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Targeting hepatic macrophages to treat liver diseases

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Summary

Our view on liver macrophages in the context of health and disease has been reformed by the recognition of a remarkable heterogeneity of phagocytes in the liver. Liver macrophages consist of ontogenically distinct populations termed Kupffer cells and monocyte-derived macrophages. Kupffer cells are self-renewing, resident and principally non-migratory phagocytes, serving as sentinels for liver homeostasis. Liver injury triggers Kupffer cell activation, leading to inflammatory cytokine and chemokine release. This fosters the infiltration of monocytes into the liver, which give rise to large numbers of inflammatory monocyte-derived macrophages. Liver macrophages are very plastic and adapt their phenotype according to signals derived from the hepatic microenvironment (*e.g.* danger signals, fatty acids, phagocytosis of cellular debris), which explains their manifold and even opposing functions during disease. These central functions include the perpetuation of inflammation and hepatocyte injury, activation of hepatic stellate cells with subsequent fibrogenesis, and support of tumor development by angiogenesis and T cell suppression. If liver injury ceases, specific molecular signals trigger hepatic macrophages to switch their phenotype towards reparative phagocytes that promote tissue repair and regression of fibrosis. Novel strategies to treat liver disease aim at targeting macrophages. These interventions modulate Kupffer cell activation (*e.g.* via gut-liver axis or inflammasome formation), monocyte recruitment (*e.g.* via inhibiting chemokine pathways like CCR2 or CCL2) or macrophage polarization and differentiation (*e.g.* by nanoparticles). Evidence from mouse models and early clinical studies in patients with non-alcoholic steatohepatitis and fibrosis support the notion that pathogenic macrophage subsets can be successfully translated into novel treatment options for patients with liver disease.

Lay summary:
Macrophages (Greek for "big eaters") are a frequent non-parenchymal cell type of the liver that ensures homeostasis, antimicrobial defense and proper metabolism. However, liver macrophages consist of different subtypes regarding their ontogeny (developmental origin), differentiation and function. Understanding this heterogeneity and the critical regulation of inflammation, fibrosis and cancer by macrophage subsets opens promising new options for treating liver diseases.

Key point

Liver macrophages display a remarkable heterogeneity, reflecting their developmental origin (resident Kupffer cells and infiltrating monocytes) and their differentiation (*e.g.* inflammatory or anti-inflammatory) in response to microenvironmental signals (*e.g.* danger signals, phagocytosis of cellular debris)

Introduction

Some of the most pressing unresolved challenges in hepatology today can be related to an imbalance of inflammatory processes: (i) functional or biological cure from hepatitis B virus (HBV) infections can probably not be achieved without an effective antiviral immune response; (ii) the progression from non-alcoholic fatty liver to non-alcoholic steatohepatitis (NASH) with fibrosis is fuelled by chronic hepatic inflammation; (iii) patients with end-stage cirrhosis are prone to life-threatening bacterial infections indicating insufficient antimicrobial responses in the liver; finally, (iv) development and progression of hepatocellular carcinoma (HCC) is the result of inadequate tumor clearance and/or suppression of anti-tumor immunity [1]. During the past decades, it has become apparent that hepatic macrophages hold central functions in initiating, perpetuating and even restricting inflammation in the liver. The tremendous progress in understanding their heterogeneity and various functions will be reviewed here and opens new perspectives for the treatment of liver diseases.

The concept of Kupffer cells and infiltrating monocytes in homeostasis and injury response

Macrophages are particularly abundant in the liver. Studies from healthy rodent livers estimated that every 100 hepatocytes are accompanied by 20–40 macrophages [2]. Although these macrophages may look quite similar by histology, they can be a very heterogeneous population with highly specialized functions in the context of liver diseases [3]. One of the fundamental discoveries in the field was the interplay between liver-resident macrophages, termed Kupffer cells, and blood/bone marrow-derived macrophages, termed monocyte-derived macrophages (Fig. 1).

