Macrophage heterogeneity in liver injury and fibrosis

Frank Tacke*, Henning W. Zimmermann

University Hospital Aachen, Department of Medicine III, Pauwelsstrasse 30, 52074 Aachen, Germany

Summary

Hepatic macrophages are central in the pathogenesis of chronic liver injury and have been proposed as potential targets in combating fibrosis. Recent experimental studies in animal models revealed that hepatic macrophages are a remarkably heterogeneous population of immune cells that fulfill diverse functions in homeostasis, disease progression, and regression from injury. These range from clearance of pathogens or cellular debris and maintenance of immunological tolerance in steady state conditions; central roles in initiating and perpetuating inflammation in response to injury; promoting liver fibrosis via activating hepatic stellate cells in chronic liver damage; and, finally, resolution of inflammation and fibrosis by degradation of extracellular matrix and release of anti-inflammatory cytokines. Cellular heterogeneity in the liver is partly explained by the origin of macrophages. Hepatic macrophages can either arise from circulating monocytes, which are recruited to the injured liver via chemokine signals, or from self-renewing embryo-derived local macrophages, termed Kupffer cells. Kupffer cells appear essential for sensing tissue injury and initiating inflammatory responses, while infiltrating Ly-6C⁺ monocyte-derived macrophages are linked to chronic inflammation and fibrogenesis. In addition, proliferation of local or recruited macrophages may possibly further contribute to their accumulation in injured liver. During fibrosis regression, monocyte-derived cells differentiate into Ly-6C (Ly6C, Gr1) low expressing ‘restorative’ macrophages and promote resolution from injury. Understanding the mechanisms that regulate hepatic macrophage heterogeneity, either by monocyte subset recruitment, by promoting restorative macrophage polarization or by impacting distinctive macrophage effector functions, may help to develop novel macrophage subset-targeted therapies for liver injury and fibrosis.

© 2014 European Association for the Study of the Liver. Published by Elsevier B.V. Open access under CC BY-NC-ND license.

Introduction

Hepatic macrophages hold a central position in the pathogenesis of chronic liver injury and have been proposed as potential targets in combating fibrosis [1]. The ambivalence of macrophage activity in experimental liver damage and the identification of functionally opposing macrophage subsets, though, have impeded the development of macrophage-based interventional strategies so far. In congruence with the fact that liver fibrosis is not an unidirectional irreversible process, hepatic macrophages can actually exert dual functions in the context of experimental liver fibrosis by either promoting or abrogating the excessive deposition of extracellular matrix [2]. Intriguing questions have arisen from this finding, and current research focuses on unscrambling mechanisms of functional diversity underlying the opposing tasks of hepatic macrophages throughout the evolution of liver scarring. Important aspects include the origin of the macrophage subsets (derived from circulating monocyte precursors vs. resident Kupffer cells), their differentiation (oftentimes classified as M1 vs. M2 polarization) as well as their effector functions in the context of liver diseases.

Macrophage heterogeneity

Macrophage heterogeneity is expressed by a high diversity in cytokines released, cell surface markers and transcriptional profiles. In order to accommodate for the broad spectrum of macrophage function and phenotypes, these cells have been classified either into ‘pro-inflammatory’ M1 or ‘immunoregulatory’ M2 macrophages, though this simple dichotomous nomenclature does not fully reflect the complex biology of macrophage subsets [3]. Consequently, M2 macrophages are now further categorized into various subtypes that pursue wound healing or anti-inflammation but may also promote inflammation in some circumstances. M1 macrophages are intimately linked to Th1 primed CD4 T-cells, whereas M2 macrophages reciprocally engage with Th2 CD4 T-cells. M1 macrophages are typically induced by IL-12, IFN-γ, and LPS in response to acute deleterious incidents, whereas M2 macrophages are controlled by IL-4 and IL-13 [4].
Key effector functions of ‘classical macrophages’ (M1) are bacterial clearance, antiviral activity and release of pro-inflammatory cytokines (such as TNF, IL-1β, IL-12, reactive oxygen species), while ‘alternatively activated macrophages’ (M2) promote defense against parasitic infections, are involved in tissue remodeling and secrete immune-modulatory mediators (such as IL-10, TGF-β, IL-4, IL-13) [3]. However, in disease conditions that are not exclusively skewed towards one end of the spectrum such as acute bacterial peritonitis (M1) or chronic helminth (M2) infection, it is very difficult to assign tissue macrophages to classical or alternative activation. In fact, liver macrophages appear to express markers of M1 and M2 differentiation simultaneously [5], indicating that this dichotomous concept cannot be entirely applied to hepatic diseases. Rodent models of injury rather indicate that the function of hepatic macrophage subsets in the context of liver diseases largely depends on their origin [6]. Therefore, we propose to distinguish between resident hepatic macrophages, termed Kupffer cells, and infiltrating bone marrow-derived macrophages, originating from circulating monocytes, to characterize macrophage heterogeneity in the liver.

