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Review Article

Review Article

Active role of the immune system in metabolic dysfunction-associated steatotic liver disease

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Abstract

Non-alcoholic fatty liver disease, recently renamed metabolic dysfunction-associated steatotic liver disease (MASLD), is a complex multifactorial disease that progresses from steatohepatitis (MASH) to liver cirrhosis and liver cancer. Recent research has revealed that crosstalk between innate immune cells and hepatic parenchymal and non-parenchymal cells is involved in the pathogenesis of liver disease in MASLD/MASH. Of particular importance, novel inflammatory mechanisms, including macrophage diversity, neutrophil NETosis, B-cell biology, auto-reactive T cells, unconventional T cells, and dendritic cell–T cell interactions, are considered key drivers for disease progression. These mechanisms and factors are potential targets for the therapeutic intervention of MASLD/MASH. In this review, we focus on recent discoveries related to liver inflammation and discuss the role of innate immune cell subsets in MASLD/MASH.

Keywords: MASLD; MASH; innate immunity; liver fibrosis; inflammatory cytokine

Introduction

With the global prevalence of obesity, diabetes, and metabolic syndrome, the burden of metabolic dysfunction-associated steatotic liver disease (MASLD) has been increasing rapidly [1]. Currently, MASLD is the most common liver metabolic disorder worldwide [2]. Projections suggest that, by 2030, >100 million individuals in the USA and 15–20 million individuals in major European countries will be affected by MASLD. The current economic costs associated with MASLD and its complications are substantial [3]. MASLD progresses from simple steatosis to metabolic dysfunction-associated steatohepatitis (MASH) and may further advance to liver fibrosis and cirrhosis [4]. MASH has become the major cause of liver transplantation in developed countries [5], with a 114% increase in registered liver transplants related to MASH in males and an 80% increase in females between 2004 and 2016 [6].

MASH is characterized by hepatic lipid accumulation, hepatocellular injury, inflammation, and varying degrees of fibrosis, progressing to cirrhosis or end-stage liver disease. Liver fibrosis is an independent predictor of disease-related mortality in patients with MASH, with mortality rates ranging from 12% to 25% [7]. There is substantial evidence that multifaceted mechanisms, including lipid toxicity, oxidative stress, and inflammation, act cooperatively to promote the progression of liver disease [8]. Metabolic dysfunction complicated with hepatic steatosis is an early event in the pathogenesis of MASH that causes hepatocyte injury and insulin resistance [9]. In addition, liver inflammation driven by lipotoxicity, as well as innate and adaptive immune responses are considered essential drivers in the complex pathophysiology of MASLD [10].

In 2023, many societies involved in liver disease in various regions and countries endorsed the nomenclature change of non-alcoholic fatty liver disease (NAFLD) to MASLD [11]. In this review, except for the description of MASLD/MASH as a liver disease, the term NAFLD/nonalcoholic steatohepatitis (NASH) is used on the basis of its use in the previous studies cited. This review focuses on the inflammatory aspects of MASLD/MASH involving innate immune cells and discusses the roles of various immune cell populations leading to the transition to hepatocellular carcinoma with potential reference to the review by Peiseler *et al.* [12].

Immunomodulation in the development of MASLD

Obese patients with metabolic abnormalities possess adipose tissues exhibiting chronic low-grade inflammation, which can secrete adipokines and inflammatory cytokines such as leptin, tumor necrosis factor (TNF), and IL-6 [13]. In addition, obese adipose tissue releases free fatty acids into the circulation, promoting ectopic fat deposition in the liver. Lipid accumulation in hepatocytes leads to lipotoxicity, mitochondrial dysfunction, reactive oxygen species generation, and endoplasmic reticulum stress [14]. Inflammatory cytokines, lipotoxicity, and products of intestinal bacterial origin promote the activation of liver resident macrophages called Kupffer cells (KCs) and the mobilization of inflammatory macrophages [15]. The activation of innate

