Annals of Gastroenterology (2023) **36,** 244-256

Innate immunity and nonalcoholic fatty liver disease

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Abstract

Nonalcoholic fatty liver disease (NAFLD), recently renamed as metabolic (dysfunction)-associated fatty liver disease (MAFLD), is a complex, multifactorial disease that progresses via nonalcoholic steatohepatitis (NASH) towards severe liver complications. MAFLD/NAFLD affects up to a third of the global population. It is connected with metabolic syndrome parameters and has been increasing in parallel with the rates of metabolic syndrome parameters worldwide. This disease entity exhibits a strong immune-inflammatory dimension**.** In MAFLD/NAFLD/NASH, a vast network of innate immune cells is mobilized that can provoke liver damage, leading to advanced fibrosis, cirrhosis and its complications, including hepatocellular carcinoma. However, our understanding of the inflammatory signals that drive the onset and progression of MAFLD/NAFLD/NASH is fragmented. Thus, further investigation is required to better understand the role of specific innate immune cell subsets in the disease, and to aid the design of innovative therapeutic agents to target MAFLD/NAFLD/NASH. In this review, we discuss current concepts regarding the role of innate immune system involvement in MAFLD/NAFLD/NASH onset and progression, along with presenting potential stress signals affecting immune tolerance that may trigger aberrant immune responses. A comprehensive understanding of the innate immune mechanisms involved in MAFLD/NAFLD/NASH pathophysiology will help the discovery of early interventions to prevent the disease, and lead to potential innovative therapeutic strategies that may limit its worldwide burden.

Keywords Innate immune response, nonalcoholic fatty liver disease, metabolic-associated fatty liver disease, liver fibrosis, metabolic syndrome

Ann Gastroenterol 2023; 36 (3): 244-256

Introduction

Nonalcoholic fatty liver disease (NAFLD), recently renamed as metabolic (dysfunction)-associated fatty liver disease (MAFLD) [1], has become one of the most significant liver diseases. The prevalence of MAFLD/NAFLD is increasing worldwide, together with the pandemic of obesity and other metabolic syndrome (MetS) parameters, affecting at least 25% of the adult population globally [2]. In morbidly obese patients its prevalence may rise to 90% [3]. Up to 20-30% of patients develop nonalcoholic steatohepatitis (NASH), and more than 30% of NASH patients may progress to cirrhosis and/or hepatocellular carcinoma (HCC) [4]. MAFLD/NAFLD/NASH is likely to become the main indication for liver transplantation in the near future; NASH is anticipated to be the leading cause of liver transplantation in the US, with a mortality rate considerably higher than the general population or in patients without this inflammatory subtype of MAFLD/NAFLD [5].

The immunological facets of MAFLD/NAFLD have become the focus of investigation, showing a critical role for innate immunity [6]. A large number of innate immune cells, parenchymal hepatocytes and liver sinusoidal endothelial cells are involved in the onset of the hepatic chronic inflammatory

Conflict of Interest: None

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Received 13 December 2022; accepted 14 February 2023; published online 8 April 2023

DOI: https://doi.org/10.20524/aog.2023.0793

process associated with MAFLD/NAFLD [7]. Complex innate immune responses involved in the pathogenesis of MAFLD/NAFLD are executed by a diverse collection of cells, such as Kupffer cells (KCs)/macrophages, neutrophils, dendritic cells (DCs), mast cells (MCs), natural killer (NK) cells and other innate-like lymphocytes that initiate a shift from an immune tolerant—in healthy livers—to an immune reactive and often aberrant phenotype [8-10]. Moreover, these cells produce an abundance of soluble proteins, cytokines and complement proteins, thereby allowing the liver to extend its immunological functions beyond its anatomical borders [9]. Accumulating data indicate that innate immunity is a driving force that contributes to NASH progression, because it is mechanistically involved in both intrahepatic histological alterations and extrahepatic comorbidities [9]. MAFLD/NAFLD is strongly associated with a higher risk of extrahepatic diseases, such as chronic kidney, cardio-cerebrovascular and neurodegenerative disorders, the endpoints of MetS-related MAFLD/NAFLD [1,11,12]. In this review, we aimed to summarize the recent advances in our understanding of how the innate immune system leads to the development and progression of MAFLD/NAFLD.

Innate immune cells

KCs/macrophages

Signals trigger various cellular receptors which can activate diverse cell types in the liver. About 15% of them are KCs, which represent the primary immune effector cells involved in maintaining immunological homeostasis. KCs represent 80-90% of hepatic tissue-resident macrophages. KCs are categorized into immunogenic M1 KCs and tolerogenic M2 KCs. In the absence of metabolic disturbances and gut dysbiosis, KCs act as M2 macrophages that produce interleukin (IL)-10 and transforming growth factor-β (TGF-β), while inducing T regulatory cells and DCs [13]. Activated KCs/macrophages (M1 type) exhibit phagocytic activity and secrete proinflammatory cytokines and reactive oxygen species (ROS). Gut dysbiosis associated with MetS parameters promotes KC activation through increased enteral absorption of bacterial products [14]. Bacterial lipopolysaccharide (LPS), and other bacterial products activate KC recognition by Toll-like receptors (TLR) 2 and 4 and induce the M1 phenotype, which produces, via an increase in nuclear factor-κB (NF-κB) translocation, several proinflammatory cytokines, chemokines—C-C motif ligand 2 (CCL2) and CCL5—and damage-associated molecular patterns (DAMPs). DAMPs promote liver damage through activation of KCs by the TLR pathway, repeating this inflammation process and leading to hepatocyte damage. TLR4 in KCs contributes to the progression of simple steatosis to NASH by inducing the ROS-dependent activation of X-box binding protein-1, an important transcription factor induced by endoplasmic reticulum stress that in turn induces NF-κB-dependent proinflammatory cytokine production [8]. Furthermore, endoplasmic TLRs, such as TLR9, bind to mitochondrial DNA from damaged hepatocytes or translocated bacterial DNA to accelerate the inflammatory response in NASH, via the TLT9/MyD88/NF-κB/IL-1β signaling pathway. The accumulation of other agents, such as free fatty acids (FFAs), a risk factor of insulin resistance (IR), oxidized lipoproteins and other lipids, is a very important issue to take into account in experimental MAFLD/NAFLD/NASH pathophysiology because of its involvement in KC activation, causing a robust response to LPS and hence disease progression. Within hepatocytes, excessive accumulation of FFAs caused by defective efferocytosis [15] favor the production of proinflammatory cytokines and microvesicles that stimulate macrophage mobilization by activating stress-responsive kinases JNK1 and JNK2, ultimately leading to liver cell death/apoptosis [15].

Increased numbers of KCs/macrophages have been detected in the liver biopsies of NASH patients, positively correlating with MAFLD/NAFLD disease severity. Macrophages contribute to oxidative stress, one of the cardinal features in the development of MAFLD/NAFLD and NASH. Moreover, oxidized phospholipids and reactive aldehydes produced by lipid peroxidation act as DAMPs in inflammasome activation and form antigenic aggregates with cellular macromolecules named oxidative-stress epitopes (OSEs). OSEs-related immune responses appear to have a B-cell-dependent activity in the pathogenesis of NASH [16].

