

# Macrophages as Key Players during Adipose Tissue–Liver Crosstalk in Nonalcoholic Fatty Liver Disease

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## Abstract

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in Western countries that could lead to serious health problems including liver failure, cancer, or death. The term NAFLD includes a spectrum of disease states with histological features ranging from simple steatosis to nonalcoholic steatohepatitis (NASH). A key aspect within this research field is the identification of pathogenic factors that trigger inflammation, thus fueling the transition from nonalcoholic fatty liver to NASH. These inflammatory triggers may originate from within the liver as a result of innate immune cell activation and/or hepatocyte injury. Additionally, they may originate from other sites such as adipose tissue or the intestinal tract. In the current review, the authors will primarily focus on events within adipose tissue which may be of importance in triggering the disease progression. They specifically focus on the role of adipose tissue macrophages during NAFLD pathogenesis and how microenvironmental factors may shape their metabolic profile. They further dissect how redirecting the macrophage's metabolic profile alters their immunological functions. Finally, they discuss the opportunities and challenges of targeting macrophages to interfere in disease progression.

## Keywords

- ▶ nonalcoholic fatty liver disease
- ▶ macrophages
- ▶ adipose tissue inflammation
- ▶ metabolism

Nonalcoholic fatty liver disease (NAFLD) is now one of the most common causes of chronic liver disease in both children and adults and the disease is predicted to become the most important indication for liver transplantation during the next decade.<sup>1</sup> NAFLD is strongly associated with obesity and metabolic syndrome, and similar to these conditions, the incidence and prevalence of NAFLD are increasing to epidemic proportions.<sup>2,3</sup> The early stages of NAFLD are hallmarked by accumulation of lipids in hepatocytes (hepatic steatosis). The majority of patients with simple steatosis will not progress to more severe liver disease. However, for reasons incompletely understood, a subset of patients will develop superimposed hepatic inflammation which is referred to as nonalcoholic steatohepatitis (NASH).<sup>4</sup> Importantly, once NASH is established patients may further progress to cirrhosis and hepatocellular carcinoma.<sup>5</sup> Currently, there are no approved pharmacological therapies that have been shown to be effective in NASH. The development of

novel strategies for NASH treatment will rely on the identification and targeting of key pathogenic pathways.<sup>6</sup>

Although the pathogenesis of NASH is complex and partially unknown, it likely encompasses multiple exogenous as well as endogenous hits resulting in the propagation of liver disease.<sup>7</sup> In the liver, the excess amounts of circulating fatty acids and carbohydrates result in the accumulation of toxic lipids, oxidative- and ER-stress responses, and eventually hepatocyte death.<sup>8</sup> A high-fat diet or nutrient overload may also trigger qualitative and quantitative changes in gut microbiota that may increase intestinal permeability and translocation of bacterial products to reach the liver through the portal vein.<sup>9</sup> The continuous exposure to danger-associated molecular patterns (DAMPs) released from necrotic liver cells and pathogen-associated molecular patterns (PAMPs) originating from the gut may sustain and amplify inflammatory events ultimately leading to fibrosis and cirrhosis development.<sup>10,11</sup>

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The onset of dyslipidemia and inflammation in the liver is closely linked to early events occurring in the adipose tissue.<sup>12</sup> In both mice and human subjects, the recruitment of macrophages within the adipose tissue compartment has been associated with the development of insulin resistance and steatohepatitis.<sup>13,14</sup> Conversely, ablation of adipose tissue macrophages, surgical removal of adipose tissue, or inhibiting peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) pathways in mice normalized insulin sensitivity and partially reversed liver inflammation.<sup>15–17</sup> We and others have shown that CD11c<sup>+</sup>CD206<sup>+</sup> and CCR2<sup>+</sup> macrophages infiltrate visceral adipose tissue, and are associated with increased production of inflammatory cytokines in NASH.<sup>18–20</sup> Interestingly, adipose tissue inflammation also preceded the appearance of inflammation in the liver, suggesting that disease-initiating triggers originate from adipose tissue rather than the liver.<sup>18,20</sup> Notably, while also other immune cells play a role in adipose tissue inflammation,<sup>21,22</sup> for the purpose of this review we specifically focus on the role of adipose tissue macrophages during NAFLD progression. We further dissect their immunological and metabolic profiles, their interaction with adipocytes as well as their plethora of secreted factors that may fuel inflammation in the liver. Finally, we discuss the translational potential of rewiring the functional or metabolic status of adipose-tissue macrophages.

