Introduction

At present, chronic liver disease accounts for approximately 2 million deaths per year worldwide. Cirrhosis is currently the 11th most common cause of death globally and liver cancer is the 16th leading cause of death; combined, they account for 3.5% of all deaths worldwide. Cirrhosis is within the top 20 causes of disability-adjusted life years and years of life lost, accounting for 1.6% and 2.1% of the worldwide burden [1].

Liver injury induces a series of events causing inflammation. Chronic inflammation results in activation and trans-differentiation of hepatic stellate cells (HSCs) into myofibroblasts. Liver macrophages secrete the fibrogenic growth factor transforming growth factor (TGF)-β. TGF-β in turn stimulates HSCs to secrete collagen. Excess extracellular matrix (ECM) deposition leads to liver fibrosis [2].

The liver has a large amount of tissue macrophages compared to other body organs. This confirms the critical role of liver macrophages in keeping liver homeostasis, but also suggests the high levels of heterogeneity that exist between these cell phenotypes [3]. Some macrophage activation markers were reported to correlate with liver injury and demonstrated good predictive ability for advanced fibrosis [4].

Liver Macrophages Heterogeneity

It was believed that macrophages are functionally classified into two distinct phenotypes the classically activated ‘pro-inflammatory’ M1 and the alternative activated ‘immune-regulatory’ M2 macrophages. However, M2 macrophages are now further categorized into different subtypes which stimulate wound healing, represent anti-inflammatory cell population and may also act as proinflammatory in some conditions. Therefore, macrophages are considered to represent a wide spectrum of phenotypes [5].

Liver macrophages heterogeneity is partly due to their different origin and subsequently their different functions according to the phase of liver injury: inflammation fibrosis or...
regression of fibrosis. They either arise from infiltrating migratory monocytes, recruited to the injured liver due to inflammatory signals, or from the local hepatic macrophages; Kupffer cells (KCs) [6].

**Resident Liver Macrophages (Kupffer Cells)**

KCs play their role to initiate inflammatory responses, while infiltrating monocyte-derived macrophages (MoMϕs) have a role both in chronic inflammation and fibrosis and in fibrosis resolution. Ly-6C high expressing monocytes act during fibrogenesis, while Ly-6C low expressing monocytes are restorative macrophages which promote resolution of fibrosis after end of the injury [7].

On the other hand, previous studies proved that KCs also exert a profibrotic role via paracrine action on HSC; the key cell in liver fibrosis. This could be due to the site of KCs in the sinusoids which allows interactions with neighbouring non-parenchymal hepatic cell populations [8]. Understanding different mechanisms orchestrating this heterogeneity may help to reach macrophage targeted approaches for treatment of liver fibrosis.

**Kupffer Cells Initiate Hepatic Inflammation and Fibrosis**

KCs are innate immune cells that phagocytose dead cells and cell debris to maintain liver homeostasis. In addition, they respond to liver injury and subsequently initiate pro-inflammatory processes [9]. KCs can sense liver damage via pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs). In the liver, PAMPs (e.g., lipopolysaccharide associated) mostly originate from bacterial translocation in the gut. However, DAMPs (e.g., adenosine triphosphate and DNA fragments) mainly originate from damaged hepatocytes [10].

The experimental model of carbon tetrachloride (CCl4)-induced liver cirrhosis initially causes hepatocellular structural derangements, then cellular metabolic changes occur which induce further damage and result in necrosis or apoptosis [11]. These changes lead to the release of DAMPs which in turn activate KCs to initiate inflammatory cascades. Activated KCs secrete a great number of chemokines and cytokines, resulting in further recruitment of monocytes and neutrophils to site of inflammation. Activated KCs secrete C-X-C motif chemokine ligand 1 (CXCL1), CXCL2, and CXCL8 (interleukin [IL]-8) [12].

Neutrophil recruitment increases reactive oxygen species (ROS) and proteases, leading to hepatocyte necrosis. In parallel, KCs secrete chemokine (C-C motif) ligand (CCL) 2 to increase circulating CCR2+Ly-6C+ monocytes that massively expand the local macrophage pool. CCR2+Ly-6C+ monocytes are critical to maintain liver inflammation and fibrogenesis [13].

KCs respond to PAMPs or DAMPs with the production of tumor necrosis factor (TNF). TNF promotes release of many other inflammatory mediators including IL-6, IL-12/23 (p40), and type I interferons (e.g., IFN-γ and TNF-α). IFN-γ is a hallmark cytokine of type 1 T-helper (Th1) cells that greatly increases the production of inflammatory mediators by macrophages. 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 3 activation, markedly increases after acute CCl4-induced hepatic damage and promote liver proliferation by stimulating release of hepatocyte growth factor. IL-6 also inhibits hepatocyte apoptosis [14].

