Journal of
Innate Immunity

Review Article

J Innate Immun
DOI: 10.1159/000518407

Received: March 22, 2021
Accepted: May 26, 2021
Published online: August 24, 2021

The Role of Innate Immune Cells in Nonalcoholic Fatty Liver Disease

Marina Nati[a, b, c] Kyoung-Jin Chung[a] Triantafyllos Chavakis[a, b, c]

[a]Institute for Clinical Chemistry and Laboratory Medicine, University Hospital and Faculty of Medicine Carl Gustav Carus, TU Dresden, Dresden, Germany; [b]Paul Langerhans Institute Dresden of Helmholtz Center Munich at the University Hospital and Faculty of Medicine Carl Gustav Carus of TU Dresden, Dresden, Germany; [c]German Center for Diabetes Research (DZD), Neuherberg, Germany

Keywords
Inflammation · Macrophages · Neutrophils · Non-alcoholic fatty liver disease

Abstract
Nonalcoholic fatty liver disease (NAFLD) is a very common hepatic pathology featuring steatosis and is linked to obesity and related conditions, such as the metabolic syndrome. When hepatic steatosis is accompanied by inflammation, the disorder is defined as nonalcoholic steatohepatitis (NASH), which in turn can progress toward fibrosis development that can ultimately result in cirrhosis. Cells of innate immunity, such as neutrophils or macrophages, are central regulators of NASH-related inflammation. Recent studies utilizing new experimental technologies, such as single-cell RNA sequencing, have revealed substantial heterogeneity within the macrophage populations of the liver, suggesting distinct functions of liver-resident Kupffer cells and recruited monocyte-derived macrophages with regards to regulation of liver inflammation and progression of NASH pathogenesis. Herein, we discuss recent developments concerning the function of innate immune cell subsets in NAFLD and NASH.

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Introduction: NAFLD and NASH

Nonalcoholic fatty liver disease (NAFLD) is the most frequent chronic liver pathology in developed countries [1]. The prevalence of NAFLD is approximately 25%; however, this prevalence is likely underestimated due to the absence of symptoms and lack of noninvasive diagnostic tools [2]. NAFLD affects up to 70% of type 2 diabetes patients [3, 4] and is present in the majority of obese individuals subjected to weight loss surgery [5, 6]. Therefore, NAFLD can be contemplated as the liver component of the metabolic syndrome [7]. Given that obesity, metabolic syndrome, and diabetes have reached a pandemic state, and that nonalcoholic steatohepatitis (NASH) is an increasing etiology for liver transplantation, it can be easily reasoned and forecasted that the prevalence of NAFLD will further increase [8, 9].

NAFLD is hallmarked by hepatic steatosis without excessive alcohol intake and in the absence of other potential causes of fat accumulation in the liver, such as infections (specifically viral hepatitis), medication-related steatosis, or hepatic autoimmune pathologies [10]. In approximately 20–30% of NAFLD patients, the disease progresses from simple steatosis to NASH [11]. NASH is characterized by necro-inflammation and ballooning of

karger@karger.com
www.karger.com/jin

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Correspondence to:
Marina Nati, marina.nati@uniklinikum-dresden.de
Triantafyllos Chavakis, triantafyllos.chavakis@uniklinikum-dresden.de

hepatocytes; NASH may progress in a subset of patients toward development of fibrosis that can further lead to cirrhosis and liver failure, as well as hepatocellular carcinoma [12–14]. NAFLD may predispose to hepatocellular carcinoma even in the absence of cirrhosis and is a continuously increasing cause for liver transplantation [2, 8, 15–17]; in the United States, it is projected that NAFLD will likely become the main indication for liver transplantation in the near future [18]. The extent and significance of NAFLD as a health burden become even more obvious when considering the absence of approved treatments despite multiple ongoing clinical trials [19, 20].

