Roles of heterogenous hepatic macrophages in the progression of liver diseases

Kyeong-Jin Lee*, Mi-Yeon Kim* & Yong-Hyun Han*
Laboratory of Pathology and Physiology, College of Pharmacy, Kangwon National University, Chuncheon 24341, Korea

Hepatic macrophages are key immune cells associated with the broad ranges of liver diseases including steatosis, inflammation and fibrosis. Hepatic macrophages interact with other immune cells and orchestrate hepatic immune circumstances. Recently, the heterogenous populations of hepatic macrophages have been discovered termed residential Kupffer cells and monocyte-derived macrophages, and identified their distinct population dynamics during the progression of various liver diseases. Liver injury lead to Kupffer cells activation with induction of inflammatory cytokines and chemokines, which triggers recruitment of inflammatory monocyte-derived macrophages. To understand liver pathology, the functions of different subtypes of liver macrophages should be regarded with different perspectives. In this review, we summarize recent advances in the roles of hepatic macrophages under liver damages and suggest hepatic macrophages as promising therapeutic targets for treating liver diseases. [BMB Reports 2022; 55(4): 166-174]

INTRODUCTION

The liver is the organ that has diverse functions such as numerous protein synthesis, metabolizing drugs and nutrients, and recycling components of red blood cells (1). Disorders of hepatocyte metabolism and other nonparenchymal cells including macrophages cause development of hepatic inflammation and fibrosis which lead to steatohepatitis and cirrhosis (2, 3). Hepatic macrophages are most abundant immune cells in the liver, estimated that 60% of hepatic immune cells are liver macrophages in healthy livers of human and mouse (4). There have been numerous studies that hepatic macrophages could modulate pathophysiological conditions of the liver including hepatoxocity, inflammation, tissue repair and fibrosis (5). Therefore, understanding the physiological actions of hepatic macrophages for liver diseases is necessary to improve perspectives on therapeutic strategies for curing liver inflammations and fibrotic diseases. Here, we highlight recent findings about roles of heterogenous hepatic macrophages in a pathologic status and open new approach for the treatment of liver diseases.

UNDERSTANDING HETEROGENEITY OF LIVER MACROPHAGES

Hepatic macrophages are most abundant immune cells in the liver. The hepatic macrophages contain different origins derived from yolk sac and bone marrow. The diverse heterogeneity of the liver macrophages are identified based on released cytokines, cell surface markers and transcriptional profiles (5, 6). The subsets of liver macrophages are separated to yolk sac-derived residential Kupffer cells (KCs) and recruited bone marrow (BM)-derived macrophages (monocyte-derived macrophages; MoMFs) (Fig. 1). The heterogenous subpopulations of liver macrophages can be separately determined by distinct expression of surface markers. In mice, KCs are F4/80high, CD11b+ and C-type lectin 4F (Clec4F)pos, while MoMFs are F4/80low, CD11bhigh, lymphocyte antigen 6 complex locus C1 (Ly6C)pos or neg, CD115pos, and Clec4Fneg (7-11) (Table 1). Human hepatic macrophages also share similar marker with murine liver macrophage such as T-cell immunoglobulin and mucin domain containing 4 (TIMD4) and macrophage receptor with collagenous structure (MARCO) (Table 2). The circulating monocytes can differentiate toward hepatic MoMFs, which are derived from BM CX3CR1posCD117posLinneg progenitor cells (12). Furthermore, hepatic MoMFs are divided to distinct subsets according to Ly6C expression: inflammatory Ly6Cpos MoMFs and restoring Ly6Cneg MoMFs (Table 1).

Kupffer cells (KCs)

KCs were named derived from the Karl Wilhelm von Kupffer who initially described these cells as hepatic sinusoidal endothelial cells (13). Later, they were corrected as macrophages by Brawic, and then were included in the mononuclear phagocyte system considered as the liver-resident monocyte-derived macrophages (14, 15). But, numerous fate-mapping studies have completely revealed the dogma of the origin of residual macrophages including KCs (16-18). The development of tissue resident macrophages occurs asyn-
Understanding the roles of hepatic macrophages
Kyeong-Jin Lee, et al.

Fig. 1. Roles of heterogenous liver macrophages during the progression of liver injury. Under the pathologic process of liver injury, the resident embryonic-derived Kupffer cells (EmKCs) located inside the sinusoidal endothelium recognize microbial pathogen-associated molecular patterns (PAMPs) and damaged cell-released damage-associated molecular pattern (DAMPs) via PRRs and inflammasome. The activated EmKCs release inflammatory cytokines and CCL2 chemokine to recruit circulating monocytes. They develop to the pre-macrophages until embryonic day 16.5. KCs are mainly yolk sac derived origins populated by second wave and marginally recapitulated by hematopoietic stem cell-derived monocytes after 1 year age mice generating heterogeneity of liver macrophages (20, 21). The definitive hematopoiesis is the third wave that hematopoietic stem cells from the aorta-gonad-mesonephros regions and umbilical/vitelline arteries move to fetal liver and differentiate into resident macrophages (19, 21).

KCs are primarily identified liver macrophage populations as CD45<sup>++</sup> F4/80<sup>++</sup> CD11b<sup>low</sup> with distinct expression of liver macrophage protein Clec4F. Based on cellular morphology analysis and single cell gene expression profiling, the researchers found two distinct heterogeneous KCs, YS-derived KCs (embryonic-derived KCs; EmKCs) and BM-derived KC (monocyte-derived KCs; MoKCs). YS-derived KCs mainly express Tim4 and MARCO, not expressed in BM-derived KCs. YS-derived KCs contain higher phagocytic and pro-inflammatory functions (22).