Resident hepatic macrophages in health and disease

Owing to its unique vascular supply the liver is constantly exposed to high concentrations of blood-borne food antigens and bacterial constituents derived from the commensal intestinal flora (Fig. 1). Therefore, highly orchestrated innate immune mechanisms in the liver are required to prevent the instigation of inflammatory responses towards these harmless substrates. Due to their potent phagocytic capacity, high density of surface scavenger and pattern recognition receptors as well as the ability to release numerous mediators that govern the local immunological milieu, resident hepatic macrophages meet the prerequisite to balance this incessant immunogenic stimulus and promote tolerogenic environments. Due to their high phagocytic and endo(pino)cytic capacity, local Kupffer cells can be relatively easy targeted by biofunctionalized nanoparticles intended to influence macrophage polarization as well as by carrier tools designed to deliver drugs directly to Kupffer cells (Table 1) [19,20]. In order to translate such concepts into clinical applications, however, the precise contribution of local macrophages to liver injury, fibrosis, and resolution in relation to invading monocyte-derived macrophages needs to be fully dissected.

Monocytes as precursors of hepatic macrophages

While circulating monocytes are likely dispensable for replenishing the hepatic macrophage pool in homeostasis, hepatic metabolic or toxic damage results in the massive infiltration of monocyte-derived macrophages into the liver (Fig. 1). Murine models revealed that ‘inflammatory’ Ly-6Chigh expressing monocytes accumulate in injured liver, dependent on the chemokine – receptor interactions CCL2/CXCR2 or CCL11/CXCR3 [21-24]. One of the major sources of CCL2 are HSCs, which are activated through TLR4 ligands and thereby guide monocyte recruitment [25]. Freshly infiltrating (monocyte-derived) macrophages are characterized as CD11b+ F4/80+ cells by FACS in mice, whereas matured monocyte-derived and resident Kupffer cells are CD11b+ F4/80+ [20,23]. Targeted deletion of macrophages in CD11b-diphtheria toxin receptor (DTR) transgenic mice ameliorates liver fibrosis similar to the abrogation of chemokine pathways that control monocyte influx [2,23,26,27], suggesting the activation of hepatic macrophages, as evidenced for the overload of lipids and certain cholesterol derivatives in Kupffer cells in models of fatty liver disease and steatohepatitis [11,12].

The central location of Kupffer cells in the sinusoids also allows intimate interactions with other non-parenchymal hepatic cell populations (Fig. 1). On the one hand, hepatic macrophages interact with other immune cells; for instance, they secrete the chemokine CXCL16 that attracts NKT cells, which in turn can activate pro-inflammatory signals in macrophages [13]. On the other hand, there is clear evidence from in vitro and in vivo studies that Kupffer cells can activate hepatic stellate cells (HSC) to transdifferentiate into myofibroblasts, the major collagen-producing cell type in hepatic fibrosis [14,15]. Kupffer cells activate HSC via paracrine mechanisms, likely involving the potent profibrotic and mitogenic cytokines TGF-β and PDGF (Fig. 1) [15]. These profibrotic functions of Kupffer cells during chronic hepatic injury remain functionally relevant, even if the infiltration of additional inflammatory monocytes is blocked via pharmacological inhibition of the chemokine CCL2 [16].

Moreover, hepatic macrophages can express several matrix metalloproteinases (MMP), including MMP-9, MMP-12, and MMP-13, that are involved in matrix degradation and thereby favor resolution of liver injury and fibrosis [17,18]. Although it appears plausible that Kupffer cells, which have tolerogenic and immune-suppressive functions in homeostasis, may undergo a phenotypic switch and promote tissue remodeling, experimental evidence assigning such antifibrotic functions to resident macrophages are currently lacking (Fig. 2).