During NASH, associated with obesity-related metabolic dysregulation, the expanded adipose tissue displays chronic low-grade inflammation and is a source for adipokines, such as leptin, and inflammatory cytokines, such as TNF or IL-6 [21]. Additionally, the obese adipose tissue releases free fatty acids (FFAs) into the circulation, which promotes ectopic fat deposition in the liver [21]. Fat accumulation in hepatocytes results in lipotoxicity, mitochondrial dysfunction, reactive oxygen species (ROS) generation, and endoplasmic reticulum stress [22]. In addition, NASH is associated with gut microbiota dysbiosis, and a dysfunctional gut barrier with enhanced permeability, resulting in the secretion of pro-inflammatory factors in the portal circulation that contribute to hepatic inflammation [23]. Pro-inflammatory cytokines, lipotoxicity, and gut-derived bacterial products promote activation of liver-resident macrophages, designated Kupffer cells (KCs), and recruitment of inflammatory macrophages [24]. Activation of innate immunity drives further hepatic infiltration and accumulation of inflammatory cells, thereby exacerbating liver inflammation and damage [25]. This inflammation-related pathological cascade leads to hepatic stellate cell (HSC) activation and their fibrogenic differentiation, culminating in liver fibrosis. Importantly, activated HSCs may further aggravate inflammation, thereby facilitating a vicious circle of inflammation and fibrosis, which can promote progression to cirrhosis [26].

In summary, understanding the molecular mechanisms triggering inflammation during NASH development and progression is a major research aim and focus of extensive investigations [25, 27–30]. Given the central role of innate immunity in NAFLD pathogenesis, the present article focuses on the role of neutrophils, macrophages, and KCs in the context of NAFLD and NASH development and progression.

The Role of Neutrophils in NAFLD

Being the most abundant leukocytes in human blood and the first responders to pathogen invasions, acute inflammation, or injury, neutrophils are principal players of the innate immune response [31–33]. Despite their extensively studied contribution in the context of acute sterile injury of the liver [34–37], less is known about their involvement in the metabolically induced chronic scenario of NAFLD.

In NAFLD patients, neutrophils are present in the portal inflammatory infiltrate and represent a source of pro-inflammatory IL-17 in progressed NASH; additionally, their number increases with NASH-related liver fibrosis [38]. Moreover, neutrophil abundance correlates with the degree of steatosis and neutrophils are often associated with steatotic hepatocytes in human NASH [39]. An elevation of the neutrophil-to-lymphocyte ratio in peripheral blood of human NAFLD patients has been suggested as a noninvasive marker for NASH and liver fibrosis severity [40]. In an attempt to clarify if neutrophil recruitment is a mere indication of the extent of liver damage or if it causally contributes to hepatocyte cytotoxicity and necro-inflammation, neutrophils were depleted, by using an antibody against Ly6G, in different murine models of NAFLD. Neutropenic mice were protected from both high-fat diet (HFD)-induced and methionine-choline-deficient (MCD) diet-induced steatohepatitis [41, 42]. The MCD diet induces steatosis, steatohepatitis, and fibrosis already after a few weeks [43]. On the other hand, the work of Calvente and colleagues [44] highlighted that neutrophils are also critical for the resolution of liver inflammation in a mouse NASH model based on MCD diet.

Several publications have underlined the potential role of neutrophil-derived factors in NAFLD progression. To immobilize and neutralize extracellular microbes, neutrophils release neutrophil extracellular traps (NETs), web-like structures comprising decondensed chromatin, nuclear, and granule proteins, in a process of self-induced death called NETosis [45]. Recently, a role of NETs in NAFLD progression was proposed. Elevated levels of myeloperoxidase (MPO)-DNA complexes, a NET biomarker, were found in the serum of NASH patients; additionally, neutrophil infiltration and NETosis were shown to promote NAFLD progression to hepatocellular carcinoma in mice [46]. Disrupting NET formation via deoxyribonuclease treatment or using peptidyl arginine deaminase type IV-deficient mice inhibited NASH-related cancer development [46]. Furthermore, the importance of NETs in promoting inflammation during early stages of

murine NASH was confirmed by Zhao and colleagues [47] using deoxyribonuclease administration in a model in which mice were fed a methionine-choline-deficient and a high-fat diet. These authors also showed that NETosis is activated in mouse fatty livers via S1P receptor 2 signaling [47].

