Liver Macrophages: Old Dogmas and New Insights

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Inflammation is a hallmark of virtually all liver diseases, such as liver cancer, fibrosis, nonalcoholic steatohepatitis, alcoholic liver disease, and cholangiopathies. Liver macrophages have been thoroughly studied in human disease and mouse models, unravelling that the hepatic mononuclear phagocyte system is more versatile and complex than previously believed. Liver macrophages mainly consist of liver-resident phagocytes, or Kupffer cells (KCs), and bone marrow-derived recruited monocytes. Although both cell populations in the liver demonstrate principal functions of macrophages, such as phagocytosis, danger signal recognition, cytokine release, antigen processing, and the ability to orchestrate immune responses, KCs and recruited monocytes retain characteristic ontogeny markers and remain remarkably distinct on several functional aspects. While KCs dominate the hepatic macrophage pool in homeostasis ("sentinel function"), monocyte-derived macrophages prevail in acute or chronic injury ("emergency response team"), making them an interesting target for novel therapeutic approaches in liver disease. In addition, recent data acquired by unbiased large-scale techniques, such as single-cell RNA sequencing, unraveled a previously unrecognized complexity of human and murine macrophage polarization abilities, far beyond the old dogma of inflammatory (M1) and anti-inflammatory (M2) macrophages. Despite tremendous progress, numerous challenges remain in deciphering the full spectrum of macrophage activation and its implication in either promoting liver disease progression or repairing injured liver tissue. Being aware of such heterogeneity in cell origin and function is of crucial importance when studying liver diseases, developing novel therapeutic interventions, defining macrophage-based prognostic biomarkers, or designing clinical trials. Growing knowledge in gene expression modulation and emerging technologies in drug delivery may soon allow shaping macrophage populations toward orchestrating beneficial rather than detrimental inflammatory responses. (*Hepatology Communications* 2019;3:730-743).

The liver is the largest solid organ and exerts vital metabolic functions. Liver diseases leading to liver cirrhosis or cancer are increasingly challenging for public health, the current trend being an augmentation of such diseases mainly caused by changes in alimentation and life habits.[1] Liver diseases are various by nature in terms of etiologies, chronicity, and chances of recovery. However, one constant feature is the presence of liver inflammation, and most remarkably, there is an apparent compulsory association of inflammation with a poor outcome for patients.[2-6]

Liver macrophages are included in the mononuclear phagocyte system and are renown cornerstones in most if not all inflammation-related liver disorders due to their ability to respond to a seemingly infinite

variety of activating signals. As a consequence, numerous reviews are available on the crucial roles of hepatic macrophages in liver cancer,[7-9] fibrosis,[6,10,11] alcoholic liver disease and bacterial infections,[12-16] nonalcoholic fatty liver disease,[17-19] viral hepatitis,[20] cholestatic diseases,[21,22] drug-induced acute liver injury,[23,24] ischemia reperfusion injury and liver transplant,[25,26] liver regeneration,[23,27,28] and also in aging liver.[29] Because our knowledge on macrophages in the context of liver disease has increased exponentially over recent years, a fresh view on this fascinating immune cell population has emerged, challenging some old dogmas and highlighting the heterogeneity and plasticity of liver macrophages.

One must keep in mind that the liver is not an isolated organ. About two thirds of its blood supply is rich in nutrients and potential pathogens coming from the intestines through the portal vein, and the remaining third is loaded with oxygen and delivered through the hepatic artery. Additionally, the liver occupies a filter barrier role for most toxic substances derived from the circulation or locally generated by the liver enzymatic arsenal (including bile acids). Finally, the liver is the largest organ located in the peritoneal cavity and is in contact with peritoneal fluids. Thus, to decipher liver inflammation, these aspects need to be kept in mind in order to fully integrate the complexity of the immune system combined with the specific aspects of liver physiology. This review mainly aims to highlight new findings on liver macrophage heterogeneity, shifting from a classical M1 versus M2 dichotomic view to a spectrum model of macrophage polarization[30] or a universe of macrophage activation states.[31] These insights help to comprehend the diverse and sometimes even opposing functions of hepatic macrophages in the context of liver diseases.

