

Role of macrophages in experimental liver injury and repair in mice (Review)

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Abstract. Liver macrophages make up the largest proportion of tissue macrophages in the host and consist of two dissimilar groups: Kupffer cells (KCs) and monocyte-derived macrophages (MoM ϕ). As the liver is injured, KCs sense the injury and initiate inflammatory cascades mediated by the release of inflammatory cytokines and chemokines. Subsequently, inflammatory monocytes accumulate in the liver via chemokine-chemokine receptor interactions, resulting in massive inflammatory MoM ϕ infiltration. When liver injury ceases, restorative macrophages, derived from recruited inflammatory monocytes (lymphocyte antigen 6 complex, locus C^{hi} monocytes), promote the resolution of hepatic damage and fibrosis. Consequently, a large number of studies have assessed the mechanisms by which liver macrophages exert their opposing functions at different time-points during liver injury. The present review primarily focuses on the diverse functions of macrophages in experimental liver injury, fibrosis and repair in mice and illustrates how macrophages may be targeted to treat liver disease.

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1. Introduction

Macrophages, originating from monocytic precursors, have multiple functions and are widely known for their phagocytic capacity, antigen-presenting function and active secretory properties. Once localized in the liver, macrophages exhibit high phagocytic activity to remove endotoxins and pernicious substances from the portal vein. Resident tissue macrophages and inflammatory monocytes recruited from bone marrow have a dual role in organ damage induced by various factors, including infection, auto-immune disorders and mechanical or toxic injuries (1). Following liver injury, the resident liver macrophages are activated and exert pro-inflammatory, pro-wound healing and restorative effects at different stages of hepatic injury and the repair response (2). Studies using animal models of chemical-induced liver injury have identified macrophages as the key regulators of liver repair and regeneration, or fibrosis. In the present review, the various functions of macrophages in hepatic toxicity are illustrated.

2. Macrophages

Macrophages are widely distributed phagocytic innate immune cells that have essential roles in tissue homeostasis and the host defence. The diverse tissue macrophage populations resident in most tissues of the body mainly originate from the yolk sac in the process of embryogenesis, and certain tissue macrophages are developed from fetal liver and hematopoietic progenitors at later time-points (3). For closed tissues, resident macrophages [e.g., lung alveolar macrophages and liver Kupffer cells (KCs)] mostly originate from fetal liver monocytes (4). Liver macrophages, accounting for 20-35% of hepatic non-parenchymal cells, make up the largest proportion (80-90%) of tissue macrophages in the host and are an essential constituent of the mononuclear phagocytic system (5). They consist of two distinct populations: 'sessile' KCs and motile liver macrophages, named as monocyte-derived macrophages (MoM ϕ). The former, 'sessile' KCs, function as a scavenger to remove microorganisms and cell debris from the blood and clear aged erythrocytes. Furthermore, in adult tissues, they undergo self-maintenance independently of hematopoietic stem cells. Phenotypes of 'sessile' KCs are characterized as F4/80^{hi}, CD11b^{lo}, CD169⁺, CD68⁺, Mac-2⁺ and CD80^{lo/-} (6-8). The latter, motile liver macrophages are distinct from 'sessile'

KCs in terms of local migration to participate in inflammatory foci (9). The major function of motile liver macrophages is immune surveillance. Furthermore, these cells directly originate from circulating monocytes. Surface expression marker profiles of motile liver macrophages include F4/80^{int}, CD11b^{hi} and CD80^{hi} (8). These characteristics suggest that liver macrophages have distinct liver-specific gene expression patterns.

In spite of the widespread use of specific terms to define macrophage activation states [i.e., classically activated (M1) and alternatively activated (M2), no experimental standards are currently available for describing their activation (10). The original terminology using M1 and M2 macrophage activation states is derived from different macrophage gene expression patterns stimulated with interferon (IFN)- γ /lipopolysaccharide (LPS) or interleukin (IL)-4/IL-13 (11). Within this terminology, classically activated M1 macrophages (activated by IFN- γ , LPS or high-mobility group protein 1) are functionally pro-inflammatory, microbicidal and tumoricidal. Furthermore, they exhibit anti-proliferative and cytotoxic activity. Virtually all of these features are produced by the release of numerous inflammatory cytokines, including tumor necrosis factor (TNF)- α , IL-1, IL-6 and IL-12/23 (p40). By contrast, alternatively activated M2 macrophages downregulate the inflammatory response and facilitate tissue repair by increasing the expression of IL-10, IL-4/IL-13 and transforming growth factor (TGF)- β , as well as vascular endothelial growth factor (VEGF)- α . Due to the complex biological characteristics of macrophage subsets, M2 macrophages are further subdivided to account for their differences: M2a, M2b and M2c activated by IL-4/IL-13, LPS/IL-1 β and IL-10/glucocorticoids, respectively (12). However, the concept of the M1 and M2 definitions requires to be revised; this should include a reproducible experimental standard, minimal reporting standards, a definition of the activators and markers of activation (10,13). In fact, macrophages display variable functions (e.g., initiation and perpetuation of inflammation, promotion of liver fibrosis and resolution of inflammation and fibrosis) in diverse microenvironments. The plasticity of macrophage activation may be elucidated by analyzing macrophage expression profiles. Furthermore, it is noteworthy that the 'restorative macrophages' in the liver fibrosis resolution phase derived from recruited lymphocyte antigen 6 complex, locus C (Ly6C)⁺ monocytes have a phenotype that is distinct from the M1/M2 definitions. Thus, the M1/M2 terminology is, at large, not applicable to liver diseases. However, whether the resolution of liver damage only requires newly recruited monocytes or hepatic macrophages capable of proliferating and switching their state of activation or that may be transformed in response to complex signals has remained to be sufficiently elucidated. In the present review, the original definition from the respective previous studies discussed is quoted when referring to mononuclear phagocyte cell types.