### Molecular Events Triggering Macrophages within the Adipose Tissue Compartment

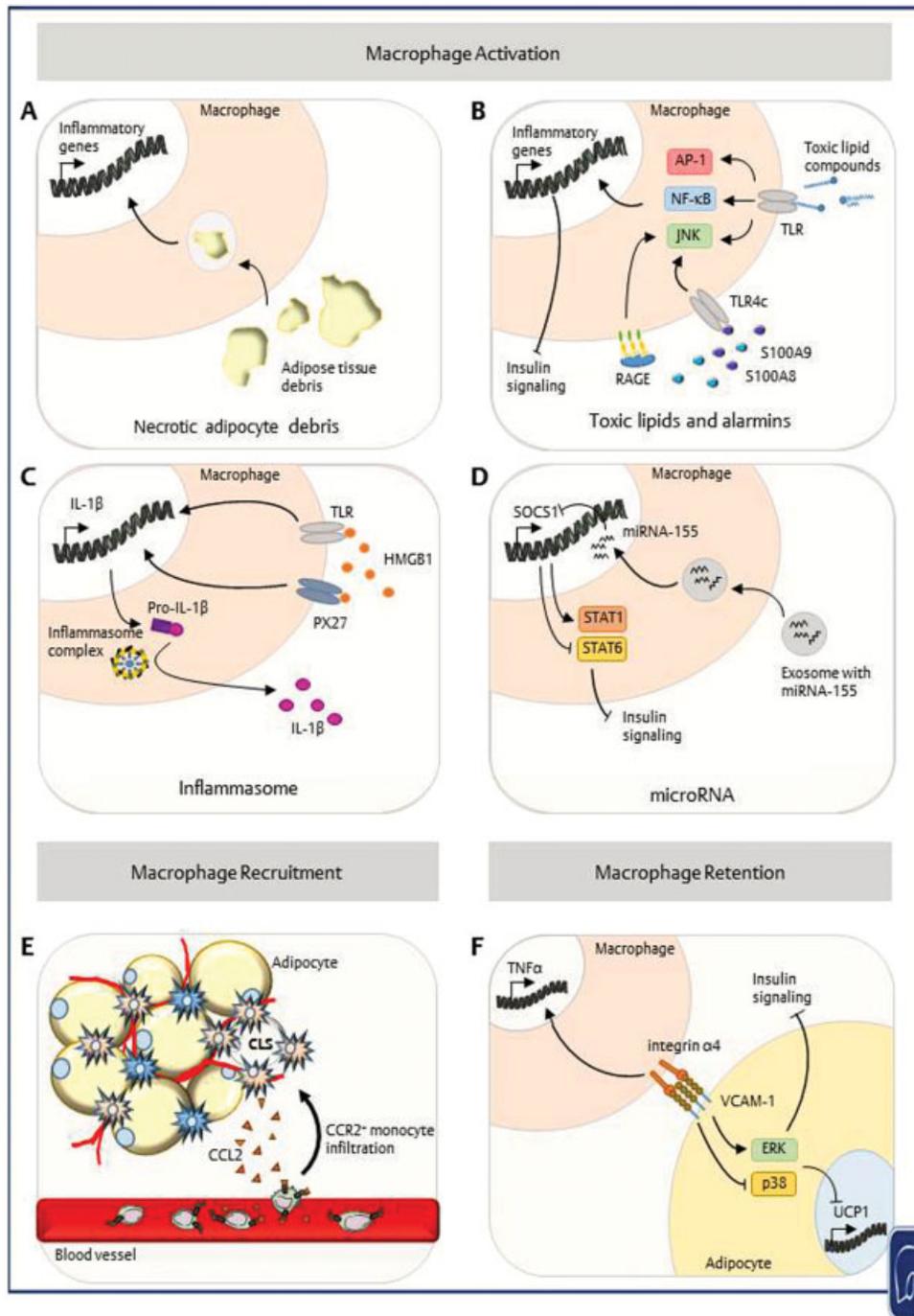
During pathological conditions such as NAFLD where chronic overnutrition prevails, the size and number of adipocytes increase to compensate for the excess lipid availability. However, this containment mechanism may ultimately fail leading to adipose tissue dysfunction, dyslipidemia, and insulin resistance. The considerable lipid burden within adipocytes triggers intracellular endoplasmic reticulum (ER) stress mechanisms, which culminate in cell death and the release of lipid contents and cellular debris.<sup>8</sup> Consequently, tissue resident macrophages are exposed to a variety of triggers such as toxic lipids, oxidative radicals, adipokines, nucleic acids, exosomes, and DAMPs derived from dying cells, and combined these biomolecules create a complex microenvironment that initiates macrophage activation.<sup>10,23</sup> For example, macrophages surrounding necrotic adipocytes within crown-like structures can become activated following engulfment of necrotic debris.<sup>24–26</sup> Additionally, danger signals such as high mobility group box protein 1 (HMGB1) can be recognized by toll-like receptors (TLRs) or P2X purinoceptor 7 (P2RX7) on the surface of macrophages, leading to the activation of inflammasomes.<sup>27–29</sup> Alternatively, toxic lipid compounds can signal macrophage activation and production of proinflammatory mediators by activating intracellular pathways involving key transcription factors such as c-Jun N-terminal kinase (JNK), activator protein 1 (AP-1), and nuclear factor- $\kappa$ B (NF- $\kappa$ B).<sup>8</sup> Furthermore, extracellular alarmins (e.g., S100A8 and S100A9) can promote human macrophage-mediated inflammation through the receptor advanced glycation end-products (RAGE) and TLR4-dependent pathways.<sup>30,31</sup> Finally,

microRNA-155 in adipose-derived microvesicles has been demonstrated to induce macrophage activation, chronic inflammation, and local insulin resistance in a murine model.<sup>32</sup> An overview of mechanisms that potentiate macrophage-mediated inflammation is depicted in **Fig. 1** and has also been reviewed elsewhere.<sup>8,10,13,14,21,33</sup>

