Macrophages in obesity and non-alcoholic fatty liver disease: Crosstalk with metabolism

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Summary

Non-alcoholic fatty liver disease (NAFLD) is the most prevalent liver disease worldwide, and a major cause of liver cirrhosis and hepatocellular carcinoma. NAFLD is intimately linked with other metabolic disorders characterized by insulin resistance. Metabolic diseases are driven by chronic inflammatory processes, in which macrophages perform essential roles. The polarization status of macrophages is itself influenced by metabolic stimuli such as fatty acids, which in turn affect the progression of metabolic dysfunction at multiple disease stages and in various tissues. For instance, adipose tissue macrophages respond to obesity, adipocyte stress and dietary factors by a specific metabolic and inflammatory programme that stimulates disease progression locally and in the liver. Kupffer cells and monocyte-derived macrophages represent ontologically distinct hepatic macrophage populations that perform a range of metabolic functions. These macrophages integrate signals from the gut-liver axis (related to dysbiosis, reduced intestinal barrier integrity, endotoxemia), from overnutrition, from systemic low-grade inflammation and from the local environment of a steatotic liver. This makes them central players in the progression of NAFLD to steatohepatitis (non-alcoholic steatohepatitis or NASH) and fibrosis. Moreover, the particular involvement of Kupffer cells in lipid metabolism, as well as the inflammatory activation of hepatic macrophages, may pathogenically link NAFLD/NASH and cardiovascular disease. In this review, we highlight the polarization, classification and function of macrophage subsets and their interaction with metabolic cues in the pathophysiology of obesity and NAFLD. Evidence from animal and clinical studies suggests that macrophage targeting may improve the course of NAFLD and related metabolic disorders.

Introduction

In parallel to the obesity pandemic, non-alcoholic fatty liver disease (NAFLD) has become the most common cause of chronic liver disease worldwide.[1,2] NAFLD, particularly in its inflammatory form of non-alcoholic steatohepatitis (NASH), can progress to fibrosis, cirrhosis and NASH-induced hepatocellular carcinoma. The prevalence of NAFLD is projected to increase further over the next 10–15 years, with a higher proportion of patients developing fibrosis,[3] the main determinant of both liver-related and overall mortality.[4,5] Tremendous advances in basic and translational research have provided insights into the pathogenic mechanisms driving the progression of NAFLD, in which inflammatory processes that are controlled by macrophages, a key component of innate immunity, play a major role.[6] Historically, NAFLD has been viewed as a consequence of insulin resistance and the metabolic syndrome.[7] In particular, depending on selection criteria, the prevalence of NAFLD in type 2 diabetic patients ranges from 30 to almost 100%.[8–11] Nevertheless, NAFLD is not only a manifestation of, but can also precede the development of other components of the metabolic syndrome, which has sparked a debate over the direction of the causal relations involved.[12] Indeed, insulin resistance and ectopic fat accumulation interconnect NAFLD with other metabolic disorders such as obesity, type 2 diabetes mellitus and atherosclerosis. While numerous pathways are involved in this pathophysiological process, chronic inflammation and macrophages have been implicated as drivers of disease at multiple stages and in various tissues during the development of metabolic disease.[13]

Metabolic inflammation is critically different from the paradigmatic acute inflammation seen in bacterial infections, where a strong immune response is followed by elimination of the pathogen and rapid resolution to baseline (if successful). Instead, metabolic inflammation is characterized by a persistent, low-grade, sterile inflammation, often termed metaflammation. From this perspective, inflammatory mediators can be thought of as metabolic hormones that regulate insulin signalling.[14]

In the last decades, macrophages have emerged as key regulators of inflammation-mediated insulin resistance.[15] The functional diversity of macrophages is reflected by the concept of "inflammation-promoting M1 macrophages" and "inflammation-suppressing M2 macrophages". Beyond this rigid (and over-simplifying) M1 *vs.* M2

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paradigm, macrophages can adapt and react to a wide variety of stimuli, including metabolic signals.[16] Upon stimulation, macrophages undergo a complex and context-dependent metabolic reprogramming that determines their polarization status. These findings have led to the emergence of immunometabolism as a research field, which investigates the crosstalk between metabolism and immune cells.[17,18]

Further progress in our understanding of the functional and tissue-specific heterogeneity of macrophages, as well as their crosstalk with metabolism could direct the development of therapies for metabolic disorders. In this review, we describe the relationship between inflammation (with a focus on macrophages) and insulin resistance, as well as the regulation of macrophage activation by metabolic processes. We elaborate on their contribution to the initiation and progression of fatty liver disease, highlighting putative macrophage-mediated mechanisms that link NAFLD and cardiovascular disease, and discussing the potential of macrophages as therapeutic targets in fatty liver disease.

Inflammation, macrophages and insulin resistance

Overnutrition leads to a positive energy balance and the accumulation of fat in the adipose tissue, which evokes an immune response that evolutionarily constitutes a physiological attempt to restore homeostasis. Nevertheless, in the long term

> **Key points**
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> Macrophages play a key role in the chronic inflammatory processes that drive NAFLD and other metabolic diseases.
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> Crosstalk between macrophages and metabolic stimuli is crucial to the development of metabolic dysfunction, inflammation and disease progression in NAFLD.
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> Kupffer cells and monocyte-derived macrophages integrate signals from the gut-liver axis, overnutrition, systemic low-grade inflammation and steatosis, driving the progression of NAFLD to NASH and fibrosis.
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> Increasing evidence from both animal and clinical studies suggests that macrophage targeting may be an effective therapeutic strategy for NAFLD and related metabolic disorders.

this response is maladaptive and leads to insulin resistance and the loss of metabolic flexibility.[19]

Initial evidence for the link between obesity, inflammation and insulin resistance was provided by seminal studies showing increased expression and production of tumor necrosis factor-α (TNF-α) in the adipose tissue of obese rodents and patients.[20,21] TNF-α was subsequently demonstrated to derive mainly from adipose tissue macrophages (ATMs). These cells represent the predominant immune population in the adipose tissue, accumulate further in obesity and are to a large extent responsible for perpetuating adipose tissue inflammation[22,23] (Fig. 1).