The opposing effects of macrophage activation in homeostasis and inflammation indicate the versatile nature of Kupffer cells that could possibly rest on heterogeneous subsets that merge into the term ‘hepatic macrophage’ or on the plasticity of the cells that may adopt various phenotypes according to the hepatic microenvironment. Due to their high phagocytic and endo(pino)cytic capacity, local Kupffer cells can be relatively easy targeted by biofunctionalized nanoparticles intended to influence macrophage polarization as well as by carrier tools designed to deliver drugs directly to Kupffer cells (Table 1) [19,20]. In order to translate such concepts into clinical applications, however, the precise contribution of local macrophages to liver injury, fibrosis, and resolution in relation to invading monocyte-derived macrophages needs to be fully dissected.
that infiltrating monocytes exert major profibrotic functions in fibrosis progression (Fig. 1). Apart from directly stimulating matrix-secreting HSC, hepatic macrophages may aggravate scarring by promoting HSC survival via IL-1 and TNF-induced NF-κB activation [15]. Interestingly, the profibrogenic effect of infiltrating monocytes in mouse models depends on the underlying genetic background. Balb/c mice, in which Th2 immune responses inherently prevail, are more protected from liver damage and ensuing fibrosis by the disruption of monocyte infiltration than Th1-dominated C57Bl/6 mice, indicating that M1 and M2 polarization might directly influence the outcome in chronic hepatic injury [21]. Another important factor determining the fate of invading monocytes as to the extent of liver damage is the fractalkine receptor CX3CR1, predominantly present on non-classical Ly-6C<sup>+</sup> monocytes, in the absence of which monocyte-derived macrophages differentiate into detrimental iNOS and TNF-α producing effector cells [28].

As outlined above, macrophages can also enhance fibrosis resolution in a phase-dependent fashion through the secretion of matrix metalloproteinases (Fig. 2) [17,29]. Hence, not only the interference
with the recruitment of ‘profibrogenic’ monocytes was tested in experimental settings, but also the cell transfer of ‘fibrolytic’ monocytes/macrophages. As such, the delivery of syngeneic mature macrophages, but not immature (precursor) monocytes, into CCl4-treated mice reduced liver scarring by attracting other immune cells resulting in enhanced levels of the antifibrotic cytokine IL-10 and increased MMP-9 and -13 activation, accompanied by the elevation of the local growth factor levels IGF-1, VEGF, and CSF-1 [30]. The fact that only terminally differentiated macrophages bear the potential to facilitate fibrosis regression in contrast to their progenitors suggests that hepatic macrophage function varies according to disease kinetics and environmental cues.

Following this concept, early infiltrating monocytes elicit organ impairment, whereas after local differentiation into resident macrophages, these cells can gain the ability to restore liver integrity (Fig. 2). Recently, ‘restorative macrophages’, characterized by Ly-6C<sup>hi</sup> expression in mice, have been identified that are competent in resolving fibrosis and accumulated in the regenerative phase after tissue damage [5]. These Ly-6C<sup>hi</sup> macrophages directly delineate from infiltrating Ly-6C<sup>lo</sup> monocytes/macrophages [5], thus undergo a functional switch in the course of liver injury due to mechanisms that warrant further elucidation. Of note, transcriptome analysis revealed that these Ly-6C<sup>lo</sup> hepatic macrophages display a profile that cannot be classified according to the M1/M2 nomenclature. A striking feature of those restorative macrophages is that they are postphagocytic, because injection of liposomes boosted the degradation of extracellular matrix [5]. Interestingly, even after cessation of liver injury, a
sustained influx of Ly-6Ch macrophages can be observed, that dampens spontaneous fibrosis regression by producing pro-inflammatory cytokines such as TNF. Blocking the CCL2-dependent influx in the phase of fibrosis resolution therefore even enhances clearance of scar fibers [31]. These findings substantiate the theory that freshly infiltrating macrophages worsen liver injury, whereas restoration is elicited by locally matured monocyte-derived macrophages.

**Proliferation of hepatic macrophages in liver injury?**

Fate mapping studies revealed that Kupffer cells originate prenatally from either the yolk sac or local precursors and maintain themselves by self-renewal through proliferation [8,9]. In line, the absence of the transcription factor Myb1, which is critical for hematopoietic stem cells, does not affect the presence of Kupffer cells, indicating that myeloid precursor cells are dispensable for the population of hepatic macrophages in homeostasis [32]. However, there is increasing evidence that even during certain types of inflammation, accumulation of macrophages derives from the division of resident cells. In Th2-dominated helminth infection alternatively activated pleural macrophages proliferate in response to IL-4 beyond physiological borders set by the availability of CSF-1 [33,34]. Importantly, IL-4 also drives replication of Kupffer cells, denoting that this phenomenon may hold true for the liver as well [33]. Of note, alternatively activated arginase-expressing macrophages are capable of constraining liver fibrosis in an archetypal Th2 inflammation model and it is conceivable that they also delineate from local precursors [35]. However, more detailed studies are necessary to assess the contribution of in situ proliferating macrophages for expanding the macrophage pool during infections and sterile liver inflammation. Moreover, it is currently unclear whether only resident Kupffer cells or also monocyte-derived macrophages are capable of proliferating upon inflammation (Fig. 1). In conditions of atherosclerosis or peritonitis, infiltrated monocyte-derived as well as tissue resident macrophages locally proliferated in response to inflammatory stimuli [36,37]. In case of progressing liver injury, the contribution of local macrophage proliferation in the liver remains to be determined.

