(1):198–214.
69 Yang J, Zhang JX, Wang H, Wang GL, Hu QG, Zheng QC. Hepatocellular carcinoma and macrophage interaction induced tumor

immunosuppression via Treg requires TLR4 signaling. *World J Gastroenterol*. 2012;18(23):2938–2947.
70 Wang B, Li X, Hu W, Zhou Y, Din Y. Silencing of lncRNA SNHG20 delays the progression of nonalcoholic fatty liver disease to hepatocellular carcinoma via regulating liver Kupffer cells polarization. *IUBMB Life*. 2019;71(12):1952–1961.
71 Han JM, Patterson SJ, Speck M, Ehses JA, Levings MK. Insulin inhibits IL-10-mediated regulatory T cell function: implications for obesity. *J Immunol*. 2014;192(2):623–629.
72 Tosello-Trampont AC, Krueger P, Narayanan S, Landes SG, Leitinger N, Hahn YS. NKp46(+) natural killer cells attenuate metabolism-induced hepatic fibrosis by regulating macrophage activation in mice. *Hepatology*. 2016;63(3):799–812.
73 Bozward AG, Warricker F, Oo YH, Khakoo SI. Natural killer cells and regulatory T cells cross talk in hepatocellular carcinoma: exploring therapeutic options for the next decade. *Front Immunol*. 2021;12:643310.
74 Syn WK, Oo YH, Pereira TA, et al. Accumulation of natural killer T cells in progressive nonalcoholic fatty liver disease. *Hepatology*. 2010;51(6):1998–2007.
75 Ihara F, Sakurai D, Takami M, et al. Regulatory T cells induce CD4(-) NKT cell anergy and suppress NKT cell cytotoxic function. *Cancer Immunol Immunother*. 2019;68(12):1935–1947.
76 Zakeri N, Hall A, Swadling L, et al. Characterisation and induction of tissue-resident gamma delta T-cells to target hepatocellular carcinoma. *Nat Commun*. 2022;13(1):1372.
77 Yi Y, He HW, Wang JX, et al. The functional impairment of HCC-infiltrating gammadelta T cells, partially mediated by regulatory T cells in a TGFbeta- and IL-10-dependent manner. *J Hepatol*. 2013;58(5):977–983.
78 Wiedemann GM, Knott MM, Vetter VK, et al. Cancer cell-derived IL-1alpha induces CCL22 and the recruitment of regulatory T cells. *Oncoimmunology*. 2016;5(9):e1175794.
79 Wei R, Hu Y, Dong F, Xu X, Hu A, Gao G. Hepatoma cell-derived leptin downregulates the immunosuppressive function of regulatory T-cells to enhance the anti-tumor activity of CD8+ T-cells. *Immunol Cell Biol*. 2016;94(4):388–399.
80 Subramanian P, Hampe J, Tacke F, Chavakis T. Fibrogenic pathways in metabolic dysfunction associated fatty liver disease (MAFLD). *Int J Mol Sci*. 2022;23(13):6996.
81 Khadem F, Gao X, Mou Z, et al. Hepatic stellate cells regulate liver immunity to visceral leishmaniasis through P110delta-dependent induction and expansion of regulatory T cells in mice. *Hepatology*. 2016;63(2):620–632.
82 Wiedemann GM, Rohrle N, Makeschin MC, et al. Peritumoural CCL1 and CCL22 expressing cells in hepatocellular carcinomas shape the tumour immune infiltrate. *Pathology*. 2019;51(6):586–592.
83 Langhans B, Nischalke HD, Kramer B, et al. Role of regulatory T cells and checkpoint inhibition in hepatocellular carcinoma. *Cancer Immunol Immunother*. 2019;68(12):2055–2066.
84 Liu Q, Du F, Huang W, et al. Epigenetic control of Foxp3 in intratumoral T-cells regulates growth of hepatocellular carcinoma. *Aging*. 2019;11(8):2343–2351.
85 Bai Y, Chen D, Cheng C, et al. Immunosuppressive landscape in hepatocellular carcinoma revealed by single-cell sequencing. *Front Immunol*. 2022;13:950536.
86 Chuckran CA, Liu C, Bruno TC, Workman CJ, Vignali DA. Neuropilin-1: a checkpoint target with unique implications for cancer immunology and immunotherapy. *J Immunother Cancer*. 2020;8(2):e000967.
87 Lee JC, Mehdizadeh S, Smith J, et al. Regulatory T cell control of systemic immunity and immunotherapy response in liver metastasis. *Sci Immunol*. 2020;5(52):eaba0759.
88 Pfister D, Nunez NG, Pinyol R, et al. NASH limits anti-tumour surveillance in immunotherapy-treated HCC. *Nature*. 2021;592(7854):450–456.