Kupffer cells highly express scavenger, complement and pattern recognition receptors (PRRs) (e.g. Toll-like receptors [TLRs]) (Fig. 1) (23). These scavenger roles of KCs functions as a guardian for the host defense against microorganisms (24). TLRs in KCs bind to pathogen-associated molecular patterns (PAMPs) such as bacteria-derived lipopolysaccharide (LPS) to drive an immune response to remove microorganisms (25). CRIg is unique complement receptor to mediate host defense of KCs via binding of the complement factors to activate phagocytose pathogens (26). Furthermore, CRIg has ability to directly capture lipoteichoic acid (LTA) from gram-positive bacteria in the liver sinusoidal circulatory systems acting as PRR, not depending on complement factors (27).

Kupffer cells regulate immune surveillance via antigen uptake and presentation to control T cell immunity as antigen presenting cells (24, 28). On the other hand, KCs suppress T cell activation via release of immunosuppressive cytokines such as interleukin-10 (IL-10) and transforming growth factor β (TGFβ) (29).

Monocyte-derived macrophages (MoMFs)
Circulating bone marrow-derived monocytes can infiltrate into the liver when hepatic injuries cause hepatic macrophage niche (6). The recruitment of MoMFs is mainly initiated via increased secretion of chemokine (C-C motif) ligand 2 (CCL2) from KCs or hepatic stellate cells (HSCs) (Fig. 1) (30-32). MoMFs include expression markers such as chemokine CX3-C motif receptor 1 (CX3CR1), Ly6C, CD11b and chemokine (C-C motif) receptor 2 (CCR2), not express Clec4F (11).

Ly6C is important expression marker to distinguish the function of two major populations of MoMFs in the mouse models...
Table 1. The markers of murine liver macrophage populations

| Markers (mouse) | Macrophages | EmKCs | MoKCs | LAMs | MoMFs | Monocytes | Neutrophils |
|----------------|-------------|-------|-------|------|-------|------------|-------------|
| CD11b          | +           | +     |       | +    | +     | +          | +           |
| CD64           | +           | +     | +     | +    | -     | +          | +           |
| F4/80          | + +         | +     |       | +    |       | - To +     | -           |
| CX3CR1         | -           | -     | -     | -    | -     | - To +     | -           |
| Clec4F         | + +         | +     | +     | +    |       | -          | -           |
| Ly6C           | +           | -     |       | +    | +     | + To +     | +           |
| Ly6G           | -           | -     | -     | -    | -     | -          | -           |
| MHCII          | - To +      | +     |       | +    |       | +          | +           |
| Tim4           | + +         | - To +| -     | - To +| -     | -          | -           |
| TREM2          | -           | +     | +     | +    | -     | -          | -           |
| CD9            | -           | +     |       | +    | -     | -          | -           |
| Clec2          | -           | +     |       | +    | -     | -          | -           |

EmKCs, embryo-derived KCs; MoKCs, monocyte-derived KCs; LAMs, lipid-associated macrophages; MoMFs, monocyte-derived macrophages.

Table 2. The markers of human macrophage populations

| Markers (human) | Macrophages | KCs | SAMs | MoMFs | Monocytes |
|----------------|-------------|-----|------|-------|------------|
| MARCO          | ++          |     | -    | -     | -          |
| TIMD4          | ++          |     |      | -    | -          |
| MERTK          | ++          |     |      | -    | -          |
| TREM2          | -           | +    |      | +    | -          |
| CD9            | -           | +    |      | +    | -          |
| SPP1           | -           | +    |      | +    | -          |
| CCR2           | -           | +    |      | ++   | +          |
| CX3CR1         | -           | -    | To + | ++   | +          |
| APOBEC3A       | -           | -    | +    | ++   | +          |
| MNDMA          | -           | -    | +    | +    | +          |
| S100A12        | -           | -    | -    | +    | +          |

SAMs, scar-associated macrophages.

(16, 33). The hepatic Ly6C<sup>+</sup> MoMFs mainly recruited by CCL2-CCR2 axis have been suggested as potent proinflammatory and profibrotic cells that induce hepatic inflammation and fibrosis, while Ly6C<sup>−</sup> MoMFs which highly express CX3CR1 may have function to restore tissue injuries (30, 34). Ly6C<sup>+</sup> MoMFs are precursor monocytes to change toward Ly6C<sup>−</sup> MoMFs (16). Unlike YS-derived tissue resident macrophages, MoMFs don't perform self-renewing and have a half-life of 2 days (Ly6C<sup>+</sup>) or 20 hours (Ly6C<sup>−</sup>) (16). The Ly6C<sup>+</sup> MoMFs migrate to the injured sites of the liver and act as inflammatory mediators via expression of PRRs and inflammatory cytokines (35). The Ly6C<sup>−</sup> MoMFs exhibit patrolling behavior in the liver and perform restoring tissue injury and scavenging waste of the liver (36, 37). The Ly6C<sup>−</sup> MoMFs are mainly derived from bone marrow and recruited to the liver via CCR2, whereas the spleen is the main source for the Ly6C<sup>+</sup> MoMFs which infiltrate into the liver through CX3CR1 (38, 39).