KCs and infiltrating macrophages are centrally involved in the recruitment and function of other immune cells in MAFLD/NAFLD by activating pattern recognition receptors (PRRs) [17] (Fig. 1). Activation of resident KCs/macrophages in the liver and increased production of proinflammatory factors promotes the recruitment and accumulation of non-resident inflammatory cells to the liver, such as B-lymphocytes, T-lymphocytes, neutrophils and monocytes, the latter giving rise to M1-activated macrophages [17]. In contrast, depletion of KCs, by administration of gadolinium chloride, significantly alleviates diet-induced NASH [18]. The depletion of KCs also

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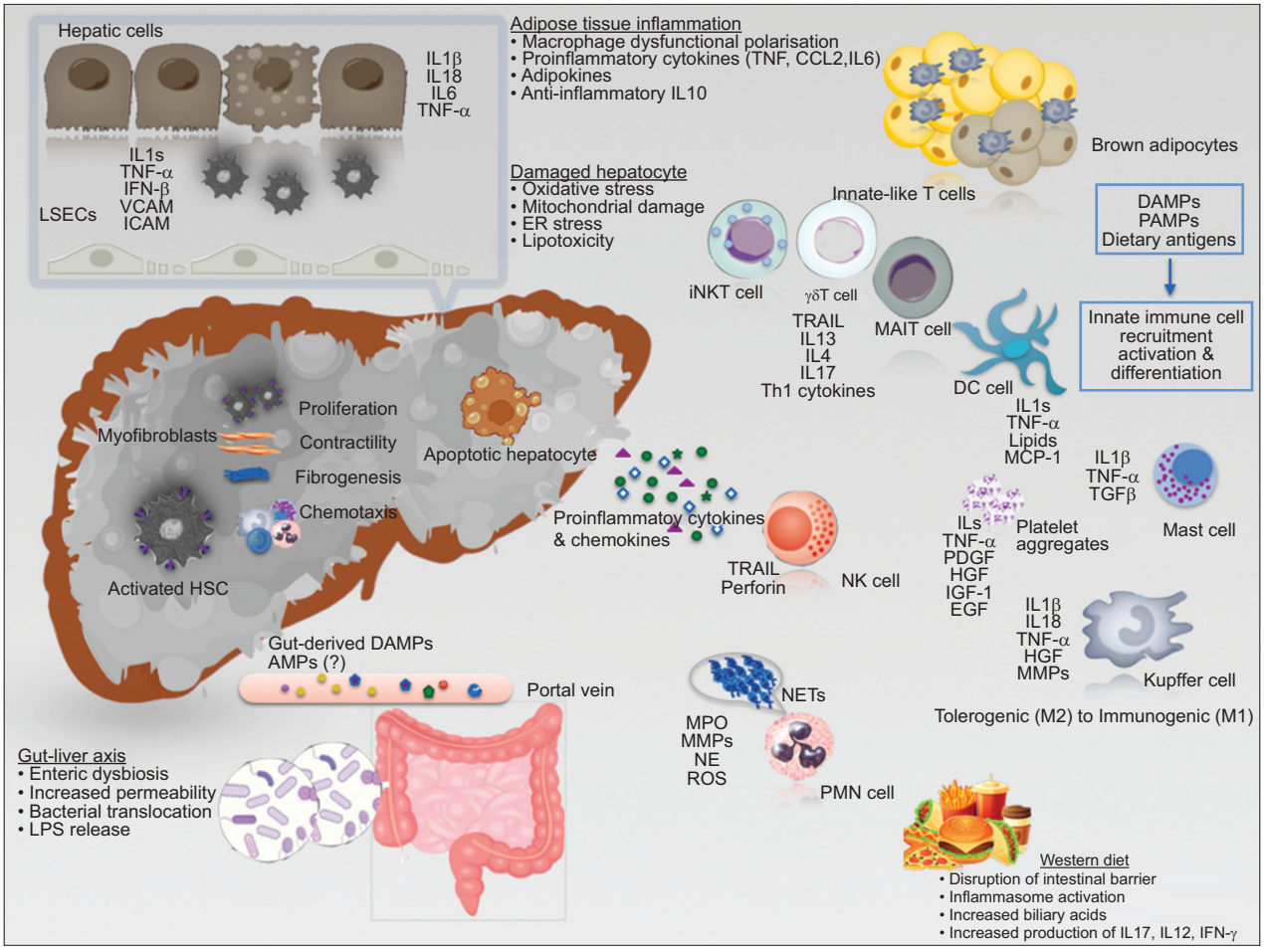

Figure 1 Innate immune modulation in MAFLD/NAFLD pathophysiology. Early reshaping of the innate immune signaling during MAFLD/NAFLD initiates an inflammatory cascade, resulting in uncontrolled liver injury and fibrogenesis. The hepatic repertoire of innate immune cells includes Kupffer cells, neutrophils, dendritic cells, hepatic stellate cells, innate immune-like resident T cells (including iNKT, γδT and MAIT cells), liver sinusoidal endothelial cells, mast cells, natural killer cells, platelets and hepatocytes that detect metabolic-associated stress signals (PAMPs, DAMPs) and elicit an immune response via the transcriptional regulation of proinflammatory mediators, including cytokines, chemokines, and cell growth factors. Adipose–liver organ crosstalk is mediated by the release of proinflammatory cytokines, chemokines, adipocytokines, miRNAs, extracellular vesicles and metabolites, promoting liver inflammation and injury. In addition, adipocyte transition during metabolic syndrome-associated events and nutrient-related signals (high-fat diet) triggers enhanced macrophage phenotypic differentiation that further aggravates disease progression. Intestinal bacterial dysbiosis drives inflammation and the production of metabolic by-products, such as ethanol and endotoxins/lipopolysaccharides, that in turn cause intestinal-mucosa barrier dysfunction and increased gut permeability. Thus, via the gut–liver axis (biliary system and the portal vein) the liver is exposed to flora metabolites and altered bile acids, promoting the development and progression of MAFLD/NAFLD

AMPs, antimicrobial peptides; γδT, γδ Tcells; CCL-2, C-C motif chemokine -2; DAMPs, damage-associated molecular patterns; DCs, dendritic cells; EGF, epidermal growth factor; ER, endoplasmic reticulum; FGF, fibroblast growth factor; HSCs, hepatic stellate cells; HGF, hepatocyte growth factor; IGF, insulin growth factor; ICAM, intercellular adhesion molecule; IFN, interferon; IL, interleukin; iNKT, invariant natural killer T cell; KCs, Kupffer cells; LPS, lipopolysaccharides; LSECs, liver sinusoidal endothelial cells; MAFLD, metabolic (dysfunction)-associated fatty liver disease; MCP-1, monocyte chemoattractant protein-1; MMPs, matrix metalloproteases; MAIT cell, mucosal associated invariant T cell; MPO, myeloperoxidase; NK, natural killer cells; PMNs, neutrophils; NAFLD, nonalcoholic fatty liver disease; NETs, neutrophil extracellular traps; PAMPs, pathogen-associated molecular patterns; PDGF, platelet-derived growth factor; ROS, reactive oxygen species; Th1, T helper 1; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; VCAM, vascular cell adhesion protein; VEGF, vascular endothelial growth factor; NE, norepinephrine

attenuates hepatic steatosis and liver IR, the key component of MetS [19], in rats fed high-sucrose or high-fat diets [18]. Depletion of macrophages also leads to decreased neutrophil infiltration. Thus, KCs and infiltrating macrophages are centrally involved in the recruitment and function of other immune cells in MAFLD/NAFLD/NASH.