Repeated injections of low doses of CCl4 in rodents lead to liver liver cirrhosis. In fibrogenesis, Ly-6C high expressing monocytes directly activate HSCs via TGF-β, upon chronic liver injury. Macrophage-derived TNF-α and IL-1β enhance the survival of activated HSCs. Activated HSCs and hepatic myofibroblasts exert pro-fibrogenic activity, as they may increase the levels of fibrotic matrix proteins, thus inhibiting fibrotic degradation [15].

**Macrophages Decelerate Fibrogenesis**

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 ECM [16]. Four cytokines with the ability to downregulate macrophage function have been identified: IL-4, IL-10, IL-13, and TGF-β1, of which IL-10 appears to have a broader and deeper effect. Importantly, IL-10 is released from macrophages, Th2 cells and stromal cells. IL-10 inhibits the production of pro-inflammatory cytokines by Th1 cells, macrophages and neutrophils, the proliferation of hepatocytes and fibrogenesis during liver repair [10]. Macrophages produce gelatinases (matrix metallopeptidase [MMP] 9, 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 [17]. Furthermore, KCs produce MMP13, which may disassemble the interstitial matrix and promote fibrosis resolution. KCs and Ly6C− ‘restorative’ macrophages, which of them are the sources of MMP13 remain elusive [18]. In addition, KCs are a major source of CX3CL9, which ameliorate liver fibrogenesis. Also, CX3CR1 is a major regulator of monocyte differentiation and survival in the liver which protects against fibrogenesis [9].

**Replenishment of Kupffer Cells in Liver Injury**

A rapid loss of KCs occurs during liver injury in models of methionine/choline-deficient diet-induced nonalcoholic steatohepatitis and hepatocellular carcinoma. With regard to KC replenishment, KCs have the capacity to self-renew through proliferation, probably due to colony stimulating factors. However, MoMϕs are considered in other studies to be the major contributors for replenishment of the macrophage pool. Selective depletion of Clec4F-expressing KCs induced recruited MoMϕs to differentiate into fully functional KCs, restoring the resident hepatic macrophages within 1 month. In a mouse model of conditional KC depletion, monocytes differentiate into KCs within days [19]. HSCs and liver sinusoid endothelial cells orchestrate monocyte engraftment and replenishment of the KC phenotype which depends on the transcription factors ID3 and liver X receptor-alpha [20].

In addition to the proliferation of KCs and the recruitment and differentiation of MoMϕs, peritoneal macrophages may be recruited through the visceral endothelium into liver tissue. In a model of sterile liver injury, mature peritoneal macrophages expressing CD102 and GATA6 migrated to liver tissue within 1 hour after injury. Furthermore, GATA6-deficient mice showed impaired macrophage recruitment and tissue regeneration [21].

Splenitic macrophages also contribute to the hepatic macrophage pool upon liver injury. The spleen serves as a reservoir of monocytes during liver damage. release of lipocalin-2 53 and CCL254 by the spleen regulates monocyte infiltration into the liver, KC activation, and overall hepatic inflammation [21, 22]. However, splenic macrophages involvement in liver diseases remains to be elucidated.

**Migrating Monocytes/Macrophages**

In normal conditions, Ly-6C high expressing monocytes migrate and accumulate in the bone marrow where they differentiate to the Ly-6C low expressing sub-population. Functionally, the Ly-6C high expressing cells have a role in replenishment of resident macrophages and Ly-6C low expressing monocytes. In case of inflammation, Ly-6C low expressing sub-population has a proinflammatory and antigen processing role [23].

Infiltrating monocytes/macrophages are critical for the initial inflammatory phase of wound healing process as in liver fibrosis. Early after cessation liver injury a remarkable recruitment of Ly-6C high expressing macrophages can be observed, which attenuates spontaneous fibrosis regression. They play a principal role in the resolution of acetaminophen-induced liver injury via ending neutrophil activity. At the necroinflammatory phase, reduced numbers of ROS-producing neutrophiis was observed after inducing ablation of circulating Ly-6C high expressing monocytes. Ly-6C low expressing macrophages directly delineate from the infiltrating Ly-6Chi monocytes/macrophages after local functional switching during monocyte maturation [24].