The inflammatory process in obesity is affected through a plethora of cytokines, receptors and signalling molecules. Apart from direct binding of cytokines to their corresponding receptors, as in the case of TNF-α, inflammation is often initiated
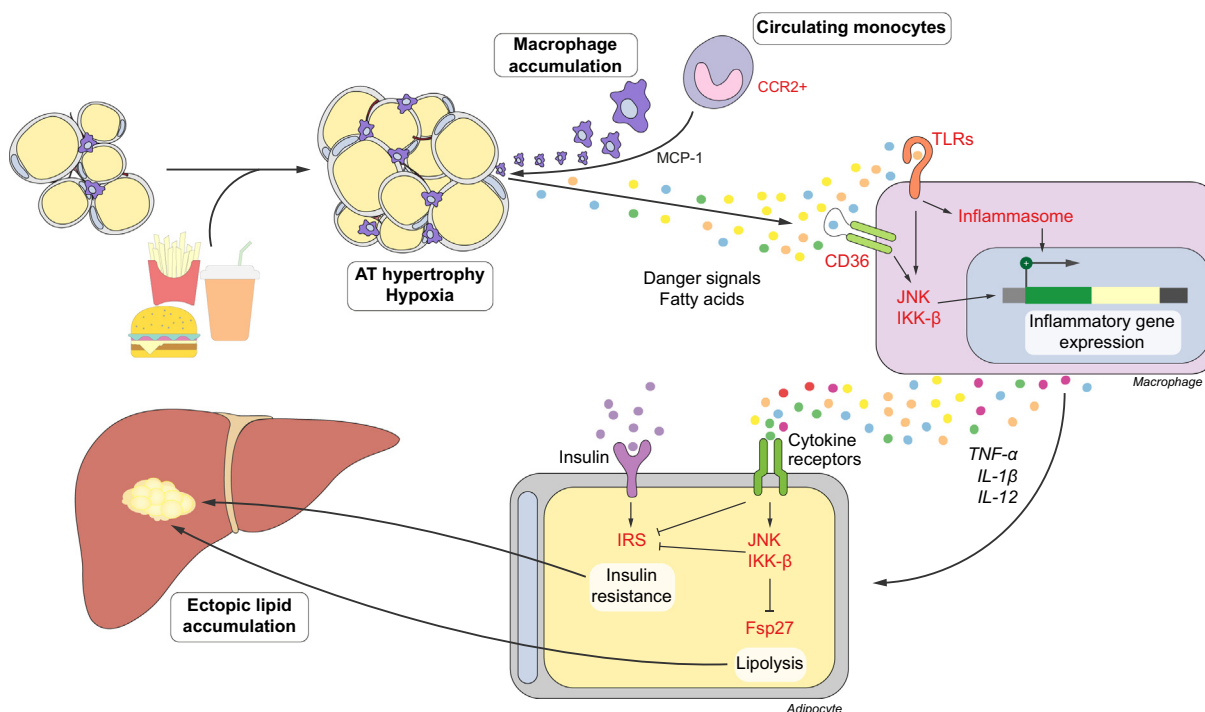

Fig. 1. Macrophages in obese adipose tissue. In obesity, chemokines stimulate the infiltration of macrophages into the AT. Various danger molecules and fatty acids promote a pro-inflammatory phenotype in ATMs, which contributes to the development of a persistent, low-grade inflammation. Pro-inflammatory mediators secreted by ATMs induce insulin resistance by signalling through the JNK and IKK-β pathways. Inhibition of lipid droplet-associated peptides, such as fsp27, enhance lipolysis. Downstream, insulin resistance and lipolysis lead to ectopic lipid accumulation in various organs, including the liver. AT, adipose tissue; ATMs, adipose tissue macrophages; IRS, insulin receptor substrates; TLRs, Toll-like receptors.

by activation of pattern recognition receptors, such as Toll-like receptors (TLRs), of which TLR4 is the most well-studied in this context.[24] Furthermore, adipose tissue expansion in obesity often exceeds the compensatory increase in oxygen delivery through angiogenesis, causing adipose tissue hypoxia and adipocyte cell death.[25] Dying cells release inflammatory cytokines and danger signals, which aggravate inflammation and activate ATMs. These various mediators then converge on inflammatory signalling pathways such as the activation of c-jun N-terminal kinase (JNK) and IκB kinase β (IKK-β). These pathways directly affect insulin signalling in adipocytes by phosphorylating the insulin receptor and insulin receptor substrates, and indirectly by modulating metabolic genes and altering energy homeostasis.[26,27]

This link between inflammation and metabolism is remarkably conserved between organisms. Eiger, the drosophila TNF-α orthologue, is produced by the specialized fat body and binds on its receptors Wengen and Grindelwald to suppress insulin action through JNK and IKK-β pathways.[28,29] Notably, knockdown of Grindelwald in the fat body reversed the insulin resistance induced by a high-sugar diet in these flies.[28]

Multiple macrophage-targeting approaches have underscored their importance in the development of insulin resistance.[15] For instance, knocking out JNK, IKK-β or TLR4 in macrophages or hematopoietic cells improved glucose tolerance and adipose tissue inflammation in mice.[24,30,31] These approaches simultaneously ameliorated hepatic inflammation and/or liver steatosis, corroborating the role of macrophages and adipose tissue inflammation in the progression of NAFLD. Furthermore, inhibiting ATM infiltration has yielded promising results. Deleting the chemokine macrophage chemoattractant protein-1 (MCP-1), also known as CCL2, strongly reduced insulin resistance,[32] and inhibition of its receptor CCR2 in diet-induced obesity improved glucose tolerance as well.[33–35] Similar results were obtained upon inhibition of other effectors of macrophage recruitment, such as the CCL5-CCR5 axis.[36,37] Accordingly, ablation of pro-inflammatory CD11c+ macrophages[38] or clodronate-mediated depletion of ATMs[39,40] improved glucose tolerance in obese mice.

Metabolic reprogramming coordinates macrophage polarization

The rapid response of macrophages to infection and inflammation is an energy-intensive process. Macrophages therefore undergo a reprogramming of their own metabolism upon stimulation. This metabolic switch is context-dependent and influences the outcome of the inflammatory response, which also holds true in metabolic disease. The relevance of these metabolic adaptations of macrophages is corroborated by the fact that classical M1 or alternatively activated M2 macrophages show striking differences in their cellular metabolism.