In addition to recruitment into injured sites, MoMFs regenerate the liver-resident macrophage pool to fill macrophage niche in the liver. In a mouse model of diphtheria toxin-mediated removal of Clec4F<sup>+</sup> KCs, MoMFs reconstituted the hepatic macrophage population and differentiated toward Clec4F<sup>+</sup> KCs within 168 hours, suggesting that monocyte can convert toward KCs by hepatic circumstances (7). This process is conducted by the combinational actions of liver sinusoid endothelial cells (LSECs), HSCs and hepatocytes which induce monocyte recruitment and imprinting of the KC signature transcriptional profiles such as inhibitor of differentiation 3 and liver X receptor α (LXRα) (40, 41). Also, MoMFs replace the liver macrophage population after microbial-induced KC death (42), bone marrow transplant into irradiated mice (22), and clodronate-mediated macrophage depletion (36).

HEPATIC MACROPHAGES IN LIVER DISEASES

Dynamic repopulation of macrophages and monocytes subsets in liver diseases

Tissue macrophages serve dual roles for the liver injuries between beneficial versus detrimental functions; friends or foes (43–45). The loss of resident macrophage KCs cause critical consequences in both the injury and recovery phases during scar processing (34, 46). However, general view of MoMFs is that recruitment of pro-inflammatory MoMFs is indispensable to activate regenerative mechanisms as well as overwhelmed inflammation, while unknown mechanisms to increase restorative macrophages recruitment induce inflammation resolution.
and tissue repair but also aberrant tissue repair to enhance hepatic fibrosis (11, 47). Indeed, a chronic loss of residential KCs is observed in methionine/choline-deficient (MCD) diet-induced nonalcoholic steatohepatitis (NASH) and hepatocellular carcinoma (HCC) models (48, 49). The recruitment of MoMFs via CCR2 exacerbates hepatic inflammation in acetaminophen-induced acute liver injury models (35). The pharmacological inhibition of CCR2 attenuates the symptoms of NASH, indicating that early replenishment of MoMFs lead to detrimental consequence to drive liver injuries (30).

It was reported that MoKCs population enhances NASH-induced hepatocyte damages via modulating hepatic triglyceride storage (50). Due to the complex situation of NASH disease, novel hepatic macrophage population-derived from MoKCs has been discovered in human and murine models. These macrophages were identified to scar-associated macrophages (SAMs) or lipid-associated macrophages (LAMs) since their localization is closely related with fibrotic and lipid droplet area (Fig. 1). SAMs highly express CD9 and triggering receptor expressed on myeloid cells 2 (TREM2), a scavenger receptor for specific glycoproteins and lipids (Table 1, 2) (51, 52). Interestingly, TREM2 expression is positively correlated with nonalcoholic fatty liver disease (NAFLD) histological features such as steatosis, inflammation, and fibrosis (52).

However, functional roles of TREM2<sup>−/−</sup> SAMs are not clearly identified to date. These CD9<sup>−/−</sup>TREM2<sup>−/−</sup> SAMs share many similarities in other inflamed tissue-specific macrophages such as adipose tissue in obesity models and brain microglia in Alzheimer’s disease (53, 54). Recently, TREM2 deficiency murine models suggest that TREM2 actually has protective roles in various liver injury models (55, 56). However, these models didn’t reflect specific depletion of SAMs, only considered the roles of TREM2 protein in the liver injury. Future studies are needed for complete understating of hepatic macrophage heterogeneity during liver injuries and will offer opportunity for novel therapeutic interventions.

**Modulation of fibrosis and inflammasome by hepatic macrophages**

Liver fibrosis is a general pathological character of most chronic liver diseases (6). Persistent liver injury lead to aberrant wound healing and tissue repair, resulting in excessive accumulation of extracellular matrix (ECM) or failure of ECM degradation. Disturbance of excessive accumulation of hepatic macrophages attenuates liver fibrosis in mice, indicating liver macrophage has profibrogenic roles (32). The several mechanisms of fibrosis progression are involved in macrophage activations. First, KCs increase secretion of CCL2 chemokines to mediate recruitment of pro-inflammatory and fibrotic MoMFs. Second, KCs directly activate HSCs via secretion of fibrotic growth factors such as TGFβ, platelet derived growth factor, and connective tissue growth factor. Third, hepatic macrophages influence HSC activation via proinflammatory cytokines and chemokines such as TNFα, IL-1β, and IL-6.

Because of their central roles in the hepatic microenvironment as recognizer for pathogens, KCs are first-line responders upon liver injury (57). KCs sense pathogens and injured cells via TLRs and damage-associated molecular pattern (DAMP) receptor (Fig. 1) (58). Activation of inflammasome assembly triggers caspase-1-mediated maturation of the inflammatory cytokines IL-1β and IL-18 (59). In the liver, gut-derived pathogens and damaged cell component DAMPs lead to inflammasome activation in KCs (60). KCs increase inflammasome-mediated secretion of IL-1β, which plays a critical role in mediating liver injuries including steatosis and inflammation. In mouse models of chronic liver diseases including MCD and western diet, NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome exacerbate liver inflammation and severity of NASH progression (61). However, inhibition of NLRP3 inflammasome via genetic deletion of NLRP3 and antibody-mediated neutralization of IL-1β didn’t show significant difference in acetaminophen-induced acute hepatotoxicity model (62).

