CrossMark

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 combatting 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.

Introduction

Hepatic macrophages hold a central position in the pathogenesis of chronic liver injury and have been proposed as potential targets in combatting 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].

ELSEVIER

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 those 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 tolerance (e.g., dampening of T cell activation) [6]. Actually, under steady state conditions the liver harbors the most abundant pool of macrophages in the whole body. It has long been debated whether circulating monocytes contribute to the Kupffer cell pool. Data from bone marrow and liver transplanted mice demonstrated that monocytes in principle can give rise to functional hepatic macrophages without overt inflammatory stimuli to the liver [7]. However, already in 1997 Naito and coworkers provided experimental data indicating that Kupffer cells almost exclusively originate from fetal yolk sac precursors and self-renew throughout adult life in homeostasis, depending on proliferative signals via M-CSF [8]. These findings could be recently recapitulated using sophisticated cell tracking techniques. These experiments revealed that Kupffer cells delineate from local precursors and constantly renew themselves dependent on the growth factors GM-CSF and M-CSF [9].

During inflammation the hepatic macrophage pool is even expanded, and a startling scientific debate is ongoing regarding the origin and underlying mechanisms of macrophage enrichment. In the early phase after a hazardous incident, sessile hepatic macrophages rapidly secrete pro-inflammatory cytokines and chemokines such as IL-1β, TNF, CCL2, and CCL5, resulting in the paracrine activation of protective or apoptotic signaling pathways of hepatocytes and the recruitment of additional immune cells that booster hepatic injury [10] (Fig. 1). In addition, not only inflammatory stimuli, but also metabolic signals may modulate 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.

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-6C^{hi} expressing monocytes accumulate in injured liver, dependent on the chemokine – receptor interactions CCL2/CCR2 or CCL1/CCR8 [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^{lo} F4/80^{hi} [20,23]. Targeted deletion of macrophages in CD11b-diphteria toxin receptor (DTR) transgenic mice ameliorates liver fibrosis similar to the abrogation of chemokine pathways that control monocyte influx [2,23,26,27], suggesting