Research Article

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Monocyte-endothelial cell interactions in the regulation of vascular sprouting and liver regeneration in mouse

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Background & Aims: Regeneration of the hepatic mass is crucial to liver repair. Proliferation of hepatic parenchyma is intimately dependent on angiogenesis and resident macrophage-derived cytokines. However the role of circulating monocyte interactions in vascular and hepatic regeneration is not well-defined. We investigated the role of these interactions in regeneration in the presence and absence of intact monocyte adhesion.

Methods: Partial hepatectomy was performed in wild-type mice and those lacking the monocyte adhesion molecule CD11b. Vascular architecture, angiogenesis and macrophage location were analyzed in the whole livers using simultaneous angiography and macrophage staining with fluorescent multiphoton microscopy. Monocyte adhesion molecule expression and sprouting-related pathways were evaluated.

Results: Resident macrophages (Kupffer cells) did not migrate to interact with vessels whereas infiltrating monocytes were found adjacent to sprouting points. Infiltrated monocytes colocalized with Wnt5a, angiopoietin 1 and Notch-1 in contact points and commensurate with phosphorylation and disruption of VE-cadherin. Mice deficient in CD11b showed a severe reduction in angiogenesis, liver mass regeneration and survival following partial hepatectomy, and developed unstable and leaky vessels that eventually produced an aberrant hepatic vascular network and Kupffer cell distribution.

Conclusions: Direct vascular interactions of infiltrating monocytes are required for an ordered vascular growth and liver regeneration. These outcomes provide insight into hepatic repair and new strategies for hepatic regeneration.

Keywords: Angiogenesis; Immunology; Innate immune system; Hepatic repair.
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Abbreviations: EC, Endothelial cell; KC, Kupffer Cells; *MCP-1*, monocyte chemotactic protein 1; NO, nitric oxide; *ICAM-1*, Intercellular Adhesion Molecule 1; TNF-α, tumor necrosis factor α.

Introduction

The liver is a unique organ in not only its capacity to regenerate but in the complex sequential activation of multiple pathways and cell types involving the entire remaining organ in recovery of mass [1,2]. What has not yet been fully delineated is how these processes interact and in particular, the converging roles of angiogenesis [3] and circulating monocytes. After hepatectomy, injured hepatocytes, liver progenitor cells and resident macrophages, that is, Kupffer cells (KCs) from portal areas recruit circulating immune cells such as monocytes to damaged liver through release of monocyte chemotactic protein 1 (*MCP-1*) [4,5]. Infiltrating monocytes can activate c-Met and Tie-2 pathways by direct interaction [6–8], and trigger the paracrine release of different cytokines and endothelial growth factors important to liver regeneration such as the family of molecules Notch and Wnt [9–11]. Either disruption of the canonical Wnt/β-catenin signaling pathway in macrophages [12] or depletion of systemic macrophages reduces liver regeneration [13,14]. KCs in particular, as resident macrophages play an elaborate control role of sinusoidal endothelium [2]]. Priming factors (e.g., IL-6 and TNFα) by KCs and hepatocytes themselves [15] stimulate hepatocytes to respond to growth factors (e.g., HGF, TGF-β, and EGF) [16]. Hepatocytes undergo DNA synthesis that peaks at ~36 h post-hepatectomy in mice [16] followed by three additional smaller waves of proliferation to eventually restore the original number of hepatocytes [17]. Proliferation of hepatocytes sequentially advances from periportal to pericentral areas of the lobule, as a wave of mitosis under circadian control [18].

A vascular response simultaneously arises and begins with endothelial budding, and is facilitated by vasodilation and uncoupling of interendothelial contacts, which allows extravasation of plasma proteins and extracellular matrix components to set down an initial scaffold for migrating ECs [19]. The vasodilator and pro-angiogenic substance nitric oxide (NO) [20] is the main factor responsible of the initial vascular effects, while Wnt and Notch family proteins provide proliferating and non-proliferating control of tip and stalk cells, respectively [21]. These factors help overcome the forces that provide vascular mechanical strength and stiffness. At the molecular level, the disruption of EC assembly includes the phosphorylation of adherens junctions such as vascular endothelial (VE)-cadherin, platelet endothelial cell adhesion molecule-1, and their corresponding cytoskeleton-linking molecules. Moreover, endothelial and