**Interplays with other liver cells**

Hepatic macrophages contribute to the pathogenesis of various liver diseases via crosstalk with other liver cell types including LSECs, neutrophils, dendritic cells, natural killer (NK) cells, and platelets. LSECs support the adhesion and migration of circulating monocytes via intercellular adhesion molecule 1 and vascular cell adhesion protein 1, and thereby promote liver regeneration and inflammation in NASH models (63, 64). Increased recruitment of neutrophils is often observed in NAFLD and these cells enhance acute inflammation with disease progression (65). KCs are the major cells to attract neutrophils and interact with these cells to regulate liver inflammation. KCs release CXCL1, CXCL2, and CXCL8 chemokines to recruit neutrophils and other immune cells during various liver injuries (66). The roles of dendritic cells (DCs) in NAFLD pathogenesis are unclear until now, but the recruitment of monocytes by CX3CR1 receptor elevates the number of DCs and affects hepatic inflammation (67). Ly6C<sup>+</sup> MoMFs highly express CXCL16 chemokines during liver fibrosis progression by carbon tetrachloride and MCD diet, which promote hepatic recruitment of NKT and T cells to exacerbate liver inflammation (68). KCs can interact with platelet through KC Clec4F and platelet glycoprotein Ib α (69, 70). The blockade of KCs-platelet interaction attenuates liver steatosis, inflammation, and fibrosis during NASH development (70).

**SINGLE CELL TRANSCRIPTOMICS TO UNDERSTANDING LIVER MACROPHAGES**

In principle, hepatic macrophages could be attractive therapeutic targets to cure liver diseases. However, the findings of the distinct heterogeneity of KCs, MoMFs, and complexity of macrophage niche have caused difficulty to study the therapeutic aspects for liver macrophages. Nevertheless, numerous studies of single cell transcriptomics are solving the key questions to
understand physiology of KCs and MoMFs (51, 71, 72). Single-cell sequencing technology could provide the obvious advantages in an objective manner via unbiased algorithms for macrophage populations. Now, the hepatic macrophage populations were deeply analyzed and finally revealed to be much more heterogeneous depending on the disease status (73). This database allows to discover overlooked clusters and potential genes for identity of hepatic macrophages.

Furthermore, single cell transcriptomics can be used to obtain insights for macrophage and other cell interactions. Xiong et al. clarified the interplay between endothelial cell, HSC, and hepatic macrophages in the progression of NASH (52). This group conducted single cell sequencing of liver non-parenchymal cells and found NASH-associated macrophages expressing TREM2 and CD9. Through analysis of vascular signaling via single cell sequencing, the author revealed that endothelial cell and liver macrophages were regulated by secretion factors “stellakines” from HSC. Also, other single cell study is focused on non-parenchymal cells obtained from human cirrhotic patients (51). They profiled the transcriptomes of over 100,000 human single cells and also observed the appearance of cirrhotic-associated TREM2 and CD9 positive hepatic macrophages. Interestingly, they conducted interaction studies between endothelial cells, macrophages and HSCs, and described pro-fibrogenic pathways by TREM2 positive macrophages which are similar populations of lipid-associated macrophages to regulate adipocyte hypertrophy and fat accumulation during obesity (51, 53).

However, we should not exclude potential hurdles of single cell transcriptomics studies. The cell numbers might be too low to conclude immune cell populations. The sequencing analysis provides superficial and subtle findings for gene signatures because this technology could not detect enough gene numbers (74). Also, difficult isolation of residential KCs due to weak survival displayed bias of analyzing results for particular immune cell populations. Therefore, massive analysis of single cell transcriptomics studies. The cell numbers might be too low to conclude immune cell populations. The sequencing analysis provides superficial and subtle findings for gene signatures because this technology could not detect enough gene numbers (74). Also, difficult isolation of residential KCs due to weak survival displayed bias of analyzing results for particular immune cell populations. Therefore, massive analysis of single-cell populations and detecting high number of genes is urgently needed to clarify roles of hepatic macrophages in liver diseases.

Multiple approaches to search novel therapies for liver diseases have been endeavored that target diverse key pathway to regulate disease progression (75). Hepatic macrophages have a role as first-line responders for liver injuries which promote or inhibit progression of liver diseases. Therefore, targeting liver macrophages are intriguing therapeutic strategy. Even if most liver macrophage reports were focused on animal-based models, there are some clinical trials already conducted. Approaches with targeting liver macrophages are categorized as inhibition of inflammatory cell recruitment, macrophage activation, and reshaping of macrophage polarization (Table 3).

### Table 3. Potential therapeutic targeting of hepatic macrophages in clinical trials

| Strategy                          | Drug           | Mode of action | Clinical trial | Ref    |
|-----------------------------------|----------------|----------------|----------------|--------|
| Monocyte recruitment blockade     | Cenicriviroc   | CCR2/CCR5 inhibitor | Phase 3 termination | (80-82) |
|                                   | Maraviroc      | CCR5 inhibitor  | Pre-clinical    | (83)   |
|                                  | Propagermanium | CCR2 inhibitor  | Pre-clinical    | (84)   |
| Inhibitor of Kupffer cell activation | Serelaxin         | TLR4 antagonist | Pre-clinical    | (90)   |
|                                   | MCC950         | NLRP3 inflammasome blocker | Phase 2 termination | (61)   |
| Reshaping of macrophage polarization | JCI-40            | RORα agonist    | Pre-clinical    | (94)   |
|                                   | Belapectin     | Galectin-3 inhibitor | Phase 2        | (98)   |
|                                   | Lanifibranor   | Pan-PPAR agonist | Phase 2        | (99)   |
Dampening of Kupffer cell activation