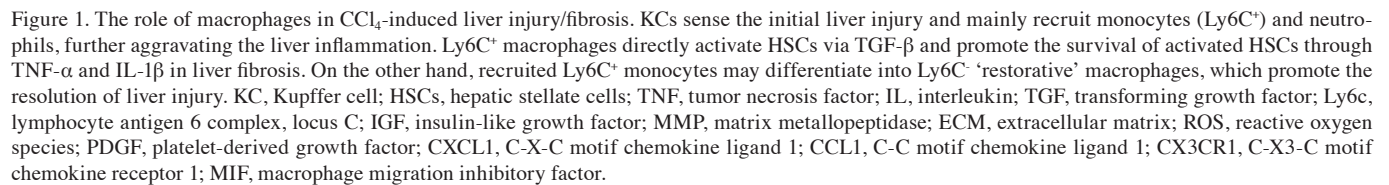
3. Role of macrophages in carbon tetrachloride-induced liver injury, fibrosis and repair.

The liver is a crucial organ of drug and toxin metabolism in the body, making it susceptible to toxic substances. When the liver is injured, monocytes and KCs are recruited and activated to

exert numerous functional roles (1). Carbon tetrachloride (CCl₄) toxicity has various distinct ultimate outcomes, including hepatocyte necrosis, liver fibrosis and cirrhosis, or even cancer (14). Tissue repair is a complex process influenced by intricate cellular signaling pathways consisting of various cytokines, chemokines, nuclear receptors and growth factors that may trigger the expression of pro-mitogenic genes and finally promote cell division (15). Hence, the mouse model of CCl₄-induced hepatic injury or fibrosis is probably the best representative experimental model for elucidating the various roles of liver macrophages in response to liver injury or fibrosis (Fig. 1).

Monocyte recruitment. Blood monocytes represent circulating precursors of tissue dendritic cells and macrophages, and may be divided into two major subsets in mice: Ly6C^{+/hi} and Ly6C^{-/low} monocytes. Ly6C⁺ mouse monocytes highly express the chemokine receptors C-C motif chemokine receptor 1 (CCR1) and CCR2, whereas murine Ly6C⁻ monocytes mainly express CCR5 and C-X3-C motif chemokine receptor 1 (CX3CR1) (16). The early recruitment of Ly6C⁺ monocytes, but not of Ly6C⁻ monocytes, to the liver upon toxic injury is mediated by CCR2 [ligand: C-C motif chemokine ligand 2 (CCL2)] and CCR8 (ligand: CCL1). Studies using CCR2-deficient (CCR2^{-/-}) and monocyte chemoattractant protein (MCP)-1^{-/-} mice or specific blockade suggested that CCR2 mediates the early accumulation of inflammatory Ly6C⁺ monocytes in the damaged murine liver (17). CCR8 is also crucial for Ly6C⁺ monocyte infiltration into the injured murine liver (18). Furthermore, Ly6C⁺ monocytes, migrating from the blood to tissues affected by infection, may differentiate into inflammatory macrophages in inflamed tissues (19). Of note, Ly6C⁻ monocytes have a more patrolling role at the endothelium in a lymphocyte function-associated antigen-1- and CX3CR1-dependent fashion, acting as scavengers and orchestrating tissue repair (20), without inflammatory stimuli. It was recently noted that Ly6C⁻ monocytes do not represent a distinct lineage, but instead originate from Ly6C⁺ monocytes regulated by CCAAT/enhancer binding protein β in the bone marrow and blood, and that the lifespan of Ly6C⁻ monocytes may be negatively controlled by Ly6C⁺ peripheral blood monocytes (21). A systematic assessment of the differential roles of these monocyte subsets and their recruitment dynamics in liver injury is required prior to the development of therapeutic strategies aimed at targeting these monocyte subsets.

Regulation of macrophage activation. The failure of CCl₄-induced liver fibrosis matrix degradation resulting from the depletion of scar-associated macrophages (SAMs) in the process of recovery and the reduction of scarring and myofibroblast activation resulting from SAM depletion during the injury stage suggest that macrophages exert distinct functions in the same tissue. Duffield *et al* (22) identified two distinct populations of SAMs: One derived from circulating monocytes and the other likely derived from KCs. They subsequently identified the CD11b^{hi} F4/80^{int} Ly6C^{lo} macrophage subset as the 'restorative macrophage group', which originated from recruited inflammatory monocytes (Ly6C^{hi} monocytes), and as the governing matrix metalloproteinase (MMP)-expressing subset during most active fibrosis resolution. Furthermore, a study on the depletion



Mediators derived from macrophages initiate hepatic inflammation and fibrosis. KCs are phagocytic innate immune cells that clear dead cells and cell debris, and maintain liver homeostasis; furthermore, they are able to sense liver injury and subsequently orchestrate pro-inflammatory processes. Similar

CXCL1, CXCL2 and CXCL8 are central chemokines that attract neutrophils mainly through the chemokine receptors CXCR1 and CXCR2. Neutrophil recruitment increases the release of reactive oxygen species (ROS) and proteases, leading to hepatocyte necrosis (29). In parallel, KCs secrete CCL2 to increase circulating CCR2⁺Ly6C⁺ monocytes that massively expand the local macrophage pool (17). CCR2⁺Ly6C⁺ monocyte migration into the liver after injury is functionally critical for the maintenance of liver inflammation and fibrogenesis.

The functionality of MoM ϕ not only depends on CCL2, but also on CCL1 and CCL25, which induce the migration of pro-inflammatory monocytes/macrophages via CCR8 and CCR9, respectively (18,30,31). Ultimately, KCs sense initial liver injury and are responsible for monocyte and neutrophil recruitment, further aggravating liver inflammation.