Stressed or dying adipocytes also contribute to the recruitment of monocytes/macrophages through the release of chemokines. Although several chemokines have been implicated in this process,<sup>34,35</sup> C-C motif chemokine ligand 2 (CCL2) and its receptor CCR2 seem to play a prominent role (**Fig. 1**).<sup>36</sup> Hereto, the absence of CCR2-blunted macrophage infiltration in an experimental model improved insulin sensitivity and hepatic steatosis.<sup>37</sup> Conversely, transgenic expression of CCL2 in murine adipose tissue promoted macrophage recruitment and obesity-induced insulin resistance.<sup>36</sup> These studies establish a prominent role for chemokine-driven recruitment of monocytes/macrophages to adipose tissue. However, whether adipocytes also actively participate in the retention of macrophages to the site of inflammation is less well characterized. Interestingly, a recent report demonstrated that adipose tissue inflammation was dependent on the physical interaction of integrin  $\alpha$ 4 on macrophages and vascular cell adhesion molecule 1 (VCAM-1), its counter-receptor on adipocytes in a mouse model.<sup>38</sup> This adhesive interaction resulted in the upregulation of extracellular-signal-regulated kinase (ERK) signaling and the promotion of insulin resistance in adipocytes.<sup>38</sup> This study shed new light on the extent by which adipocytes sustain adipose tissue inflammation. Additionally, netrin-1 has recently been identified as a macrophage retention molecule (**Fig. 1**).<sup>39</sup> Finally, also the production of macrophage migration inhibitor factor (MIF) could be potentially important in both recruiting and retention of macrophages to the adipose tissue site.<sup>40,41</sup> Combination, recruitment, retention, and activation of macrophages perpetuate a vicious loop of events leading to exacerbated inflammation.<sup>42</sup>

### Elucidating the Spectrum of Adipose Tissue Macrophage Phenotypes

Macrophages are highly versatile cells, with key functions in the initiation as well as resolution of inflammation.<sup>43</sup> Such functions include phagocytosis of apoptotic/necrotic cells and pathogens, elaboration of immune-effector molecules and growth factors, and remodeling of the extracellular matrix.<sup>44</sup> Furthermore, they are equipped with a wide range of surface and nuclear receptors to appropriately scale the molecular threat in their microenvironment and can respond accordingly.<sup>45</sup> If one considers the diversity of signals that these cells encounter *in vivo*, it is plausible that they exist as a continuum of different activation statuses.<sup>43</sup> Nevertheless, based on *in vitro* studies, the extremes of this continuum have been classified in two main groups, namely classically activated/proinflammatory (M1-like) and alternatively activated/anti-inflammatory (M2-like) macrophages.<sup>46,47</sup> The M1-like macrophage phenotype is driven by proinflammatory mediators such as lipopolysaccharide (LPS) and interferon gamma (IFN $\gamma$ ) and is characterized by increased production of proinflammatory



**Fig. 1** Molecular mechanism leading to macrophage activation, recruitment, and retention leading to exacerbated adipose tissue inflammation. Macrophages are exposed to several biomolecules initiating their activation (A–D). (A) Necrotic adipocyte debris is engulfed by the surrounding macrophages causing increased expression of proinflammatory genes. (B) Binding of toxic lipid compounds and several alarmins such as S100A8 and S100A9 to TLRs and RAGE elevates the expression of proinflammatory genes via AP-1, NF-κB, and JNK-mediated signaling and thereby worsening insulin resistance. (C) Binding of the danger signal HMGB1 to TLRs and P2RX7 can activate the inflammasome complex which will cleave pro-IL-1β into IL-1β. (D) Lastly, adipose tissue-derived exosomes contain elevated levels of miRNA-155 which will bind the SOCS1 promoter and inhibit its transcription. This leads to increased STAT1 and decreased STAT6 signaling causing defective insulin signaling. Mechanism triggering macrophage recruitment (E). Necrotic adipocytes produce CCL2 leading to recruitment of CCR2<sup>+</sup> monocytes from the circulation into the adipose tissue. These monocytes differentiate to proinflammatory macrophages surrounding the dying adipocytes forming CLS. Mechanisms promoting macrophage retention at the site of inflammation (F). The physical interaction of integrin α4 on macrophages with VCAM-1 on adipocytes causes increased ERK signaling and decreased p38 signaling leading to inhibition of the UCP1 gene and worsening of insulin signaling. Moreover, TNFα transcription in the macrophage is stimulated because of this cell–cell adhesion. AP-1, activator protein 1; CCL/R2, C-C motif chemokine ligand/receptor 2; CLS, crown-like structures; ERK, extracellular-signal-regulated kinase; HMGB1, high mobility group box protein 1; IL-1β, interleukin-1β; JNK, c-Jun N-terminal kinases; miRNA, microRNA; NF-κB, nuclear factor-κB; P2RX7, P2X purinoceptor 7; RAGE, receptor for advanced glycation end-products; SOCS1, suppressor of cytokine signaling 1; STAT, signal transducer and activator of transcription; TLR, toll-like receptor; TNFα, tumor necrosis factor α; VCAM-1, vascular cell adhesion molecule 1; UCP1, uncoupling protein 1.