Kupffer cells initiate inflammatory cascades in the liver by several mechanisms. KCs can be activated through DAMPs/PAMPs-induced NF-κB signaling and inflammasome activation. Also, bacterial translocation via disruption of the gut barrier promotes to TLR4-dependent KC activations (83). Therefore, broad spectrum antibiotics and influencing intestinal permeability could reduce endotoxin-mediated steatohepatitis, fibrosis and hepatocarcinogenesis in mice models (86-88). The TLR4 is a critical receptor for PAMP-induced liver macrophage activation, showing that genetic knockout of TLR4 displayed protective effects on NAFLD and NASH (88, 89). Thus, TLR4 antagonists serelaxin (RLX030) displayed additional effects combined with the PPARγ agonist rosiglitazone to ameliorate liver fibrosis (90). Blockade of NLRP3 inflammasome with treatment of MCC950 effectively reduced liver inflammation and fibrosis in the experimental murine NASH model (61). Hepatocytes and liver macrophages contain similar intracellular pro-inflammatory signaling pathways such as NF-κB, JNK, or ASK1 (91). In particular, ASK1 inhibitor, selonsertib, showed effect on macrophage inactivation, and tested even in human NASH patients. In a phase 2 trial, selonsertib reduced severity of NASH patients, but did not show promising results in phase 3 randomized studies in NASH patients with cirrhosis (92, 93).

Reshaping of macrophage polarization and programming

Due to the dynamic change of macrophage polarization, therapeutic strategies to reshape from the proinflammatory status to regenerative polarization could be a beneficial option for the treatment of hepatic diseases. Nuclear receptor RORγt could induce polarity switch of KCs from proinflammatory to antiinflammatory M2 phenotypes to resolve hepatic inflammation in NASH diseases (94). The oral administration of RORγ agonists, JC1-40 and Maresin 1, dramatically recovered from high fat diet-induced NAFLD symptoms via activation of M2 liver macrophages (94, 95). The galectin-3, a β-galactoside-binding lectin mainly expressed on macrophages, has been identified to induce inflammatory signaling and HSC activation (96). The galectin-3 inhibitor, belaplatin, positively alleviates liver fibrosis in preclinical NASH mouse models (97). However, clinical trials didn’t show positive results to reduce hepatic fibrosis in NASH patients (98). PPARs are also strong inducer for M2 macrophage phenotypes. Lanifibranor, a pan-PPAR agonist, decreased steatois, inflammation, and fibrosis with anti-inflammatory action of murine macrophages (99). Also, the accumulation of pro-inflammatory MoMFs was reduced by treatment of lanifibranor in preclinical choline-deficient, amino acid-defined high-fat diet (CDAA-HFD) and western diet model (99). Now, lanifibranor is under phase 2 clinical trial for NASH patients (NCT03008070). Diverse murine models and cutting-edge technology allow to identify novel molecular mechanisms underlying polarity switch and reprogramming of pro-inflammatory macrophages into restorative functions in the liver, which leads to expand new translational therapeutic approaches.

CONCLUDING REMARKS

In summary, numerous studies in recent years have improved our insights of hepatic macrophages and their homeostatic functions in the liver diseases. These researches give the opportunity to plan novel therapeutic strategy targeting hepatic macrophages. There are some advantages to develop therapeutic targeting of hepatic macrophages: i) Macrophages are crucial drivers for liver inflammation and fibrosis. ii) Major pathways of MoMFs recruitment and macrophage activation are well conserved between human and mouse. iii) Specific targeting to hepatic macrophages can be easily performed due to liver macrophage-specific surface molecules or scavenging functions. However, developing hepatic macrophage-mediated therapy against liver diseases has some challenges: i) Animal models has a limitation to reflect human macrophage functions in diseases stage. ii) The heterogeneity of human hepatic macrophages is less defined compared to mouse models. iii) Technical hardness to isolate human and mouse hepatic macrophages cause bias to interpret results.

In spite of these challenges, the rapid development of knowledge associated with the mechanisms of hepatic macrophages has unraveled the large spectrum of macrophage heterogeneity and immunomodulatory functions in liver diseases. Taken together, the integrated understanding of hepatic macrophages in liver diseases can offer promising points for therapeutic interventions to treat liver diseases in the clinic.

ACKNOWLEDGEMENTS

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2021R1C1C1004023 and 2021R1A4A3031661).

CONFLICTS OF INTEREST

The authors have no conflicting interests.