Early studies already indicated that classical M1 macrophages, differentiated *in vitro* by lipopolysaccharide (LPS) or interferon gamma (IFNγ) stimulation, rely on aerobic glycolysis to satisfy the increased need for ATP and metabolic precursors.[41] More recent reports have revealed a much more complex interplay between metabolic programming and polarization. Enhanced flux through the pentose phosphate pathway further secures building blocks for biosynthesis, while mitochondrial oxidative phosphorylation and fatty acid oxidation are repressed in LPS-stimulated macrophages[42–44] (Fig. 2A). This rewiring is in essence identical to the Warburg effect observed in proliferating tumour cells almost a century ago.[45]

Hypoxia-inducible factor (HIF)-1α is a master regulator of glycolysis in macrophages. Besides linking hypoxia with macrophage activation, HIF-1α is also activated through hypoxia-independent mechanisms.[46] For instance, the metabolic rewiring induced by LPS and/or IFNγ generates elevated levels of the citric acid cycle intermediate succinate. Succinate inhibits the oxygen-sensing prolyl hydroxylases, leading to HIF-1α stabilization and a state of pseudo-hypoxia.[43,47] Downstream, HIF-1α increases the expression of the glucose transporters GLUT1 and GLUT3, and stimulates the conversion of pyruvate to and subsequent secretion of lactate.[48,49] The enzyme pyruvate dehydrogenase kinase, the expression of which is also regulated by HIF-1α, channels glucose metabolism away from oxidative phosphorylation towards glycolysis.[42,50] Notably, inhibition of either glycolysis or HIF-1α signalling suppresses pro-inflammatory gene production and impairs bacterial killing by macrophages, demonstrating the functional importance of metabolic rewiring.[43,46,51,52]

In line with the hypoxia-induced adipose tissue dysfunction during obesity,[25] ATM HIF-1α is involved in the development of insulin resistance. Deletion of myeloid HIF-1α in obese mice reduced adipose tissue inflammation and restored glucose tolerance. Paradoxically, macrophage HIF-1α suppressed adipose tissue angiogenesis, which in turn stimulated hypoxia in a vicious circle.[53] Furthermore, macrophage HIF-1α was increased in mice and patients with NASH, inducing pro-inflammatory cytokines and aggravating hepatic steatosis and inflammation.[54] In contrast, macrophage HIF-2α was reported to ameliorate insulin resistance and adipose tissue inflammation through induction of M2 polarization.[55]

Alternatively activated M2 macrophages, obtained *in vitro* after interleukin (IL)-4 stimulation, rely on mitochondrial fatty acid oxidation instead[56,57] (Fig. 2B). The lysosomal breakdown of exogenous triacylglycerol substrates is the main fatty acid source supporting this metabolic programme, with *de novo* fatty acid synthesis as a complementary pathway.[56] Importantly, inhibition of fatty acid oxidation has been shown to suppress M2 polarization.[56,57] Moreover, *in vitro* LPS+IFNγ-stimulated macrophages inhibit fatty acid phosphorylation, which confers resistance to
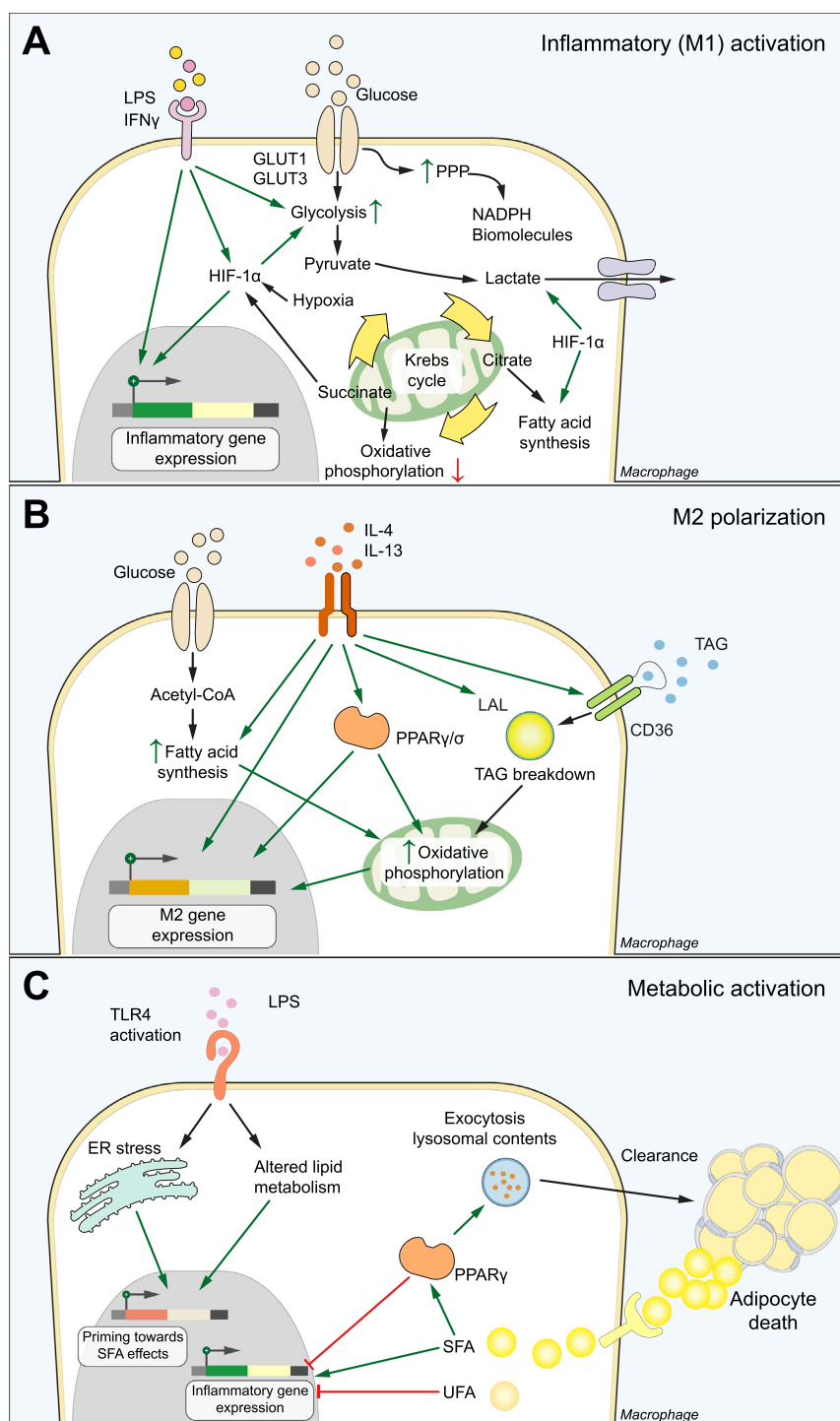
Fig. 2. Metabolic coordination of macrophage activation. (A) Inflammatory (M1) activation. LPS and/or IFNγ stimulation increases glucose consumption, which is converted to lactate instead of entering the Krebs cycle as pyruvate. The pentose phosphate pathway and fatty acid synthesis are activated to generate biomolecules. These metabolic alterations depend in part on HIF-1α, which stimulates glucose entry and conversion to lactate and directly promotes M1 gene expression. (B) M2 polarization depends on increased β-oxidation and oxidative phosphorylation of fatty acids. This is fuelled by the uptake and lysosomal breakdown of TAGs and the *de novo* synthesis from glucose. PPARγ and PPARδ bind these intracellular lipids and are key effectors of the metabolic and phenotype switch upon IL-4 stimulation. (C) Macrophages display a metabolically activated phenotype upon stimulation with SFAs. In the adipose tissue, one mechanism of SFA uptake is through the digestion of dead adipocytes at crown-like structures. Binding of LPS primes macrophages towards the pro-inflammatory effects of SFAs, leading to a distinct macrophage phenotype that secretes inflammatory mediators such as IL-1β. PPARγ has a dual role in stimulating the clearance and breakdown of dead adipocytes, and inhibiting the production of inflammatory cytokines. ER, endoplasmic reticulum; LPS, lipopolysaccharide; PPP, pentose phosphate pathway; SFA, saturated fatty acid; TAGs, triacylglycerides; UFA, unsaturated fatty acid.