With inflammatory stimuli, the program of cytokine production by KCs, triggered by TLR signaling, is more complex than the simple result from the activation of transcription factors. KCs are poised to swiftly respond to PAMPs or DAMPs with the production of TNF. In KCs, the production of TNF usually precedes and generally promotes the carefully orchestrated release of many other inflammatory mediators including IL-6, IL-12/23 (p40) and type I interferons (e.g., IFN- γ and TNF- α) (32). IFN- γ is a hallmark cytokine of Th1 cells that greatly increases the production of inflammatory mediators by macrophages. Consequently, KCs activated by IFN- γ express numerous pro-inflammatory cytokines (e.g., IL-6 and TNF- α) with the enhancement of nuclear factor (NF)- κ B gene expression. While these pro-inflammatory signals may lead to enhanced liver inflammation and injury, they also have protective effects on the liver. IL-6 signaling, via signal transducer and activator of transcription (STAT)3 activation, markedly increases after acute CCl₄-induced hepatic damage and promotes liver proliferation by upregulating the expression of growth factors (e.g., hepatocyte growth factor), increases hepatocyte responsiveness and also inhibits hepatocyte apoptosis. TNF- α has a major role in the regulation of IL-6 secretion and liver regeneration via induction of NF- κ B (33,34).

Severe liver damage caused by high doses of CCl₄ may cause death or liver self-healing. However, repeated injections of low doses of CCl₄ in rodents lead to liver fibrosis or liver cirrhosis. In fibrogenesis, Pradere *et al* (35) revealed that liver macrophages promote myofibroblast survival in an NF- κ B-dependent manner, and macrophage-derived TNF- α and IL-1 β enhance the survival of activated hepatic stellate cells (HSCs). Activated HSCs and hepatic myofibroblasts exert pro-fibrogenic activity, as they may increase the levels of fibrotic matrix proteins, thus inhibiting fibrotic degradation (36). Furthermore, Karlmark *et al* (17) demonstrated that CCR2 has a crucial role in the migration of Ly6C^{hi} monocytes, which directly activates HSCs via TGF- β , upon chronic liver injury.

Aside from TGF- β , platelet-derived growth factor (PDGF) expressed in liver macrophages also contributes to liver fibrogenesis (37). PDGF promotes HSC proliferation and activation through PDGF receptor and does not have this effect after neutralization (38). However, the fibrosis-associated TLR signaling of KCs and HSCs remains elusive. Perugorria *et al* (39) reported that once TLR is activated, elevated Tpl2 may be identified as an essential signal transducer in KCs and HSCs, and promotes fibrogenic gene expression.

Mediators derived from macrophages suppress liver inflammation and decelerate fibrogenesis. KCs have an important role in the initiation and perpetuation of liver inflammation, which are necessary for the host defence, but if not controlled, they may cause hepatic damage, fibrosis and cirrhosis. Four cytokines with the ability to downregulate macrophage function have been identified: IL-4, IL-10, IL-13

and TGF- β 1, of which IL-10 appears to have a broader and deeper effect. Importantly, IL-10 is released from macrophages, type 2 T-helper (Th2) cells and stromal cells. IL-10 inhibits the expression of NF- κ B, the production of pro-inflammatory cytokines by Th1 cells, macrophages and neutrophils, the proliferation of hepatocytes and fibrogenesis during liver repair (40,41). Hepatic macrophages not only promote hepatic fibrosis by activating HSCs in chronic hepatic damage, but also contribute to the resolution of fibrosis by degrading the extracellular matrix (ECM) (26). Macrophages produce gelatinases (MMP9, MMP12 and MMP13) under different circumstances, resulting in complex ECM degradation. During fibrosis regression, recruited Ly6C⁻ monocytes differentiate into Ly6C⁺ 'restorative' macrophages, with upregulation of MMPs (MMP9 and MMP12), downregulation of pro-inflammatory cytokines and chemokines, enhanced expression of insulin-like growth factor 1 (IGF-1) and genes associated with anti-inflammatory or antifibrotic effects, including CX3CR1, CD74 and macrophage migration inhibitory factor, and a reduction in TGF- β , thus promoting recovery from injury. Furthermore, SAMs produce MMP13, which may disassemble the interstitial matrix and promote fibrosis resolution (23,42). However, whether KCs or Ly6C⁻ 'restorative' macrophages are the source of MMP13 remains elusive. In addition, KCs are a major source of CXCL9, which ameliorate liver fibrogenesis (43). Furthermore, CX3CR1 is a major regulator of monocyte differentiation and survival in the liver and protects against liver fibrosis (44).

4. Role of macrophages in other non-CCl₄ induced liver injury animal models

Macrophages in cholestatic liver injury, fibrosis and repair. Bile duct ligation (BDL) is a commonly used animal model of cholestatic liver disease. Ligation of the common bile duct performed at a standardized site prevents bile flow and causes bile reflux followed by cholangitis, coagulation defects and hepatic damage, and may even result in biliary fibrosis and cirrhosis (45). The absence of bile salts and bile in the intestines after BDL may promote translocation of endotoxins and growth of endotoxin-producing bacteria, resulting in cholangitis (45). KCs are a major type of defence cell for the liver, as they remove endotoxins (e.g., LPS) and phagocytose bacteria under normal conditions. However, the intracellular bactericidal function of KCs in the BDL model is impaired as a result of high levels of hydrophobic bile acids in the serum. The altered sensitivity of KCs to endotoxins in BDL induces overproduction of TNF- α and IL-6, which leads to liver injury, but KC blockade may suppress systemic cytokine production and improve survival under these conditions (46). Furthermore, toxic bile salts directly cause rodent hepatocyte apoptosis through activation of Fas (47), which leads to apoptotic body formation and results in HSC activation through phagocytosis of apoptotic bodies and enhancement of fibrogenesis (48). The engulfment of hepatocyte apoptotic bodies by KCs has also been reported to promote liver inflammation and fibrogenesis, which is mediated by death ligands and cytokines, including TNF- α , TNF-related apoptosis-inducing ligand (TRAIL), Fas and TGF- β (49). However, later studies demonstrated that KCs abrogate cholestatic liver injury via IL-6 and

acid sphingomyelinase-dependent mechanisms (50,51). In conclusion, KCs may have protective and promoting roles in cholestatic liver injury.