REFERENCES

1. Sanyal A, Boyer T, Terrault N et al (2017) Zakim and Boyer’s hepatology: a textbook of liver disease. Philadelphia, PA: Elsevier
2. Koyama Y and Brenner DA (2017) Liver inflammation and fibrosis. J Clin Invest 127, 55-64
3. Lopez BG, Tsai MS, Baratta JL et al (2011) Characterization of Kupffer cells in livers of developing mice. Comp Hepatol 10, 2
4. Chen Y and Tian Z (2021) Innate lymphocytes: pathogenesis and therapeutic targets of liver diseases and cancer. Cell Mol Immunol 18, 57-72
5. Tacke F (2017) Targeting hepatic macrophages to treat...
liver diseases. J Hepatol 66, 1300-1312
6. Tacke F and Zimmermann HW (2014) Macrophage heterogeneity in liver injury and fibrosis. J Hepatol 60, 1090-1096
7. Scott CL, Zheng F, De Baetselier P et al (2016) Bone marrow-derived monocytes give rise to self-renewing and fully differentiated Kupffer cells. Nat Commun 7, 10321
8. Stutchfield BM, Antoine DJ, Mackinnon AC et al (2015) CSF1 restores innate immunity after liver injury in mice and serum levels indicate outcomes of patients with acute liver failure. Gastroenterology 149, 1896-1909.e1814
9. Nascimento M, Huang SC, Smith A et al (2014) Ly6Chi monocyte recruitment is responsible for Th2 associated host-protective macrophage accumulation in liver inflammation due to schistosomiasis. PLoS Pathog 10, e1004282
10. Zigmond E, Samia-Grinberg S, Pasmanik-Chor M et al (2014) Infiltrating monocyte-derived macrophages and resident kupffer cells display different ontogeny and functions in acute liver injury. J Immunol 193, 344-353
11. Rillich-O and Tacke F (2017) Liver macrophages in tissue homeostasis and disease. Nat Rev Immunol 17, 306-321
12. Fogg DK, Sibon C, Miled C et al (2006) A clonogenic bone marrow progenitor specific for macrophages and dendritic cells. Science 311, 83-87
13. Kupffer C (1876) Ueber intravasculäre Zellen in den Blutcapillaren der Leberacini. Archiv für mikroskopische Anatomie 12, 353-358
14. Browicz (1899) Ueber intravasculäre Zellen in den Blutcapillaren der Leberacini. Archiv für mikroskopische Anatomie 55, 420-426
15. van Furth R, Cohn ZA, Hirsch JG et al (1972) The mononuclear phagocyte system: a new classification of macrophages, monocytes, and their precursor cells. Bull World Health Organ 46, 845-852
16. Yona S, Kim KW, Wolf Y et al (2013) Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. Immunity 38, 79-91
17. Hoeftel G, Chen J, Lavin Y et al (2015) C-Myb(+) erythro-myeloid progenitor-derived fetal monocytes give rise to adult tissue-resident macrophages. Immunology 42, 665-678
18. Mass E, Ballesteros I, Farlik M et al (2016) Specification of tissue-resident macrophages during organogenesis. Science 353, aaf4238
19. Mass E (2018) Delineating the origins, developmental programs and homeostatic functions of tissue-resident macrophages. Int Immunol 30, 493-501
20. Gomez Perdiguerro E, Klapproth K, Schulz C et al (2015) Tissue-resident macrophages originate from yolk-sac-derived erythroid-myeloid progenitors. Nature 518, 547-551
21. Bertrand JY, Jalil A, Klaine M et al (2005) Three pathways to mature macrophages in the early mouse yolk sac. Blood 106, 3004-3011
22. Beattie L, Sawtell A, Mann J et al (2016) Bone marrow-derived and resident liver macrophages display unique transcriptomic signatures but similar biological functions. J Hepatol 65, 758-768
23. Heymann F and Tacke F (2016) Immunology in the liver--from homeostasis to disease. Nat Rev Gastroenterol Hepatol 13, 88-110
24. Heymann F, Peusquens J, Ludwig-Portugall I et al (2015) Liver inflammation abrogates immunological tolerance induced by Kupffer cells. Hepatology 62, 279-291
25. Strnad P, Tacke F, Koch A et al (2017) Liver - guardian, modifier and target of sepsis. Nat Rev Gastroenterol Hepatol 14, 55-66
26. Helmy KY, Katschke KJ Jr, Gorgani NN et al (2006) CR3g: a macrophage complement receptor required for phagocytosis of circulating pathogens. Cell 124, 915-927
27. Zeng Z, Surewaard BG, Wong CH et al (2016) CR3g functions as a macrophage pattern recognition receptor to directly bind and capture blood-borne Gram-positive bacteria. Cell Host Microbe 20, 99-106
28. Jenne CN and Kubes P (2013) Immune surveillance by the liver. Nat Immunol 14, 996-1006
29. Zheng M and Tian Z (2019) Liver-mediated adaptive immune tolerance. Front Immunol 10, 2525
30. Baeck C, Wehr A, Karlmark KR et al (2012) Pharmacological inhibition of the chemokine CCL2 (MCP-1) diminishes liver macrophage infiltration and steatohepatitis in chronic hepatic injury. Gut 61, 416-426
31. Karlmark KR, Weiskirchen R, Zimmermann HW et al (2009) Hepatic recruitment of the inflammatory Gr1+ monocyte subset upon liver injury promotes hepatic fibrosis. Hepatology 50, 261-274
32. Miura K, Yang L, van Rooijen N et al (2012) Hepatic recruitment of macrophages promotes nonalcoholic steatohepatitis through CCR2. Am J Physiol Gastrointest Liver Physiol 302, G1310-G1321
33. Varol C, Landsman L, Fogg DK et al (2007) Monocytes give rise to mucosal, but not splenic, conventional dendritic cells. J Exp Med 204, 171-180