Neutrophils

Neutrophils are important effectors of the innate immune system, with high phagocytic potential, and possess a large number of antimicrobial molecules. Beyond clearing pathogens, neutrophils may also exacerbate macrophage

cytotoxicity and promote a chronic inflammatory state [20]. Mechanistic studies have revealed that excessive activation of neutrophils induces liver damage, mainly via release of proteases, such as myeloperoxidase, neutrophil elastase, proteinase 3, cathepsins, and matrix metalloprotease-9, into the extracellular environment [21] (Fig. 1). Neutrophils also activate KCs and endothelial cells, resulting in upregulation of cellular adhesion molecules and triggering downstream recruitment of other leukocytes. Therefore, these cells are important in the initiation of liver inflammation.

Related studies have identified a potential role of neutrophils in the pathophysiology of MAFLD/NAFLD and NASH. Neutrophil abundance correlates with the degree of steatosis, and neutrophils are often linked with steatotic hepatocytes in human NASH. Likewise, neutrophils present in the portal inflammatory infiltrates produce proinflammatory IL-17 in progressed NASH [22]. Moreover, an increase of the neutrophil-to-lymphocyte ratio in peripheral blood of human MAFLD/NAFLD patients has been proposed as a noninvasive marker for NASH and liver fibrosis severity. Mouse studies have identified that human neutrophil peptides can enhance hepatic fibrosis in fatty liver by inducing proliferation of hepatic stellate cells (HSCs). In contrast, the deletion of elastase, secreted by neutrophils, in high fat diet (HFD)-induced obese mice was found to improve hepatic histologic inflammation, with a reduction of neutrophil and macrophage infiltration [23]. Likewise, neutropenic mice were protected from both HFD- and methionine choline-deficient diet-induced NASH (Table 1).

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 [24]. Circulating levels of markers of NETs increase in NASH patients and the liver NET formation occurs at the early stage of the MAFLD/NAFLD in mice. A role of NETs in MAFLD/NAFLD progression has also been proposed. In contrast, inhibition of the NET formation protects mice from hepatic inflammation, liver injury and NASH-driven HCC [25]. Therefore, NETosis could be considered as a potential pharmacological target in future preclinical and clinical studies.

Another main microbicidal component of neutrophils are human neutrophil peptides (HNPs) also known as α-defensins. Human defensins form 2 genetically distinct α and β subfamilies involved in innate immunity via killing microbial pathogens or neutralizing bacterial toxins, and in adaptive immunity by serving as chemoattractants and activators of immune cells. The α-defensins comprise 4 HNPs (1–4) present in neutrophils and 2 human α-defensins (HD-5 and HD-6), predominantly expressed in the small intestine Paneth cells [26]. The α-defensins display a broad spectrum of antimicrobial actions against Gram-positive and Gram-negative bacteria, certain fungi, parasites or viruses [26]. Moreover, HNPs appear to be serum indicators of the necro-inflammatory process seen in MAFLD/NAFLD obese patients, whereas their circulating levels are considerably reduced by bariatric surgery-induced amelioration of NASH [27].

Dysfunction of Paneth cells is associated with a noticeable decrease in the expression of antimicrobial peptides (AMPs) and gut dysbiosis [28]. Moreover, NETs activation by gut dysbiotic microbial populations may be involved in the gut inflammatory process linked to inflammatory bowel disease, especially given the combined load of gut dysbiosis and neutrophil recruitment, the marks of active disease [29].

In this regard, increasing evidence supports an important role for gut–liver axis dysfunction in the pathogenesis of MAFLD/NAFLD and NASH. The associated changes in the microbiome, referred to as dysbiosis, have been associated with MAFLD/NAFLD and HCC in both animal experiments and human studies: gut dysbiosis is strongly linked to fatty liver disease, type 2 diabetes mellitus and other MetS-related disorders [30]; in cirrhosis the underlying changes of portal hypertension influence intestine transit and permeability, resulting in the so called "leaky gut" also seen in MAFLD/NAFLD-related cirrhosis [31]; tight junction disruption in mice and MAFLD/NAFLD patients increases intestinal permeability and bacterial translocation involved in the pathogenesis of complications of chronic liver disease, including hepatic encephalopathy [19,32]; and gut dysbiosis in patients with NAFLD/NASH promotes IR, *de novo* lipogenesis in liver, and also increases intestinal permeability, which promotes chronic pathogen-associated molecular patterns (PAMP) exposure and oxidative stress caused by increased endogenous ethanol.

In this respect, several reports support favorable effects of various AMPs, such as regenerating islet-derived lectins and cathelicidin-related AMP [33], which ameliorate liver damage through mechanisms including reduction of bacterial colonization of mucosal surfaces, prevention of bacteria/bacterial product translocation through the gut, and abrogation of hepatic inflammasome activation, among others. Of particular interest are the structurally similar human β defensins (hβDs), a subclass of AMPs consisting of 6 identified isoforms (numbered hβD-1-6) secreted by leukocytes and epithelial cells in the intestine, skin, lungs, liver and other organs. The ability of hβD-2 to ameliorate liver inflammation by modification of gut microbiota requires further evidence. However, oral administration of hβD-2 in mice [34] attenuates the increase of certain microbial genera that comprise part of the typical gut microbiota signature in NASH and steatosis, while favoring a shift towards a more diverse phenotype, similar to healthy controls.