**Macrophages Classification Variants**

It was revealed that Ly-6C low expressing restorative macrophages accumulated in the resolution phase after tissue damage in experimentally induced liver cirrhosis, and they were found to exert a significant role in regression of fibrosis. During the phase of fibrosis regression, resolution of fibers could be accelerated by blocking the CCL2. As, Ly-6C high expressing monocytes/macrophages are CCL2-dependent [25]. The restorative macrophage phenotypes do not lie under the M1/M2 categories, denoting the limitations of this M1/M2 classification.

This phenotype transition of infiltrating macrophages from Ly-6C high expressing and to Ly-6C low expressing, plays an essential role in the liver fibrosis regression. The switch to the restorative macrophage population associated with increased expression of matrix-degrading enzymes such
as MMP-9 and MMP-12, the antifibrotic cytokine IL-10 and the growth factor levels IGF-1 and VEGF [9]. Splenectomy attenuated liver fibrosis by upregulation of the macrophage switch to an anti-inflammatory Ly-6C low expressing phenotype via activation of ERK1/2 pathway [26].

It was revealed that the restorative Ly-6C low expressing macrophages are not in phagocytic phase but postphagocytic. They increased after removal of cell debris. The resolution of fibers was associated with decrease in hepatocyte apoptosis and improvement of liver functions. Apoptotic debris was mostly bound to the cell surface in the proinflammatory Ly-6Chi macrophages. Whereas the debris had been ingested in the restorative macrophages [17].

**Variants of the Classification of Macrophages**

Several variants of the classification of macrophages have been described. The classification based on the level of expression of CD14 and CD16 molecules on the cell surface is the closest scheme to a functional classification. Within the framework of this classification, three large groups are distinguished: classical (CD14highCD16−), intermediate (CD14highCD16low) and non-classical macrophages. Occasionally, non-classical and transitional macrophages are grouped together (CD14lowCD16low). CD14+CD16− and CD14–CD16+ monocytes in human matched with Ly-6C high expressing and Ly-6C low expressing murine monocytes, respectively [27].

The classical macrophages (CD14+CD16−) show phagocytic activity, while nonclassical (CD14–CD16+) macrophages are pro-inflammatory subset which secretes TNF-α [28]. The expression of CD206 is involved in the mechanism regulating the immune response and tissue remodeling [29].

In addition, another classification based on differences in FcγRI (CD64) expression has been described. The CD64+CD16+ macrophages combine both the properties of macrophages and dendritic cells (DCs). CD64–CD16+ cells express the major histocompatibility complex II (MHC II) molecule at high levels and display a pronounced antigen-presenting function [30].

Three monocyte subsets in humans are described: intermediate and non-classical monocytes which emerge sequentially from the pool of classical monocytes. Experimentally induced endotoxemia resulted in rapid loss of all monocyte subsets. However, classical monocyte numbers were restored from the bone marrow or margined pools first, with intermediate and non-classical monocytes following. Intermediate and non-classical followed a peak in CCL2, CCL3, and CCL4 blood levels, in contrast to classical monocytes which were sensitive to CX3CL1 [31]. In mice, monocyte development clearly occurs in the bone marrow where granulocyte-monocyte and monocyte-DC progenitor pools produce functional monocytes. Furthermore, during infections, monocyte progenitor reprogramming happens already in the bone marrow [32].

**New Phenotypes of Macrophages in Fatty Liver Diseases**

Recent studies have identified new phenotypes, such as metabolically activated M, oxidized, hemoglobin-related macrophages (Mhem and MHb), M4 and neuroimmunological macrophages, which directly and indirectly affect energy metabolism in adipose tissue. Thus, they may have a role in fatty liver diseases.

Macrophages represent a crosstalk between adipose tissue and liver in fatty liver disease. Insulin resistance activates hepatic macrophages in fatty liver disease via fatty acids accumulation in the liver. Macrophage recruitment in this case occurred earlier in adipose tissue compared to the liver. In animal models, ablation of adipose tissue macrophages resulted in decreased insulin resistance, while adipose tissue macrophages from obese visceral adipose tissue increased hepatic inflammation via stimulation of increased hepatic macrophage infiltration [3].

**Conclusion**

Macrophages are considered to represent a wide spectrum of phenotypes. Restorative Ly-6C low expressing macrophages have a role in resolution of fibrosis and can be a target for therapeutic approaches of liver fibrosis. New phenotypes associated with fatty liver can also be targeted for therapeutic purposes.

