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Hepatic macrophages in liver fibrosis: pathogenesis and potential therapeutic targets

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ABSTRACT

Hepatic macrophages account for the largest non-parenchymal cell population in the liver. Recent studies have found that hepatic macrophages have different functions in different stages of experimental liver fibrosis. Some studies found that there are different types of hepatic macrophages in the liver, although others have suggested that hepatic macrophages could switch to different phenotypes in different environments. Many studies demonstrated that while hepatic macrophages promoted fibrosis through the recruitment of proinflammatory immune cells, and the secretion of proinflammatory cytokines and chemokines in the early stages, these also promoted the resolution of hepatic fibrosis through the secretion of matrix metalloproteinases in the late stages. This article will review the current role played by hepatic macrophages in liver fibrosis and the potential therapeutic targets that modulate hepatic macrophages.

INTRODUCTION

Hepatic fibrosis is a dynamic process of repairing chronic liver injuries that may lead to cirrhosis and significant morbidity and mortality.[1] Chronic necroinflammation activates hepatic stellate cells (HSCs) into myofibroblast-like cells, and the latter cells produce excessive extracellular matrix (ECM). Hepatic macrophages are a heterogeneous population of immune cells that perform diverse functions in homeostasis, and the progression and regression of chronic liver diseases. Recent studies with animal models of toxic or cholestatic liver fibrosis showed that hepatic macrophages can promote fibrogenesis via the initiation of fibrosis and sustain the phases of liver fibrosis,[2] and can also promote fibrinolysis in the resolution phase.[3]

In this review, we will summarise the current understanding of the ambivalent roles played by macrophages in liver fibrosis, and will explore the potential targets of hepatic macrophages for treating liver fibrosis.[4]

The roles of hepatic macrophages in the pathogenesis of liver fibrosis

Hepatic macrophages play a central role in the pathogenesis of chronic liver injury, including inflammation and fibrosis.[5] The phagocytic receptors in hepatic macrophages can be divided into membrane surface receptors and intracellular receptors.[6] All of these receptors recognise and activate downstream molecules through different signalling pathways, thereby becoming involved in the processes of inflammation and fibrosis.[7 8]

Macrophages have different effects if their target cells are different. For example, phagocytosis of red blood cells causes iron deposition and induces oxidative stress reactions, which in turn promote inflammation and fibrosis; phagocytosis of collagen-producing cells and cell debris reduces inflammation and liver fibrosis. Furthermore, phagocytosis of apoptotic liver cells does not change the secretion of proinflammatory factors, although phagocytosis of necrotic liver cells causes the secretion of proinflammatory cytokines.[9] This phenomenon may explain why macrophages do not promote fibrotic responses in normal conditions despite the fact that apoptosis of liver cells happens every day,[10 11] whereas hepatic macrophages produce inflammatory responses and liver fibrosis when hepatocyte necrosis occurs.[12–17]

A recent study showed that macrophage migration inhibitory factor (MIF) plays an important role in the early stages of liver fibrosis.[6 18] CCL4-induced liver fibrosis was more severe in MIF gene knockout mice than in wild-type mice. Some studies also found that sustained activation of hepatic nuclear factor κB (NFκB) in macrophages led to liver inflammation and fibrosis,[8 19] whereas killing hepatic macrophages significantly reduced NFκB activity and inflammation and fibrosis in the liver.[7]

Under the effects of tumour necrosis factor (TNF) and transforming growth factor

β1 (TGF-β1), and C-C motif chemokine receptor 9 (CCR9) and C-C motif chemokine ligand 25 (CCL25), blood mononuclear cells accumulate in the liver and turn into classical macrophages (M1).[20 21] Some other molecules, such as CCL2[15 22] and monocyte chemotactic protein 1 (MCP-1) are also involved in the chemotaxis of M1 proinflammatory macrophages, and thereby play an important role in the recruitment of Ly-6C+ monocytes in liver fibrosis induced by CCL4.[22 23] Other studies also suggest that hepatic macrophage promoted liver fibrosis is mediated by CCL2, CCR8 and CCR9, and maintains NFκB activation in the early stage.[24 25]

Many studies have suggested that hepatic macrophages have a two-way regulatory function[2] in liver fibrosis; hepatic macrophages promote fibrosis through the recruitment of proinflammatory immune cells and the secretion of proinflammatory cytokines and chemokines in the early stages, whereas in the late stages, they promote the resolution of hepatic fibrosis through the secretion of matrix metalloproteinases (MMPs).

The classification of hepatic macrophages in liver fibrosis

Studies have suggested that macrophage activation may be classified into classic and alternative pathways—the M1 and M2 types of macrophages with helper T cell 1 (Th1) and helper T cell 2 (Th2) immune responses, respectively.[26 27] Both types of hepatic macrophages express the molecules CD68, CD163, and the monocyte-specific molecule CD14; there is generally no expression or low expression of CD86 (dendritic cells) and CD3 (T cells).[26]

M1 cells favour the Th1 response; secrete mainly TNF-α, interleukin 1 (IL-1), IL-12, and reactive oxygen species (ROS) to remove bacteria; exert antiviral effects; and are also known as classical macrophages.[28] M1 cells are induced by interferon-γ and IL-12, and these strongly express inducible nitric oxide syntheses (iNOS) and transglutaminase-2 (TGM-2) and weakly express arginase.[29] M1 cells also have phagocytic functions, promote inflammation and are involved in liver fibrogenesis.