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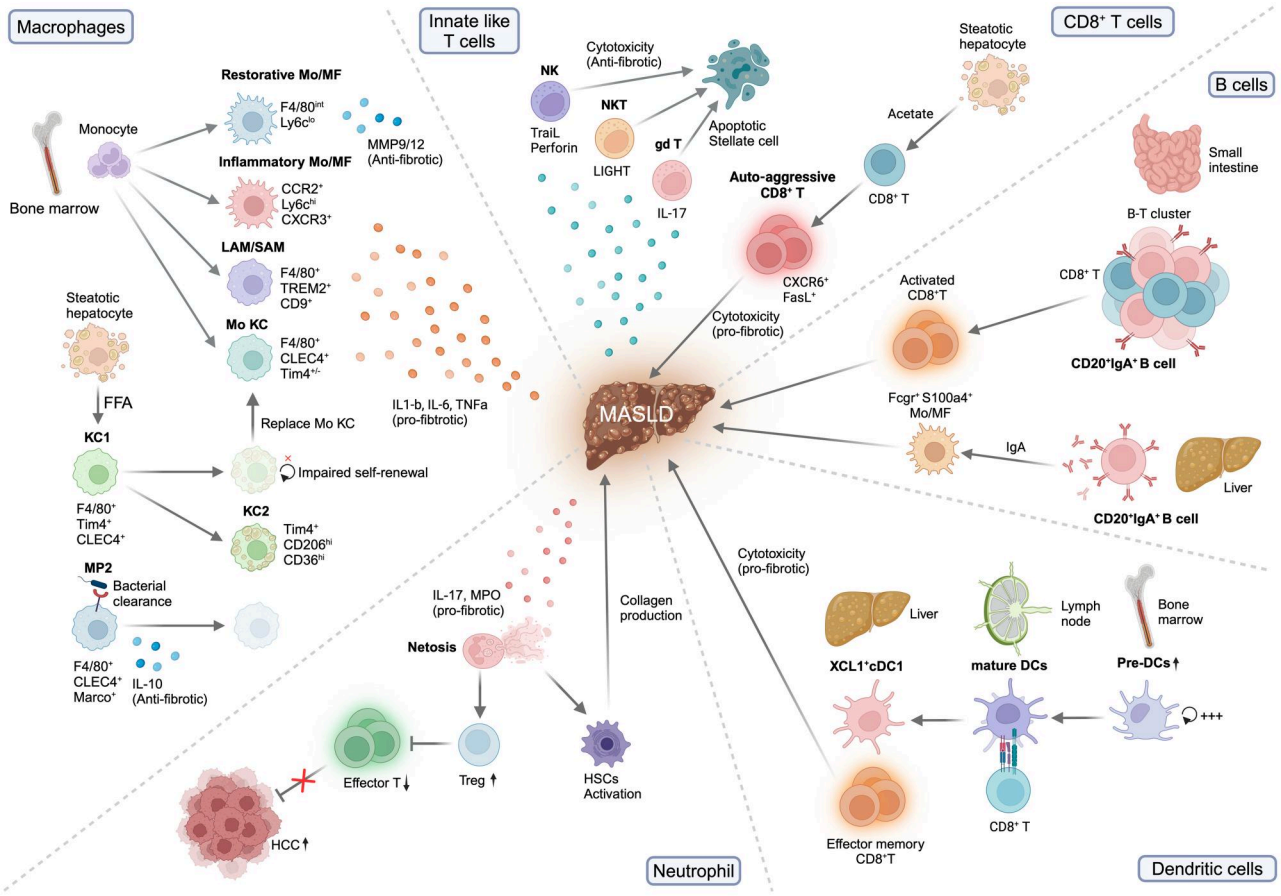

Figure 1. Recently reported mechanisms of the immune cell-mediated regulation of MASLD. This figure illustrates the involvement of immune cells in MASLD, based mainly on recent findings using mouse models. Bone-marrow-derived monocytes sense hepatocyte death signals and differentiate into restorative or inflammatory Mo/MF. Simultaneously, monocytes differentiate into LAMs or emerge as SAMs in fibrotic niches upon interaction with steatotic hepatocytes. Regarding KCs, the resident type KC1 is converted into KC2, a phenotype with high CD36 expression, due to impaired self-renewal by FFAs that are derived from steatotic hepatocytes. MoKCs with a KC-like phenotype also emerge from monocytes and replace the impaired KC1 population. While MP2 is responsible for immunosuppression by sensing bacteria via Marco in a steady state, it decreases in MASLD. Neutrophils accumulate early in MASLD and promote disease progression via cytokine production. NETosis induces HSCs activation and suppresses tumor immunity by inhibiting effector T cells through the induction of Tregs. Pre-DC precursors that increase in the bone marrow mature in the hepatic lymph nodes and differentiate into XCL1$^+$ cDC1 in the liver. Activated effector memory CD8$^+$ T cells by cDC1 are involved in the pathogenetic progression of MASLD. CD20A$^+$IgA$^+$ B cells that are induced in the small intestine form clusters with CD8$^+$ T cells and induce the activation of CD8$^+$ T cells. These B cells also induce Fcgr$^+$S100a4$^+$ Mo/MF in the liver via IgA production and are involved in the progression of MASLD. CD8$^+$ T cells are converted into CXCR6$^+$FasL$^+$Foxo^{low} auto-aggressive T cells by acetate, a metabolic product of fatty hepatic cells, and contribute to the development of MASH. While innate-like T cells play a role in promoting fibrosis through the release of cytotoxic factors in MASH, they also contribute to the apoptosis of hepatic stellate cells (anti-fibrosis). MASLD = metabolic dysfunction-associated steatotic liver disease, Mo/MF = monocyte/macrophage, KCs = Kupffer cells, LAM= lipid-associated macrophages, SAMs = scar-associated macrophages, MoKCs = monocyte-derived Kupffer cells, MP2 = Marco$^+$ Kupffer cell subset, FFAs = free fatty acids, NETosis = neutrophil extracellular traps-osis, MPO = myeloperoxidase, HSCs = hepatic stellate cells, Tregs = regulatory T cells, HCC = hepatocellular carcinoma, cDC = conventional dendritic cell, NK = natural killer, NKT = natural killer T, γδ T = gamma delta T.