Liver Macrophages: Multiple Players on the Same Team

Not so long ago, the term Kupffer cells (KCs) was synonymously used for hepatic macrophages, and relatively approximate methods (e.g., immunohistochemistry for F4/80 or cluster of differentiation [CD]68) were used to identify these cells in liver sections (Fig. 1). It is now well recognized that macrophages observed in the liver following injury are heterogeneous and may derive from different origins: liver-resident macrophages or KCs and two patrolling populations of bone marrow monocyte-derived macrophages (MoMFs) as well as peritoneal macrophages for subcapsular regions of the liver. Different cell origins have been linked to discrepancies in cell functionality as well as in responsiveness toward activating and recruiting signals, directly influencing the immune response outcome.

KUPFFER CELLS

KCs are the liver-resident macrophages. They are located at the luminal side of the hepatic sinusoidal endothelium and are sensing their microenvironment through long cytoplasmic expansions. Moreover, KCs do not seem to patrol the liver but rather to occupy a fixed position over time.[32-35] KCs exert crucial functions during homeostasis, such as clearance of systemic or intestine-derived pathogens and iron metabolism regulation, and they are among the first responders following liver injury.[36] KCs are primarily identified as $CD45^{positive\,(+)}$ $F4/80^{+}$ $CD11b^{intermediate\,(int)}$ cells

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FIG. 1. Old dogmas versus new insights on liver macrophages. Liver macrophages have long been regarded as a homogeneous population and designated as F4/80+ KCs. Innovative new techniques, such as cell tracking, multi-omics phenotyping, and single-cell RNA sequencing, unraveled a previously unrecognized heterogeneity in liver macrophage origins and functions. The simplistic dichotomic view of M1- versus M2-polarized macrophages also appears outdated as macrophages of virtually all intermediate phenotypes have been described depending on the pathology or activating signals they are exposed to. Considering the multifaceted roles of liver macrophages in promoting or preventing tissue damage and repair, immunomodulating (e.g., gene expression modulation, chemokine receptor antagonism, à la carte activating signals) rather than immunodepleting approaches need to be considered.

in the mouse liver, and C-type lectin domain family 4, member f (Clec4F) appears to be the most specific murine KC marker identified so far.[37-39] Alternatively, KCs have been shown to be distinguishable from MoMFs based on their expression of the T cell immunoglobulin (Ig) and mucin domain containing 4 (Timd4) and stabilin 2 (Stab2) gene receptors.[40] Additional KC and MoMF markers have been reviewed.[41] Although mainly thought to have immunomodulatory functions at the steady state, recent data based on single-cell RNA sequencing obtained from human liver claimed the identification of two KC subsets, one of which is indeed mainly immunoregulatory while the second has a proinflammatory gene signature.[39] Tolerogenic KCs were identified as macrophage receptor with collagenous structure (MARCO)-expressing and reported to be mainly located in the periportal area.[39] However, confirmation of this data is pending as in-depth analysis may, for instance, reveal different ontogenies of these cell populations; the proinflammatory "KC" population could potentially derive from recruited MoMFs.

It is now widely accepted that KCs derive from colony-stimulating factor 1 receptor (CSF1R)+ erythro-myeloid progenitors in the yolk sac that migrated to the liver around embryonic day (E)10.5 in mice.[42-44] This has been demonstrated using *Csf1r* reporter mice and refined by a later study that showed KCs derived from hematopoietic stem cells that migrated to the liver at E10.5, using KIT proto-oncogene receptor tyrosine kinase (*Kit*) for fate mapping.[43,45] KCs, like other tissue-resident macrophages, self-renew at steady state independently of bone marrow progenitors at least up to 9 months of age in mouse.[46] However, recent findings indicated a potential role of MoMFs for being an alternate source of KCs (discussed below).