Kupffer cells, named after the German anatomist Karl Wilhelm von Kupffer [4], represent the self-renewing, resident and principally non-migratory phagocyte population in the liver. Kupffer cells originate from yolk sac-derived specific progenitor cells that seed the liver during embryogenesis (during embryonic days 9.5–12.5 in mice) [5–7]. Kupffer cells are highly effective phagocytes that recognize, ingest and degrade cellular debris, foreign material or pathogens [8]. They thereby act as critical sentinels that ensure liver homeostasis and eliminate antibodies, debris or dead cells. Unlike liver phagocytes arising from monocytes or infiltrating peritoneal macrophages, Kupffer cells are stationary and do not migrate [9–11]. In healthy livers, Kupffer cells are exclusively located in the intravascular compartment (mainly within the hepatic sinusoids), whereas dendritic cells (and possibly also monocyte-derived macrophages) can be located extravascularly [12].

In homeostatic conditions, the ingestion of particles results in antigen-processing by Kupffer cells and induction of regulatory T cells, corroborating that Kupffer cells support primarily tolerogenic immune responses in homeostasis [11]. However, Kupffer cells are equipped by scavenger, complement and pattern recognition receptors (*e.g.* Toll-like receptors [TLRs]) [1]. The latter allows them to become activated upon infectious or non-infectious threats in order to induce immunogenic T cell responses [11]. Kupffer cells express the complement receptor of the immunoglobulin superfamily (CRIg), which binds complement fragments C3b and iC3b and allows phagocytosis of complement C3-opsonized particles [13]. Using CRIg, Kupffer cells are capable of catching bacteria under flow conditions [14]. For instance, *Staphylococcus aureus* bacteria are cleared by Kupffer cells within hours after systemic injection in mice [15], corroborating the importance of Kupffer cells for antimicrobial defense [16].

The macrophage pool of the liver can be rapidly expanded by infiltrating phagocytes that mainly originate from monocytes (Fig. 1). In mice, two major populations of circulating monocytes exist: Ly-6C high (Ly-6C^{hi}) and Ly-6C low (Ly-6C^{lo}) expressing monocytes. Whereas the Ly-6C^{hi} monocytes express inflammatory chemokine receptors (like CCR2), pattern-recognition receptors and cytokines [17,18], the Ly-6C^{lo} monocytes show a patrolling behavior in the liver and express more scavenging receptors [11,12,19]. The bone marrow is the primary source of the (relatively immature) Ly-6C^{hi} monocytes [20], whereas the spleen serves as a reservoir for Ly-6C^{lo} monocytes [21]. As a consequence of tissue injury, Kupffer cells and other liver cells (stellate cells, hepatocytes) secrete chemokines like CCL2 that provoke the massive infiltration of Ly-6C^{hi} monocytes to the injured liver [9,18,22]. This provides a rapid and transient mechanism to expand the macrophage pool in the liver by inflammation-prone phagocytes. As an example, in a mouse model of hemolytic anemia, monocyte-derived macrophages were found to accumulate quickly and "on demand" in the liver, where they protect the liver from iron toxicity by ingesting senescent and dying erythrocytes [23]. More recently, the infiltration of phagocytes from the peritoneal cavity, characterized by expression of the transcription factor GATA6, has been described in mouse models of sterile liver injury [10]. It is currently unclear if this mechanism might be restricted to subcapsular liver lesions in proximity to the peritoneal cavity.

Importantly, the compartment of hepatic myeloid cells is not simply dichotomic (Kupffer cells *vs.* monocyte-derived macrophages). Monocytes or specialized hematopoietic precursors give rise to several subsets of hepatic dendritic cells in the liver [12,24,25]. Moreover, monocytes can replace Kupffer cells, if they are experimentally depleted or reduced as a consequence of liver injury [12,26–28]. Monocyte-derived macrophages can then acquire a phenotype that is virtually indistinguishable from Kupffer cells [26]. Finally, Kupffer cells and monocyte-derived macrophages are very plastic and adapt their phenotype according to the signals derived from the hepatic microenvironment [27,29].

The heterogeneity of liver macrophages is less well defined in humans compared to mice. However, different monocyte, macrophage and dendritic cell populations exist in human livers as well [30–32], and several markers including CD14, CD16 or CD68 have been proposed to distinguish these populations [1].

Beyond M1 and M2: Liver macrophage polarization in the context of injury

Traditionally, macrophage functions have been assigned as inflammatory or "M1" *vs.* anti-inflammatory or "M2" [33]. This concept is originally based on cell culture experiments, showing that monocyte-derived macrophages can differentiate towards M1 cells by interferon-γ or towards M2 cells by interleukin (IL)-4, which results in typical

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