immunity promotes further hepatic infiltration and accumulation of inflammatory cells, which exacerbates hepatitis and liver injury [16]. Recent advances in single-cell transcriptomics have allowed a better understanding of how the immune cell repertoire is reorganized during MASH in mouse [17–20] and human cirrhotic livers [17, 21–23]. For example, significant changes in the myeloid compartment were observed with a marked influx of monocytes and monocyte-derived cells [17]. In complex diseases including MASLD and MASH, there is the phenomenon of pleiotropy, in which multiple genetic variants are involved in the susceptibility of multiple traits [24]. Recent analyses have shown that multiple single nucleotide polymorphism are jointly associated with metabolic and/or inflammatory traits [25]. Such changes in hepatic immune cells are likely to contribute to a disordered inflammatory environment that promotes liver injury [26]. Complex crosstalk between diverse immune cell populations and hepatocytes, hematopoietic stem cells, and liver sinusoidal endothelial cells (LSECs) is critical for liver disease [17, 27]. Previous reports of immune cell populations involved in the progression of MASLD are described below (Figure 1).

Macrophages

Macrophages—innate immune cells—are an abundant resident cell population in the liver [28]. Liver macrophages are broadly divided into KCs and monocyte-derived macrophages (MoMFs). While all macrophages in the human liver can be identified on the basis of their expression of *CD68*, scRNA-seq studies have revealed that these can be further split into distinct subsets [23]. Two populations of macrophages have been identified in healthy human liver tissue, which are distinguished by their expression of *MARCO* and *TIMD4*. The development and homeostasis of KCs are involved in the etiology of MASLD, which is supported by

findings that the number of KCs/macrophages in human liver biopsy samples correlated with the severity of disease [29]. An increase in portal vein macrophages was reported in the early stages of MASLD livers, the counts of which were associated with subsequent inflammatory events in patients [30].

Mouse liver macrophages are broadly divided into KCs and MoMFs. Mouse KCs, defined as F4/80^{hi}CD11b^{int} cells, express T-cell immunoglobulin mucin (TIM) 4 and C-type lectin domain family 4 member F (CLEC4F), whereas mouse MoMFs have a CD11b^{hi}F4/80^{int} phenotype and tend to express CX3C motif chemokine receptor (CX3CR) 1 and C–C chemokine receptor type 2 (CCR2) [31]. Recent studies reported that the number of KCs was reduced in mouse MASH livers and that KCs were replaced by MoMFs of hematopoietic origin [32]. Activated KCs produce chemokines and regulate inflammatory cell recruitment [33]. Blériot *et al.* [34] found that, in addition to the major CD206^{lo}ESAM KC population, a secondary CD206^{hi}ESAM subpopulation was found in healthy subjects and obese mice. Fibrosis causes a KC to lose contact with parenchymal cells, downregulating "KC identity" and rendering it unable to eliminate bacteria, but infiltrating monocytes form multinucleated cells (syncytia) and are responsible for antimicrobial defense [35]. Such infiltrative macrophage populations are distinct from resident KCs and their abundance in the livers of patients correlates with the severity of MASH and the stage of fibrosis. Importantly, Miyamoto *et al.* [36] identified a Marco^{+} immunosuppressive macrophage subset, MP2, which is enriched in the periportal region and develops in a gut microbiota-dependent manner in the MASLD mouse. In patients, the number of MP2 cells in the MASH (more severe) group was markedly decreased compared with those in the MASLD (less severe) group.

Among the chemokines, C–C motif ligand 2 (CCL2) has an important role in the pathogenesis of MASH [37]. In a mouse model of diet-induced MASH, Ly6Chi monocytes accumulated in the liver via interactions between CCL2 and its receptor CCR2 [38]. These infiltrating monocytes that derive from bone marrow hematopoietic cells give rise to the unique phenotypically different macrophage population called MoMFs. Morinaga *et al.* [39] showed that MoMFs infiltrating the fatty liver of obese mice expressed higher levels of CCR2 and lower levels of CCL2 compared with KCs. In the livers of MASLD patients, an increase in CCR2-expressing MoMFs was also observed [40]. Krenkel *et al.* [20] reported the proliferation of MoMFs characterized by a unique inflammatory phenotype in MASH livers using a Western diet mouse model. Xiong *et al.* [17] identified a MASH-specific macrophage subpopulation that highly expressed trigger receptor 2 (TREM2), which is known to be expressed by bone marrow cells. This population has been named MASH-associated macrophages and is present in human and mouse MASH. In addition, TREM2 characterizes adipose tissue lipid-associated macrophages (LAMs) that are related to obesity [41]. The TREM2^{+}CD9^{+} subpopulation of macrophages was also found in human MASH and named scar-associated macrophages (SAMs) because of their fibrogenic phenotype [42]. Spatial analysis of human MASLD revealed that aggregation of IBA1^{+}CD16^{low}CD163^{low} macrophages occurs in the non-parenchymal cell region in correlation with the disease state and these macrophages show clear spatial proximity to ductular cells [43].