Neutrophil activation and NETosis result in the release of granule proteins, key activators of the innate immune response [48]. Among others, neutrophil elastase (NE) participates in the generation of NETs, cathelicidins interact with and modulate toll-like receptor (TLR) activation, bactericidal/permeability-increasing protein exerts antimicrobial activity, whereas MPO stored in azurophilic granules, is a lysosomal enzyme with bactericidal activity that promotes ROS generation [49–52]. There is a consistent body of literature investigating the role of MPO in NAFLD. MPO levels are elevated in the circulation and in livers of NASH subjects compared to patients with simple steatosis and correlates with severity of liver inflammation [39]. Circulating neutrophils isolated from NASH patients are more prone to generate ROS than neutrophils from healthy subjects [53]. Whether the enhanced preparedness for ROS generation of neutrophils from NASH patients indicates the involvement of trained innate immunity, a form of innate immune memory, which can lead to increased inflammatory responses of innate immune cells, including neutrophils [54–56], in NASH pathogenesis remains to be elucidated. In a similar context, intracellular ROS generation may also promote migration and activation of human HSCs/myofibroblasts, thereby contributing to liver damage and fibrosis [57]. Importantly, MPO-triggered oxidative stress may result in increased DNA damage and accumulation of genomic mutations that increase the risk of malignant transformation and hepatocellular carcinoma development in NAFLD patients [58]. MPO may also participate in a neutrophil-HSC cross talk in NASH. Specifically, hepatic MPO is increased in NAFLD livers and MPO inactivation protects mice from NASH development, HSC activation, and fibrogenesis; in turn activated HSC-derived GM-CSF and IL-15 may enhance neutrophil survival, and thereby contribute to a feed-forward loop connecting perpetuation of liver inflammation and fibrosis [59–61].

The release of neutrophil granule contents into the liver extracellular matrix has been studied extensively in the context of hepatic injury and NASH pathogenesis [48]. Human neutrophil peptides (HNPs) also known as α-defensins, are a major microbicidal component of neutrophils. Treating human HSCs with HNP-1 in vitro increases their proliferation. Consistently, transgenic overexpression of HNP-1 promotes HSC proliferation and liver fibrosis in mouse NASH triggered by feeding with a choline-deficient, L-amino acid-defined diet [62]; the choline-deficient, L-amino acid-defined diet is a frequently used diet that induces steatohepatitis and fibrosis [43]. Circulating levels of NE, proteinase 3, and lipocalin 2 (LCN2) are increased in patients with NASH [63–66]. Consistently, a treatment with sivelestat, an NE inhibitor, ameliorated inflammation, and liver damage during NASH in $APOE^{-/-}$ mice fed with high-fat, high-cholesterol diet [42]. Moreover, NE secretion has been linked to insulin resistance. Treatment of primary mouse and human hepatocytes with NE directly degrades insulin receptor substrate-1, thereby increasing insulin resistance [67]. Pharmacological and genetic inactivation of NE mitigates liver insulin resistance and hepatic steatosis in obese mice [67, 68]. Additionally, NE and proteinase 3 may also promote HFD-triggered NAFLD pathogenesis, via their propensity to enzymatically activate IL-1β [69, 70]. Ye et al. [65] showed that neutrophil-derived LCN2 is increased in circulation and liver of mice subjected to 2 diet-induced NASH models, the MCD and the high-fat, high-cholesterol dietary models. LCN2 promotes the expression of the CXCR2 chemokine receptor and drives macrophage accumulation in the liver via a neutrophil-dependent mechanism. Consequently, deficiency of LCN2 protects mice from NASH development while chronic infusion of recombinant LCN2 enhances inflammation and liver damage [65]. Taken together, NETosis and the release of neutrophil-derived granule proteins resulting upon neutrophil activation may participate in the development of NASH and hepatic fibrosis and could be considered as a potential pharmacological target in future preclinical and clinical studies.