Similar to that in cholestatic liver injury, KCs also possess a dual role in BDL-induced liver fibrosis. Although KCs generate death ligands to promote liver inflammation (49) and produce acid sphingomyelinase to activate AKT in hepatocytes, which is required for regeneration (51), higher expression of TGF- β , a fibrogenic cytokine, has also been observed (49,51). Liver injury is associated with increased hepatic exposure to LPS. In contrast to KCs, LPS mainly targets TLR4 in HSCs, and makes HSCs sensitive to TGF- β via the MyD88/NF- κ B-dependent pathway (52). Furthermore, high levels of IL-17A have been detected in fibrotic livers (53). IL-17A is generated predominantly by effector CD4⁺ T (Th17) cells, which differentiate from Th0 cells, and is regulated by IL-6, TNF, TGF- β and IL-23 (54). IL-17 stimulates KCs to further upregulate IL-17A levels, increasing IL-17 receptor A expression and induces the production of the pro-inflammatory cytokines TNF- α , IL-6 and IL-1 β , as well as the fibrogenic cytokines TGF- β 1 and PDGF (53). IL-6 and TGF- β 1 further facilitate the differentiation and expansion of Th17 cells (53). In addition, PDGF promotes HSC proliferation and activation (55). However, IL-17 production may be inhibited by activation of cannabinoid receptor 2 in macrophages (56). Furthermore, CD68⁺ KCs may clear apoptotic cholangiocytes via phagocytosis, resulting in the upregulation of MMP3, MMP8 and MMP9, which contribute to the reversal of biliary fibrosis (57).

Role of macrophages in thioacetamide-induced liver injury, fibrosis and repair. Thioacetamide (TAA), a centrilobular hepatotoxin, is widely applied to induce acute or chronic hepatic disease (58). Initial lesions in the liver begin with a cytochrome P450 family 2 subfamily E member 1-mediated two-step bioactivation of TAA into thioacetamide sulfoxide and further to thioacetamide-S,S-dioxide (58), which is mainly distributed in zone 3 (centrilobular area), resulting in cytotoxicity. Injured or necrotic hepatocytes release S100 proteins, high-mobility group box proteins (HMGBs) and heat shock proteins (HSPs) as DAMPs (59). HMGBs and S100 proteins may be recognized by TLR-2 and TLR-4 (60) to produce pro-inflammatory cytokines IL-6, TNF α and IL-1 β . However, HSP25 may act to protect liver cells by macrophages invading the hepatic lesion (61). Gadolinium chloride (GD) is used to selectively inactivate KCs, resulting in decreased serum levels of TNF- α and IL-6, reduced TAA-induced liver injury, as well as enhanced metallothionein and HSP70 expression (62). Furthermore, GD treatment decreases the numbers of CD68⁺ and CD163⁺ macrophages and inhibits TGF- β 1 expression in macrophages (63). CD68⁺ cells with maintained high levels of MCP-1 expression have been detected in injured perivascular areas for up to 20 days, whereas CD163⁺ cells gradually decreased in number in the mid-zonal areas after day 3 (64). In addition, depletion of M1 (expressing CD68 and major histocompatibility complex class II) and M2 (expressing CD163 and CD204) macrophages by liposomal clodronate, may aggravate and prolong coagulation necrosis of hepatocytes. This has been reported to be primarily due to the depletion of M2 macrophages, revealing a remodeling stage dominated by M2 (65). However, KCs may release nitric oxide

and trigger post-necrotic hepatocyte regeneration following TAA treatment (66). Therefore, KCs may also possess a dual role in TAA-induced hepatic injury.

Repeated injection of TAA or the addition of TAA to drinking water has been widely used to induce hepatic fibrosis. Similarly, HSCs also have a critical role in TAA-induced fibrosis (67). PDGF-B may be the major cytokine for HSC activation in TAA-induced liver fibrosis (68). Furthermore, galectin-3 (Gal-3)⁺ macrophages are also involved in the initiation of fibrosis via the activation of HSCs (69). However, Gal-3⁺ macrophages possess M1 and M2 properties in the advanced stage of liver fibrosis (70). To remodel tissue in the fibrotic liver, a splenectomy may be effective due causing an accumulation of Ly6C^{lo} macrophages and the disappearance of hepatic progenitor-like cells (71).

Role of macrophages in acetaminophen-induced acute liver injury and repair. Acetaminophen (APAP) overdose-induced liver injury is the most common cause of drug-induced hepatotoxicity in Western countries. An APAP overdose may result in mitochondrial dysfunction, ATP depletion and DNA fragmentation, which eventually cause cell necrosis (72). In addition to initial hepatocyte necrosis, the release of DAMPs, including DNA fragments, HMGB-1 and HSPs, induces KC activation and an inflammatory response, which also contributes to the disease (73). Although HMGB-1 has several separate receptors, including TLR2, TLR4 and TLR9, TLR4 is a pivotal receptor for macrophage activation and cytokine production (TNF, CXCL2, IL-6, IL-1 β and IL-10) (74). HMGB-1/TLR4-dependent activation of IL-23 release from macrophages promotes $\gamma\delta$ T cells to produce IL-17A, which recruits neutrophils participating in sterile inflammation (75). Furthermore, neutrophil infiltration to necrotic areas also depends on the CXCR2/formyl peptide receptor 1 axis (76). However, the role of neutrophils in liver damage is controversial, as neutrophils may either aggravate or have no effect on liver injury (77,78).