**Heterogeneity of human hepatic macrophages and translation into clinical applications**

A major obstacle for the development of novel therapies for liver fibrosis targeting macrophages is the significant paucity of functional data in man. Distinct macrophage populations can also be found in human liver, including ‘classical’ CD14++CD16− and ‘non-classical’ CD14−CD16+ monocyte/macrophages as well as CD16++ cells that include atypical macrophages and dendritic cells [38]. The progression of chronic liver diseases from hepatitis to fibrosis and eventually to cirrhosis is closely associated with an enrichment of ‘non-classical’ CD14−CD16+ monocyte-derived macrophages in the liver of patients with various disease etiologies [39]. The accumulation of these cells is likely based on two mechanisms: facilitated recruitment across inflamed sinusoidal endothelium and local transdifferentiation from CD14++CD16− precursor cells [38], reminiscent of the maturation of murine Ly-6C+ to Ly-6Clo macrophages in experimental rodent liver injury [5,23].

Circulating human CD14−CD16+ monocytes share phenotypic features with murine Ly-6Clo monocytes [40], but intrahepatic human CD14−CD16+ macrophages are strikingly similar to mouse inflammatory monocyte-derived macrophages with respect to functional aspects, as these cells release proinflammatory as well as fibrogenic mediators [38]. On the other hand, human intrahepatic CD14−CD16+ macrophages possess a high phagocytic activity, which has been identified as a feature of 'restorative' (Ly-6Clo) hepatic macrophages in mice [5]. These partly overlapping and partly opposing features of human and mouse macrophage subsets require further translational research. Very likely, our current definitions of the distinct hepatic macrophage subsets remain to be deciphered.

Another impediment in translating findings from mouse models to human disease is the wide spectrum of chronic liver disorders in humans. It is very likely that the functionality of hepatic macrophage subsets is influenced by the nature of the underlying liver disease (e.g., lipid overload in fatty liver disease; antigen-specific responses in autoimmune hepatitis; impact of bile acids in cholangiopathies). Current studies have focused on the stage of disease progression (fibrosis, cirrhosis) rather than the nature...
of the underlying injury [38,39]. Hence, further translational research is warranted to address disease-specific characteristics of hepatic monocyte and macrophage heterogeneity.

Nevertheless, many of the pathways characterized in mice for monocyte recruitment and macrophage effects are also strongly regulated in patients with liver diseases, suggesting well-conserved mechanisms of macrophage activation across species [41]. Such pathways may possibly serve as novel targets for therapeutic approaches (for a summary of selected potential targets, see Table 1). For instance, activation of the CCL2-CCR2 axis is associated with monocyte infiltration in patients with chronic liver diseases and parallels fibrogenesis [39,42]. Also, CD14+CD16+ monocyte-derived macrophages are capable of activating human stellate cells in vitro, partially dependent on TGF-β release [39]. The way human hepatic macrophage subsets shape the outcome of chronic liver disease is still unresolved and needs to be clarified before new therapeutic agents aiming at macrophage infiltration or polarization can enter clinical trials.

Key Points

- Macrophages exert critical functions in liver homeostasis, in the initiation of inflammation in response to hepatic injury and induction of fibrogenesis, but also in resolution of inflammation and fibrosis.
- Animal models from experimental liver injury revealed a remarkable heterogeneity of hepatic macrophages with diverse functions.
- Important aspects of hepatic macrophage heterogeneity include the origin of the macrophage subsets (derived from circulating monocyte precursors vs. resident Kupffer cells), their differentiation (often termed M1 vs. M2 polarization) as well as their effector functions in the context of liver diseases.
- Macrophage subpopulations with distinct functional properties have also been identified in human livers from patients with chronic liver diseases and fibrosis.

Financial support

This work was supported by the German Research Foundation (DFG Ta434/2-1 & SFB/TRR57) and by the Interdisciplinary Center for Clinical Research (IZKF) Aachen.

Conflict of interest

Work in the laboratory of Frank Tacke has been supported by materials from Noxxon, Gilead and Genentech.

Acknowledgments

This work was supported by the German Research Foundation (DFG Ta434/2-1 & SFB/TRR57) and by the Interdisciplinary Center for Clinical Research (IZKF) Aachen.

References


[JOURNAL OF HEPATOLOGY]
Clinical Application of Basic Science