34. Ramachandran P, Pellicoro A, Vernon MA et al (2012) Differential Ly-6C expression identifies the recruited macrophage phenotype, which orchestrates the regression of murine liver fibrosis. Proc Natl Acad Sci U S A 109, 3186-3195
35. Messina J, Krenkel O, Ergen C et al (2016) Chemokine (C-C motif) receptor 2-positive monocytes aggravate the early phase of acetaminophen-induced acute liver injury. Hepatology 64, 1667-1682
36. David BA, Rezende RM, Antunes MM et al (2016) Combination of mass cytometry and imaging analysis reveals origin, location, and functional repopulation of liver myeloid cells in mice. Gastroenterology 151, 1191-1201
37. Affray C, Fogg D, Garfa M et al (2007) Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior. Science 317, 666-670
38. Serbina NV and Pamer EG (2006) Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. Nat Immunol 7, 311-317
39. Swinski FK, Nahrrendorf M, Etzrodt M et al (2009) Identification of splenic reservoir monocytes and their deployment to inflammatory sites. Science 325, 612-616
40. Bonnardel J, T’Jonck W, Gaublomme D et al (2019) Stellate cells, hepatocytes, and endothelial cells imprint the Kupffer cell identity on monocytes colonizing the liver macrophage niche. Immunity 51, 638-654.e639
41. Sakai M, Troutman TD, Seidman JS et al (2019) Liver-
derived signals sequentially reprogram myeloid enhancers to initiate and maintain Kupffer cell identity. Immunity 51, 655-670.e658
42. Lériot C, Dupuis T, Jouvin G et al (2015) Liver-resident macrophage necroptosis orchestrates type 1 microbicidal inflammation and type-2-mediated tissue repair during bacterial infection. Immunity 42, 145-158
43. Braga TF, Agudelo JS and Camara NO (2015) Macrophages during the fibrotic process: M2 as friend and foe. Front Immunol 6, 602
44. Gao B and Tsukamoto H (2016) Inflammation in alcoholic and nonalcoholic fatty liver disease: friend or foe? Gastroenterology 150, 1704-1709
45. Grunhut J, Wang W, Aylut B et al (2018) Macrophages in nonalcoholic steatohepatitis: friend or foe? Eur Med J Hepatol 6, 100-105
46. Duffield JS, Forbes SJ, Constandinou CM et al (2005) Selective depletion of macrophages reveals distinct, opposing roles during liver injury and repair. J Clin Invest 115, 56-65
47. Ritz T, Krenkel O and Tacke F (2018) Dynamic plasticity of macrophage functions in diseased liver. Cell Immunol 330, 175-182
48. Devischer L, Scott CL, Lefere S et al (2017) Non-alcoholic steatohepatitis induces transient changes within the liver macrophage pool. Cell Immunol 322, 74-83
49. Lefere S, Degroote H, Van Vlierberghe H et al (2019) Unveiling the depletion of Kupffer cells in experimental hepatocarcinogenesis through liver macrophage subtype-specific markers. J Hepatol 71, 631-633
50. Tran S, Baha J, Poupel L et al (2020) Impaired Kupffer cell self-renewal alters the liver response to lipid overload during non-alcoholic steatohepatitis. Immunity 53, 627-640.e5
51. Ramachandran P, Dobie R, Wilson-Kanamori JR et al (2019) Resolving the fibrotic niche of human liver cirrhosis at single-cell level. Nature 575, 512-518
52. Xiong X, Kuan H, Ansari S et al (2019) Landscape of intercellular crosstalk in healthy and NASH liver revealed by single-cell secretome gene analysis. Mol Cell 75, 644-660.e5
53. Jaitin DA, Adlung L, Thaiss CA et al (2019) Lipid-associated macrophages control metabolic homeostasis in a Trem2-dependent manner. Cell 178, 686-698.e14
54. Wang Y, Cellia M, Mullinson K et al (2015) TREM2 lipid sensing sustains the microglial response in an Alzheimer’s disease model. Cell 160, 1061-1071
55. Hou J, Zhang J, Cui P et al (2021) TREM2 sustains macrophage-hepaticocyte metabolic coordination in nonalcoholic fatty liver disease and sepsis. J Clin Invest 131, e135197
56. Perugorria MJ, Esparza-Baquer A, Oakley F et al (2019) Non-parenchymal TREM-2 protects the liver from immune-mediated hepatocellular damage. Gut 68, 533-546
57. Guillot A and Tacke F (2019) Liver macrophages: old dogmas and new insights. Hepatol Commun 3, 730-743
58. Wen Y, Lambrecht J, Ju C et al (2021) Hepatic macrophages in liver homeostasis and diseases-diversity, plasticity and therapeutic opportunities. Cell Mol Immunol 18, 45-56
59. Franchi L, Eigenbrod T, Muñoz-Planillo R et al (2009) The inflammasome: a caspase-1-activation platform that regulates immune responses and disease pathogenesis. Nat Immunol 10, 241-247
60. Szabo G and Petrasek J (2015) Inflammasome activation and function in liver disease. Nat Rev Gastroenterol Hepatol 12, 387-400
61. Mridha AR, Wree A, Robertson AAB et al (2017) NLRP3 inflammasome blockade reduces liver inflammation and fibrosis in experimental NASH in mice. J Hepatol 66, 1037-1046
62. Zhang C, Feng J, Du J et al (2018) Macrophage-derived IL-1α promotes sterile inflammation in a mouse model of acetaminophen hepatotoxicity. Cell Mol Immunol 15, 973-982
63. Melgar-Lesmes P and Edelman ER (2015) Monocyte-endothelial cell interactions in the regulation of vascular sprouting and liver regeneration in mouse. J Hepatol 63, 917-925