M2 repolarization,[44] implying macrophage metabolism at the centre of their functional plasticity. Accordingly, transcription factors that modulate lipid metabolism, such as peroxisome proliferator-activated receptors (PPARs), are key effector molecules regulating this switch.

PPARs, members of the nuclear receptor superfamily, act as fatty acid sensors and modulate lipid transport, storage and expenditure. PPARα is mainly expressed in metabolically active tissues such as the liver, skeletal muscle and brown adipose tissue.[58] In addition to their roles in other cells, including adipocytes, PPARγ and PPARβ/δ are expressed in macrophages in a stimulus- and tissue-dependent manner.[58,59] Although PPARγ but not PPARδ was required for the metabolic switch upon stimulation with IL-4,[60] ablation of either isoform has been shown to impair IL-4-stimulated alternative macrophage activation.[60–62]

These findings are of translational significance in the context of obesity and NAFLD. Both adipose tissue and hepatic macrophages displayed an impaired M2 polarization upon PPARγ or PPARδ deletion in a high-fat diet mouse model, resulting in insulin resistance and hepatic steatosis.[60–63] Furthermore, the response to pioglitazone and rosiglitazone, PPARγ agonists with well-known therapeutic effects on insulin resistance and NAFLD, was impaired following macrophage PPARγ deletion.[61,63]

Fatty acids direct adipose tissue macrophage polarization in obesity

Although most data on the interplay between macrophage metabolism and polarization have been obtained in M1 and M2 polarized macrophages, this classical paradigm is too simplistic and cannot fully describe the many distinct phenotypes observed in tissue macrophages.[64] Among other stimulants, metabolites can induce unique activation states.[16] One exemplar characterized in recent years is the effect of fatty acids on macrophage polarization. The plasticity of macrophages in tissues such as the liver or fat makes it difficult to accurately capture their ontogeny, polarization and function by characteristic markers, although some phenotypic features have been consistently reported in mouse models (Table 1).

In the healthy adipose tissue, ATMs display an M2-like phenotype and produce anti-inflammatory cytokines such as IL-10. The development of obesity leads to a shift in polarization, which has classically been described as a pro-inflammatory M1 polarization that contributes to metabolic dysregulation.[65,66] However, an unbiased transcriptomic analysis could not confirm a typical M1 signature in obese adipose tissue.[67] Specifically, ATMs from obese mice or humans were shown to display a 'metabolically activated' (MMe) phenotype that is distinct from M1 or M2 macrophages (Fig. 2C). This phenotypic switch can be reproduced in vitro using saturated fatty acids (SFAs) such as palmitate.[68] Genes involved in lipid metabolism and lysosome biogenesis, such as perilipin-2 (Plin2) and lysosome-associated membrane protein 2 (Lamp2), were found to be significantly upregulated in these macrophages.[67,68] Glycolysis and oxidative phosphorylation are similarly activated after stimulation with SFAs[69], indicating a unique metabolic signature in MMe macrophages.

This metabolic reprogramming of ATMs is closely linked to the increased production of pro-inflammatory cytokines in obesity.[69,70] SFAs promote the expression of inflammatory cytokines and chemokines such as IL-1β, IL-6 and MCP-1, whereas unsaturated fatty acids counteract these effects.[71–73] PPARγ promotes lipid metabolism while negatively regulating pro-inflammatory gene expression in MMe macrophages as well,[68] suggesting the possibility of uncoupling these key functions as a strategy to reduce insulin resistance.

One source of SFA delivery to macrophages is through digestion of dead adipocytes.[74] Because adipocytes are generally much larger than macrophages, classical phagocytosis is not possible. Instead, ATMs form hydrolytic synapses with dead adipocytes, in which they secrete lysosomal contents, mostly at sites known as crown-like structures.[74] The resulting uptake of fatty acids has been shown to reproduce the metabolically activated ATM phenotype.[70] Interestingly, these data have been validated using a single-cell sequencing approach. Hill et al. were able to distinguish between different ATM populations in high-fat diet-induced obesity. CD9+ ATMs resided within crown-like structures, were lipid-laden and upregulated lysosomal metabolism and inflammatory genes. Conversely, Ly6C+ ATMs were dispersed throughout the adipose tissue and were phenotypically distinct, expressing genes involved in tissue organization and angiogenesis.[75]

Table 1. Selected markers in mouse macrophage subsets.

M1 macrophages	M2 macrophages	MMe ATMs	Kupffer cells	Liver MoMFs
CD80	Mannose receptor (CD206)	ABCA1	F4/80^{high}	CD11b^{high}
CD11c	CD301	PLIN2	CD11b^{int}	F4/80$^{int/high}$
Inducible NO synthase	IL-4 receptor (CD124)	LAMP2	Tim4$^+$	Ly6C$^{+/-}$
TNF-α	Arginase 1	NPC1	Clec4f$^+$	CCR2$^+$
S100A8/A9 (calprotectin)			CD68$^+$	CX$_3$CR1$^{+/-}$

Expression of the macrophage polarization markers is context-dependent and these should ideally be used in combination. MMe ATMs: metabolically activated adipose tissue macrophages; MoMFs, monocyte-derived macrophages.

Further evidence for the importance of cellular metabolism in directing macrophage polarization by fatty acids comes from a recent study showing that, contrary to the accepted paradigm, SFAs do not directly activate TLR4. Intriguingly however, TLR4 activation by other signals resulted in an altered intracellular lipid composition, which was necessary for SFA-induced inflammation.[76] In the context of obesity, SFAs may thus act as a 'second hit' in macrophages primed towards a pro-inflammatory response.