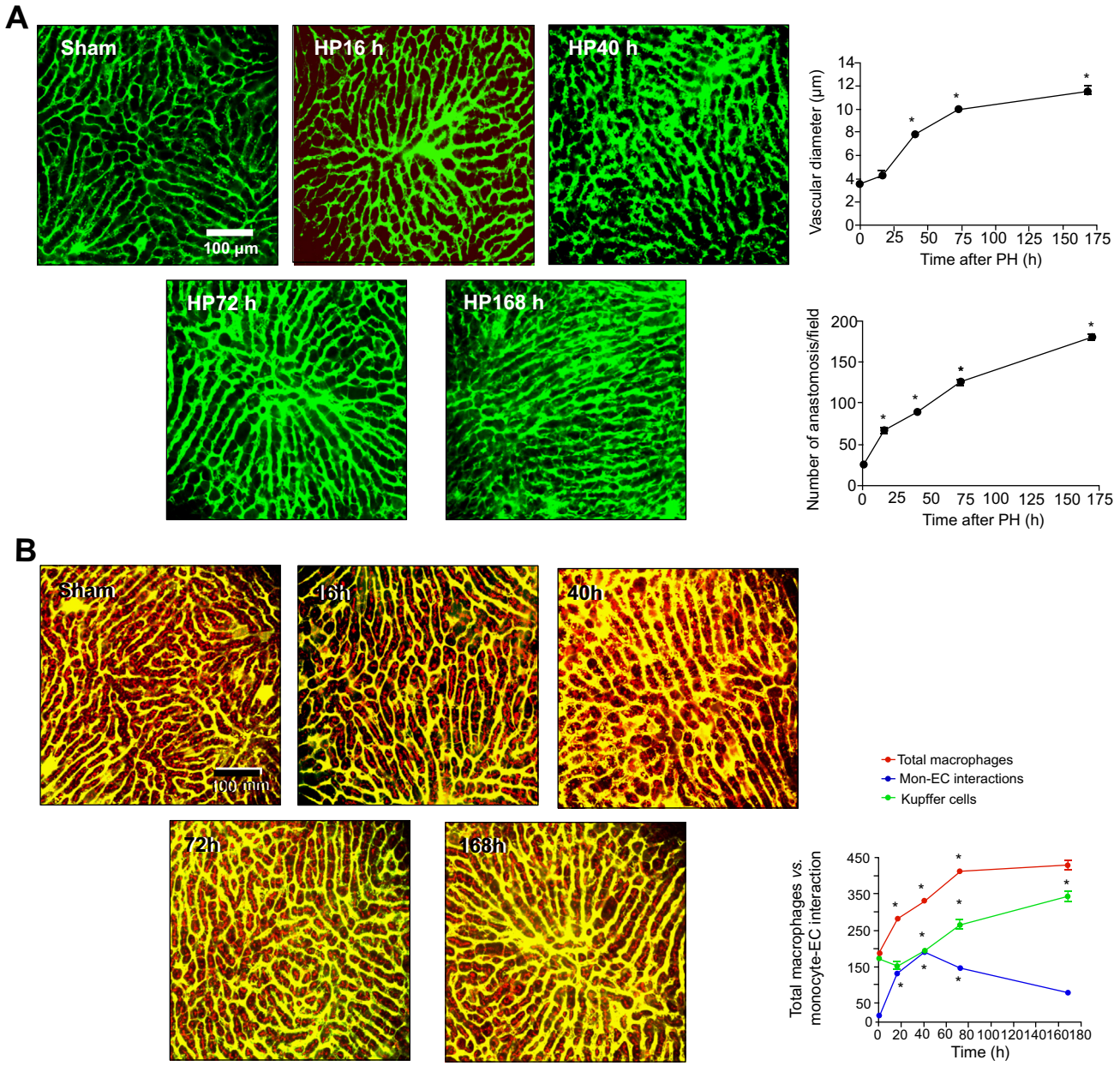

Fig. 1. Monocyte/macrophage interactions with liver sinusoidal endothelial cells during liver regeneration. (A) C57BL/6 mice underwent 70% hepatectomy and samples of liver right lobe were examined at sequential time points (0, 16, 40, 72, 168 hours post-op). Vasodilation (initiated in portal areas) and anastomoses were analyzed by angiography (vascular perfusion of FITC-dextran, MW 2×10^6 Da) using multiphoton microscopy. (B) The total number of macrophages and monocyte-vascular interactions were quantified in whole liver images obtained from mice intravenously injected with 70 kDa Texas red-dextran two hours before angiography with FITC-dextran and multiphoton microscopy, and sacrifice. Intravascular and extravascular monocyte-endothelium interactions and non-interacting macrophages highlighted in red were quantified in every z-stack from every field. One representative Z image of liver from every time point is shown n = 10 at every time point; $^*p$ <0.05 *vs.* former time point.

inducible NO synthases are upregulated after partial hepatectomy by c-Met activation and released NO facilitates the uncoupling of endothelial junctions [22,23]. Likewise, c-Met receptor has recently been described as a co-receptor of the leukocyte intercellular adhesion molecule 1 (*ICAM-1*) [24] and this latter signaling pathway triggers liver regeneration by stimulating KC to release tumor necrosis factor α (TNF-α) and IL-6 in mice [25].

The regulation of interactions of infiltrating monocytes with endothelial cells is critical for the optimization of angiogenesis in order to synchronize vascular growth to parenchymal