64. Guo Q, Furuta K, Lucien F et al (2019) Integrin β1-enriched extracellular vesicles mediate monocyte adhesion and promote liver inflammation in murine NASH. J Hepatol 71, 1193-1205
65. Cai J, Zhang XJ and Li H (2019) The role of innate immune cells in nonalcoholic steatohepatitis. Hepatology 70, 1026-1037
66. Soehnlein O and Lindbom L (2010) Phagocyte partnership during the onset and resolution of inflammation. Nat Rev Immunol 10, 427-439
67. Sutti S, Bruzzi S, Heymann F et al (2019) CX3CR1 mediates the development of monocyte-derived dendritic cells during hepatic inflammation. Cells 8, 1099
68. Wehr A, Baecck C, Heymann F et al (2013) Chemokine receptor CXCR6-dependent hepatic NK T cell accumulation promotes inflammation and liver fibrosis. J Immunol 190, 5226-5236
69. Jiang Y, Tang Y, Hoover C et al (2021) Kupffer cell receptor CLEC4F is important for the destruction of desialylated platelets in mice. Cell Death Differ 28, 3009-3021
70. Malehmir M, Pfister D, Gallage S et al (2019) Platelet GP Ibα is a mediator and potential interventional target for NASH and subsequent liver cancer. Nat Med 25, 641-655
71. Seidman JS, Troutman TD, Sakai M et al (2020) Niche-specific reprogramming of epigenetic landscapes drives myeloid cell diversity in nonalcoholic steatohepatitis. Immunity 52, 1057-1074.e7
72. Daemen S, Gainullina A, Kalugotla G et al (2021) Dynamic shifts in the composition of resident and recruited macrophages influence tissue remodeling in NASH. Cell Rep 34, 108626
73. Chakarov S, Lim HY, Tan L et al (2019) Two distinct interstitial macrophage populations coexist across tissues in specific subtissular niches. Science 363, eaau9064
74. Chen G, Ning B and Shi T (2019) Single-cell RNA-Seq technologies and related computational data analysis. Front Genet 10, 317
75. Lambrecht J, van Grunsven LA and Tacke F (2020) Current and emerging pharmacotherapeutic interventions for the treatment of liver fibrosis. Expert Opin Pharmacother 21, 1637-1650
76. Ju C and Tacke F (2016) Hepatic macrophages in homeostasis and liver diseases: from pathogenesis to novel thera-
peutic strategies. Cell Mol Immunol 13, 316-327
77. van der Heide D, Weiskirchen R and Bansal R (2019) Therapeutic targeting of hepatic macrophages for the treatment of liver diseases. Front Immunol 10, 2852
78. Krenkel O, Puengel T, Govaere O et al (2018) Therapeutic inhibition of inflammatory monocyte recruitment reduces steatohepatitis and liver fibrosis. Hepatology 67, 1270-1283
79. Kruger AJ, Fuchs BC, Masia R et al (2018) Prolonged cenicriviroc therapy reduces hepatic fibrosis despite steatohepatitis in a diet-induced mouse model of nonalcoholic steatohepatitis. Hepatol Commun 2, 529-545
80. Friedman SL, Ratziu V, Harrison SA et al (2018) A randomized, placebo-controlled trial of cenicriviroc for treatment of nonalcoholic steatohepatitis with fibrosis. Hepatology 67, 1754-1767
81. Ratziu V, Sanyal A, Harrison SA et al (2020) Cenicriviroc treatment for adults with nonalcoholic steatohepatitis and fibrosis: final analysis of the phase 2b CENTAUR study. Hepatology 72, 892-905
82. Anstee QM, Neuschwander-Tetri BA, Wong VW et al (2020) Cenicriviroc for the treatment of liver fibrosis in adults with nonalcoholic steatohepatitis: AURORA Phase 3 study design. Contemp Clin Trials 89, 105922
83. Pérez-Martínez L, Pérez-Matute P, Aguilera-Lizarraga J et al (2014) Maraviroc, a CCR5 antagonist, ameliorates the development of hepatic steatosis in a mouse model of non-alcoholic fatty liver disease (NAFLD). J Antimicrob Chemother 69, 1903-1910
84. Mulder P, van den Hoek AM and Kleemann R (2017) The CCR2 inhibitor propagermanium attenuates diet-induced insulin resistance, adipose tissue inflammation and non-alcoholic steatohepatitis. PLoS One 12, e0169740
85. Albillos A, de Gottardi A and Rescigno M (2017) The gut-liver axis in liver disease: pathophysiological basis for therapy. J Hepatol 72, 558-577
86. Dapito DH, Mencin A, Gwak GY et al (2012) Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. Cancer Cell 21, 504-516
87. Schneider KM, Bieggs V, Heymann F et al (2015) CX3CR1 is a gatekeeper for intestinal barrier integrity in mice: Limiting steatohepatitis by maintaining intestinal homeostasis. Hepatology 62, 1405-1416
88. Seki E, De Minicis S, Osterreicher CH et al (2007) TLR4 enhances TGF-beta signaling and hepatic fibrosis. Nat Med 13, 1324-1332
89. Carpino G, Del Ben M, Pastori D et al (2020) Increased liver localization of lipopolysaccharides in human and experimental NAFLD. Hepatology 72, 470-485
90. Bennett RG, Simpson RL and Hamel FG (2017) Serelaxin increases the antifibrotic action of rosiglitazone in a model of hepatic fibrosis. World J Gastroenterol 23, 3999-4006
91. Weiskirchen R and Tacke F (2016) Liver fibrosis: from pathogenesis to novel therapies. Dig Dis 34, 410-422
92. Lefere S, Puengel T, Hundertmark J et al (2020) Differential effects of selective- and pan-PPAR agonists on experimental steatohepatitis and hepatic macrophages. J Hepatol 73, 757-770