A total of three distinct macrophage subsets were identified during APAP-induced acute liver injury and repair (Fig. 2): Resident KCs, Ly6C^{hi} monocytes/macrophages and Ly6C^{lo} MoM ϕ . Although KCs produce TNF- α and IL-1 β , there is no credible evidence that KCs directly cause liver cell damage (79). Furthermore, the recently discovered Mer tyrosine kinase-expressing macrophage phenotype that has hepatoprotective effects, including neutrophil apoptosis and clearance, is mainly derived from the KC population (80). It is worth noting that the number of KCs is decreased upon APAP application, and then increased by self-renewal. In addition, Ly6C^{hi} monocytes may be recruited into the liver and trans-differentiated into the Ly6C^{lo} MoM ϕ phenotype through CX3CL1/CX3CR1, MCP-1/CCR2 and in a monocyte colony-stimulating factor-dependent manner. In addition, Ly6C^{hi} monocytes and Ly6C^{lo} MoM ϕ contribute to liver recovery. However, Zigmond *et al* (81) and Mossanen *et al* (82) demonstrated that CCR2Ly6C^{hi} monocytes/macrophages promote APAP hepatotoxicity at an early stage. Another study has indicated that Ly6C^{hi} monocytes and Ly6C^{lo} MoM ϕ are likely to work together: The former promote apoptosis of ROS-producing neutrophils and the latter promote neutrophil clearance (83). In addition, Ly6C^{hi} monocytes have higher levels of VEGF-A,

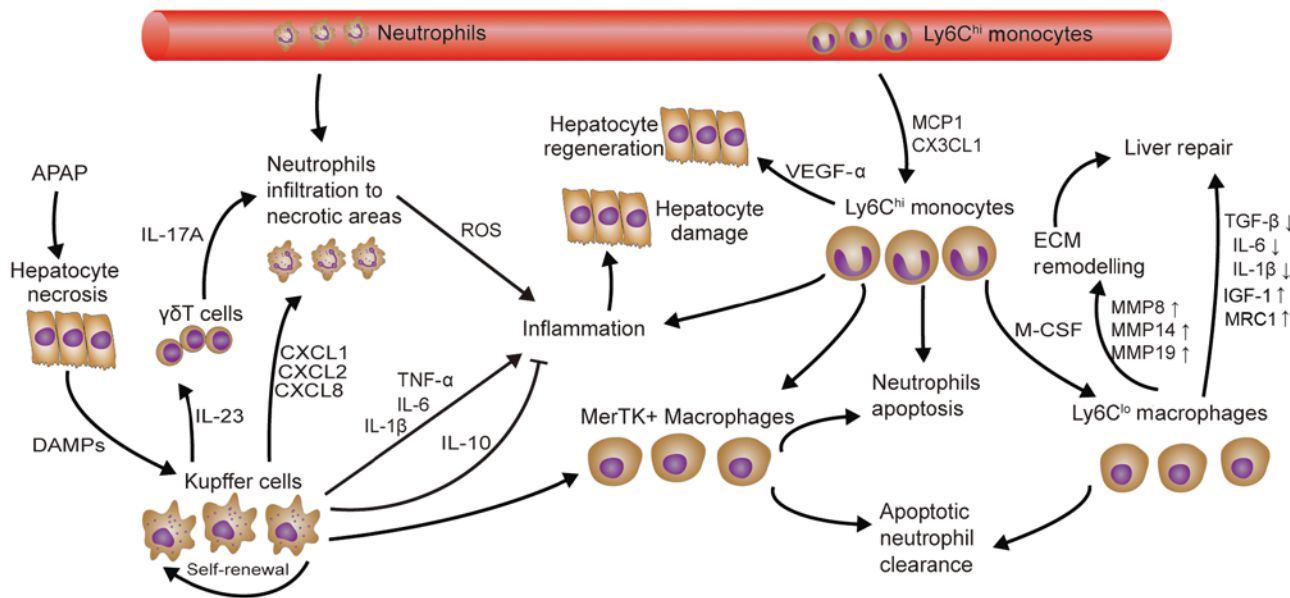


Figure 2. Role of macrophages in APAP-induced acute liver injury. APAP-induced liver injury is aggravated by the activation of KCs as a result of DAMP release from necrotic hepatocytes, causing neutrophil infiltration and subsequent Ly6C^{hi} monocyte recruitment. Ly6C^{hi} monocytes and their Ly6C⁺ macrophage descendants then promote neutrophil apoptosis and clearance, hepatocyte regeneration and remodeling of the ECM. MerTK⁺ macrophages derived from KCs and monocyte-derived macrophages may also promote neutrophil apoptosis and clearance. KC, Kupffer cell; TNF, tumor necrosis factor; IL, interleukin; TGF, transforming growth factor; Ly6c, lymphocyte antigen 6 complex, locus C; IGF, insulin-like growth factor; MMP, matrix metalloproteinase; ECM, extracellular matrix; CXCL1, C-X-C motif chemokine ligand 1; CX3CR1, C-X3-C motif chemokine ligand 1; DAMP, damage-associated molecular pattern; APAP, acetaminophen; M-CSF, monocyte colony-stimulating factor; MCP1, monocyte chemoattractant protein 1; VEGF, vascular endothelial growth factor; MerTK, Mer tyrosine kinase; MRC1, mannose receptor C-type 1.

TGF- β 1, IL-6, IL-1 β and MMP18 gene expression, whereas Ly6C^{lo} MoM ϕ express higher levels of pro-restorative genes, including IGF-1 and mannose receptor 1. In comparison with KCs, Ly6C^{hi} monocytes and Ly6C^{lo} MoM ϕ also express high levels of MMP8, MMP14 and MMP19, which are important for ECM re-modeling (81).