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cytokines, while the M2-like macrophage phenotype is driven by anti-inflammatory cytokines such as interleukin-4 (IL-4).<sup>48</sup>

Applying this basic classification in the context of NAFLD resulted in the identification of macrophages with an M2-like phenotype in lean adipose tissue and their function to maintain insulin sensitivity through the anti-inflammatory actions of IL-10 and signal transducer and activator of transcription 3 (STAT3).<sup>25</sup> Furthermore, they activate expression of immunosuppressive factors and PPAR $\gamma$  which promote tissue remodeling and resolve inflammation.<sup>48</sup> They further produce factors such as insulin-like growth factor 1 (IGF1) and macrophages expressing the receptor of IGF1 have been implicated as negative regulators of inflammation.<sup>49</sup> Along the same line of investigation, the loss of GPR120, a G protein-coupled receptor with immunoregulatory actions, led to the loss of the inhibitory effect on adipose tissue inflammation which promoted insulin resistance in an experimental mouse model.<sup>50</sup>

M1-like macrophages on the other hand have been described in adipose tissue from NAFLD subjects and they are characterized by the expression of the surface marker CD11c, the secretion of proinflammatory cytokines such as tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) and IL-6, as well as the generation of reactive oxygen and nitrogen intermediates.<sup>18,51</sup> In a murine model, macrophages that secrete proinflammatory cytokines induced adipokine dysregulation that impaired insulin action to confer systemic insulin resistance.<sup>25</sup> The importance of these events in disease pathogenesis has been demonstrated by the fact that ablation of the proinflammatory signaling molecule IKK $\beta$  in murine myeloid cells reduces myeloid cell-mediated inflammation in adipose tissue, resulting in preservation of insulin sensitivity.<sup>52</sup> Similarly, macrophage-specific deletion of stress-activated protein kinases, JNK, protects against high-fat diet-induced obesity and insulin resistance, and reverts M1-like polarization in mice.<sup>53</sup>

Notably, phenotypes of adipose tissue macrophages distinct from classical activation (M1-like) or alternative activation (M2-like) have recently been described. For example, obese adipose tissue macrophages featured increased liposomal biogenesis and lipid catabolism most probably due to chronic lipid overloading *in vivo*.<sup>54</sup> Another report described that treating macrophages with a cocktail of glucose, insulin, and fatty acids (palmitate) triggers a metabolically activated state (MMe).<sup>55</sup> Interestingly, the phenotype of MMe macrophages was driven by the NADPH oxidase 2 complex and they participated in both detrimental and beneficial functions during obesity by promoting inflammatory cytokine production as well as lysosomal exocytosis to adipocytes.<sup>56</sup> Moreover, another macrophage phenotype termed Mox has also recently been reported in a murine model to be present in lean adipose tissue as a consequence of exposure to truncated oxidized lipids.<sup>57</sup> Interestingly, these “redox-regulatory” Mox macrophages feature antioxidant gene expression and a quiescent metabolism.<sup>57</sup> It would be intriguing to further characterize and understand the relevance of these macrophage phenotypes during human disease. Nevertheless, these interesting new findings imply that harnessing

macrophage metabolism could represent a promising approach to interfere in NAFLD and associated comorbidities.

## Macrophage Metabolic Programs That Govern Immune Function

Intracellular energy metabolism of macrophages has recently been highlighted as a regulator of their immunological functions.<sup>58,59</sup> For example, M2-like macrophages, presumed to be present in lean adipose tissue, exhibit a metabolic program that relies on fatty acid oxidation to fuel tricarboxylic acid (TCA) cycle-coupled oxidative phosphorylation. Importantly, the events leading to increased oxidative phosphorylation are orchestrated by the transcription factor STAT6, which in turn induces expression of PPAR $\delta$ , PPAR $\gamma$ , and the coactivator protein PGC-1 $\beta$ .<sup>48,60</sup> As demonstrated in a mouse model, M2-like macrophages also maintain insulin sensitivity by the anti-inflammatory actions of IL-10 and STAT3.<sup>61</sup> Additionally, a role for the mammalian target of rapamycin complex 2 (mTORC2) and interferon regulatory factor 4 (IRF4) signaling axis in M2-like polarization has been demonstrated.<sup>62</sup> Hereto, the upregulation of both mTORC2 and IRF4 increases glucose-dependent oxidative phosphorylation, which subsequently stimulates the expression of M2-like target genes such as arginase 1 or resistin-like molecule  $\alpha$ .<sup>62</sup>

Inflammatory-type macrophages, presumed to be present in inflamed adipose tissue, on the other hand are characterized by increased glucose uptake and glycolytic flux, along with impaired oxidative phosphorylation via the TCA cycle.<sup>63</sup> M1-like macrophages feature an interrupted TCA cycle whereby intermediates such as citrate and succinate accumulate within the cell.<sup>64</sup> Importantly, these metabolites have been shown to directly affect immune responses. For example, the build-up of citrate in the cytoplasm can promote fatty acid synthesis and production of the antimicrobial metabolite itaconic acid.<sup>64</sup> Additionally, succinate accumulation leads to stabilization of hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ), a master transcriptional regulator of proinflammatory and glycolytic genes.<sup>65</sup>

However, as mentioned above, we are only starting to grasp the extent of these macrophage phenotypes and their accompanying metabolic signatures especially during NAFLD pathogenesis in an *in vivo* situation. For instance, it is possible that lipids are excessively engulfed by resident adipose tissue macrophages giving rise to the unique macrophage phenotypes as described above as well as potential novel phenotypes. Key transcription factors that control lipid metabolism in macrophages include PPARs, liver X receptors (LXRs), CCAAT enhancer-binding proteins (C/EBPs), and sterol regulatory element-binding proteins (SREBPs).<sup>66</sup> However, how exactly these pathways are dysregulated during NAFLD pathogenesis whereby adipose tissue macrophages fail to cope with the lipid overload remains to be further investigated. Additionally, key molecules that could reverse defective lipid metabolism within these pathways may be highly relevant as disease interventional strategies.