BONE MARROW/MONOCYTE-DERIVED MACROPHAGES

The precursors of MoMFs, the monocytes, circulate in the bloodstream and are principally generated from a chemokine (C-X3-C motif) receptor 1 (CX3CR1)+ CD117+ lineage-negative (Lin−) bone marrow progenitor population.[47] The main functions of MoMF depend on their ability to rapidly accumulate and to be activated following virtually any organ injury where they can further adapt toward a plethora of phenotypes that direct their functionality and

their influence on other cell types (Fig. 1). Thus, they harbor fascinating plasticity that truly defines them as major immune response orchestrators.

Two main populations of MoMFs have been identified in the healthy mouse based on lymphocyte antigen 6 complex, locus C1 (Ly6C) expression.[48,49] This distinction of tissue MoMFs is founded on the principal observation that circulating monocytes consist of two populations in mice that are differentiated by their Ly6C (previously termed Gr1) and chemokine receptor expressions.[50] Blood monocytes can be distinguished between CX3CR1^{lo}CD62L$^+$ chemokine (C-C motif) receptor 2 (CCR2)$^+$ Gr1^{high} (Ly6C/G) immature/inflammatory and CX3CR1^{hi} CCR2$^−$ Gr1^{low} mature/patrolling subsets, having the ability to differentiate toward macrophages or dendritic cells *in vivo*, while human monocytes were similarly defined as CX3CR1^{lo} CD14$^+$ CD11b^{hi} CD11c$^+$ CD62L$^+$ CD16$^−$ or CX3CR1^{hi} CD14^{lo} CD16$^+$ CD11b$^+$ CD11c^{hi} subsets.[50,51] In analogy, tissue Ly6C$^+$ MoMFs have been proposed as potent proinflammatory cells that are primarily responsible for acute inflammation, while Ly6C$^−$ cells may serve as precursors for dendritic cells.[49] Ly6C$^+$ MoMFs have also been proposed to represent precursors for Ly6C$^−$ MoMFs.[48] At the steady state, MoMFs are distinct from self-renewing tissue-resident macrophages and were reported to have a half-life of 2 days (Ly6C^{lo} cells, mouse) or 20 hours (Ly6C^{hi}, mouse).[48,52] Ly6C$^+$ MoMFs were also reported to express higher levels of T cell Ig mucin 3 (Havcr2), toll-like receptor (TLR) 2 gene (*Tlr2*), the C-type lectin genes *Clec4d, Clec4e*, and *Clec5a*, dendritic cell-specific intercellular cell adhesion molecule 3 (ICAM3)-grabbing nonintegrin isoform Cd209a, and the C1q receptor CD93 in a murine model of acute liver injury induced by N-acetyl-p-aminophenol.[40] Morphologically, MoMFs have few cytoplasmic expansions and remain relatively circular while they patrol the liver as opposed to KCs that have a more stellate appearance.[53]

Monocytes are recruited to the liver when TLR signaling is being activated in immune-sensing cells, such as KCs or hepatic stellate cells,[54] and drives an increase in chemokine (C-C motif) ligand 2 (CCL2) and chemokine (C-X-C motif) ligand 1 (CXCL1) levels, considered to be the main drivers of monocyte infiltration into the liver.[55-57] Consequently, CCR2 and/or CCR5 antagonism has led to decreased inflammatory MoMF numbers in the liver in a variety of disease models.[53,55,58-60]

Following injury or massive KC depletion, MoMFs have been reported to have the capacity of regenerating the liver-resident macrophage pool. In a mouse model of diphtheria toxin-induced depletion of *Clec4f*-expressing KCs, MoMFs replenished the liver macrophage population and differentiated toward fully functioning and self-renewing KCs within 2 weeks to 1 month.[37] This study also suggested that MoMFs participate in the establishment of the KC pool shortly after birth. MoMFs were also shown to repopulate the liver after *Listeria monocytogenes*-induced KC death,[61] irradiation followed by bone marrow transplant,[62] and clodronate-mediated macrophage depletion.[63] On the other hand, following the reduction of KC numbers as commonly observed in acute liver injury after acetaminophen poisoning, remaining KCs proliferated to restore the resident macrophage pool while recruited MoMFs participated at early stages in tissue injury and later in tissue regeneration as their depletion using an anti-CCR2 MC21-depleting antibody delayed recovery.[40]