Lipogenized hepatocytes secreted cytokines, extracellular vesicles, and chemokines including CCL2 and C–X–C motif chemokine ligand (CXCL)10, which activate non-parenchymal cells including hepatic stellate cells (HSCs), LSECs, and liver macrophages [44]. Macrophage populations have an active role in lipid metabolism in the MASLD liver, particularly in fatty acid metabolism via CD36 expression [34]. In contrast, activated KCs that secrete pro-inflammatory cytokines including IL-1β and TNF are involved in hepatic lipid metabolism through the peroxisome proliferator-activated receptor (PPAR)-α pathway [45]. KCs inhibit the expression of lipid metabolism genes in hepatocytes and promote hepatocellular lipodystrophy. Indeed, the inhibition of KCs in mice that were fed a high-fat diet (HFD) led to a reduction in hepatic adiposity and inflammation [46]. Tran *et al.* [47] also showed that Ly6C^{+} monocyte-derived Kupffer cells (MoKCs) appeared in MASLD as the disease progressed and increased in response to the death of embryo-derived KCs (EmKCs). EmKCs promoted triglyceride accumulation more efficiently than MoKCs during MASH, while MoKCs exacerbated liver injury, highlighting functional differences between KCs of different origins.

Macrophages are considered the most attractive therapeutic target cells in the innate immune system of MASLD/MASH livers. Because KCs/macrophages possess dichotomous ability in liver inflammation, leading to the aggravation or alleviation of symptoms, it is crucial to identify more specific therapeutic targets of disease-associated macrophages for their practical use as regulators of inflammation.

Neutrophils

Neutrophils are important first responders of the innate immune system but, in chronic inflammatory diseases, their ability to release toxic molecules such as proteases, oxidants, cytokines, and neutrophil extracellular traps (NETs) may contribute to tissue damage [48, 49]. The hepatic expression of neutrophil chemoattractants, such as CXCL1, IL-8, and E-selectin, is higher in patients with MASH compared with those with adiposity [50] and the plasma levels of neutrophil elastase correlated with the increased severity of MASLD [51]. The depletion of neutrophils from a MASH-like mouse model that was fed a methionine/choline-deficient diet (MCD) and a high-fat, high-cholesterol diet (HFHCD) alleviated the histological findings of MASH, supporting the idea that neutrophils are involved in its pathogenesis [52]. Mice that were deficient in myeloperoxidase or neutrophil elastase had reduced liver damage in MCD and Western diet models [53, 54]. Importantly, in models of MASH–hepatocellular carcinoma (HCC) that lacked a response to the immune checkpoint inhibitor, the combination of a CXCR2 antagonist with anti-PD1 reduced the tumor burden and extended survival [55]. Furthermore, the overexpression of hepatic CXCL1 promoted the progression from fatty liver to MASH in mice that were fed an HFD through the activation of neutrophil-derived reactive oxygen species and stress kinases, which was reversed by IL-22 treatment [56].

NETosis is a recently discovered mechanism of neutrophil killing [57] and there is increasing evidence for the involvement of NETs in MASH [58]. Markers of NET formation were elevated in patients with MASLD and correlated with the severity of MASLD [59, 60]. In a recent study, NETs were present in the early stages of a mouse MASH model and the concurrent administration of DNase alleviated the severity of MASH and HCC development [61]. Of note, a positive correlation was observed between increased NETs and hepatic regulatory T-cell (Treg) levels. In that study, NETs promoted Treg activity and regulated MASH pathogenesis by affecting the gene expression profile of naive CD4^{+} T cells in mice [62]. NETs play critical role in the development of MASH hepatic fibrosis by inducing metabolic reprogramming of HSCs through the toll-like receptor 3/cyclooxygenase-2/cyclooxygenase-2 pathway [63]. In contrast, the inhibition of NET formation in mice that were fed an HFD did not suppress adipose tissue

inflammation or hepatic steatosis [64]. Furthermore, the reduction of neutrophils in a mouse model of toxic liver injury did not affect the development of fibrosis in the mice [65]. Despite the findings of these studies and the frequent presence of neutrophil infiltrates with pro- and anti-inflammatory functions in the MASH liver, their actual role is not fully understood. The single-cell analysis of neutrophils in the livers of MASLD/MASH patients is expected to address this issue.

Natural killer cells

Natural killer (NK) cells are the major cytotoxic effector cells in innate immunity. Cytotoxic activity of NK cells is important in the hepatic immune system, as it induces cell death and the subsequent clearance of target cells such as infected and tumor cells [66]. NK cells are regulated by the integrated signals from an array of activating or inhibitory receptors and cytokines [67]. Human NK cells are classified into $CD56^{dim}$ subsets with high cytotoxic activity and $CD56^{bright}$ subsets with a preference for cytokine secretion [68]. We previously reported that, in patients with MASLD, the expressions of NK-cell activation markers including NKp46 and NKp30 were significantly reduced in a peripheral blood $CD56^{dim}$ NK-cell subset. In contrast, the expressions of exhaustion or inhibitory markers including PD-1 and immunoglobulin-like transcript 2 (ILT2) on $CD56^{dim}$ NK cells were increased [69]. In HCC patients, ILT2 is a signature molecule for cancerous $CD56^{dim}$ NK cells with impaired cytolytic capacity [70]. In the livers of MASLD patients, a dramatic numerical increase in $CD56^{bright}$ NK cells and high expression of activation markers including NKG2D, CD69, and CD38 were observed [69]. Liver fibrosis in MASH patients was associated with an increase in hepatic NKG2D-positive NK cells and the elevated expression of its ligand MICA/B [71]. NKG2D expression is activated by cytokines (i.e. IL-2 and IL-15) [72] and high NKG2D expression in NK cells may be associated with increased IL-15 expression [73]. In addition, NKG2D ligands such as MICA/B in humans and MULT-1, RAE, and HS90 in mice [74] were activated in stressed or injured lipid-loaded hepatocytes [75], suggesting that NKG2D–NKG2D ligand interactions may be involved in the pathogenesis of MASH.