The Role of KCs and Monocyte-Derived Macrophages in NAFLD

KCs, the liver-resident macrophages, are yolk sac-derived, self-renewing macrophages located inside the hepatic sinusoids, in close proximity with endothelial cells [71–75]. C-Type Lectin Domain Family 4 Member F (CLEC4F) has been identified as a KC-specific marker [76, 77]; additionally, T-cell immunoglobulin and mucin domain containing 4 (TIM4, a receptor mediating efferocytosis of apoptotic cells [78], is also a marker of KCs [77, 79, 80]. Several studies suggest a critical regulatory participation of KCs in human NASH. Increased numbers of KCs/macrophages have been observed in liver biopsies of

NASH patients, positively correlating with NAFLD disease severity; moreover, KCs and macrophages may form hepatic "crown-like structures" in NASH; crown-like structures represent macrophage aggregates surrounding steatotic hepatocytes that usually contain large lipid droplets [81, 82]. Enhanced numbers of portal macrophages are found at the early stages of human NASH, preceding subsequent inflammatory events [38]. In addition to KCs that are F4/80^{hi} and CD11b^{int}, recruited CD11b^{high} monocyte-derived macrophages (MoMFs) have also been implicated in NAFLD and NASH development and progression [24, 83]. In a series of studies in rodents subjected to different diet-induced NASH models, global depletion of KCs and macrophages via clodronate liposomes or gadolinium chloride protected against the development of steatosis, necro-inflammation, and fibrosis, hence suggesting that liver KCs/macrophages are a component of NASH pathogenesis [84–91].

The mechanisms underlying the role of KCs and macrophages in NASH are multifaceted. Hepatic macrophages/KCs may be activated by FFAs originating from the obese adipose tissue, in a manner that involves TLR signaling; in this regard, palmitate and TLR2 may collaborate to induce KC/macrophage inflammasome activation, while palmitate interacts with the TLR4/MD2-complex stimulating ROS generation in inflammatory macrophages [21, 92–94]. Additionally, trans-fatty acids and peroxidized lipids derived from an unbalanced diet can promote activation of KCs [95, 96]. In experimental models leading to NAFLD/NASH, including genetic deficiency of leptin in ob/ob mice or feeding a HFD followed by carbon tetrachloride administration to induce steatohepatitis, the adipocytokine leptin, deriving from the obese adipose tissue, stimulates KC production of pro-inflammatory and pro-fibrogenic cytokines [97–99]. At the same time, the decreased levels of adiponectin in obesity promote steatohepatitis in mice, as adiponectin exerts anti-inflammatory actions on KCs [100, 101].

NASH is also associated with altered gut microbiota composition and related elevated intestinal barrier permeability; in this context, bacteria or their products, such as endotoxin, may reach the liver via the portal circulation and contribute to KC/macrophage activation [102]. Bacterial lipopolysaccharide (LPS) and bacterial DNA interact with TRL4 and TLR9, respectively, thereby promoting pro-inflammatory and pro-fibrotic activity in KCs/macrophages in rodent NASH models; consistently, in humans with NAFLD, TLR4 expression correlated with portal inflammation and fibrosis [86, 103–106]. Once activated, KCs may contribute to steatosis development by regulating hepatocyte lipid metabolism. Stienstra et al. [107] showed that KCs stimulate fat accumulation in hepatocytes by reducing their fatty acid oxidation via IL-1β-dependent suppression of peroxisome proliferator-activated receptor α activity. Moreover, TNF is involved in mediating the KC-dependent impaired fatty acid oxidation and enhanced triglyceride accumulation, as shown in rat KC-hepatocyte co-cultures using antibodies blocking TNF [84].