M2 cells favour the Th2 response; secrete mainly the immunomodulatory cytokines TGF-β1, IL-4, IL-13, and IL-10 to protect against parasite infections; and are also known as 'alternatively activated macrophages'.[27] These are induced by IL-4 and IL-13 or the hepatitis B virus.[30] Rodent hepatic macrophages express chitinase-3-like-protein 4 (YM-2) molecules, whereas human hepatic macrophages express CD206 and TGM-2 molecules. M2 cells secrete anti-inflammatory cytokines, including IL-10, to maintain the homeostasis of the intracellular environment and synthesise TGF-β1 to maintain and promote the progression of liver fibrosis.[30 28]

There are at least two sources of hepatic macrophages: the liver itself (Kupffer cells) and circulating monocytes. Some studies found another group of macrophages different from these types of macrophages—the hepatic macrophages that may be derived from blood Ly-6C^{high} mononuclear cells.[23] Under the action of CX3CL1, these cells mature and turn into Ly-6C^{low} macrophages,[31] which promote apoptosis of activated HSCs, and secrete MMP-12 and MMP-13 to promote ECM degradation.[32] In clinical studies, patients with hepatitis or cirrhosis exhibited CD14+CD16+ atypical macrophages in liver fibrosis tissue, and these cells may be derived from classical macrophages exhibiting CD14++CD16− in the microenvironment of the liver.[27] This process appears similar to that of murine models, where Ly6C^{high} mononuclear cells convert to Ly6C^{low} hepatic macrophages in the liver.[23]

It is difficult to strictly separate the hepatic macrophages into these two subgroups because some of the cells express M1 and M2 molecules, suggesting that this classification is not fully applicable. Therefore, some scholars have suggested that hepatic macrophages may be functionally divided into defensive, restorative, and regulatory macrophages.[27]

Defensive macrophages, similar to M1 macrophages, are mainly activated by engulfing foreign pathogens; promote the production of inflammatory cytokines IL-1, IL-6 and IL-23; and promote T cell differentiation to Th17 cells, thus leading to inflammatory cell infiltration and causing apoptosis or necrosis. These cells may also cause some autoimmune diseases such as inflammatory bowel disease.[33]

Restorative macrophages induce IL-4 and CX3CL1. Tissue and cell injury, and natural killer (NK) cell activation can promote the differentiation of hepatic macrophages into this cell type. Restorative macrophages cannot effectively maintain their function by themselves because they do not sustainably secrete IL-4. Instead, these cells depend on the continuous secretion of IL-4 by eosinophils or antigen-specific T cells. Restorative hepatic macrophages are believed to be related to liver fibrosis regression.[27 34]

Regulatory macrophages are similar to M2 cells and are characterised by high expression of TGF-β1 and IL-10, and can be activated by both phagocytosis and toll-like receptors (TLR). Although they express TGF-β1, hepatic macrophages do not promote the progression of fibrosis because they also express IL-10, suggesting that the net effect of macrophages in liver fibrosis is governed by the overall levels of different cytokines.

However, it is not possible to fully distinguish various types of macrophages because one type of macrophage can be transformed into another type. Some hepatic macrophages secrete proinflammatory and anti-inflammatory cytokines at the same time. Therefore, some scholars believe that macrophage subtypes do not exist, because cell types always change.

Potential targets of hepatic macrophages to treat liver fibrosis

Hepatic macrophages engage in close interactions with other non-parenchymal cells of the liver, especially HSCs.[35 36] TGF-β1 and platelet derived growth factor (PDGF) secreted by hepatic macrophages can activate HSCs to fibroblasts, and the latter can proliferate and

secrete abundant collagens and other ECM, thereby causing liver fibrosis.[37]

As hepatic macrophages have such great variability and huge numbers (10–15% of total liver cells), and also play important regulatory roles, these cells offer potential targets for treating liver fibrosis.

The first target involves preventing the infiltration of inflammatory mononuclear cells (Ly-6C+) through the inhibition of CCL2 (MCP-1) by RNA molecular technology,[38] cleaning the intestinal tract with antibiotics to reduce the exposure of the liver to endotoxin, thereby reducing the infiltration of inflammatory cells.[15 38–40]

The second target involves antagonising the inflammatory cytokines released from hepatic macrophages, such as IL-1 and TNF-α,[41] or promoting the apoptosis of activated HSCs, thereby attenuating hepatic fibrosis.[42 43]

The third target involves modulating the functional switch of hepatic macrophages via biological engineering of macrophages by nanoparticles[44 45] or targeting drugs (dexamethasone vesicles) to control the functional transformation of hepatic macrophages.[46]

The fourth target involves promoting the functional restoration of macrophages by using CX3CL1 and IL-4 to accelerate the resolution of liver fibrosis.[31] An ex vivo approach entails culturing peripheral blood monocytes in vitro under conditions favouring restorative hepatic macrophages[47] or other desired subtypes[48] and then intravenously infusing the cells back into patients to alleviate liver fibrosis.[49]

Hepatic macrophages play a key role in the progression and regression of fibrosis, although there are still many unanswered questions that need further investigation. Finally, tailored yet standardised methods for the purification and identification of functionally heterogeneous hepatic macrophages are urgently needed to yield reproducible and communicable results that shed light on the pathogenesis of liver fibrosis and offer novel potential therapeutic targets for liver fibrosis.[3 30]

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