## Promising New Therapeutic Approaches during NAFLD Progression

### Macrophage Recruitment and Activation Status

There is a major unmet need for effective therapies in fatty liver disease and this is of high clinical relevance considering escalating disease prevalence. Macrophages have key regulatory roles in both inflammation and metabolism, which underscores targeting them for disease intervention (see also overview of new strategies in **Table 1**).<sup>67</sup> In fact, interference with chemokine pathways to restrict proinflammatory monocyte/macrophage recruitment using the CCR2/CCR5 antagonist ceniciviroc is one of the most advanced treatments of NASH-related fibrosis (phase IIb) and a phase III

trial is currently ongoing to confirm the efficacy and safety of this drug (the Aurora study).<sup>68–70</sup> Another key strategy is to promote anti-inflammatory macrophage polarization and consequently also the amelioration of NASH progression by promoting signaling through PPAR pathways. Indeed, recent work in animal models has demonstrated blunted inflammation and reversal of fibrosis is triggered by the PPAR $\alpha$ /PPAR $\gamma$  agonist saroglitazar.<sup>71</sup> Also in clinical studies, elafibranor, an agonist of PPAR $\alpha$  and  $\delta$ , has been shown to attenuate inflammation in the liver without adversely affecting fibrosis in patients with NASH.<sup>72</sup> Alternative approaches include targeting activating signals of myeloid-derived cells in liver disease. In this regard, exciting new research implicated a role for inositol-requiring enzyme 1 $\alpha$  (IRE1 $\alpha$ ), the

**Table 1** Overview of novel therapeutic strategies targeting macrophages during NAFLD

Target	Agent	Mechanism of action	Experimental phase
Chemokines	Ceniciviroc	CCR2/CCR5 antagonist reducing M1 and improving fibrosis	Phase III
	Maraviroc	CCR5 antagonist reducing inflammation and improving insulin sensitivity	Preclinical
	NIBR2130	CXCR3 antagonist reducing inflammation	Preclinical
IRE1 $\alpha$	4 $\mu$ 8C	IRE1 $\alpha$ inhibition promoting M2 polarization	Preclinical
FXR	Obeticholic acid	FXR agonist resulting in shift to M2 macrophages and reduction of inflammation	Phase III
	Isoxazoles	FXR agonist improving insulin sensitivity and inflammation	Phase II
	EGCG	FXR agonist reducing oxidative stress, inflammation, and fibrosis	Preclinical
TGR5/FXR	INT-767	TGR5/FXR agonist promoting M2 polarization	Preclinical
PPAR	Saroglitazar	PPAR $\alpha$ and PPAR $\gamma$ agonist, leading to increase of M2 macrophages	Phase III
	Pemafibrate	PPAR $\alpha$ and PPAR $\delta$ agonist modulating lipid turnover and reducing inflammation	Phase II
	Elafibranor	PPAR $\alpha$ and PPAR $\delta$ agonist improving insulin sensitivity and inflammation	Phase II
	L-165041	PPAR $\alpha$ and PPAR $\delta$ agonist improving insulin sensitivity and inflammation	Preclinical
ASK1	Selonsertib	ASK1 inhibitor improving fibrosis and reducing inflammation by increasing M2 polarization	Phase III
SKY	R406-PLGA	R406-PLGA nanoparticle, inhibiting SKY pathway in macrophages, improving fibrosis and inflammation	Preclinical
microRNA	AntimiR-221/222	Inhibition of microRNA-221 and microRNA-222, improving fibrosis, and reducing inflammation	Preclinical
	MRG-201 (miRagen)	microRNA-29 mimic, improving fibrosis	Phase I
	Lac-PDMAEMA/miR-146b mimic	Targeted delivery of micro-RNA146b mimic via nanoparticles to hepatocytes, reducing inflammation	Preclinical
mTORC1	Rapamycin	mTORC1 inhibition improves inflammation and insulin sensitivity	Preclinical
NOTCH1	Curcumin	NOTCH1 inhibition improves inflammation and insulin sensitivity	Preclinical
AMPK	Tamoxifen	AMPK activation inhibits M1 polarization and improves inflammation	Preclinical
SIRT1/2	SRT1720	SIRT1/2 activation improves inflammation by inhibiting NF- $\kappa$ B signaling	Preclinical

Abbreviations: AMPK, 5' AMP-activated protein kinase; ASK1, apoptosis signal-regulating kinase 1; FXR, farnesoid X receptor; IRE1 $\alpha$  inositol-requiring enzyme 1 $\alpha$ ; mTORC, mammalian target of rapamycin complex; NF- $\kappa$ B, nuclear factor- $\kappa$ B; PPAR, peroxisome proliferator-activated receptor.