The important role of cytotoxic effector cells in the progression of MASH is relatively clear in animal models. NK cells including innate lymphocytes type 1 (ILC1s) have been implicated in tissue injury and inflammation during MASH progression as well as metabolic abnormalities such as insulin resistance in mice [76, 77]. Hepatic NK cells in MCD-induced MASH mice had increased expressions of NKG2D, CD107a, and IFN-γ, and decreased expression of inhibitory NKG2A [78]. Furthermore, the genetic depletion or antibody suppression of NK cells in MASH mice suppressed cytokine levels and JAK-STAT1/3 activity in the liver and significantly alleviated MCD-induced steatohepatitis. In contrast, $DX5^{+}NKp46^{+}$ NK cells are increased during MASH and have a role in polarizing macrophages toward the inflammatory (M1-like) phenotype. The depletion of $NKp46^{+}$ cells promoted the development of fibrosis and the increased expressions of pro-fibrogenic genes with skewed M2 phenotypes of hepatic macrophages in mice [79]. Hepatic NK cells attenuated the fibrosis progression of MASH via CXCL10-mediated recruitment in an MCD-induced mice model [80]. Of particular importance, NK-cell populations are phenotypically and functionally distinct according to their localization in the body. Peripheral blood NK-cell subsets in patients with MASLD tend to be exhausted, whereas hepatic NK cells are relatively activated and contribute proactively to the progression of MASLD.

Natural killer T cells

Natural killer T (NKT) cells are a population of innate immune-like T cells that are characterized by the expression of T cell receptors composed of α and β chains, similarly to conventional T cells, in addition to surface markers that are specific for NK cells [81]. They are divided into two main subsets: invariant natural killer T (iNKT) cells and natural killer T type II (NKTII) cells [82]. Both of these subpopulations recognize lipid antigens presented by CD1d [83]. Although NKT cells represent a small proportion of lymphocytes, they exhibit innate and adaptive immunological features and have a profound immunomodulatory role in a variety of diseases [84, 85]. Liver NKT-cell numbers appear to be increased in MASLD patients and tend to increase as the disease progresses [86]. Among the various populations of hepatic infiltrating cells in MASLD, only the number of $CD56^{+}$ cells was significantly increased with increased disease activity, and the expression of CD1d, the ligand for NKT cells, was also increased with increased MASLD activity scores (NAS) [87]. An increased frequency of $CXCR3^{+}IFN\text{-}\gamma^{+}T\text{-}bet^{+}$ and $IL\text{-}17A^{+}$ iNKT cells was found in peripheral blood mononuclear cells from MASH patients compared with non-alcoholic fatty liver patients or healthy controls [88]. CD206-mediated crosstalk between iNKT and KC-1 cells maintains IL-10 expression in KC-1 cells affecting hepatic immune balance [89].

In a MASH mouse model, the inhibition of NKT cells by genetic defects/therapeutic interventions suppressed the activation of HSCs, reduced neutrophil, KC, and $CD8^{+}$ T cell infiltration, and suppressed inflammatory and fibrogenic gene expressions [88]. Similarly, a mouse MASLD model using CD1d-deficient mice lacking NKT cells was protected against fibrosis whereas mice that were genetically engineered to have an increased accumulation of NKT cells developed exacerbated liver fibrosis [90]. A mice study reported a regulatory mechanism whereby NKT cells promoted hepatocyte lipidosis via the secretion of LIGHT (TNFSF14) and cooperated with $CD8^{+}$ cells to induce liver injury [91]. Macrophage infiltration and macrophage-derived inflammatory cytokines IFN-γ, TNF-α, and IL-4 were also reduced in the fibrotic livers of $Cxcr6^{-/-}$ mice, supporting the idea that hepatic NKT cells provide essential cytokine signals that perpetuate hepatitis and fibrosis [92]. Overall, the depletion or suppression of NKT cells appears to inhibit the progression of MASH and might be a novel treatment for MASH.

Gamma delta T cells

Gamma delta (γδ) T cells are the prototype "unconventional" T cells and represent a relatively small subset in the peripheral blood. γδ T cells have broad functional plasticity following the recognition of infected/transformed cells mediated by the production of cytokines (IFN-γ, TNF-α, IL-17) and cytolysis of infected or transformed target cells by perforin, granzymes, and TNF-related apoptosis-inducing ligand [93]. The interaction of γδT cells with other cells, including epithelial cells, monocytes, dendritic cells (DCs), neutrophils, and B cells, has been reported [94]. The frequency of hepatic γδ T cells was comparable between MASLD patients and healthy individuals [95]. Furthermore, the liver pathology was more severe in MASH patients with a higher frequency of $IL\text{-}17A^{+}$ γδ T cells in the blood compared with those with a lower frequency of cells [75]. In patients with MASLD, the periportal accumulation of $CCR6^{+}$ mononuclear cells and induction of CCL20 by liver parenchymal cells were observed [96].