**ORCID**

Sherine Ahmed Elsherif: https://orcid.org/0000-0002-5878-4704

Ahmed Salah Alm: https://orcid.org/0000-0002-6078-5669
Author Contributions

Conceptualization: SAE, ASA. Data acquisition: SAE, ASA. Data analysis or interpretation: SAE, ASA. Drafting of the manuscript: SAE, ASA. Critical revision of the manuscript: SAE, ASA. Approval of the final version of the manuscript: all authors.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

References

1. Asrani SK, Devarbhavi H, Eaton J, Kamath PS. Burden of liver diseases in the world. J Hepatol 2019;70:151-71.
2. Tanwar S, Rhodes F, Srivastava A, Tremling PM, Rosenberg WM. Inflammation and fibrosis in chronic liver diseases including non-alcoholic fatty liver disease and hepatitis C. World J Gastroenterol 2020;26:109-33.
3. Alharthi J, Latchoumanin O, George J, Eslam M. Macrophages in metabolic associated fatty liver disease. World J Gastroenterol 2020;26:1861-78.
4. Kazankov K, Barrera F, Moller HJ, Rosso C, Bugianesi E, Davids E, Younes R, Esmaili S, Eslam M, McLeod D, Bibby BM, Vilstrup H, George J, Gronbaek H. The macrophage activation marker scd163 is associated with morphological disease stages in patients with non-alcoholic fatty liver disease. Liver Int 2016;36:1549-57.
5. Martinez FO, Gordon S. The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000Prime Rep 2014;6:13.
6. Tacke F, Zimmermann HW. Macrophage heterogeneity in liver injury and fibrosis. J Hepatol 2014;60:1090-6.
7. Baekc C, Wei X, Bartneck M, Fech V, Heymann F, Gassler N, Hittatiya K, Eulberg D, Luedde T, Trautwein C, Tacke F. Pharmacological inhibition of the chemokine C-C motif chemokine ligand 2 (monocyte chemoattractant protein 1) accelerates liver fibrosis regression by suppressing Ly-6C(+) macrophage infiltration in mice. Hepatology 2014;59:1060-72.
8. Tacke F. Targeting hepatic macrophages to treat liver diseases. J Hepatol 2017;66:1300-12.
9. Dong X, Liu J, Xu Y, Cao H. Role of macrophages in experimental liver injury and repair in mice. Exp Ther Med 2019;17:3835-47.
10. Wree A, Marra F. The inflammasome in liver disease. J Hepatol 2016;65:1055-6.
11. Weber LW, Boll M, Stampfl A. Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. Crit Rev Toxicol 2003;33:105-36.
12. Marra F, Tacke F. Roles for chemokines in liver disease. Gas.
13. Chu PS, Nakamoto N, Ebimuna H, Usui S, Saeki K, Matsumoto A, et al. C-C motif chemokine receptor 9 positive macrophages activate hepatic stellate cells and promote liver fibrosis in mice. Hepatology 2013;58:337-50.
14. Li Y, Schwabe RF, DeVries-Seimon T, Yao PM, Gerbod-Gianne no MC, Tall AR, Davis RJ, Flavell R, Brenner DA, Tabas I. Free cholesterol-loaded macrophages are an abundant source of tumor necrosis factor-alpha and interleukin-6: model of NF-kappaB- and map kinase-dependent inflammation in advanced atherosclerosis. J Biol Chem 2005;280:21763-72.
15. Pradere JP, Kluwe J, De Minicis S, Jiao JJ, Gwak GY, Dapito DH, Jang MK, Guenther ND, Mederacke I, Friedman R, Dragomir AC, Aloman C, Schwabe RF. Hepatic macrophages but not dendritic cells contribute to liver fibrosis by promoting the survival of activated hepatic stellate cells in mice. Hephatology 2013;58:1461-73.
16. Ramachandran P, Iredale JP, Fallowfield JA. Resolution of liver fibrosis: basic mechanisms and clinical relevance. Semin Liver Dis 2015;35:119-31.
17. Ramachandran P, Pellicoro A, Vernon MA, Boulter L, Aucott RL, Ali A, Hartland SN, Snowden VK, Cappon A, Gordon-Walker TT, Williams MJ, Dunbar DR, Manning JR, van Rooijen N, Fallowfield JA, Forbes SJ, Iredale JP. Differential Ly-6C expression identifies the recruited macrophage phenotype, which orchestrates the regression of murine liver fibrosis. Proc Natl Acad Sci U S A 2012;109:E3186-95.