upstream regulator of a part of the unfolded protein response, in aggravating inflammation and the obesity-associated symptoms by reprogramming macrophage function.<sup>73</sup> Mechanistically, IRE1 $\alpha$  plays a role in suppressing IRF4 and KLF4, both key transcription factors promoting M2-like polarization. Consequently, ablation of IRE1 $\alpha$  in mice attenuated the shift towards the proinflammatory M1-like phenotype, and simultaneously promoted the anti-inflammatory M2-like phenotype.<sup>73</sup> Similarly, a dual agonist for G-protein coupled BA receptor 5 and farnesoid X receptor (TGR5/FXR) triggered elevated frequencies of anti-inflammatory monocytes/macrophages and protected against steatohepatitis in a murine model.<sup>74</sup> Moreover, the semisynthetic bile acid analogue obeticholic acid is a strong FXR agonist with promising results from an early phase clinical trial in patients with NASH.<sup>75</sup>

### Shifting the Macrophage Metabolic Program

Additionally, approaches aiming at metabolic rewiring of macrophages to regulate their immune function may be of equal importance and this concept is already well established in other disease fields. With relevance to NAFLD, a promising study demonstrated that inhibition of the key metabolic regulator, mTORC1, improved high-fat diet-induced steatohepatitis through modulation of lipid metabolism, macrophage polarization, inflammatory response, and autophagy.<sup>76</sup> Mice with a selective deficiency of mTORC1 in macrophages portrayed a predominantly M2-like phenotype, reduced ER stress, reduced inflammation in the liver, and improved insulin sensitivity.<sup>76</sup> Another interesting study also implicates a role for the NOTCH1 pathway in promoting mitochondrial oxidative phosphorylation and reactive oxygen species as well as the expression of M1-related genes. Importantly, conditional deficiency of NOTCH1 in myeloid cells attenuated M1-like activation of hepatic macrophages and inflammation in a murine model of alcoholic steatohepatitis.<sup>77</sup> Mechanistically, ligand binding to the Notch receptor triggers proteolytic cleavage of its receptor, resulting in the release of Notch intracellular domain (NICD). In turn, NICD translocates to the nucleus and binds to recombining binding protein suppressor of hairless (RBP-J), resulting in the release of IRF8 and NF- $\kappa$ B. Moreover, the sedoheptulose kinase of the pentose phosphate pathway, carbohydrate kinase-like (CARKL) protein, plays a role in regulating a metabolic switch toward glycolysis in M1-like macrophages.<sup>78</sup> Consequently, knock down of CARKL removed the negative regulation on glycolysis and produced a clear macrophage phenotype. In turn, overexpression of CARKL blunted the expression of IL-6 and TNF $\alpha$  while augmenting IL-10.<sup>78</sup> Finally, 5' AMP-activated protein kinase (AMPK) was shown to induce an anti-inflammatory phenotype in macrophages through inhibiting proinflammatory polarization and crucially contributes to immune function in macrophages.<sup>79</sup>

### Epigenetic Regulation and Innate Immune Memory

Another concept that opens a window of opportunity for new interventions stems from studies demonstrating that epige-

netic mechanisms can regulate macrophage function by imprinting them with a memory response towards future stimuli.<sup>80,81</sup> Notably the concept of innate immune memory has been established in infection studies whereby macrophages modify their histone acetylation and methylation traits, to become either 'trained' or 'tolerant' upon exposure to subsequent stimulation.<sup>82</sup> For example, during trained memory a metabolic switch from oxidative phosphorylation to aerobic glycolysis occurs through activation of the mTOR–HIF1 $\alpha$  pathway. Mechanistically, this was achieved through increased trimethylated histone H3K4 and acetylated histone H3K27 in the promoter region of the main mTOR-target gene and thereby permitting its transcription.<sup>83</sup> In contrast, macrophages that exhibit endotoxin tolerance upon TLR4 activation undergo a metabolic switch from glycolysis to oxidative phosphorylation through activation of the histone deacetylases sirtuin-1 and sirtuin-6 (SIRT1/6) and consequent inhibition of inflammatory gene transcription.<sup>84</sup> Notably, these pathways are potentially targetable since inhibitors of the histone deacetylases SIRT1/2 have been shown to have the capacity to reverse immune paralysis in experimental sepsis models.<sup>85,86</sup> Importantly, a recent study also demonstrated that macrophages can exhibit a molecular memory after digesting apoptotic cells.<sup>87</sup> The mechanism thereof was dependent on JNK-induced upregulation of the damage signal Draper leading to imprinting of an anti-inflammatory response following corpse engulfment.<sup>87</sup> Further support of the concept that innate memory can also be induced by danger signals within a sterile inflammation environment stems from studies within the atherosclerosis field. For example, monocytes exposed to oxidized low-density lipoproteins exhibit increased expression of inflammatory cytokines following secondary challenge with triggers of activation, and this effect was reversed by treatment with a methylation inhibitor.<sup>88</sup> Whether such a molecular memory imprinting of tolerant or anti-inflammatory macrophages can be achieved during NAFLD might be highly relevant to investigate in the future.