In mice that were fed an HFHCD, hepatic γδ T cells were increased and the specific depletion of IL-17 in γδ T cells alleviated MASH, suggesting that IL-17A is a disease-promoting factor [97]. Similarly, IL-17 released from γδ T cells by NKG2D-mediated activation mobilized inflammatory cells to the liver resulted in enhanced MASH [75]. In the liver, CCR6 was mainly expressed by γδ T cells after chronic injury and CCR6-deficient mice had exacerbated liver fibrosis, inflammation, and chronic liver injury in an

MCD-diet-induced MASH model [96]. That study reported that the adoptive transfer of γδ T cells alleviated liver fibrosis, presumably by promoting the apoptosis of activated HSCs in mice [96].

DCs

DCs recognize pathogens and danger signals, and have an important role as a bridge between innate and adaptive immune responses. Hepatic DCs represent <1% of all hepatic myeloid cells [98] and are mainly localized in the portal region [99, 100]. DCs migrate from the blood to lymph nodes via the hepatic sinusoids, where they act as an important enrichment zone for hepatic DCs [101]. DCs can be divided into plasmacytoid DCs (pDCs) characterized by PDCA1$^+$ and conventional DCs (cDCs)/myeloid DCs (mDCs) [102]. Human cDCs are subtyped further into cDC1 (CD11c^{int} XCR1$^+$) and cDC2 (CD11c^{hi} XCR$^-$) and cDC1 is divided further into CD103$^+$CD11b$^-$ mDC1 and CD103$^-$CD11b$^+$ mDC2 [103]. cDC1 is regulated by the transcription factors *Batf3* and *IRF8* whereas cDC2 is regulated by *IRF4* [104]. In healthy livers, DCs predominantly have an immature phenotype. Immature DCs are characterized by a low capacity to endocytose antigens and stimulate T lymphocytes, with high production of kynurenine and IL-10 [105], and the ability to promote the differentiation of CD4$^+$ T cells into Tregs [106]. Under inflammatory conditions, DCs differentiate into mature phenotypes, mobilize monocytes, promote the production of inflammatory cytokines and chemokines, develop an important capacity to respond to Toll-like receptors (TLRs), activate NKT cells, and promote T-cell proliferation [107]. DCs are also a source of inflammatory cytokines such as TNF-α and IL-6, which activate stellate cells [108]. The role of DCs in MASLD/MASH varies between animal feeding models and situations, and conflicting data have been reported.

In a recent human study, the frequency of hepatic DC CD11c$^+$ expression in liver tissues was higher in MASLD patients than in mildly obese or non-obese patients [109]. DCs steadily accumulate in the liver in the early stages of the disease and produce significant amounts of the pro-inflammatory cytokines TNF-α, IL-6, and MCP-1 and the anti-inflammatory cytokine IL-10 [110], suggesting that hepatic DCs are involved in sustaining the inflammatory process in MASH. The analysis of livers from patients on the MASH spectrum showed that cDC1 was more abundant and activated during disease, and that the genetic depletion of cDC1 or anti-XCL1 treatment in a MASH mouse model suppressed liver pathology [111]. A recent transcriptional and immune-profiling study of patients with MASLD showed a positive correlation between cDC2 and the progression of MASLD [112]. In contrast, the proportion of cDC2 in total leukemic cells in non-alcoholic fatty liver (NAFL) and MASH patients was comparable with those of healthy individuals [111]. The increased differentiation of CX3CR1$^+$ monocyte-derived DCs in a mouse model of MASH promoted hepatitis through the upregulation of a fractalkine (CX3CL1) associated with the proliferation of monocyte-derived DCs [113].

Even in mice models, the increased differentiation of CX3CR1$^+$ monocyte-derived DCs in a mouse model of MASH promoted hepatitis through the upregulation of a fractalkine (CX3CL1) associated with the proliferation of monocyte-derived DCs [113]. In contrast, a study that examined the role of cDC1s in *Batf3*-deficient mice demonstrated that *Batf3*-deficient mice rapidly progressed to steatohepatitis and had an increased influx of chemokines and inflammatory immune cells [114]. In addition, the adoptive transfer of CD103$^+$ cDC1s to *Batf3*-deficient mice reduced inflammatory macrophage accumulation and metabolic disturbances [114]. Similarly, other studies of MASH have shown that DCs limited CD8$^+$ T-cell expansion and restricted TLR expression and cytokine production in innate immune effector cells, including KCs, neutrophils, and inflammatory monocytes [110]. Taken together, these data suggest that hepatic DCs may have a dichotomous role in the progression or resolution of MASLD.