Role of macrophages in D-galactosamine-induced acute liver failure. D-galactosamine (D-GalN) may inhibit the synthesis of mRNA and proteins that cause hepatocyte necrosis. Furthermore, D-GalN-induced necrosis was reported to increase gut permeability and cause endotoxemia in a rat model but not in a mouse model (84). This is also thought to contribute to hepatotoxicity. Thus, D-GalN/LPS co-administration is commonly used to induce liver injury in mice (85). In fact, D-GalN increases the sensitivity of rabbits, rats and mice to LPS, possibly by upregulating TLR4 expression in liver macrophages and producing lethal toxicity (86,87). Along these lines, depletion of TLR4 leads to a decreased inflammatory response and hepatic injury (88). Under LPS stimulation, KCs secrete TNF- α , and cause hepatocyte apoptosis and the release of other cytokines (IL-1 and IL-6) (85). However, TNF- α appears to be the major pro-inflammatory mediator, as macrophage autophagy may inhibit the production of IL-1 β . In addition, IL-6 treatment was reported to have a beneficial effect and reduce the expression of TNF- α and MCP-1, and promote macrophage polarization towards M2 in D-GalN/LPS induced liver injury models (89,90). Besides LPS, cytosine-phosphate-guanine DNA and polyinosinic:polycytidylic acid also exert hepatic toxicity mediated by TNF- α in D-GalN-sensitized mice (91). The depletion of macrophages and the inhibition of TNF- α production protected against D-GalN/LPS-induced hepatic injury (92,93).

Furthermore, increasing the production of IL-10 in macrophages via treatment with IL-35 also provided a therapeutic effect (94).

Role of macrophages in liver ischemia/reperfusion injury and repair. Ischemia/reperfusion injury (IRI) may be divided into two types, namely those with 'warm' ischemia and 'cold' ischemia. Warm ischemia may occur during liver surgery, shock or trauma, whereas cold ischemia may develop in liver transplants. However, the two types share a common mechanism in inflammatory immune regulation (95). HMGB1 may be released from hepatocytes in response to hypoxia/ischemia and remain at high levels for up to 24 h after reperfusion, resulting in liver inflammation and injury mediated by TLR4 (96). Inhibition of TLR4 and KCs simultaneously was reported to reduce TNF- α , IL-6, CXCL2 production and IRI (97,98). Conversely, KCs also exert protective effects in IRI through IL-10 and heme oxygenase-1 (HO-1) (99,100). Furthermore, HO-1 modified MoM ϕ may prevent IRI, possibly via the HO-1/STAT3 axis (101,102). In addition, neutrophils recruited by CXCL2 and CD4⁺ T cells, which regulate macrophage and neutrophil function via IFN- γ and IL-10, also have an important role in IRI (103).

Role of macrophages in non-alcoholic fatty liver disease. Non-alcoholic fatty liver disease (NAFLD), which encompasses simple fatty liver (SFL), non-alcoholic steatohepatitis (NASH) and associated cirrhosis, are usually linked to obesity, insulin resistance and hyperlipidemia (104). The phenotypic switch of adipose tissue macrophages (ATMs) from M2 ATMs to M1 ATMs contributes to insulin resistance (IR) and hepatic steatosis in obese mice (105). IR, as well as concomitant hyperglycemia and hyperinsulinemia, may lead to increased production of free fatty acids (FFAs), but reduced FFA