Fecal transplantation, via a beneficial influence on the gut microbiota, improves HFD-induced NASH in mice by decreasing hepatic lipid accumulation and proinflammatory cytokine levels [35]. Likewise, cathelicidin suppresses lipid accumulation and hepatic steatosis via the inhibition of the CD36 receptor [36]; CD36, also known as FAT, is an FFA transporter that facilitates FFA uptake and has a profound influence on the development of MAFLD/NAFLD. In this respect, vitamin D-induced autophagy is mediated by the expression of human cathelicidin, which displays a direct antimicrobial action [37]. Vitamin D signaling appears to play a major role in maintaining eubiosis via induction of Paneth cell-specific α-defensins. In contrast, lacking vitamin D or

Table 1 MAFLD / NAFLD / NASH main types of mouse models

Type	Subtype	Features	Comments
Metabolic models	HFD	Obesity, IR, Steatosis, Hepatic ballooning, NASH, Fibrosis	Variable degree of steatosis in HFD models due to dietary and genetic factors (strain specific)
	Fructose diet	Obesity, IR, Steatosis	Reproducibility issues
	Cholesterol & cholate	Weight loss, Hepatic IR, Steatosis, Hepatic ballooning, NASH, Fibrosis	Variable metabolic status (e.g., striking hyperinsulinemia)
	MCD diet	Weight loss, Hepatic IR, Hepatic IR Hl Steatosis, NASH, Fibrosis	Limited use due to significant differences from the MetS profile of human NASH, however it replicates NASH histological features in a relatively shorter feeding time than other dietary models.
	CDAA diet	Weight loss, Steatosis, NASH, Fibrosis, HCC	Failure to elicit features of the human MetS. It should not be used for examining the metabolic profile of the disease.
	ALIOS diet	Obesity, IR, Steatosis, NASH, Fibrosis, HCC	Serves as robust and reproducible model for human MAFLD/NAFLD studies
	DIAMOND diet	Obesity, IR, Steatosis, NASH, Fibrosis, HCC	Suitable as a preclinical model in pharmaceutical studies for NASH
Genetic models	*db/db* and *ob/ob* mice	Obesity, IR, Steatosis	Leptin deficient and leptin receptor deficient mice. Commonly used genetic models for studying steatosis in MAFLD/NAFLD and related systemic metabolic changes despite that leptin mutations in humans are not common
	PTEN knockout mice	Steatosis, Hepatic ballooning, NASH, Fibrosis, HCC	PTEN deficiency results in liver insulin hypersensitivity with decreased fasting glucose levels that contrasts human NASH
	SREBP-1c transgenic mice	Weight loss, IR, Steatosis, NASH, Fibrosis, Hepatic ballooning	Lipodystrophy-associated steatohepatitis model
	KK-Ay/a mice	Obesity, IR, Steatosis	Additional stimulus such as MCD diet can induce steatohepatitis
	Gankyrin (Gank)-deficient mice	Steatosis, NASH	Use of this model supports the notion that liver proliferation drives fibrosis, while steatosis might play a protective role.
	AOX knockout mice	Steatosis, transient NASH, HCC	Marked fibrosis is absent, despite the development of HCC
	apoE knockout mice	Steatosis, NASH (rapid), Hepatocellular ballooning, Fibrosis	HFD stimulus is necessary. Mice possess the characteristics of NASH and MetS, making it a valuable tool in NASH research
	ALR-H-KO mice	Steatosis, NASH, Fibrosis, HCC	Suitable for studies on the pathogenesis of steatohepatitis, its complications and progression to HCC
	Mat1a knockout mice	Steatosis, NASH, Fibrosis, HCC	Evaluates the contribution of impaired lipid homeostasis to MAFLD/NAFLD/NASH/HCC progression
Chemical/ pharmacological models	Steptozotocin +/- HFD	Obesity, IR, Steatosis, Hepatic ballooning, NASH, Fibrosis, HCC	Relevant to oxidative stress
	CCL_4	Steatosis, Hepatic ballooning, NASH, Fibrosis, HCC	Combined with Western diet or HFD. Model animals are susceptible to death by poisoning
	STAM model	Steatosis, NASH, Fibrosis, HCC	This model represents NASH with diabetic background
	Tetracycline	Steatosis, Hepatic ballooning, NASH, Fibrosis, HCC	Similar to, but less toxic than CCL_4

MAFLD, metabolic (dysfunction)-associated fatty liver disease; ALIOS, American lifestyle induced obesity syndrome; ALR-H-NO, augmenter of liver degeneration hepatic knockout; AOX, acyl-coenzyme A oxidase; apoE, apolipoprotein E; CCL4, carbon tetrachloride; CDAA, choline deficient L-amino acid defined; DIAMOND, diet-induced animal model of nonalcoholic fatty liver disease; HCC, hepatocellular carcinoma; HFD, high fat diet; IR, insulin resistance; Mat1a, methionine adenosyltransferase 1a; MCD, methionine and choline deficient; MetS, metabolic syndrome; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PTEN, phosphatase and tensin homolog; SREBP-1c, sterol regulatory element-binding protein 1c

vitamin D signaling pathways may impair the intestinal innate immunity, including downregulation of Paneth cell defensins, leading to bacterial translocation, endotoxemia, systemic inflammation, IR, and hepatic steatosis. Epidemiological studies show an association between the prevalence of vitamin D deficiency and MetS-related obesity, type 2 diabetes mellitus, and MAFLD/NAFLD, signifying potential linkages. In contrast, vitamin D supplementation improves MetS, although the impact on NAFLD is not known [38]. Thus, further research is warranted to elucidate this topic in depth.

DCs

DCs, which belong to the innate immune system, are a specific type of hematopoietic cells that can sense characteristics of the local environment, recognize pathogens and danger signals, and play a substantial role in bridging innate and adaptive immune responses [9]. Specifically, DCs are the most functional and specialized antigen-presenting cells, acting as a cellular connector between innate and adaptive immune system. Hepatic DCs are involved in antigen presentation to lymphocytes and modulation of hepatic immune responses. DCs migrate from the blood to the lymph nodes via the hepatic sinusoids. Therefore, hepatic sinusoids can serve as a significant enrichment area for hepatic DCs (Fig. 1) [39]. Hepatic DCs account for <1% of total liver myeloid cells and are mainly localized in the portal area. They can be categorized according to the expression of specific markers as following: plasmacytoid-like DCs (PDCA-1$^+$; pHepaticDCs) and myeloid or classical DCs (PDCA-1$^-$; cHepaticDCs/mHepaticDCs); the latter are further subdivided into CD103$^+$/CD11b$^-$ type 1 (mHepaticDCs1) and CD103$^-$/CD11b$^+$ type 2 (mHepaticDCs2) cells. DCs can switch from a tolerogenic state to an immunogenic phenotype, depending on cues in the hepatic microenvironment of the liver and the DCs' cellular lipid contents [9].

The role of DCs in MAFLD/NAFLD/NASH remains unclear, since diverse studies have reported contradictory data. For instance, some studies show that hepatic DCs play a proinflammatory role in NASH processes [9]. When a liver injury occurs, hepatic DCs proliferate and activate as efficient antigen-presenting cells, able to specifically stimulate CD4+ T cells and producing large amounts of proinflammatory cytokines [40]. This response involves a subset of DCs with high lipid content and is triggered by TLR4 stimulation induced by the release of HMGB1 protein from damaged hepatocytes. Antigen presentation to CD4+ T helper cells supports cytotoxic CD8+ T cell and B cell responses, which in NASH are further costimulated by additional signals provided by molecules of the B7 or tumor necrosis factor (TNF) families, expressed in activated T cells, such as OX40/CD134. Interestingly, levels of soluble OX40 correlate positively with the severity of steatohepatitis [41]. In contrast, depletions of CD11c$^+$ DCs or CD103$^+$ DCs reduce proinflammatory cytokine and chemokine expression, thereby decreasing MAFLD/NAFLD-related liver fibrosis [42]. Recent data indicate that MAFLD/NAFLD patients with coexistent morbid obesity display hepatic DCs (CD11c$^+$) on liver tissue more frequently than patients with less severe or no obesity, thereby suggesting the involvement of hepatic DCs in the perpetuation of the inflammatory process in MAFLD [43]. However, CD103$^+$/mHepaticDCs1 appear to display an anti-inflammatory ability, affecting the conversion from steatosis to steatohepatitis. It has been suggested that different subsets of mHepaticDCs may display opposite effects in regulating lobular inflammation in human MAFLD/NAFLD/NASH [44]. Likewise, other studies show that CD11c$^+$ populations play a protective role against the intrahepatic fibro-inflammatory process in NASH. These contradictory data could partially be explained by differences in the NASH models introduced or diets applied in the studies, given that the DC phenotypes noticed in NASH could switch between immune-tolerant and immunogenic settings in a manner dependent on dietary factors. Therefore, the role of hepatic DCs in the development and/or progression of MAFLD/NAFLD disease warrants further investigation.