Adipose tissue macrophages fuel hepatic lipid accumulation

Insulin resistance in adipose tissue and the subsequent inability to store the surplus energy as fatty acids lead to ectopic lipid accumulation. Given the impact of ATMs on insulin resistance, it is not surprising that many of the macrophage-targeting approaches to counteract insulin resistance cited in previous sections, such as inhibition of macrophage infiltration or of pro-inflammatory signalling, also ameliorated hepatic lipid accumulation.[15] Beside their promotion of insulin resistance, pro-inflammatory macrophages also directly stimulate lipolysis, which is key in increasing lipid flux to the skeletal muscle and liver.[77] Mechanistically, pro-inflammatory cytokines secreted by ATMs down-regulate lipid droplet-associated peptides, thereby decreasing lipid droplet stability and permitting lipolysis.[78,79] Indeed, adipose triglyceride lipase inhibition was shown to simultaneously reduce insulin resistance and hepatic steatosis.[80] In mice, transplanting visceral adipose tissue from obese mice increased hepatic macrophage accumulation and worsened steatohepatitis, compared with adipose tissue transplanted from lean mice.[81] This effect was dependent on CD11c^{+} inflammatory ATMs that secrete manifold cytokines including chemotactic signals for neutrophils and macrophages.[81]

Furthermore, inflammation can interfere with beige adipogenesis, a process critical for energy dissipation through thermogenesis and prevention of ectopic lipid accumulation.[82] Activated ATMs were recently shown to express $\alpha 4$ integrin, which can bind vascular cell adhesion molecule-1 (VCAM-1) on adipocytes. These adhesive interactions down-regulate uncoupling protein-1, a key mediator of adipose tissue thermogenesis, whereas genetic or pharmacological $\alpha 4$ inhibition reduced macrophage retention and improved insulin sensitivity. Adipocyte VCAM-1 induction by ATMs and, conversely, ATM TNF-α induction by VCAM-1 binding constitutes a self-sustaining pro-inflammatory loop.[83] The upregulation of VCAM-1 expression in the adipose tissue of patients with NAFLD and fibrosis compared to those without[84] suggests this mechanism may contribute to the link between obesity, ATM activation and NAFLD progression.

The importance of ATMs in NAFLD was further corroborated in humans, as both the adipose tissue expression of pro-inflammatory genes as well as the number of ATMs were associated with the progression of NAFLD to NASH and fibrosis.[85,86]

Hepatic macrophage subsets play distinct roles in metabolism

The liver contains the largest proportion of macrophages in any solid organ.[87] These hepatic macrophages are highly heterogeneous with respect to cellular origin, functionality and interaction with other liver cells. Specifically, the resident Kupffer cells (KCs) can be distinguished from bone marrow-derived monocytes, which receive cues from the local micro-environment that can prompt their differentiation into infiltrating macrophages (Table 1). KCs are self-renewing macrophages that line the sinusoidal endothelium and scavenge cellular debris, pathogens and gut-derived products. In the healthy liver, they promote tolerance towards these potential particulate antigens, in part via an expansion of regulatory T-cells.[88] KCs originate from yolk-sac derived progenitor cells, which then give rise to archetypical pre-macrophages that colonize the embryo during early organogenesis and activate tissue-specific transcriptional programmes, in this case under the control of inhibitor of DNA binding 3 (ID3).[89,90]

Under homeostatic conditions, monocyte-derived macrophages are present in relatively low numbers, where they fulfil metabolic functions.[87] In reaction to any type of liver injury, however, hepatocytes as well as non-parenchymal cells secrete chemokines, which can induce a massive infiltration of monocytes, which, in mice, express Ly6C. The phenotype of these macrophages is again highly plastic, with classical pro-inflammatory and restorative macrophages at the edges of a continuous spectrum.

Whereas KCs do not derive from monocytes in the steady state, several reports have now shown that upon their experimental depletion, monocytes can differentiate into bona fide KCs (moKCs), repopulating the now available KC niche.[91,92] Notably, a similar process occurs in experimental NASH, although these moKCS appeared to be rather short-lived after recovery,[93] and it is unclear at this point if they are capable of self-renewal.

While these subsets have been difficult to distinguish *in vivo*, transcriptomic approaches have recently enabled the identification of selective markers. Mouse KCs are characterized by C-type lectin domain family 4 member F (Clec4f) and T-cell immunoglobulin and mucin domain containing 4 (Tim4) surface expression.[92,93] Infiltrating macrophage marker expression is correlated with their polarization status, with pro-inflammatory monocyte-derived macrophages typically positive for CCR2, while more restorative macrophages upregulate CX$_3$CR1.[94] Although these subsets have not been characterized to the same extent in humans, markers such as CD14 and CD68 have been proposed to distinguish these populations, and this remains an area of active investigation.[95] For instance, a recent report using single-cell RNA sequencing of healthy human liver cells identified 2 CD68+ macrophage populations, of which one displayed features of an inflammatory phenotype (Lysozyme, S100A8/A9, CD74), while the other

population may represent a more regulatory subset (Marco, CD5L, CD163).[96]

In keeping with their specialized scavenging capacity, KCs perform accessory roles in lipid and iron metabolism.[97] Although lipid handling is a function of macrophages in general, the KC expression profile is particularly enriched for these genes compared to other tissue-resident macrophages.[92] The liver X receptor α (LXRα), a nuclear receptor critical for lipid metabolism, is differentially expressed by KCs early on in development.[90] LXRα seems essential for the maintenance of KC identity, as targeted deletion leads to a selective loss of KCs and their replacement by moKCs.[98] In macrophages, LXRα regulates cholesterol efflux and reverse cholesterol transport from peripheral tissues to the liver via high-density lipoprotein (HDL) particles. Moreover, LXRα provides another link between macrophage metabolism and inflammation, as its activation reduces the production of pro-inflammatory mediators.[99,100] LXRα modulation to stimulate lipid export from vessels is therefore an appealing pharmacological target in cardiovascular and metabolic disease. However, early LXRα agonists simultaneously stimulated fatty acid synthesis, resulting in hepatic steatosis and hypercholesterolemia.[101] New LXRα agonists have recently been developed that do not exhibit these side effects. Interestingly, they achieve this in part by selectively activating LXRα in macrophages, including KCs, while having almost no biological effect on hepatocytes, in contrast to the older compounds.[102]

In addition, the liver plays a major role in whole-body iron metabolism. KCs are well-positioned for this task and express multiple genes involved in the uptake and processing of iron under homeostatic conditions.[92,103] In circumstances of increased iron delivery however, such as haemolytic anaemia, monocyte-derived macrophages infiltrate the liver and differentiate to express ferroportin-1. These macrophages protect against the deleterious effects of iron deposition in both the liver and other iron-sensitive organs such as the kidney.[104] Of note, NALFD and insulin resistance are associated with serum and hepatic iron overload in a subset of patients,[105] which has been termed the dysmetabolic iron overload syndrome. Studies have suggested that iron accumulation specifically in macrophages correlates with increased cell death and fibrosis in NAFLD, possibly mediated through oxidative stress,[106,107] although conflicting data exist.[108]