PERITONEAL MACROPHAGES

This less well-studied population distinct from blood circulating or bone marrow-derived myeloid cells may be recruited to the subcapsular regions of the liver following specific circumstances, such as trauma, infection, or cancer. The peritoneal cavity contains self-renewing macrophages that were prenatally established and that are termed peritoneal macrophages.[48] Of note, peritoneal macrophages are often used in cell culture models to study primary macrophage functions because they can be easily isolated by washing the abdominal cavity.[64] Intraperitoneal thioglycollate injection induces a strong peritonitis in mice, facilitating the retrieval of activated macrophages from the peritoneal cavity.[65] These macrophages can also be pre-activated to proinflammatory or anti-inflammatory phenotypes by using lipopolysaccharide (LPS) or combined thioglycollate and interleukin (IL)-4 injections, respectively, prior to their isolation.[65,66]

Similar to the dichotomy of KCs and MoMFs, at least two macrophage populations seem to be present in the peritoneal cavity: a large peritoneal population making up to 90% of peritoneal macrophages in a healthy condition that are F4/80^{hi} and CD11b^{hi} and small peritoneal macrophages F4/80^{lo} CD11b^{lo} major histocompatibility complex (MHC)-II^{hi} mainly observed after LPS or thioglycollate injection.[65,67] Large peritoneal macrophages also uniquely express the transcription factor GATA-binding protein 6 (GATA6).[68]

Limited data are available on the role of peritoneal macrophages in liver disease. However, following focal thermal injury at the liver surface, peritoneal F4/80^{hi} GATA6$^+$ CD11b$^+$ mature macrophages (large peritoneal macrophages) were shown to accumulate within 1 hour at the site of hepatic injury.[69] In the same study, the authors reported that CCR2 and CX3CR1-expressing MoMFs did not infiltrate the injury site in this model but rather surrounded it. These findings were further corroborated by intraperitoneal clodronate injection leading to a preferential peritoneal macrophage depletion rather than KC and MoMF depletion when the liposomes are intravenously injected. However, it has to be kept in mind that the hepatic capsule harbors a unique cellular network of macrophages phenotypically distinct from KCs that restricts the hepatic dissemination of intraperitoneal bacteria.[70]

OTHER POTENTIAL SOURCES

Besides the bone marrow, the spleen is also a reservoir for monocytes that can exit the spleen to accumulate in peripheral sites of injury.[71] Splenic myeloid cells may, therefore, directly contribute to the hepatic MoMF populations observed following liver injury.[72] However, it is unknown whether such hepatic MoMFs from extramedullary sources are functionally distinct from bone marrow-derived liver phagocytes. In addition, splenic macrophages were found to promote KC activation in fibrosis models, which in turn facilitates monocyte recruitment and the establishment of an inflammatory hepatic macrophage phenotype, suggesting that splenic macrophages may indirectly influence hepatic inflammation by the release of signaling mediators into the portal vein.[73,74]

Kupffer Cells and Monocytes: The Sentinels and the Emergency Response Team

Due to their location in the sinusoids, their cytoplasmic expansions that allow them to sense both the blood circulation and the hepatocytes, and their

high phagocytic abilities, KCs are among the primary cells exposed to and able to respond to liver insult.[36] Key metabolic functions are undisputedly attributed to KCs. Most notably, they are pivotal in the phagocytosis of aging red blood cells and iron metabolism and have more recently been linked to lipid metabolism.[75,76] During homeostasis, KCs can uptake particulate peptide antigens to induce tolerogenic T-cell responses.[35] Following experimental liver injury, KC depletion dramatically prevents inflammation initiation and attraction of MoMFs, thus highlighting their role as sentinels in the liver.