T cells

CD8$^+$ T cells are effector cells of the adaptive immune system that are important for killing cancer cells and infected cells by an major histocompatibility complex I-restricted, antigen-specific mechanism [115]. Lymphocytic infiltrates are frequently observed in liver biopsies of patients with MASLD, often as focal lymphocyte aggregates that are composed of T cells [116]. Hepatic CD8$^+$ T lymphocytes were associated with lobular inflammation, ballooning, and the transcriptomic signature of patients with MASH [112]. Furthermore, circulating CD8$^+$ T cells were markedly activated with the increased expressions of perforin, IFN-γ, and TNF-α. A recent study that used single-cell RNA sequencing identified the expansion of CXCR6$^+$PD-1$^+$Gzmb$^+$CD8$^+$ T cells in the livers of MASH patients and mice [19]. The mechanism by which this cell population was generated involved the downregulation of the transcription factor FOXO1 by increasing IL-15 signaling in fatty livers, ultimately inducing direct hepatocyte killing via the high expression of FasL. In a mouse model, steatosis, liver injury, and inflammation induced by choline-deficient/HFD feeding were reduced in Rag1$^{-/-}$ mice that lacked T cells [91]. CD8$^+$ T cells and NKT cells cooperated to promote liver injury [91] and a deficiency of CD8$^+$ T cells or NKT cells was associated with milder fatty hepatitis [117]. The liver accumulated pathogenic CD8$^+$ T-cell subsets, which controlled hepatic insulin sensitivity and gluconeogenesis in mice with diet-induced obesity [118]. The interaction between CD8$^+$ T cells and monocyte-derived macrophages via inducible T-cell costimulator/ligand is important for maintaining TREM2$^+$-expressing cells, which contributes to the progression of non-alcoholic steatohepatitis [119].

CD4$^+$ T helper (Th) cells are broadly classified as Th1, Th2, Th17, and Tregs. Their balance is important for maintaining hepatic immune tolerance, and the dysregulation of regulatory and effector T helper cells is a hallmark of chronic liver disease [120]. Compared with healthy controls, higher frequencies of IFN-γ$^+$ and/or IL-4$^+$ cells were detected among CD4$^+$ T cells in the peripheral blood of patients with MASH and to a lesser degree in those with NAFL [121]. In a humanized mouse model that was fed an HFHCD, CD4$^+$ T cells accumulated in the liver and their depletion suppressed liver inflammation and fibrosis [122]. Intrahepatic Th17 (ihTh17) cells were increased throughout human MASLD and were more frequent in those with MASH than in those with NAFL [121]. In mice that lacked IL-17, feeding with HFD or MCD diets did not affect adiposity, but reduced hepatocellular damage and hepatitis [123, 124]. The CXCR3$^+$Th17 subset (ihTh17) exacerbated MASLD pathogenesis in a mouse model [125] and the ihTh17 cells had increased metabolic activity and production of inflammatory cytokines including IFN-γ, TNF-α, and IL-17.

Tregs are important immune regulatory cells that were reported to be decreased in MASH patients compared with healthy controls [121]. More Tregs were apoptotic and had a decreased frequency in patients with steatohepatitis, and the adoptive transfer of Tregs suppressed subsequent liver inflammation in mice [126]. However, BALB/c mice that were fed an HFD had increased intrahepatic Tregs, but the adoptive transfer of Tregs exacerbated experimental MASH [127]. Patients with MASLD/MASH or mouse models of MASH showed a marked imbalance

between ihTh17 cells and Tregs [121, 128]. It is mechanistically unclear whether such an imbalance was related to local immune dysregulation or extrahepatic factors. Interestingly, recent mice studies have identified microbial bile-acid metabolism as an important regulator of the Th17/Treg balance in the gut [129, 130].

B cells

B cells are a crucial component of the adaptive immune system and are particularly susceptible to antigens that are derived from the intestinal flora and oxidative stress [131]. There is some evidence to support a pathogenic role for B cells in MASLD, although it is more limited than for other immune cells [132]. An increase in hepatic B cells has been observed in both humans and mice with MASLD [133, 134]. Accordingly, B-cell deficiency-ameliorated MASH progression and the adoptive transfer of B cells from MASH livers recapitulates the disease in mice [133, 134]. B-cell activation during MASLD occurs through the detection of damage-associated molecular patterns or pathogen-associated molecular patterns by pattern recognition receptors such as TLR4 or through the binding of antigens that are derived from the microbiota to B-cell receptors [133]. The progression of MASLD is associated with an increase in the concentration of BAFF [135], a B-cell activation factor, and a deficiency of BAFF suppressed hepatic steatosis in a mouse model of MASLD [136]. In addition, IgA that was derived from metabolically activated B cells in the small intestine contributes to the activation and inflammation of myeloid cells in the mouse liver [137].

Development of novel therapies and their potential for therapeutic immune intervention against MASLD

Specific treatments for MASLD are still lacking, despite positive results in early-stage clinical trials [138]. The suppression of inflammatory pathways or promotion of anti-inflammatory pathways induced by innate immune cells described in this review may lead to more specific immunomodulatory strategies. Preclinical and clinical data showed that cenicriviroc—a dual CCR2/CCR5 antagonist that targets monocyte and lymphocyte recruitment in patients with MASH—was a promising drug [139]; however, it lacked efficacy when used to treat liver fibrosis in patients with MASH in the AURORA phase III randomized study [140]. ASK-1 activates p38 mitogen-activated protein kinase (p38) and c-Jun N-terminal kinase, which in turn induce tissue apoptosis, inflammation, and fibrosis in MASH [141]. This finding led to a phase III study in patients with stage 3 and stage 4 fibrosis, but no significant efficacy was achieved when ASK-1 was administered alone [141]. Galectin 3 is highly expressed by macrophages and is involved in liver fibrosis [142]. A recently published phase IIb study that evaluated the galectin-3 inhibitor belapectin did not demonstrate an improvement in portal hypertension or fibrosis in patients with MASH cirrhosis [143].