Involvement of other innate immune cells in MAFLD/NAFLD pathophysiology

NK cells

NK cells are important effectors of the innate immune system and represent the first line of defense against pathogens affecting the host. They act via direct cytotoxic mechanisms or via modulation of other immune cells by secreting cytokines and chemokines [45]. Hepatic NK cells predominate over other innate lymphoid cell subsets and are situated inside the sinusoidal lumen, adhering to KCs and endothelial cells. They represent a heterogeneous population, both phenotypically and functionally, that includes liver-resident NK cells (CCR5+CXCR+CD69+), memory-like NK cells (CXCR6+CD94/NKG2C+), and transient conventional NK cells, which mainly consist of recirculating NK cells (predominantly CD56dimCD16+ NK cells) in the liver's blood system [46]. NK cells are activated and contribute to limiting tissue damage and fibrosis during NASH by polarizing macrophages into a proinflammatory M1 phenotype via interferon (IFN)-γ, without affecting their cytolytic function [47] (Fig. 1), and help to clear senescent activated HSCs [48]. Following recruitment into the liver via mechanisms including chemokine (C-X-C motif) ligand 10 (CXCL10), they display antifibrotic activity by stimulating HSC apoptosis—a capacity that seems to be more relevant at early stages of fibrosis but is impaired at more advanced stages—antagonizing HSC-derived myofibroblasts, or acting in synergy with other immune cells (e.g., KCs/macrophages and DCs) to limit fibrotic functions of HSCs in the CCL4-treated liver fibrosis model [48]. The functions of NK cells are strongly regulated by the stimulation of multiple surfaces of activated as well as inhibited receptors. Thus, NK cells appear to play a controversial role in NASH pathophysiology; this is partially attributed either to the inability to distinguish between liver-resident and circulating NK cells, or to tissue-specific regulation

of NK cell function [49]. Some studies connect their activation by different cytokines and ligands to MAFLD/NAFLD/NASH, while others show a reduction in their cytotoxic activity in MAFLD/NAFLD/NASH [10]. Specifically, a variety of studies show that activation of NK cells in NASH may be linked to elevated levels of several NK-activating cytokines, such as IL-2, IL-12 and IFN-α/β [10]. While most of the studies support the concept of NK cell recruitment to the liver during the NASH stage, where they promote an antifibrotic effect [49], NK cells in MAFLD/NAFLD patients may also be reduced [50]. This reduction correlates with impaired functional activity leading to less granzyme/perforin and IFN-γ production, eventually reducing NK cytotoxicity. In this respect, NK cells may potentially lose their cytotoxic activity during NASH, leading to exacerbation in NASH patients in later stages of liver diseases [51]. Thus, further research is needed to clarify this contradictory field.

Innate-like T cells

NKT cells

NKT cells comprise a unique, innate-like T cell subtype, which co-expresses specific NK surface receptors, and an antigen receptor typical of conventional T cells. NKT cells recognize lipid antigens presented either by CD1d-expressing antigen-presenting cells or HSCs, and secrete a variety of cytokines (IL-4, IL-10, IFN-γ, and TNF) that in turn promote T helper (Th)-1, and Th-2 lymphocytes and CD4+CD25+ regulatory T cell activities [52] (Fig. 1). Liver NKT cell numbers seem to be elevated in MAFLD/NAFLD patients, and they tend to increase as the disease progresses [53]; the level of liver NK-T cells is positively associated with disease, and NK-T cells can accumulate in progressive NASH, thereby promoting the fibrotic process. NKT cell expansion is evident in advanced NASH, concomitant with enhanced secretion of TNFSF14, IFN-γ, and IL-17A. Interfering with NKT cells during advanced NASH effectively improves hepatic parenchymal injury, inflammation and fibrosis in different experimental models of the disease [54]. While NKT cells can both stimulate and suppress immune and/or inflammatory responses, recent evidence suggests that type I or invariant NKT (iNKT) cells, representing the vast majority (95%) of liver NKT cells [52], favor liver steatosis and, together with CD8+ T cells, induce liver damage through their ability to increase liver production of osteopontin and secrete IFN-γ, IL-4 and IL-17, known to support collagen production and extracellular matrix deposition [55]. Therefore, depletion of these cells appears to reduce NASH progression and thus may represent a novel therapeutic approach for the treatment of NASH.

γδ T cells

γδ T cells are a type of innate-like T cells, mainly characterized by the expression of γδ T-cell receptors that recognize lipid antigens and the rapid secretion of proinflammatory cytokines, such as IL-17A, in a microbiota-dependent manner. Specifically, hepatic γδT cells are liver-resident cells that mostly produce a high level of IL-17A, and their homeostasis is maintained by the intestine microbiota in a lipid antigen/CD1d-dependent manner, including cell activation, survival and proliferation [56] (Fig. 1).

Animal model studies reveal that hepatic γδ T cells, activated by increased bacterial loads of *Lactobacillus gasseri*, promote liver injury and fibrosis. In HFD-fed or high-fat/high-carbohydrate diet-fed mice, significantly raised numbers of liver γδT17 cells appear to contribute to liver damage, steatohepatitis and glucose dysmetabolism via the production of IL-17A [56]. Likewise, during HFD-induced obesity, adipose tissue-resident γδT cells seem to promote an inflammatory response by regulating macrophages and raising systemic IR. In steatohepatitis, augmented γδT cells display a distinctly activated phenotype with reduced expression of Vγ1, CD27 and CD69, whereas the expression of programmed cell death-1, CD1d and CD36, increases. The recruitment of γδT cells, predominantly IL-17^{high} Ly6C^{-} CD44^{+} γδT cells, seems to occur in a CCR2-dependent manner in the liver with steatohepatitis, which results in promoting the influx of inflammatory monocytes and lessening IFN-γ production in CD4^{+} T cells [57]. Moreover, during the progression of NASH, hepatic γδT cells may play a related pathophysiological role by linking the innate and adaptive immune responses [58]. Nevertheless, the pathogenic role of hepatic γδT cells in MAFLD/NAFLD/NASH patients warrants additional research.