During hepatic steatosis, excessive lipid metabolism in hepatocytes and increased oxidative stress further reinforce KC activation and inflammation development; a major lipotoxic molecule in the context of NASH is free cholesterol [108]. In both NASH patients and mouse models, KCs form crown-like structures, similar to those present in the inflamed adipose tissue [82, 109, 110]; such crown-like structures surround dying steatotic hepatocytes, which contain cholesterol crystals [111]. KCs engulf modified lipids, for instance, oxidized low-density lipoproteins, mainly via CD36 and scavenger receptor A, and become pro-inflammatory lipid-laden "foamy cells"; in this context, NLRP3 inflammasome stimulation by cholesterol crystals may represent a mechanism underlying KC activation [112–116]. Interestingly, cholesterol-loaded KCs are more prone to produce inflammatory cytokines and chemokines in response to LPS stimulation [117]. Whether this may reflect innate immune memory in KCs has not been addressed; however, oxidized low-density lipoproteins can induce innate immune memory in macrophages [118]. The combined effect of lipotoxicity and inflammation during steatohepatitis results in hepatocyte damage and necroptosis, which in turn further perpetuates KC/macrophage pro-inflammatory activation, hence, representing a possible feed-forward loop in NASH development [119].

Engulfment of hepatocyte-derived apoptotic bodies by KCs promotes the production of death ligands and TNF [120]. Dying hepatocytes release damage-associated molecular patterns (DAMPs) capable of enhancing inflammation by activating their respective pattern recognition receptors on KCs and by driving recruitment of inflammatory cells, such as monocytes and neutrophils [121]. Along this line, microparticles containing the DAMP mitochondrial DNA, deriving from steatotic hepatocytes, activate a pro-inflammatory response in KCs/macrophages in a TLR9-dependent manner, as shown in a mouse HFD model [122]. Another DAMP released from damaged hepatocytes is extracellular adenosine triphosphate [123]. In in vitro studies, adenosine triphosphate contributed to LPS-induced IL-6 secretion by mouse KCs

[123]. Thus, DAMPs derived from hepatocyte death may potentiate hepatic inflammation in NASH.

Activation of resident KCs/macrophages in the liver and increased production of pro-inflammatory factors promotes the recruitment and accumulation of nonresident inflammatory cells to the liver, such as B lymphocytes, T lymphocytes, neutrophils, and monocytes, the latter giving rise to macrophages [90, 91, 117, 120, 124]. Chemokines produced by activated KCs regulate the recruitment of inflammatory cells; among them, C-C motif ligand 2 (CCL2) plays a crucial role in NASH development. In diet-induced NASH models in mice, CCL2 interacting with its cognate receptor CCR2 facilitates the accumulation of $Ly6C^{hi}$ monocytes in the liver [90, 91]. These infiltrating monocytes give rise to a distinct recruited hepatic macrophage population, MoMFs [124]. MoMFs originate from bone marrow hematopoietic cells and differ phenotypically from KCs [125]. Morinaga et al. [124] showed that the MoMFs infiltrating the steatotic liver of obese mice had higher expression of CCR2 but lower expression of CCL2 than KCs. Increased abundance of CCR2-expressing MoMFs was also identified in human NAFLD [126]. This infiltrating population is distinct from resident KCs and its abundance in the liver of patients correlates with severity of NASH and the stage of fibrosis [126]. In the recovery phase of carbon tetrachloride-induced fibrosis in mice, macrophages promote matrix degradation and repair [127]. Alternatively activated KCs/macrophages with an anti-inflammatory phenotype may promote apoptosis in M1-polarized pro-inflammatory KCs/macrophages, and thereby limit NAFLD-related liver injury in mice [128].