Inflammatory macrophages drive non-alcoholic fatty liver disease progression

Although KCs induce tolerogenic responses under homeostatic conditions, they express multiple pattern recognition receptors and are capable of immune stimulation upon infectious or sterile liver injury[88,95] (Fig. 3). KCs of mice fed a high-fat diet accumulated toxic lipids, resulting in altered expression of genes involved in lipid
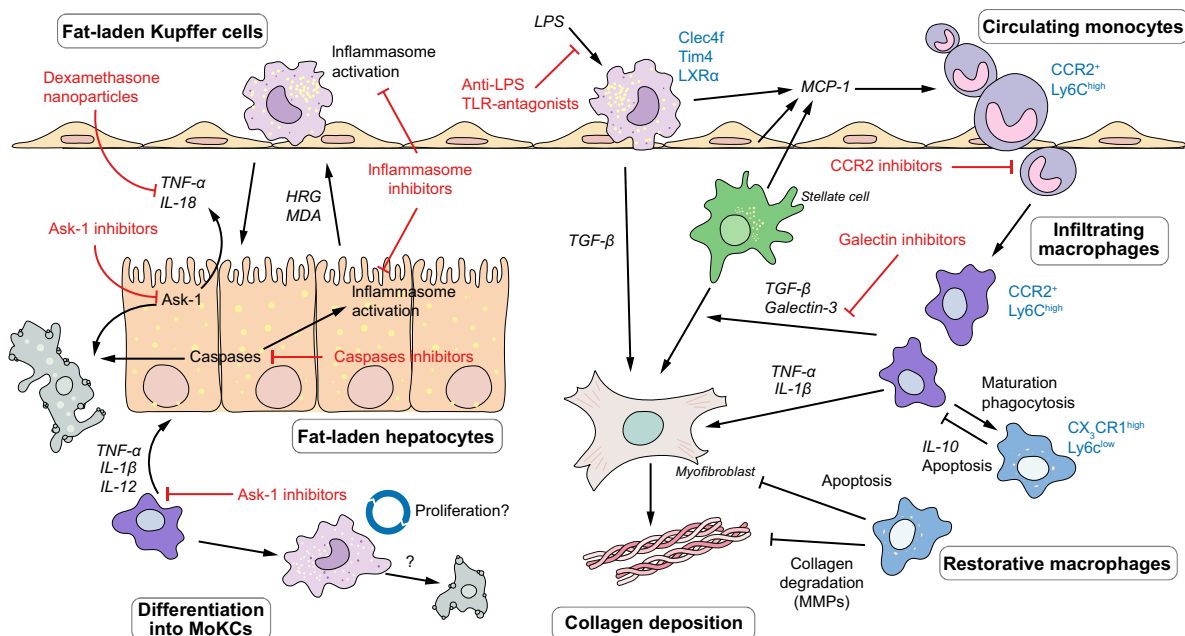

Fig. 3. Macrophage-mediated inflammation as a therapeutic target in NASH progression. In NASH, KCs are activated by bacterial LPS and by danger signals from fat-laden hepatocytes, leading to the production of pro-inflammatory mediators that promote disease progression. Chemokines induce the infiltration of monocytes, which differentiate into monocyte-derived macrophages. These cells contribute to the progression of NASH by aggravating hepatic steatosis, activating stellate cells and promoting fibrosis. Ly6C^{high} macrophages can mature into Ly6C^{low} restorative macrophages that endorse disease resolution through secretion of anti-inflammatory cytokines and collagen degrading factors. Monocyte-derived macrophages can differentiate into KCs (monocyte-derived KCs, MoKC), although it is unclear at this point if this population is capable of long-term self-renewal or if they are short-lived. Selected drugs that target inflammatory pathways and which are currently under investigation are indicated. HRG, histidine-rich glycoprotein; KC, Kupffer cell; LPS, lipopolysaccharide; MDA, malonyldialdehyde; MMPs, matrix metalloproteinases; MoKC, monocyte-derived Kupffer cell; NASH, non-alcoholic steatohepatitis; TLR, Toll-like receptor.

metabolism and the production of pro-inflammatory mediators,[109] similar to the mechanism in ATMs discussed earlier. KC-mediated inflammation is also fuelled by increased binding of bacterial products reaching the liver via the portal vein, secondary to the gut dysbiosis and impaired intestinal barrier observed in obesity.[110] In addition, hepatocyte damage due to lipid overload results in the release of danger signals. For example, histidine-rich glycoprotein (HRG), produced by hypoxic hepatocytes,[111] induces macrophage M1 polarization, whereas HRG knock-out mice were protected from experimental steatohepatitis.[112] The formation of altered lipid products such as malondialdehyde through oxidative stress is another consequence of hepatic fat accumulation which promotes macrophage activation.[113]

KC-derived IL-1β was shown to aggravate hepatocyte triglyceride accumulation through downregulation of PPARα, leading to reduced fatty acid oxidation.[114] Importantly, KC depletion decreased steatosis and hepatic insulin resistance,[114,115] providing direct evidence that KCs act as drivers of early NAFLD.[13]

Bone marrow-derived pro-inflammatory Ly6C^{+} monocytes infiltrate the murine liver following the initiation of injury, constituting a major pathophysiological mechanism in NASH progression. In analogy to the recruitment to dysfunctional adipose tissue, various studies have identified the MCP-1/CCR2 axis as the main effector of monocyte homing to the liver.[35,116,117] MCP-1 is secreted by activated KCs, but also hepatocytes, stellate cells and sinusoidal endothelial cells. The latter respond to inflammation or lipid accumulation by upregulating leukocyte adhesion molecules and facilitating VCAM-1/very late antigen-4 (VLA-4)-dependent monocyte adhesion and migration.[118] Nevertheless, other chemokine pathways have been identified that also regulate myeloid cell recruitment, including CCL25/CCR9 and CCL1/CCR8.[119,120]