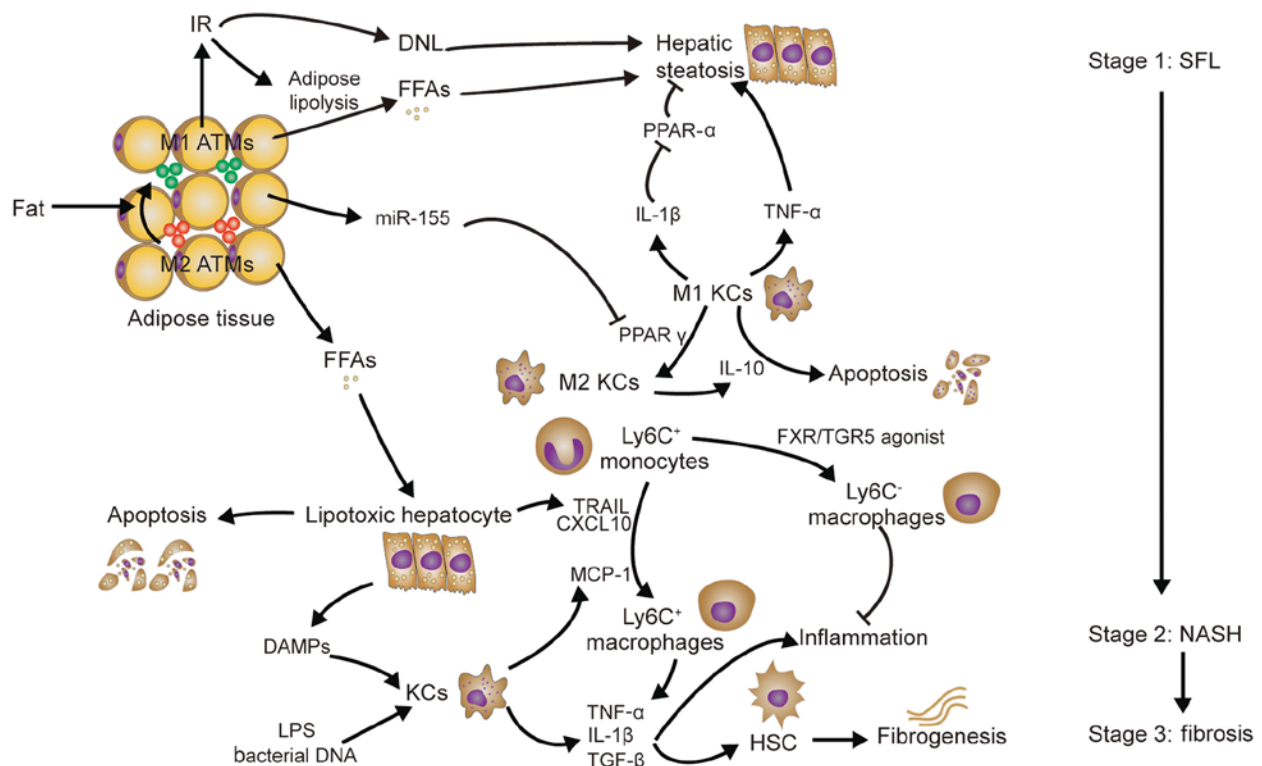


Figure 3. Role of macrophages in non-alcoholic fatty liver disease. Obesity-associated IR and KCs contribute to hepatic steatosis. LPS and bacterial DNA derived from the intestine, as well as DAMPs released from FFA-induced lipotoxic hepatocytes, activate KCs, resulting in Ly6C⁺ macrophage chemotaxis and the release of pro-inflammatory cytokines (TNF- α and IL-1 β), which promote liver inflammation. In addition to promoting liver inflammation, TNF- α and IL-1 β may activate HSCs to upregulate the production of TIMP1, an inhibitor of MMPs that causes fibrogenesis. KC, Kupfer cell; ATMs, adipose tissue macrophages; DNL, *de novo* lipogenesis; PPAR, peroxisome proliferator activated receptor; TNF, tumor necrosis factor; IL, interleukin; MMP, matrix metalloproteinase; FFA, free fatty acids; TIMP1, tissue inhibitor of metalloproteinases-1; TRAIL, TNF-related apoptosis-inducing ligand; SFL, simple fatty liver; NASH, non-alcoholic steatohepatitis; DAMP, damage-associated molecular pattern; LPS, lipopolysaccharide; miR, microRNA; IR, insulin resistance; HSCs, hepatic stellate cells; TGF, transforming growth factor; PPAR peroxisome proliferator-activated receptor; FXR, farnesoid X receptor; TGR5, Takeda G-protein receptor 5; TRAIL, TNF-related apoptosis-inducing ligand; CXCL, C-X-C motif chemokine ligand; Ly6c, lymphocyte antigen 6 complex, locus C.

oxidation and increased *de novo* lipogenesis, which results in lipid accumulation in hepatocytes and hepatic steatosis (106). In addition, KCs promote hepatic steatosis (Fig. 3). MicroRNA-155 produced by ATMs under obesity conditions may target hepatocytes and reduce the expression of peroxisome proliferator activated receptor (PPAR) γ , which is required for KC polarization to the M2 phenotype (107,108); this results in an M1-predominant phenotype of KCs. M1 KCs produce IL-1 β , which inhibits PPAR α expression and leads to a suppression of fat oxidation and an aggravation of hepatic steatosis (109). Depletion of KCs and inhibition of TNF- α reduces hepatic steatosis in rats fed a high-fat/sucrose diet (110). Conversely, M2 KCs produce IL-10 to promote M1 KC apoptosis and protect against NAFLD (111).

The mechanisms of NASH have been described by two different hypotheses: The 'two-hit' hypothesis (112) and the 'multiple parallel hits' hypothesis (113). However, the identification of KCs as promoters of hepatic steatosis (as mentioned above) challenges the latter hypothesis. Indeed, palmitic acid and stearic acid (saturated fatty acids), and oleic acid (a monounsaturated fatty acid) may all prompt hepatocyte apoptosis (lipotoxicity) (114). Furthermore, lipotoxic hepatocytes may release TRAIL and CXCL10 to induce macrophage chemotaxis (115,116). Furthermore, FFAs may also prompt hepatocytes to release HMGB1 (117). However, Ly6C⁺ macrophage infiltration may be mainly dependent on MCP-1

produced by KCs, as only early depletion of KCs prevents the development of NASH (118). In addition, inhibition of MCP-1 may also inhibit the development of steatohepatitis (119). Ultimately, macrophage accumulation aggravates liver inflammation via the release of TNF- α and IL-1 β (120). However, bile acid receptor farnesoid X receptor/Takeda G-protein receptor 5 agonist was reported to contribute to the intra-hepatic monocyte phenotype Ly6C^{low} (121). Endotoxin and bacterial DNA derived from the intestine may also activate the release of TLR4 and TLR9 from KCs separately, resulting in IL-1 β expression. In addition to promoting liver inflammation, TNF- α and IL-1 β activate HSCs to upregulate the production of tissue inhibitor of metalloproteinases-1, which inhibits MMPs and causes fibrogenesis (122,123). Leptin produced by activated HSCs may also further promote liver fibrosis, which is possibly mediated by TGF- β 1 expression (124).

5. Therapeutic potential of macrophages

Understanding the different roles and mechanisms of macrophages in hepatic damage, fibrosis and repair is critical for the development of novel therapies for hepatic diseases. When the liver is injured, KCs sense initial liver injury and initiate inflammatory cascades. Subsequently, inflammatory monocytes accumulate in the liver via chemokine-chemokine receptor interactions. When liver injury ceases, restorative macrophages

Table I. Diverse roles of macrophages in liver diseases.