18. Fallowfield JA, Mizuno M, Kendall TJ, Constandinou CM, Be nyon RC, Duffield JS, Iredale JP. Scar-associated macrophages are a major source of hepatic matrix metalloproteinase-13 and facilitate the resolution of murine hepatic fibrosis. J Immunol 2007;178:5288-95.
19. Beattie L, Sawtell A, Mann J, Frame TCM, Teal B, de Labastida Rivera F, Brown N, Walwyn-Brown K, Moore JWJ, MacDonald S, Lim EK, Dalton JE, Engwerda CR, MacDonald KP, Kaye PM. Bone marrow-derived and resident liver macrophages display unique transcriptomic signatures but similar biological functions. J Hepatol 2016;65:758-68.
20. Sakai M, Troutman TD, Seidman JS, Ouyang Z, Spann NJ, Abe Y, Ego KM, Brunni CM, Deng Z, Schlachetzki JCM, Nott A, Bennett H, Chang J, Vu BT, Pasillas MP, Link VM, Texari L, Heinz S, Thompson BM, McDonald JG, Geissmann F, Glass CK. Liver-derived signals sequentially reprogram myeloid enhancers to initiate and maintain Kupffer cell identity. Immunity 2019;51:655-70.e8.
21. Wang J, Kubis P. A reservoir of mature cavity macrophages that can rapidly invade visceral organs to affect tissue repair. Cell 2016;163:668-78.
22. Li L, Wei W, Li Z, Chen H, Li Y, Jiang W, Chen W, Kong G, Yang J, Li Z. The spleen promotes the secretion of CCL2 and supports an M1 dominant phenotype in hepatic macrophages during liver fibrosis. Cell Physiol Biochem 2018;51:557-74.
23. Orekhov AN, Orekhova VA, Nikiforov NG, Myasoedova VA, Grechko AV, Romanenko EB, Zhang D, Chistiakov DA. Mono-
cyte differentiation and macrophage polarization. Vessel Plus 2019;3:10.
24. Graubardt N, Vugman M, Mouhadeb O, Cailari G, Pasmanik-Chor M, Reuveni D, Zigmond E, Brazowski E, David E, Chappell-Maar L, Jung S, Varol C. Ly6C<sup>hi</sup> monocytes and their macrophage descendants regulate neutrophil function and clearance in acetaminophen-induced liver injury. Front Immunol 2017;8:626.
25. Song P, Zhang J, Zhang Y, Shu Z, Xu P, He L, Yang C, Zhang J, Wang H, Li Y, Li Q. Hepatic recruitment of CD11b<sup>+</sup>Ly6C<sup>+</sup> inflammatory monocytes promotes hepatic ischemia/reperfusion injury. Int J Mol Med 2018;41:935-45.
26. Zheng Z, Wang H, Li L, Zhang S, Zhang C, Zhang H, Ji F, Liu X, Zhu K, Kong G, Li Z. Splenectomy enhances the Ly6C<sup>low</sup> phenotype in hepatic macrophages by activating the ERK1/2 pathway during liver fibrosis. Int Immunopharmacol 2020;86:106762.
27. Wong KL, Tai JJ, Wong WC, Han H, Sem X, Yeap WH, Kourilsky P, Wong SC. Gene expression profiling reveals the defining features of the classical, intermediate, and nonclassical human monocyte subsets. Blood 2011;118:e16-31.
28. Jakubzick CV, Randolph GJ, Henson PM. Monocyte differentiation and antigen-presenting functions. Nat Rev Immunol 2017;17:349-62.
29. Röszer T. Understanding the mysterious M2 macrophage through activation markers and effector mechanisms. Mediators Inflamm 2015;2015:816460.
30. Kapellos TS, Bonaguro L, Gemünd I, Reusch N, Saglam A, Hinkley ER, Schultze JL. Human monocyte subsets and phenotypes in major chronic inflammatory diseases. Front Immunol 2019;10:2035.
31. Tak T, van Groenendael R, Pickkers P, Koenderman L. Monocyte subsets are differentially lost from the circulation during acute inflammation induced by human experimental endotoxemia. J Innate Immun 2017;9:464-74.
32. Askenase MH, Han SJ, Byrd AL, Morais da Fonseca D, Bouladoux N, Wilhelm C, Konkel JE, Hand TW, Lacerda-Queiroz N, Su XZ, Trinchieri G, Grainger JR, Belkaid Y. Bone-marrow-resident NK cells prime monocytes for regulatory function during infection. Immunity 2015;42:1130-42.