The mostly studied pro-inflammatory cytokines produced by liver KCs/macrophages during NASH are TNF [90, 129, 130] and IL-1β, the latter deriving from caspase-1 activation [131]. Macrophage-derived TNF and IL-1β may promote survival of activated HSCs in a manner that involves actions of the nuclear factor-kappaB, while TNF stimulates expression of tissue inhibitor of metalloproteinase 1 in HSCs as well, as shown in mouse models [132, 133]. Activated KCs/macrophages may also promote HSC transdifferentiation into collagen-secreting pro-fibrotic myofibroblast-like cells via TGF-β1 secretion, thereby aggravating hepatic fibrosis [134–136]. Interestingly, activin-A, a member of the TGF-β family, stimulates expression of TNF and TGF-β1 in mouse KCs thereby reinforcing the paracrine cross talk of KCs with HSCs [134]. Together, the interaction of KCs/macrophages with HSCs may promote NASH-related hepatic fibrosis contributing to progression of steatohepatitis toward liver cirrhosis.

Recent utilization of RNA sequencing and single-cell RNA sequencing techniques allowed a better investigation and understanding of the properties of distinct hepatic macrophage populations and resulted in functional diversification between KCs and MoMFs [137, 138]. Using a Western diet mouse model, Krenkel et al. [138] identified in NASH-livers expansion of MoMFs characterized by a unique inflammatory phenotype. Xiong et al. [139] identified a NASH-specific macrophage subset highly expressing triggering receptors expressed on myeloid cells 2 (TREM2). This population was designated as NASH-associated macrophages and is present both in human and mouse NASH [139]. Moreover, TREM2 also characterizes the lipid-associated macrophages (LAMs) of the adipose tissue in obesity [140]. A $TREM2^{+}CD9^{+}$ subpopulation of macrophages was also discovered in human NASH and named scar-associated macrophages due to their pro-fibrogenic phenotype [141]. Furthermore, recent studies demonstrated that KC numbers decrease in the NAFLD/NASH liver of mice and are replaced by recruited MoMFs of hematopoietic origin [142, 143]. In mice fed with a Western diet, high in fat, cholesterol, and sugar, liver-infiltrated MoMFs comprised 2 subpopulations, of which one is reminiscent of KCs and the other had LAM-like properties [143]. The latter population was characterized by osteopontin expression and was predominantly present in hepatic regions displaying fibrosis and low KC abundance [143]. Another study confirmed that TIM4-positive and CLEC4F-positive KCs are reduced, while abundance of infiltrated TIM4-negative MoMFs increases in the liver of mice upon NAFLD induction with a high-fat, high-sucrose-diet [79]. A subset within these recruited MoMFs expressed *Trem2* and other markers of LAMs, such as *Cd63*, *Cd9*, and *Gpmnb*; another transitional subpopulation expressed *Cx3cr1* and *Ccr2* and were designated C-LAMs. LAMs and C-LAMs were localized primarily in macrophage aggregates and crown-like structures of the NASH liver and might operate in a fashion that protects against NASH-related fibrosis [79]. A further recent mouse study identified a monocyte-derived KC population with decreased self-renewal and a pro-inflammatory phenotype that aggravates liver injury during NASH [144]. Despite the heterogeneity in markers and nomenclature, it can be stated that MoMFs play a significant role in NASH progression by promoting inflammation in a multitude of likely synergistically acting ways, including production of chemokines, cytokines, or ROS, although, as recently suggested, MoMF-derived LAMs may also protect against fibrosis [79, 141, 144–147].