### Adipose Tissue–Liver Crosstalk

The key question in the disease pathogenesis of NASH is to understand the interorgan relationship of adipose tissue inflammation, which drives the development of steatohepatitis in the liver. Notably, it is well established that adipose tissue engages in crosstalk with the liver influencing whole body metabolism and insulin resistance. For example, it is known that signaling molecules (e.g., microRNAs, adipokines, lipotoxic molecules, cytokines/chemokines, DAMPs, and metabolites) released from the adipose tissue into the portal vein can potentially trigger inflammation in the liver. Of note, also molecules derived from the intestinal compartment can have similar repercussions on pathological events in the liver; however, this will not be addressed here as this aspect has been extensively reviewed elsewhere.<sup>33,89</sup> The key issue is to define what types of molecule direct disease-relevant events at a distant site such as the liver, as well as the mechanistic insight into the targeted molecular pathways.

### Toxic Lipids

During obesity, adipocytes lose the capacity to efficiently store triglyceride, leading to free fatty acid release into the circulation and drainage to the liver.<sup>8</sup> Hepatic fatty acid availability induces accumulation of lipid intermediates, especially diacylglycerol, which activates specific isoforms of protein kinase C and hampers insulin receptor activation and insulin-stimulated glycogen synthesis.<sup>90</sup> Establishment of insulin resistance further increases the flux of substrates that promote lipogenesis and gluconeogenesis (► Fig. 2).<sup>8</sup> Furthermore, free fatty acids activate transcription of SREBP-1c further promoting lipogenesis and hepatic steatosis.<sup>91</sup> Additionally, some lipid moieties such as palmitic acid, ceramides and lysophosphatidylcholine can also hamper the function of intracellular organelles such as the ER and the mitochondria, triggering cellular stress or even hepatocyte death.<sup>8</sup> Besides their effects on hepatocytes, lipotoxic agents can influence the activation status of hepatic macrophages and Kupffer cells (KCs; ► Fig. 2). For example, palmitic acid can activate TLR2 and TLR4 in macrophages, resulting in upregulated expression of proinflammatory cytokines, via NF- $\kappa$ B, AP-1, and activation of the JNK pathway.<sup>92</sup>

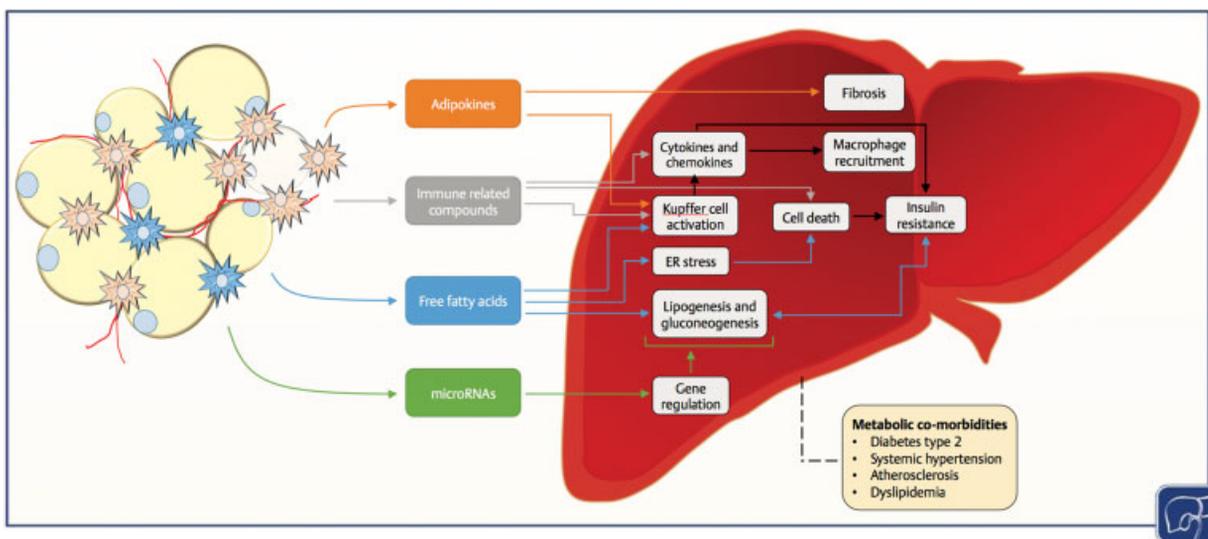
### miRNAs

A typical example of powerful adipose-tissue-derived messenger molecules that regulate gene expression in other organs such as the liver is microRNAs (miRNAs). miRNAs are snips of noncoding RNA produced intracellularly and are secreted into the circulation either as free entities or packaged into small vesicles called exosomes.<sup>93</sup> They mediate their effects either by mRNA cleavage, translational repression, or mRNA destabilization following binding to target transcript sequences (► Fig. 2).<sup>93</sup> As proof of concept, the ability of adipose-tissue derived miRNA

to directly regulate expression of FGF21 in the liver has recently been demonstrated in a mouse model.<sup>94,95</sup> More recent studies also implicate a role for miRNAs in disease progression from simple steatosis to NASH, further highlighting their relevance as potential therapeutic targets. For example, adipose tissue macrophages from obese animals secrete miRNA-containing exosomes that can influence local and systemic insulin resistance. In contrast, treatment of obese recipients with adipose tissue macrophage-derived exosomes from lean mice leads to a significant improvement in insulin sensitivity. Finally, a role for miRNA-155 in inhibiting insulin signaling has been implicated through a mechanism related to suppression of its target gene, PPAR $\gamma$ .<sup>96</sup> Furthermore, also miRNA-221 and miRNA-222 have been shown to be elevated in NAFLD and importantly, anti-miRNAs of miRNA-221/222 inhibited fibrosis and improved insulin signaling in a preclinical NAFLD model.<sup>97</sup>