Mucosa-associated invariant T (MAIT) cells

MAIT cells represent the most abundant innate-like T cell population in the human liver, mainly activated by microbial riboflavin, which is presented in a major histocompatibility complex (MHC)- or cytokine-dependent manner. MAIT cells recognize vitamin B metabolites by the presentation with MHC class I-like molecule MR1, while MAIT cells, which are augmented in human livers, constitute up to 20–50% of all intrahepatic T cells, and are predominantly located in bile ducts in the portal tracts [59,60] (Fig. 1).

In patients with obesity, preferentially recruited MAIT cells are located in adipose tissue and exhibit a strong production of IL-17, which leads to a significantly reduced presence of MAIT in the peripheral blood. In patients with MAFLD/NAFLD/NASH-related cirrhosis, while the proportion of circulating MAIT cells is decreased, these cells display an activated phenotype; furthermore, the proportion of hepatic MAIT cells is also decreased, but these cells accumulate in the mesenchymal space within the fibrotic septa. The pro-fibrogenic functions of MAIT cells have been established in CCl_4-induced liver fibrosis, using MAIT cell-deficient mice and Vα19 TCR Tg-mice with a 10-fold increase in MAIT cells [61]. MAIT cells seem to enhance the fibrogenic functions of hepatic myofibroblasts by promoting their proliferation in an MR1-dependent manner and increasing their proinflammatory properties via the

production of TNF-α [61]. However, in a methionine- and choline-deficient diet-induced NASH model, MAIT cells are augmented in the liver and may protect against its inflammatory process via the production of IL-4 and IL-10, and the induction of anti-inflammatory M2 macrophages [62]. Therefore, such controversial data signify that the precise role of MAIT cells in the pathophysiology of NASH requires further illumination.

HSCs

HSCs, quiescent pericytes that reside in the perisinusoidal space of Disse (Fig. 1) and store vitamin A, participate in the synthesis of extracellular matrices and matrix-degrading metalloproteinases, and regulate sinusoidal circulation. Trans-differentiation of HSCs into proliferative or fibrogenic myofibroblasts is now well established as a central driver of hepatic fibrosis. Specifically, in response to liver injury, lipid peroxides and apoptotic bodies that accumulate in damaged hepatocytes initiate HSC activation in a process mediated by Fas and TNF-related apoptosis-inducing ligand [63]. This activation process is initiated by pro-fibrogenic cytokines (for example TGF-β), fibronectin, platelet-derived growth factor, ROS and apoptotic bodies derived from neighboring cells, immune cells and platelets. Subsequently, HSC undergo characteristic phenotypic changes and resemble myofibroblasts, leading to liver fibrosis**.** Downregulation of TGF-β pseudoreceptor BAMBI also occurs in HSCs by TLR4 ligation and activates TGF-β signaling, resulting in increased extracellular matrix production by HSCs, thereby leading to fibrosis. Related interesting data and the continued discovery of novel pathways and mediators, including autophagy, endoplasmic reticulum stress, oxidative stress, retinol and cholesterol metabolism, epigenetics and receptor-mediated signals, suggest the possible complex involvement of HSCs in MAFLD/NAFLD pathophysiology. In this regard, for instance, leptin and adiponectin, 2 of the most prominent adipokines connected with MetS and its related-MAFLD/NAFLD, interact with liver KCs/macrophages but also directly with HSCs. Levels of leptin are increased in MAFLD/NAFLD and are connected with disease severity; leptin aggravates hepatic inflammation and fibrosis in MAFLD/NAFLD [64]. Conversely, leptin acts via its receptor and appears to display anti-steatotic effects by promoting fatty acid oxidation and suppressing hepatic *de novo* lipogenesis [64]. Unlike leptin, adiponectin is reduced in human MAFLD/NAFLD, whereas high levels appear to be protective against obesity, MAFLD/NAFLD and NASH [65]; adiponectin reduces hepatic inflammatory and fibrotic processes by suppressing the proliferation and migration of activated HSC, among other effects [65].

MCs

MCs are a subset of immune cells first found to mediate allergic and anaphylactic reactions; they represent innate immune system effector cells playing a fundamental role in innate immune responses and modulating adaptive immunity [66]. These cells express multiple PRRs that allow them to respond to diverse stimuli and release several cytokines, such as TNF-α, TGF-β, and ILs. MCs can lead to microvesicular steatosis, ductal reaction, biliary senescence, inflammation, angiogenesis, and liver fibrosis during MAFLD/NAFLD/NASH [67] (Fig. 1). MC numbers are elevated in portal areas and fibrous septa during different categories of hepatic injuries and late stages of fibrosis, though more predominantly in NASH, thereby signifying their potential trigger role for progression of MAFLD/NAFLD to NASH with worsening phenotypes [68]. Upon activation, MCs may participate in the pathophysiology of fibrosis by releasing mediators; MCs contain many mediators and cytokines in their cytoplasmic granules, such as TGF-β, which is responsible for activation of HSCs to produce extracellular matrix components, as well as tryptase and chymase, which promotes collagen synthesis via fibroblasts and fibroblast growth [69]. A chymase inhibitor seems to efficiently ameliorate experimental NASH through the decrease of oxidative stress, inflammatory and fibrotic processes, thereby suggesting chymase as a potential therapeutic agent for improvement in NASH. Other data, however, suggest that MCs might play an antifibrotic role in liver disease by secreting HLA-G [70], which mediates the reduction of type I collagen; thus, inhibiting the activation of MCs may represent a possible therapeutic option for MAFLD/NAFLD/NASH [71]. Therefore, once again, further research is needed to elucidate this controversial topic in depth.

Complement system

The human complement system, an essential component of innate immunity, acts as an association between innate and adaptive immunity [72]. It is one of the first lines of defense in innate immunity, activated in a sequential manner via one of 3 major pathways (alternative, classical or lectin pathways), which converge at the activation of complement component 3 (C3). Several studies have shown that circulating complement components (C3 and its activation product C3a) are increased in MAFLD/NAFLD patients [73]. Moreover, the terminal complement system is activated in MAFLD/NAFLD/NASH patients, resulting in hepatic accumulation of membrane attack complex (MAC) [74]. It has been proposed that this complement activation is linked with hepatic immune cell infiltration and fibrogenesis. Specifically, this system is activated, among other stimuli, after liver damage, contributing to the development of NASH and HCC. The synthesis of C3 is involved in MAFLD/NAFLD pathogenesis. Likewise, C1q is involved in the development of HCC [75]. Additional data demonstrated an attenuation of liver MAC deposition, steatosis and inflammation in the absence of C5, thereby providing a further indication for a causal role of complement in NASH pathogenesis [76]. Beyond C3 and C5, other complement components are also linked with the risk and severity of MAFLD/NAFLD [77]. Therefore, the inhibition of a permanently activated complement system could attenuate the liver damage in MAFLD/NAFLD/NASH disease [78].