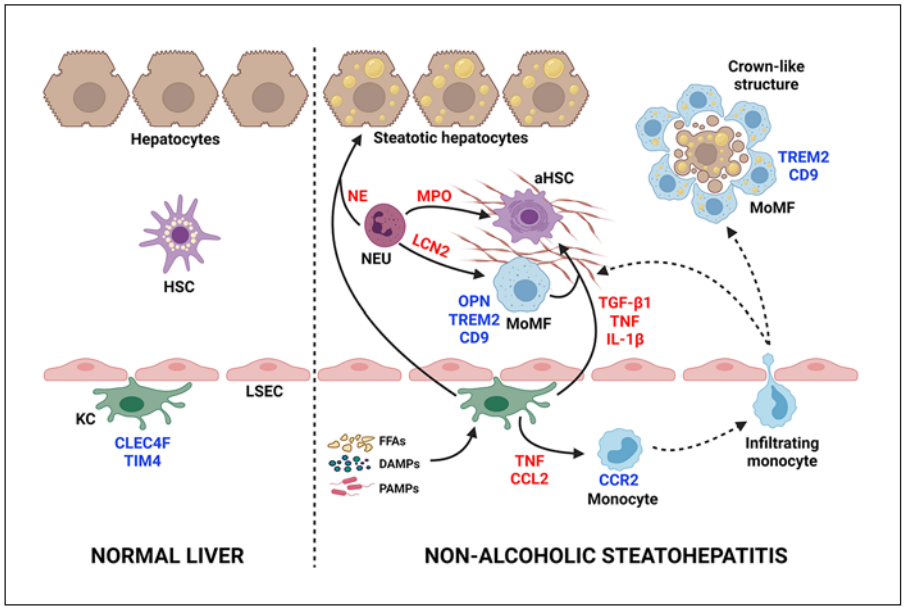

Fig. 1. Role of innate immune cells in NASH. KCs, characterized by TIM4 and CLEC4F expression, exert a homeostatic function in the healthy liver. However, during NASH development, KCs can be activated by circulating FFAs and cytokines deriving from the inflamed obese adipose tissue, dysbiotic microbiota-derived PAMPs, and DAMPs released from damaged hepatic cells. Activated KCs contribute to hepatic steatosis by modulating hepatocyte lipid metabolism. Moreover, activated KCs propagate the inflammatory response by secreting TNF and CCL2, thereby promoting CCR2-dependent recruitment of monocytes to the liver. Infiltrated monocytes can give rise to pro-inflammatory MoMFs contributing to NASH progression and fibrogenesis. A scar-associated TREM2$^+$CD9$^+$ subpopulation of MoMFs that may also produce osteopontin promotes HSC survival and activation. In this context, TNF, IL-1β, and TGF-β1 derived from activated KCs/macrophages contribute to HSC activation. In crown-like structures, surrounding dying hepatocytes, TREM2$^+$CD9$^+$ MoMFs may facilitate the clearance of dying steatotic hepatocytes and might thereby also exert protective functions in NASH-related fibrosis. Neutrophils are also recruited to the liver and accumulate in the chronically inflamed tissue in NASH. NETosis and neutrophil activation, resulting in the release of granule proteins, such as NE, MPO, and LCN2, can promote hepatic steatosis, exacerbation of inflammation and fibrosis. For details and relevant references, see the Text. KC, Kupffer cell; HSC, hepatic stellate cell; aHSC, activated HSCs; LSEC, liver sinusoidal endothelial cells; MoMF, monocyte-derived macrophages; NEU, neutrophils; FFAs, free fatty acids; DAMPs, damage-associated molecular patterns; PAMPs, pathogen-associated molecular patterns; NE, neutrophil elastase; MPO, myeloperoxidase; LCN2, lipocalin 2; OPN, osteopontin; TREM2, triggering receptors expressed on myeloid cells 2; TGF-β1, transforming growth factor-beta 1, TNF, tumor necrosis factor; IL-1β, interleukin 1 beta; CCL2, chemokine (C-C motif) ligand 2; CCR2, C-C chemokine receptor type 2; CLEC4F, C-Type Lectin Domain Family 4 Member F; TIM4, T-cell immunoglobulin and mucin domain containing 4.

Additionally, under certain circumstances, cells of hematopoietic origin, specifically monocytes derived from the bone marrow, may contribute to the replenishment of the KC niche giving rise to self-renewing and fully differentiated KCs [77, 148]. Upon acute depletion of KCs in mice, recruited macrophages acquired KC identity in a manner requiring Notch, TGF-β and liver-X-receptor signaling [149]. Consistently, a parallel study demonstrated that early TNF- and IL-1-dependent inflammatory signaling following KC depletion in mice activated HSCs and the endothelium to upregulate adhesion receptors and chemokines, thereby triggering monocyte recruitment. Recruited monocytes, in turn, gave rise to new KCs associated with induction of liver-X-receptor α expression, stimulated by interaction with HSCs and endothelial cells and involvement of NOTCH and bone morphogenetic protein (BMP) signaling [150].