An example of this sentinel role for KCs was demonstrated in a mouse model of steatohepatitis combining a high-fat diet with alcohol feeding.[77] In this model, sorted KCs did not show any tumor necrosis factor (*Tnf*)*a*, *Il1b*, or nitric oxide synthase 2, inducible (*Nos2*) gene expression induction, unlike MoMFs that were strongly activated toward a proinflammatory phenotype in a Notch homolog 1, translocation-associated (NOTCH1)-dependent manner.[77] On the other hand, KCs are known to respond to gut-derived LPS through TLRs following increased intestinal permeability caused by alcohol.[78,79] In this situation, KCs produce high amounts of IL-6, monocyte chemoattractant protein 1/CCL2, and TNFα that drive inflammatory cell recruitment, as well as hepatocyte production of alarmins, acute phase proteins, and chemokines, thus increasing the inflammatory reaction.[78-82] Interestingly, alcohol-induced and gut-derived LPS effects do not seem to be solely attributable to KCs. Inokuchi et al.[83] demonstrated that wild-type (WT) KC-depleted mice receiving TLR4-deficient bone marrow or TLR4-deficient mice receiving WT bone marrow showed an intermediate liver injury and inflammation compared to that of WT and TLR4-deficient mice exposed to ethanol challenge. The authors concluded that both MoMFs/KCs and hepatic stellate cells were implicated in the pathogenesis of alcoholic liver disease caused by LPS through TLR4 activation. Interestingly, it has also been nicely demonstrated that KCs potently produce IL-10 and relatively low amounts of TNF following LPS challenge compared to peritoneal and splenic macrophages.[84,85] KCs were also shown in this study to have higher phagocytic capacities and to express higher levels of TLR inhibitory molecules, attributes that attest to their chronic exposure to

endotoxins, their crucial roles in blood clearance, and their steady-state immunotolerant phenotype.[85]

The "task diversification" between KCs and MoMFs is more difficult to assess in continuous chronic injury models because MoMFs may, at least in part, replace KCs in such conditions (see above). Nonetheless, studying the methionine/choline-deficient dietary model of steatohepatitis revealed gene expression changes in sorted KCs that are mainly related to innate immune activation and metabolism. Contrastingly, MoMFs from steatotic livers had a higher expression level of fibrogenic and angiogenic genes than KCs from steatotic livers or cells sorted from control diet-fed animals.[60] Additionally, cell sorting from mice subjected to a combined fibrosis–cancer model induced by diethylnitrosamine followed by repetitive CCl_4 injections revealed distinct coexisting MoMF populations with specific gene expression patterns, suggesting inflammatory capacity (immature myeloid infiltrate), angiogenic and fibrogenic activity (classical tumor-associated macrophages), or immune-suppressive functions (myeloid-derived suppressor cells).[86]

While KCs are stationary and crucial for initiating inflammation, drastic changes occur in the MoMF compartment following injury. MoMFs have been shown to be recruited to the injury site just a few hours after acute injury[40,53,87] and account for the majority of hepatic macrophages in models of chronic liver injury.[57,60,88] Their initial phenotype following recruitment in mice is characterized by high expression levels of pattern recognition receptors, multiple "polarization" markers, and flags of immaturity (e.g., Ly6C, CCR2, Csf-receptor), supporting their ability to respond to signals and further mature at the site of injury.[40,53]

Multifaceted Roles of Macrophages During Liver Injury and Regeneration

Despite the undisputable association between inflammation and tissue damage, it would be misleading to consider macrophages as foes during liver injury. In fact, macrophage depletion had detrimental consequences on liver disease resolution.[89,90] Several reviews pondered beneficial versus detrimental roles of macrophages during liver injury.[91-94] A generally

accepted view is that proinflammatory macrophages, derived from MoMFs, are required for activating regenerative mechanisms but may increase tissue injury through uncontrolled inflammation while restorative macrophages promote inflammation resolution and tissue repair but are also implicated in aberrant tissue repair mechanisms, namely fibrosis and cancer.[95,96]