The recruitment of infiltrating monocyte-derived macrophages is also observed in human fatty liver disease, especially in patients with more advanced stages of fibrosis, where these cells accumulate mainly in clusters around the portal areas.[121,122] Indeed, both KCs and inflammatory macrophages are profibrogenic during active NASH. They promote the activation and differentiation of hepatic stellate cells to myofibroblasts by secreting mediators such as transforming growth factor β.[119,123] Furthermore, macrophage-derived IL-1β and TNF-α promote stellate cell survival.[124] Nevertheless, infiltrating monocytes and macrophages can mature and assume a restorative phenotype characterized by reduced Ly6C and increased CX$_3$CR1 surface protein expression (Ly6C^{low}CX$_3$CR1^{high}) and the expression of matrix degrading metalloproteinases.[94,125] These fibrolytic macrophages accumulate preferentially upon the cessation of injury by ingestion of apoptotic material, although studies inhibiting autophagy also suggest this mechanism to be

relevant to limit scar formation during active fibrogenesis.[125,126] Accordingly, the timing of macrophage depletion influences the outcome of the fibrotic process, as depletion of fibrolytic macrophages during the recovery phase inhibits the resolution of fibrosis.[125]

Related to this, the balance between macrophage polarization states is a crucial determinant in the progression of steatohepatitis. Anti-inflammatory macrophages can affect IL-10-driven apoptosis of their M1 counterparts, which has been linked to reduced disease severity in patients with alcoholic and non-alcoholic fatty liver disease.[127] Moreover, the beneficial effects of macrophage IL-10 include stimulating lipid catabolism and thereby limiting inflammation in hepatocytes.[128] Interestingly, Arginase-2 knock-out mice lacked M2 polarized macrophages and developed spontaneous steatohepatitis, which was again mitigated by KC depletion.[129] As discussed, this anti-inflammatory programme is under the transcriptional control of PPARγ and δ,[61,62] the former of which was notably activated in fibrolytic Ly6C^{low} macrophages as well.[125]

NAFLD and atherosclerosis: a putative role for macrophages in cardiovascular risk elevation

A large body of evidence now indicates that NAFLD increases the risk for fatal and non-fatal cardiovascular events. Moreover, NAFLD correlates with markers of (subclinical) atherosclerosis such as endothelial dysfunction, coronary arterial calcifications and carotid artery intima-media thickness (reviewed in 12). However, the underlying pathophysiological mechanisms have not been fully elucidated, despite a number of potential pathways being put forward.[130] Macrophage-mediated inflammation is one plausible overarching mechanism, given the crucial role of macrophages in NAFLD progression as well as in atherosclerosis development.

Foam cells had been observed over a century ago by Virchow, and were later identified as macrophages that had accumulated cholesterol esters.[131] Classical scavenger receptors that promote lipid uptake, such as macrophage scavenger receptor-1 (Msr1) and CD36, are implicated in macrophage proliferation, inflammatory gene expression and cell death in atherosclerosis,[132] indicating a profound influence of lipid handling on macrophage behaviour. Interestingly, haematopoietic deletion of Msr1 and/or CD36 has also been shown to reduce the severity of inflammation and fibrosis in experimental NASH, in part through alterations in the intracellular fat distribution in foamy macrophages.[133,134]

Related to this, plaque and hepatic macrophages are regulators of whole-body cholesterol flux. The efflux of macrophage cholesterol to HDL particles, mediated by LXRα, rather than the absolute HDL concentration, inversely correlates with the incidence of cardiovascular events in patients.[135] This efflux is counteracted by the enzyme cholesterol ester transfer protein (CETP),

which regulates the transport of cholesterol from HDL to atherogenic very-low-density and low-density lipoproteins. Circulating CETP derives mainly from KCs, and depletion of KCs has been shown to ameliorate the lipid profile in mice fed a western diet.[136]

The risk of cardiovascular disease correlates with the severity of the systemic inflammation that is characteristic of metabolic disease.[132] In support of this concept, studies have demonstrated an increased cardiovascular risk across a range of chronic inflammatory disorders such as rheumatoid arthritis.[137] NAFLD, and especially NASH, are associated with elevated circulating levels of inflammatory markers secreted by activated KCs or monocyte-derived macrophages and might therefore aggravate metabolic inflammation. This has been confirmed in a number of human studies, although the results for individual cytokines are sometimes discordant. For example, IL-6 serum levels have been reported as elevated in NAFLD[138] or selectively in patients with NASH,[139] whereas this was not confirmed in another report.[86] Multiple studies have suggested elevations of TNF-α and IL-8 in NAFLD.[86,138,140] Similar increases have been reported for the

adhesion molecules intercellular adhesion molecule-1 (ICAM-1) and VCAM-1, which mediate leukocyte infiltration and serve as markers of endothelial dysfunction.[84,141] Moreover, the macrophage marker soluble CD163 correlated with the severity of histological fatty liver disease in 2 independent cohorts and was predictive of liver fibrosis.[142]

Taken together, these data suggest that NAFLD contributes to a pro-atherogenic environment. These data also highlight a shared interaction between lipid metabolism and macrophage activation in NAFLD and atherosclerosis. Nevertheless, it is inevitably difficult to establish the causality of elevated inflammatory markers on the incidence of cardiovascular events, and to assess the precise hepatic contribution to systemic cytokine levels in metabolic diseases.

Macrophages and innate immunity are therapeutic targets in NAFLD

Macrophages, and innate immune responses in general, represent attractive therapeutic targets for the treatment of fatty liver disease, given their role as drivers of hepatic steatosis, inflammation and fibrosis, and their contribution to

Table 2. Selected compounds under investigation for NAFLD/NASH, acting on macrophages and innate immunity components.

Compound	Molecular target	Study population	Primary outcome	Trial number	Trial status/results
Phase III					
Cenicriviroc (AURORA)	CCR2/5 inhibitor	Histological NASH with stage F2-F3 fibrosis	Improvement in fibrosis without worsening NASH	NCT03028740	Recruiting
Selonsertib (STELLAR-3)	ASK-1 inhibitor	Histological NASH with stage F3 fibrosis	Improvement in fibrosis without worsening NASH	NCT03053050	Active, not recruiting
Selonsertib (STELLAR-4)	ASK-1 inhibitor	Histological NASH cirrhosis	Improvement in fibrosis without worsening NASH	NCT03053063	Active, not recruiting
Phase II					
Emricasan (Encore-NF)	Caspase inhibitor	Histological NASH with stage F1-F3 fibrosis	Improvement in fibrosis without worsening NASH	NCT02686762	Active, not recruiting
Emricasan (Encore-LF)	Caspase inhibitor	NASH cirrhosis + history of variceal bleeding and/or moderate/severe ascites	Event-free survival	NCT03205345	Active, not recruiting
Emricasan (Encore-PH)	Caspase inhibitor	NASH cirrhosis with HVPG >12 mm	Change in HVPG	NCT02960204	Active, not recruiting
GR-MD-02 (NASH-CX)	Galectin-3 inhibitor	Compensated NASH cirrhosis with HVPG >6 mm	Improvement in HVPG	NCT02462967	Completed
IMM-124E	Polyclonal anti-LPS	Histological NASH	Improvement in liver fat content on MRI	NCT02316717	No significant changes in liver fat content, decreased serum ALT and LPS
JKB-121	TLR4 antagonist	Histological NASH	Improvement in liver fat content on MRI and/or serum ALT	NCT02442687	No significant differences compared to placebo
SGM-1019	Inflammasome inhibitor	Histological NASH with stage F1-F3 fibrosis	Safety and tolerability	NCT03676231	Recruiting
Phase II trials with drug combinations					
Cenicriviroc + Tropifexor (TANDEM)	CCR2/5 inhibitor + FXR agonist	Histological NASH with stage F2-F3 fibrosis	Safety; improvement in fibrosis	NCT03517540	Recruiting
Selonsertib + GS-0976 + GS-9674 (ATLAS)	ASK-1 inhibitor + ACC inhibitor + FXR agonist	Histological NASH with stage F3-F4 fibrosis or Fibroscan stiffness value ≥14.5 kPa or ELF™ score ≥9.8	Safety; improvement in fibrosis without worsening NASH	NCT03449446	Active, not recruiting