Taking the aforementioned recent studies into account, it can be stated that bone marrow-derived MoMFs seemingly play an important pathogenic role in NASH, including promotion of inflammation and fibrosis; on the other hand, KCs may be more important in liver homeostasis [72, 125]. In fact, recent studies that identified markers distinguishing KCs and MoMFs also suggest that NASH-associated pathogenic functions that were previously ascribed to activated KCs may rather be mediated by recruited MoMFs. Additionally, in contrast to previ-

ously prevailing ideas that both KC and MoMF numbers increase during NASH, recent studies have changed our view by suggesting that enhanced MoMF infiltration may be accompanied by a reduction of KCs in different models of dietary NASH and that NASH is linked with a dysfunction of KC homeostasis [79, 144, 151]. In conclusion, due to their central role in regulating steatosis, inflammation, and fibrosis in NASH pathogenesis, macrophages may represent therapeutic targets for NASH development and progression.

Conclusions and Future Perspectives

Animal models and clinical studies have shown a critical role of cells of the innate immunity, particularly myeloid cells, such as neutrophils or MoMFs in initiation and propagation but also modulation and amelioration of hepatic inflammation in the context of NASH development, progression, and resolution [41, 42, 72, 79, 127, 141, 143] (Fig. 1). Therefore, myeloid cells and products thereof might represent potential therapeutic targets and noninvasive markers for assessing disease severity. However, due to their dual role in both contributing to and protecting against NASH pathogenesis, it is important to be able to target distinct myeloid cell subsets with pathological or pro-resolving properties specifically.

In recent years, advances in single-cell RNA sequencing allowed to explore the heterogeneity of MoMFs and KCs revealing the limitations and inadequacy of previously used markers. Novel markers and pathogenic players were identified in the context of NASH by characterizing distinct hepatic macrophage subpopulations on the basis of their transcriptional signatures [138, 139, 141]. Widely used traditional markers, such as CD68, F4/80 or CD11b, are clearly not sufficient to distinguish between resident and bone marrow-derived macrophages; novel markers, such as CLEC4F and TIM4, have been identified for KCs [77, 79, 80] or TREM2 and CD9 for inflammatory and pro-fibrotic MoMFs [141]. Due to these recent findings, researchers have shifted their focus from KCs to recruited MoMFs. Along this line, inhibiting the chemokine-dependent infiltration of monocytes seems like a promising therapeutic strategy. CCR2 and CCR5 are key players in the monocyte/macrophage and leukocyte trafficking, and cenicriviroc, a dual CCR2–CCR5 antagonist, ameliorated steatohepatitis, and fibrosis in different diet-induced mouse models of NASH [126]. Moreover, in a phase II b study, twice as many patients treated for 1 year with cenicriviroc presented fibrosis reduction when compared with the placebo group [152] and a phase III trial is in progress in NASH patients with stage F2 or F3 fibrosis [153]. Furthermore, Cenicriviroc is also tested in combination with Tropifexor, an agonist of the bile acid receptor, farnesoid X activated receptor [154]. Future studies utilizing innovative methodologies, such as single-cell RNA sequencing, and focusing on specific cellular subsets will help elucidate the exact role of different innate immune cell subsets in the complex pathophysiology of NASH and will likely provide novel therapeutic targets.

Acknowledgments

The figure was created using Biorender.com.

Conflict of Interest Statement

The authors have no conflicts of interest to declare in relation to this work.

Funding Sources

T.C. is supported by funds from the European Research Council (ERC), the German Research Foundation (DFG), the National Institute of Dental and Craniofacial Research/National Institutes of Health (NIDCR/NIH), the German Ministry of Research and Education (BMBF), and the Else Kröner Fresenius Center for Digital Health Dresden.

Author Contributions

M.N. wrote the first draft; K.-J.C. and T.C. reviewed and edited the manuscript.

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