| Injury | | |
|------------------|--|--|
| Animal model | Mφ | Role (Refs.) |
| CCl ₄ | CCR9 ⁺ MoMφ | Contribution to the induction of acute liver inflammation and promotion of liver fibrosis (30,31) |
| | CCR8 ⁺ MoMφ | Promotion of hepatic inflammation and fibrosis progression (18) |
| | CD11b ⁺ F4/80 ⁺ Ly6C ⁺ MoMφ | Differentiate preferentially into M1-type Mφ; promotion of fibrogenesis (17) |
| | F4/80 ⁺ CD11b ⁺ /Mφ | Mixed M1/M2 phenotype, promotion of myofibroblast survival (35) |
| BDL | CCR8 ⁺ MoMφ | Promotion of hepatic inflammation and fibrosis progression (18) |
| | F4/80 ⁺ CD11b ⁺ /Mφ | Mixed M1/M2 phenotype; promotion of myofibroblast survival (35) |
| | KCs | Promotion of liver inflammation and fibrogenesis mediated by death ligands and cytokines (TNF-α, TRAIL, Fas and TGF-β) (49) |
| | F4/80 ⁺ KCs | Promotion of liver fibrogenesis by TGF-β (51) |
| TAA | KCs | Promotion of liver injury (62) |
| | CD68 ⁺ /CD163 ⁺ Mφ | Promotion of hepatic fibrosis by TGF-β1 (63) |
| APAP | Gal-3 ⁺ Mφ | Participation in acute liver lesions and fibrosis (69) |
| | F4/80 ^{hi} CD11b ^{lo} KCs | Promotion of liver inflammation and neutrophil infiltration (74-76) |
| | Ly6C ^{hi} MoMφ | Promotion of hepatic inflammation (82) |
| | M1 KCs | Promotion of hepatic steatosis (109,110) |
| NAFLD | KCs | Induction of Ly6C ⁺ macrophage chemotaxis and release of pro-inflammatory cytokines TNF-α and IL-1β (118) |
| | Ly6C ⁺ MoMφ | Contribution to release of pro-inflammatory cytokines TNF-α and IL-1β (120) |
| Repair | | |
| Animal model | Mφ | Role (Refs.) |
| CCl ₄ | CX3CR1 M/MoMφ | Major regulator of differentiation and survival of monocytes in the liver; protection against liver fibrosis (44) |
| | F4/80 ⁺ scar-associated macrophages | Source of MMP13 that mediates matrix remodeling in liver fibrosis (22,42) |
| | CD11b ^{hi} F4/80 ^{int} Ly6C ^{lo} MoMφ | Promotion of fibrosis resolution with increased expression of MMP9, MMP12 and MMP13 (23,24) |
| | KCs | Major source of CXCL9 that may ameliorate liver fibrogenesis (43) |
| BDL | CX3CR1 M/MoMφ | Major regulator of differentiation and survival of monocytes in the liver and protection against liver fibrosis (44) |
| | CD68 ⁺ KCs | Clearance of apoptotic cholangiocytes via phagocytosis resulting in upregulation of MMP3, MMP8 and MMP9 to contribute to the reversal of biliary fibrosis (57) |
| | F4/80 ⁺ KCs | Abrogation of cholestatic liver injury by IL-6 and ASMA-dependent mechanisms (50,51) |
| | KCs | Release of nitric oxide that may trigger post-necrotic hepatocellular regeneration (66) |
| TAA | CD163 ⁺ CD204 ⁺ Mφ | Release of IL-4 and IL-10, phagocytosis of apoptotic cells and synthesis of TGF-β1 (65) |

Table I. Continued.

| Animal model | Repair | | Role | (Refs.) |
|--------------|---------------------------|---|------|---------|
| | Mø | | | |
| APAP | Ly6C ^{lo} MoMø | Tissue remodeling in fibrotic liver | | (71) |
| | MerTK ⁺ KCs/Mø | Promotion of neutrophil apoptosis and clearance | | (80) |
| | Ly6C ^{hi} M | Promotion of hepatocyte regeneration and neutrophil apoptosis | | (81,83) |
| | Ly6C ^{lo} MoMø | Promotion of apoptotic neutrophil clearance and remodeling of the ECM | | (81,83) |
| NAFLD | M2 KCs | Production of IL-10 to promote M1 KC apoptosis | | (111) |
| | Ly6C ⁺ MoMø | Attenuation of intrahepatic inflammation | | (121) |

Mø, macrophages; MoMø, monocyte-derived macrophages; M, monocytes; CCR, C-C chemokine motif receptor; CCL, C-C chemokine motif ligand; CX3CR, C-X3-C motif chemokine receptor; MMP, matrix metalloproteinase; KCs, Kupffer cells; CXCL, C-X-C chemokine motif ligand; CXCR, C-X-C motif chemokine receptor; NKT, natural killer T cells; VEGF, vascular endothelial growth factor; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; TGF, transforming growth factor; IL, interleukin; ASMA, acid sphingomyelinase; NAFLD, non-alcoholic fatty liver disease; APAP, acetaminophen; Ly6c, lymphocyte antigen 6 complex, locus C; Gal-3, galectin-3; MerTK, Mer tyrosine kinase; BDL, bile duct ligation; TAA, thioacetamide; ECM, extracellular matrix.

promote the resolution of hepatic damage and fibrosis. Thus, novel methods to improve liver injury or fibrosis focus on targeting liver macrophages. These interventions regulate the activation of KCs (e.g., by inhibiting bacterial translocation or changing the composition of bile acid), inflammatory monocyte migration (e.g., against various chemokines or chemokine receptors), or macrophage polarization and differentiation (e.g., by targeted delivery of nanoparticles) (125). However, different interventions lack scientific comparison and require further evaluation of their effectiveness. Numerous studies only provided an immune function analysis at the organ level and did not specify the phenotypes of liver macrophages. Furthermore, the underlying mechanisms and triggers of hepatic macrophage phenotype switching during different stages of liver injury have not been well studied. Therefore, an in-depth study of the genomes and phenotypes of liver macrophages is required.

6. Conclusions

Liver macrophages, termed KCs and MoMø, regulate liver repair following injury (Table I). They may worsen hepatic damage by producing ROS and other inflammatory mediators that induce leukocyte aggregation and exacerbate hepatic injury. However, they also produce a variety of anti-inflammatory cytokines, including IL-4, IL-10, IL-13 and TGF- β , that downregulate macrophage function and switch their phenotype to that of restorative macrophages with pro-resolving activity. Consequently, liver macrophages may serve as potential targets for liver disease treatment, and studies focusing on this strategy will become increasingly frequent.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

XD wrote the manuscript and constructed the figures. JL and YX revised the manuscript. HC designed and revised the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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