An emerging strategy is to simultaneously target metabolism and inflammation during the development of MASH by using drugs with multiple mechanisms of action, such as PPAR agonists, farnesoid X receptor (FXR) agonists, and thyroid hormone receptor-β (THR-β) agonists [144]. These nuclear receptors (NRs) not only restored metabolic disturbances, but also had multiple effects on immune cell function and improved the inflammatory environment in preclinical studies of MASLD [145]. The PPAR agonist lanifibranor, which targets all PPAR isoforms, had direct anti-inflammatory effects on liver macrophages via PPAR β/δ and direct anti-fibrotic effects on stellate cells via PPARγ in a mouse model of MASH [146]. In a phase IIb trial of patients with active MASH, the proportion of patients with at least a two-point reduction in steatosis, activity, and fibrosis scores without worsening fibrosis was significantly higher in the lanifibranol group than in the placebo group [147]. FXRs are bile-acid-activated NRs that regulate bile-acid, lipid, and glucose metabolism with a central role in metabolic homeostasis [148]. FXR signaling modulated inflammatory pathways by inhibiting the production of pro-inflammatory cytokines, activating inflammasomes, and upregulating anti-inflammatory mediators [149]. Importantly, the steroidal FXR agonist obeticholic acid has shown promise in clinical trials of patients with MASH [150]. This includes the ongoing phase III REGENERATE trial, which reported a significant improvement in fibrosis, although it did not lead to the remission of MASH [151]. Because of their potential to restore bile-acid metabolism and reduce inflammation, pharmacological FXR agonists remain an important target of future combination therapy in MASH [152].

The thyroid hormone modulates hepatic glucose and lipid metabolism [153]. THR-β agonists can reverse steatosis by many mechanisms, including the improved hepatic conversion of T4 into T3 and enhanced mitochondrial function [154]. The efficacy of resmetirom, a THR-β agonist, was reported in the ongoing phase III MAESTRO-MASH trial [155]. Resmetirom was superior to placebo on two primary end-points: MASH resolution without worsening of fibrosis and improvement (reduction) in at least one level of fibrosis without worsening of the MASLD activity score. Resmetirom (which will be marketed under the name “Rezdiffra”) was recently conditionally approved by the Food and Drug Administration as a treatment for adult patients with non-cirrhotic MASH who have moderate to severe fibrosis. Previous analyses have shown that the severity of MASH is strongly correlated with the risk of liver-related mortality and non-transplant survival [156] and this approval should be seen as a landmark event for MASH patients.

Another promising future treatment for MASLD may be the targeting of T cells. Auto-aggressive $CD8^+$ T cells that expand in the MASH liver have an exhausted profile and metabolic disturbances are associated with the development of this cell population [19]. The restoration of glycolytic pathways and mitochondrial function, as seen in T cells that are exhausted by chronic viral infection [157], may reduce auto-aggression. In addition, inhibitors of IL-17 and IL-23 signaling are expected to be effective treatments for inflammatory diseases that share the same immunopathological features as MASLD/MASH [158] and IL-17 inhibitors have been shown to be effective in improving NAS scores and FIB-4 indices in patients with MASLD/MASH [159], suggesting that inhibition of this signaling pathway may also be useful as a therapeutic agent.

A deeper understanding of the pathogenesis of the onset, propagation, and resolution of inflammation and fibrosis in MASLD highlights the complexity of potential anti-inflammatory and anti-fibrotic targets. However, despite the association of inflammation and fibrosis with MASLD-related outcomes, no “pure” anti-inflammatory or anti-fibrotic drugs for the treatment of MASH have been approved to date. This is because of the individual variations in and complexity of the processes that cause inflammation and fibrosis. Detailed insights into the complex signaling circuitry between metabolism, inflammation, and fibrosis in MASLD and MASH should ultimately enable better stage-specific and personalized treatment in the near future.

Conclusions and future perspectives

MASLD is a common liver disease worldwide that encompasses a variety of conditions ranging from basic lipidosis and metabolic

dysfunction-associated fatty liver to MASH-related fibrosis, cirrhosis, and hepatocellular carcinoma. Hepatic immunology research has led to a greater understanding of the contribution of macrophages and other innate immune cells to the progression of MASLD. These studies have established that immune abnormalities have an important role in the progression of MASLD, various immune cell populations are involved in the pathogenesis of MASLD, and organized communication between them may contribute to the formation of the hepatitis environment that is observed during the pathogenesis of MASLD. Future studies should stratify patients with specific metabolic and immune disorders more appropriately to determine which mouse models reflect the specific human MASLD subtypes. With the recent advent and application of state-of-the-art technologies, a better understanding of the dynamic accumulation and activation of immune cells is expected, which will pave the way for innovative therapeutic strategies against MASLD. The stratification of patients based on stage, risk profile, and modifying factors will allow us to design more personalized treatment options, resulting in a decrease in the global burden of MASLD.

Author contributions

T.M. and T.K. made substantial contributions to the conception and design, acquisition of data, drafting of the article, and critical revision of the article for important intellectual content. All authors have read and approved the final version of the manuscript.

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Conflicts of Interest

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