PRRs

PRRs family, including TLRs, nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), and other types of nucleic acid sensors [79], play a fundamental role in the initial stage of the innate immune response and the consequent activation of adaptive immunity (Table 2) [80]. The innate immune system provides an adequate response to pathogens and tolerance to harmless microflora via PRRs, located on the surface of cell membranes and within cells that recognize PAMPs. Another significant function of PRRs is the maintenance of homeostasis in stressful settings and the use of dead cells via the recognition of DAMPs of endogenous origin. In this respect, hepatocytes and liver sinusoidal endothelial

Table 2 Main pattern recognition receptors (PRRs) involved in MAFLD/NAFLD

PRRs	Origin	Mechanism involved / Endpoint effect
	Toll-like receptors (TLRs)	
TLR1	Hepatocytes, KCs, HSCs, LSECs, NKs, DCs	Induces upregulation of MHC class II, T-cell proliferation and IFN-γ production. Possible upregulation by plasma circulating bacterial toxins
TLR2	Hepatocytes (weak expression), KCs, HSCs, LSECs, NKs, DCs	Induces production of proinflammatory cytokines, IL-10 & IFN-γ and T-cell proliferation; TGF-β1 mediated TLR2 transcription promotes cell apoptosis
TLR3	Hepatocytes (weak expression), KCs, HSCs, LSECs, NKs, DCs	Induces production of proinflammatory cytokines & IFN-β and modulation of TLR4-induced liver injury
TLR4	Hepatocytes (weak expression), KCs, HSCs, LSECs, NKs, DCs	Induces production of proinflammatory cytokines, IFN-γ & MIP-2, T-cell proliferation and activation of downstream signaling of NF-κB, AP-1 & IRFs (upon prolonged LPS exposure)
TLR5	Hepatocytes (strong expression), HSCs, LSECs, NKs	Induces production of proinflammatory cytokines and protection against gut dysbiosis & diet-induced NAFLD
TLR6	Hepatocytes, KCs, HSCs, LSECs, NKs, DCs,peripheral monocytes & B-cells	Induces production of proinflammatory cytokines and activation of KC inflammasome; exhibits augmented inflammation by TLR2/TLR6 heterodimers
TLR9	Hepatocytes (weak expression), KCs, HSCs, LSECs, NKs, DCs	Induces production of proinflammatory cytokines and hypoadiponectinemia; exerts negative feedback on metabolic and autophagy signals; involved in DCs response to DAMPs through the production of the anti-inflammatory IL-10
	NOD-like receptors (NLRs)	
NOD1	Hepatocytes and innate immune cells	Induces hepatocyte activation, lymphocyte chemoattraction, IR, lipid accumulation and inflammation
NOD2	Hepatocytes and innate immune cells	Exhibits proinflamatory effects, synergistic with other TLRs; Possible regulatory role affecting immune cells infiltrating the liver
NLRP3	KCs, LSECs, periportal myofibroblasts and HSCs (moderately expressed)	Induces gut dysbiosis, increased gut permeability, increased sensitivity of hepatocytes to endotoxin, liver inflammation and fibrosis; mediates trained immunity following western diet; though exhibiting controversial conclusions based on genetic deletions of NLRP3 or its components
NLRP6	HSCs, innate immune cells	Induces profibrotic effect (HSCs); exhibits negative regulation of NAFLD/NASH progression and MetS via modulation of the gut microbiota
	Oligoadenylate Synthase (OAS)-like receptors (OLRs)	
OAS family & cGAMP synthase (cGAS)		Exhibits fatty acid-induced lipotoxicity and inflammation induced by DNA damage
	Advanced Glycation End-Products (AGEs) and Receptor of AGE (RAGE)	
Induces chronic inflammation in multiple types of hepatic cells; oxidative stress, hepatic steatosis, IR, and fibrosis		
	RIG-I like receptors (RLRs)	
RIG-I	Hepatocytes	Expression of RIG-I and the ratios of *RIG-I/Cardif* and *RIG-I/RNF125* genes are significantly higher in patients with steatosis than those without.
MDA5	Hepatocytes	Both experimental and human studies indicate that expression of MDA5 may be involved in the inflammation of NASH

MAFLD, metabolic (dysfunction)-associated fatty liver disease; AP-1, activator protein-1; DAMPs, damage-associated molecular patterns; DCs, dendritic cells; HSCs, hepatic stellate cells; IR, insulin resistance; IFN-γ, interferon-γ; IRFs, interferon regulatory factors; IL-10, interleukin-10; NF-κB, nuclear factor κ-light-chain-enhancer of activated B cells; KCs, Kupffer cells; LPS, lipopolysaccharides; LSECs, liver sinusoidal endothelial cells; NKs, natural killer T-cells; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; MDA5, melanoma differentiation-associated gene 5; MIP-2, macrophage inflammatory protein-2; MHC, major histocompatibility complex; MetS, metabolic syndrome; NLRP3/6, NOD-, LRR- and pyrin domain-containing protein 3/6; RIG-I, retinoic acid-inducible gene I; TGF-β1, transforming growth factor β1

cells, not formally innate immune cells, are capable of sensing PAMPs and DAMPs, as well as excessive metabolite levels, and triggering inflammatory events and metabolic dysfunction.

In the healthy mammalian liver, immune cells generally have a "resting" or tolerogenic phenotype. Nevertheless, during the transition to NASH, danger signals and proinflammatory mediators released by damaged and/or dying hepatocytes reshape the molecular signatures of resident immune and liver parenchymal cells towards proinflammatory phenotypes via PRR engagement [81,82]. This, combined with monocyte and neutrophil infiltration from the periphery, further augments liver dysfunction and fibrosis in MAFLD/NAFLD. Specifically, the TLR family is a significant and well characterized class of cell surface or intracellular PRRs, highly expressed in several liver cells under metabolic stress, such as KCs, HSCs, biliary epithelial cells and sinusoidal endothelial cells. Certain members of the TLR family (TLR2, TLR4, TLR5, TLR6, TLR7 and TLR9) are linked with MAFLD/NAFLD pathogenesis. In contrast, genetic deletion of TLR2, TLR4, or TLR9 (but not TLR5) in hepatocytes seems to resolve diet-induced hepatic inflammation and co-occurring oxidative stress, steatosis, and IR [83].

The TLR signal induces inflammatory processes in fat tissue and liver, activating transcription factors such as NF-κB and several proinflammatory cytokines. TLR4 is closely linked with MAFLD/NAFLD. It binds to the myeloid differentiation factor 2 protein, and this connection confers responsiveness to LPS. In this regard, animal models and clinical studies have observed augmented levels of circulating LPS in MAFLD/NAFLD, due to the intrinsic endotoxemia induced by factors including gut microbiota, gut permeability, and high fat and/or sugar diet [84]. Moreover, the TLR4-LPS pathway is involved in the progression of MAFLD/NAFLD to HCC. Considering that TLR2 recognizes peptidoglycan, a gram-positive bacterial component, its blockade in animal models exhibits a protective effect in developing IR, strongly linked with MAFLD pathogenesis. Some data indicate that mice with TLR2 deficiency express lower levels of proinflammatory cytokines by unchaining the inflammasome in KCs. TLR2 frequently forms heterodimers with TLR6, another extracellular receptor elevated in hepatocytes of NASH patients. This heterodimer also occurs in lobular inflammation. Thus, TLR6 is proposed as a potential biomarker in the development of NASH in MAFLD/NAFLD obese patients [85]. Likewise, TLR7 is associated with liver fibrosis, though its role in MAFLD/NAFLD has not been entirely clarified. Finally, TLR9 might be the only intracellular receptor involved in MAFLD/NAFLD pathogenesis. Related experimental data indicate that its activation leads to IL-1β production; thus, along with TLR2 and TLR4, it is intensely involved in NASH and the liver's fibrotic process.