In opposition to the old dogma that macrophage-mediated inflammation is generally detrimental, a recent paper demonstrated that blocking Ly6C$^+$ monocyte recruitment by using a chemokine receptor CCR2 antagonist delayed hepatitis B virus clearance in mice while it was enhanced by KC depletion using clodronate-loaded liposomes.[58] KC depletion led to increased Ly6C$^+$ monocyte recruitment, thus accelerating virus clearance.[58]

Another situation in which liver macrophages were shown to be beneficial is following liver regeneration. Clodronate-mediated KC depletion was reported to delay liver regeneration and to increase liver damage following partial hepatectomy in mice and rats.[97-101] These effects seem to be partly due to the absence of IL-6 production by KCs and are reversed after IL-6 administration.[101] Similarly, clodronate-mediated macrophage depletion reduced the alternative liver regeneration pathway through liver progenitor cell (LPC) activation by reducing LPC accumulation in rats in a 2-acetylaminofluorene/partial hepatectomy model.[102] Low-dose clodronate was used to deplete KCs and not MoMFs; this treatment reduced MoMF infiltration and tissue injury concomitantly with LPC accumulation in an LPC-driven liver regeneration model (choline-deficient ethionine-supplemented diet),[103] although another study reported that KC depletion reduced parenchymal invasion but not LPC proliferation in the same model.[104] Alternatively, intravenous bone marrow-derived macrophage injection has been shown to be sufficient to initiate regenerative mechanisms by inducing a ductular reaction in the healthy mouse liver through TNF-related weak inducer of apoptosis (TWEAK).[105] Macrophages have also been designated as the source of Wnt3a, thus favoring liver progenitor cell differentiation toward the hepatocyte fate during tissue regeneration.[106] Human autologous bone marrow cell infusion also seems to be beneficial for chronic liver disease treatment.[107] These effects may be attributed to immunomodulatory actions of transplanted cells and their ability to phagocytose cellular debris,[108] arguing once more for the high relevance of the immune system in tissue repair. However, data on the clinical efficacy of macrophage cell therapy in patients are still awaited. Collectively, it is well documented that MoMFs and KCs play major roles in hepatocyte- and LPC-mediated liver regeneration. Therefore, the use of macrophage-suppressive drugs should be considered cautiously.

In contrast, KC inhibition using gadolinium chloride hexahydrate prevented liver cancer stem cell occurrence and tumor development in rats exposed to diethylnitrosamine, through inhibition of liver progenitor cell activation and proliferation.[109] In a nonalcoholic steatohepatitis (NASH) model using melanocortin-4 receptor-deficient mice fed a Western diet followed by a low-dose CCl$_4$ injection, it was reported that CD11c$^+$ liver macrophage depletion prevented fibrogenesis.[110] Similarly, the use of a CCR2/CCR5 antagonist that prevents MoMF recruitment to the liver in patients with NASH and murine models shows promising results notably by preventing fibrosis.[111] Another good representation of these apparently conflicting macrophage functions is time-controlled macrophage depletion. As such, selective CD11b-expressing macrophage depletion using a diphtheria toxin receptor system during tissue scarring reduced collagen deposition, whereas macrophage depletion during the reparative phase led to reduced extracellular matrix degradation in the CCl$_4$ model.[89]

Spectra and Fluidity of Macrophage Polarization States

Macrophages present at a given time at an injury site are heterogeneous by nature. Recent unbiased approaches, such as single-cell RNA sequencing, revealed the coexistence of various and mixed activation phenotypes, even in healthy human liver.[39] This heterogeneity may also reflect the versatility of macrophages (Fig. 1). For instance, exposure of human MoMFs *in vitro* with typical extracellular stimuli, like cytokines, fatty acids, or danger signals, does not induce a well-defined M1 or M2 phenotype but a broad spectrum of activation states.[30] Similarly, feeding mice a Western diet (rich in carbohydrates, cholesterol, and fat) induced the transcriptomic and epigenomic

