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Role of macrophages in liver cirrhosis: fibrogenesis and resolution

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Abstract: At present, chronic liver disease accounts for approximately 2 million deaths per year worldwide. Liver injury induces a series of events causing inflammation. Chronic inflammation ends in liver fibrosis. A stage of fibrinolysis occurs on stopping insult. Kupffer cells play their role to initiate inflammatory responses, while infiltrating monocyte-derived macrophages 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. Recent studies have identified new phenotypes, such as metabolically activated M, oxidized, which may have a role in fatty liver diseases.

Key words: Liver cirrhosis, Macrophages, Fibrosis, Liver cirrhosis, Macrophages

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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 transdifferentiation 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 'immunoregulatory' 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

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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

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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 CXCL9, 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].

Splenic 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/Macrohages

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 neutrophils 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 FcγR1 (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 marginated 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.

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Conflicts of Interest

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

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