### Adipokines

Leptin released by the adipose tissue compartment typically augments hepatic transforming growth factor- $\beta$  (TGF- $\beta$ ) and promotes the fibrotic response, a process orchestrated by the interplay between KCs and stellate cells (► Fig. 2).<sup>98</sup> Leptin can also promote acute inflammation by triggering the release of KC-derived TNF $\alpha$  and trigger KC activation through oxidative stress mechanisms (e.g., iNOS and NADPH oxidase).<sup>98–100</sup> Leptin also prompted elevated expression of the LPS receptor CD14 through triggering STAT3 signaling in KCs and thereby increased their responsiveness towards danger signals.<sup>101</sup> On the other hand, adiponectin could play a more protective role through decreasing KC sensitivity to danger signals or exhibiting an antiproliferative effect on hepatic stellate cells.<sup>102</sup> An extensive overview of all adipokines and their downstream functions has been recently documented.<sup>23</sup>



**Fig. 2** Mechanisms involved in adipose tissue–liver crosstalk during NAFLD. Inflamed adipose tissue secretes a diverse array of molecules, which transfer to the liver via the portal circulation, causing a cascade of inflammatory, metabolic, and profibrotic events. For example, the secretion of adipokines exhibits a profibrotic effect and activates KCs. Immune compounds can trigger recruitment of innate immune effector cells and their activation. Toxic-free fatty acid moieties can initiate ER stress and cell death pathways and can contribute to KC activation. The flux of lipid species to the liver further increases intrahepatic lipogenesis and gluconeogenesis causing hepatic insulin resistance. Combined, these effects can lead to a metabolic dysregulation at a systemic level, thereby promoting the development of comorbidities such as diabetes type 2, dyslipidemia, systemic hypertension, and atherosclerosis. MicroRNAs are able to regulate gene expression in all the different hepatic cell types. ER, endoplasmic reticulum; KCs, Kupffer cells; NAFLD, nonalcoholic fatty liver disease.

### Immune-Related Compounds

The adipose tissue compartment also secretes danger signals or inflammatory parameters into the portal circulation that can promote pathogenic events in the liver (►Fig. 2). For example, adipose tissue-derived S100A8 and S100A9 can potentially trigger local and ectopic macrophage activation through TLR4 and NLRP3 signaling and IL-1 $\beta$  production.<sup>103</sup> Similarly, TNF $\alpha$  secreted from adipose tissue macrophages induces cell death via JNK pathways and promotes KC activation, further perpetuating inflammation in the liver.<sup>104</sup> Interestingly, recent animal studies also highlight that macrophages recruited to obese visceral adipose tissue contribute to neutrophil recruitment in the liver, and consequently, the development and progression of NASH.<sup>105</sup> The data suggest that the elevated hepatic neutrophilic inflammation was potentially mediated by an increase in the neutrophil chemotactic factors CXCL14 and CXCL16 by adipose tissue macrophages.<sup>105</sup> Finally, the release of plasminogen activator inhibitor-1, a serine protease inhibitor that suppresses the breakdown of blood clots, can promote shunting of free fatty acids to ectopic sites such as the liver and contributes to systemic insulin resistance and the thrombosis risk.<sup>106</sup> Combined, these factors may not only promote disease progression of NAFLD but also the pathogenesis of cardiovascular diseases and type 2 diabetes typically associated with the disease (►Fig. 2).

### Conclusion

Increasing evidence highlights the close association between macrophage function and metabolism and its importance during NAFLD. As outlined above, approaches such as functional or metabolic rewiring and targeting key epigenetic regulators within macrophages could potentially hold promise in designing novel therapeutic strategies. Of note, the availability of small molecules capable of manipulating metabolic and epigenetic traits may prove useful in these studies. Furthermore, key techniques such as single-cell RNA sequencing, single-nucleus sequencing, and/or single-molecule mRNA fluorescent in situ hybridization will be crucial in unraveling these complex mechanisms. In fact, highly exciting new research using single-cell RNA sequencing has revealed distinct populations of liver-resident monocytes/macrophages in mouse and human tissue.<sup>107,108</sup> It is plausible that these intrahepatic macrophages are receptive to signals and metabolites released from the adipose tissue and therefore play an equally important role in the crosstalk between the adipose tissue and the liver.<sup>33</sup> It will therefore be crucial for future studies to map these interorgan transcriptome signatures at a single-cell level, not only in a naïve condition, but also during the different stages of NAFLD. The latter may reveal molecular mechanisms that could potentially be exploited to combat this disease with its epidemic proportions.

#### Conflicts of Interest

None.

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