NLRs can induce inflammasomes and result in cell death. NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) is an intracellular multi-protein complex consisting of the cytosolic sensor NLR, the adapter ASC (apoptosis-associated speck-like protein containing a C-terminal caspase recruitment domain) and the effector procaspase-1. This effector mediates the processing of caspase-1 and finally results in the release of mature IL-1β, which plays an important role in the innate immune system, triggering several inflammatory components. NLRP3 inflammasome links lipid sensing with the induction of inflammation, and is therefore central in the pathogenesis of obesity-related disorders. The NLRP3 inflammasome has been shown to sense diverse DAMPs, including adenosine triphosphate, uric acid, necrotic cells and saturated fatty acids [86]. In this respect, oral administration of sulforaphane, a specific NLRP3 inhibitor, improves hepatic steatosis after an HFD, and this effect was accompanied by inhibition of saturated fatty acid-induced activation of the NLRP3 inflammasome [87]. The genetic ablation or inhibition of NLRP3 inflammasome decreases hepatocyte pyroptosis and, in consequence, inflammatory and fibrotic processes in animal models with NASH. In contrast, certain studies in humans and murine models deficient in NLRP3, caspase 1, IL-1β and IL-18, demonstrated enhanced liver damage and accelerated progression to NASH, caused by an increased influx of TLR4/TLR9 agonists through the portal circulation, altering intestinal microbiota and resulting in hepatic TNF-α expression [13]. Moreover, hypofunctional NLRP3 mutations dysregulate β-defensin expression, thereby impairing tissue bactericidal activity and causing dramatic alterations in intestinal microbiota, with potential detrimental effects on hepatic homeostasis. The NLRP3 inflammasome pathway was found to have a principal role in the induction of myeloid cell reprogramming (trained immunity), thus creating a sustained intracellular stress state that, in the context of a western diet, is able to instigate liver pathologies [88]. The aforementioned contradictory effects of NLRP3 warrant additional clarification.

Apart from TLRs and NLRs, retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs), including melanoma differentiation-associated protein 5 (MDA5) [79], might also be involved in MAFLD/NAFLD pathophysiology. Although currently controversial, recent data indicate that MDA5 could be involved in inflammation in patients with NASH [89]. Thus, RIG-I activity might be a strategy to treat MAFLD/NAFLD.

Cytokines and other immune cell inflammatory factors

Related experimental data indicate the involvement of several cytokines in MAFLD/NAFLD, including IL-1β, IL-6, TNF-α and/or IFN-α. These data are further supported by clinical studies, though some controversy is still present. IL-1β and IL-6 concentrations are significantly higher in patients with NASH compared with simple fatty liver and control arms [90]. Likewise, serum IL-6 concentrations are higher in patients with advanced than in patients with mild or no fibrosis [91]. Moreover, TNF mRNA expression is augmented in the hepatic and adipose tissue of NASH patients; in MAFLD/NAFLD patients, TNF-α displays a strong correlation with transaminase concentrations and histological severity, and has been proposed as a biomarker of disease progression [92]. Nevertheless, early studies in humans investigating TNF blockade as a therapeutic target in metabolic diseases did not demonstrate any beneficial effect. However, those trials were not well conducted and the clinical design had some problems, such as dosing, duration or presence of confounding factors.

Recent data indicate that levels of circulating proinflammatory cytokines are variable in MAFLD/NAFLD patients with or without obesity, but when patients are distributed according to the occurrence of circulating bacterial antigens, significantly higher serum TNF-α and IL-6 levels are observed in MAFLD/NAFLD patients [93].

Finally, several nuclear transcription factors and certain intracellular signaling pathways are implicated in MAFLD/NAFLD pathophysiology. In this respect, NF-κB and c-Jun N-terminal kinase are particularly remarkable in NASH proinflammatory pathways [94]. In MAFLD/NAFLD, main molecules in the non-canonical NF-κB signaling pathway are abnormally elevated. NF-κB is activated by TLRs and induces the transduction of IL-1β, IL-2, IL-6 and TNF-α [95]. In addition, JNK overactivation is strongly involved in the development of MAFLD/NAFLD [96]; thus, modulating JNK activation might be a therapeutic approach for the treatment of MAFLD/NAFLD. Furthermore, the activation of these pathways connects MAFLD/NAFLD with extra-hepatic comorbidities, such as the MetS-related cardio-cerebrovascular outcomes [1,97,98].

Concluding remarks

A vast network of innate immune system components can be mobilized by various endogenous agents that induce systemic inflammation and thus drive MAFLD/NAFLD progression. Metabolic dysregulation appears as a *sine qua non* event in the pathophysiology of early MAFLD/NAFLD stages, whereas lipotoxicity emerges as a detrimental driver of liver inflammation and fibrosis. Collective evidence highlights multiple interactions between diet-induced metabolic pathways, gut microbiome alterations, which serve as a pool of metabolites and inflammatory signals in patients with or without a "leaky" gut, and innate immunity in experimental and human MAFLD/NAFLD. This complexity of the MAFLD/NAFLD pathogenesis puzzle does not permit the portrayal of mechanisms individually, given their often dual protective and pathological properties. Gaining new insights into the role of innate immunity in MAFLD/NAFLD will enhance our understanding of disease pathogenesis, enable the identification of biomarkers able to select patients at risk, and distinguish those more likely to progress to cirrhosis, HCC and/or extrahepatic complications. Utilizing quantitative and molecular phenotypic changes in peripheral innate immune cells among MAFLD/NAFLD patients as a diagnostic tool to determine NASH progression and patient stratification appears as a promising noninvasive approach [99]. Innate immunity can be therapeutically manipulated, at the level of epigenetic modifiers, cellular metabolism and signaling pathways. An increasing number of inhibitors suppressing different stages of the innate immune response, such as ligand recognition or dimerization of TLR/NLR receptors, signal transduction molecules, and terminal proinflammatory mediators (including TNF, IL-6R and IL-1β), have been studied, some of which have already been applied for the treatment of autoimmune diseases [100]. However, further mechanistic studies of innate immunity regulation are necessary to address the efficacy, safety and cost-effectiveness in depth, and thus provide the rationale for novel innate immunomodulatory therapeutic strategies against MAFLD/NAFLD, which represents a growing global public health problem.

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