ALT, alanine aminotransferase; ELF, Enhanced Liver Fibrosis; HVPG, hepatic venous pressure gradient; LPS, lipopolysaccharide; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis.

the metabolic syndrome. Several of the pathways involved can be targeted by novel or repurposed drugs, and various compounds have indeed advanced to clinical evaluation (Table 2; Fig. 3).[143]

Inhibiting the chemokine-regulated infiltration of blood monocytes is one therapeutic strategy supported by preclinical studies. For instance, pharmacological inhibition of MCP-1 with the RNA aptamer mNOX-E36 attenuated murine steatohepatitis.[116] Regarding this mode of action, the dual chemokine receptor CCR2/CCR5 inhibitor cenicriviroc has been studied in most depth so far. Cenicriviroc was able to reduce hepatic monocyte infiltration and fibrosis in mouse steatohepatitis models.[122,144] Results from the CENTAUR phase II trial showed that although cenicriviroc did not lead to resolution of NASH, it doubled the percentage of patients in whom fibrosis was improved by at least 1 stage after 1 year, compared with placebo.[145] Based on these results, a phase III trial is currently underway. Other chemokine-axis inhibitors are under development for the treatment of diabetes or related disorders, and trials evaluating these compounds in NAFLD are eagerly awaited.[95]

Macrophages, especially KCs, readily phagocytose circulating nanoparticles such as liposomes. Liposomal delivery of dexamethasone, a derivative of corticosterone, reduced experimental liver fibrosis. The tailored delivery of nanoparticles to macrophages is therefore an interesting possibility, although practical issues such as the most effective drug delivery systems, active compounds and dosing regimen have not been established.[146]

Galectin-3 is a protein secreted by macrophages which stimulates fibrogenesis in the lung, kidney and liver.[147] It functions as a mitogen for fibroblasts and stimulates the formation of lipid oxidation products which serve as danger molecules.[147,148] Galectin-3 ablation attenuated experimental steatohepatitis,[148] and inhibitors are being tested in clinical trials for their potential to reduce fibrosis and its complications.

Macrophage activation entails the activation of intracellular effector pathways such as the inflammasome, which regulates the production of IL-1β and IL-18 and contributes to insulin resistance.[149] A clinical trial including over 10,000 patients found that targeting subclinical inflammation with the IL-1β antagonist canakinumab reduced the number of cardiovascular events in secondary prevention.[150] This clinical benefit correlated strongly with the magnitude of initial reduction in C-reactive protein levels. However, despite these anti-inflammatory effects, there was no reduction in incident diabetes.[151]

Inflammasome activation is also involved in chronic liver disease, forming the rationale for the clinical evaluation of the inflammasome inhibitor SGM-1019 in NAFLD. Caspase inhibitors are of particular interest as they could prevent both hepatocyte cell death and caspase 1-mediated inflammasome activation.[152] The pan-caspase inhibitor emricasan decreased serum alanine aminotransferase and apoptotic fragments after 28 days in an exploratory trial in patients with NAFLD,[153] and it is currently being investigated in multiple phase II trials. Similarly, inhibition of apoptosis signal-regulating kinase 1 (ASK1), an upstream mediator of inflammation in both hepatocytes and macrophages, is a promising approach. Selonsertib, a small molecule ASK1 inhibitor improved liver fibrosis but not histological inflammation or ballooning in a 24-week proof-of-concept study,[154] and is now being evaluated in 2 phase III trials, enrolling patients with NASH and advanced fibrosis or cirrhosis. Given the shared activation of these inflammatory pathways in various macrophage subsets in the liver and adipose tissue, these drugs presumably have pleiotropic effects in various stages of the pathogenesis of metabolic disease.

Of note, many of the drugs currently undergoing clinical evaluation for the treatment of NASH have direct and/or indirect effects on macrophages and innate immune responses, such as farnesoid X receptor or PPAR agonists, that not only impact hepatocyte metabolism but also inflammatory processes.[143] It is currently unclear, yet a subject of intense investigation, to what extent combining different modes of action would act synergistically to improve metabolic disorders and halt NAFLD progression.

Conclusion

Obesity and metabolic diseases can be characterized as low-grade inflammatory disorders, in which pro-inflammatory molecules can function as metabolic hormones and impact on insulin signalling. Macrophages integrate metabolic cues to adapt their phenotype to the local environment, which is especially relevant in tissues sensitive to nutrient changes, such as adipose tissue and the liver. Indeed, fatty acids induce a unique polarization status in macrophages, which contribute to ectopic lipid accumulation as well as local and systemic inflammation. KCs and monocyte-derived macrophages represent ontologically distinct phagocyte populations in the liver that perform a range of metabolic functions in homeostasis and disease. Hepatic macrophages are central players in the progression of NAFLD and may link this condition with cardiovascular disease.

Thus, macrophage targeting holds promise as a therapeutic strategy in metabolic disease. Recent advances in this field, as described in this review, might guide the development of new treatment options to counter these increasingly prevalent diseases. Various approaches, such as inhibiting monocyte infiltration or influencing their polarization, are already advancing from preclinical to clinical trials. Studies combining innovative approaches, such as single-cell sequencing and mouse lines targeting specific macrophage subsets, will further help unravel the complex pathophysiology of NAFLD and the roles of macrophages therein.

Conflict of interest

SL has no disclosures to report. FT's laboratory has received funding from Allergan, Galapagos, Inventiva and Bristol Myers Squibb.

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

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2019.02.004.

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