reprogramming of myeloid progenitor cells, resulting in increased proliferation and enhanced innate immune responses.[112] The ability of macrophages to be re-educated, reprogrammed, or repolarized is a fascinating new area, especially for diseases in which the liver is highly populated with macrophages. Indeed, it seems to be possible to re-educate macrophages from a classical so-called M1 to M2 phenotype and vice versa.[113] New technologies (sequencing techniques, imaging methods, unbiased big data analyses) allow for an exciting and fresh view on macrophage heterogeneity, for instance, regarding origin, polarization, localization in tissue, and function in relation to the disease state.[114] However, it is important to accurately describe the experimental setting from which conclusions are drawn to allow comparability between models and researchers.[115] Understanding the activating signals and intracellular signaling pathways that determine functional responses of macrophages or macrophage subsets in the liver holds great potential for advancing therapeutic options in liver diseases.

Extracellular vesicles are emerging as a significant intercellular communication tool, leading, for instance, to the activation of liver macrophages (both KCs and MoMFs) toward a proinflammatory and pathologic phenotype in alcoholic liver disease, notably characterized by TNFα and IL-12/23 expression.[116] This macrophage activation was partly attributed to increased heat shock protein 90 (HSP90) levels in hepatocyte-derived extracellular vesicles. Extracellular vesicles may contain proteins as well as DNA and microRNAs (miRNAs) influencing gene expression in the cells that uptake vesicles. On the other hand, macrophages were shown to differentiate toward a matrix-degrading phenotype when phagocytosing cell debris in culture or liposomes *in vivo*, thus accelerating fibrosis resolution.[90] It is hence of crucial importance for extracellular vesicle-related studies to be well designed in order to prevent data falsely attributed to exosome content.[117]

Bile acid accumulation in the liver during cholestasis, a widespread consequence of bile duct damage occurring during chronic liver disease or cholangiopathies, has also been shown to serve as an activating signal for liver macrophages. Interestingly, manipulating bile acid composition seems to represent a promising area of research because in the multidrug resistance protein 2 (Mdr2)-deficient model of cholangitis, pharmacologic inhibition of the

ileal apical sodium-dependent bile acid transporter (ASBT) reduced liver fibrosis progression, cholestasis, as well as alanine aminotransferase and bilirubin levels, presumably by reduced toxic hydrophobic bile acid concentration.[118] Along the same line, ursodeoxycholic acid (UDCA) treatment is currently the U.S. Food and Drug Administration-approved first-line therapy to limit primary biliary cholangitis progression.[119] UDCA is a hydrophilic bile acid that is thought to limit bile toxicity to liver cells. UDCA and 24-nor-UDCA have notably been shown to reduce liver inflammation in a *Schistosomiasis mansoni* cercariae infection model.[120] UDCA was also shown to reduce TNFα-induced IL-8 production by macrophages associated with a reduction in TNFα receptor–associated factor 2 (TRAF2) phosphorylation[121] and to reduce LPS-induced macrophage activation.[122-124]

Although all cells in one organism possess the same genetic material, gene expression modulation through epigenetic modifications controlled by histone remodeling, transcription factors, miRNAs, and long noncoding RNAs results in a plethora of distinct cell types. Recent studies have highlighted that macrophages are not exempt from these epigenetic modifications because they directly affect the polarization state of macrophages.[125-129] Aside from macrophage-depleting agents used in mice, such as clodronate-loaded liposomes or KC depletion by gadolinium chloride, several approaches have been proposed to influence the macrophage phenotype. Macrophage-targeting delivery methods for immunomodulatory drugs or silencing RNAs (siRNAs) include the use of nanoparticles, liposomes, glucan shell microparticles, and oligopeptide complexes.[22,130,131] For instance, glucan-encapsulated siRNAs against mitogen-activated protein kinase kinase kinase kinase 4 (Map4k4) prevented mortality in LPS-induced sepsis by inhibiting TNFα and IL-1β production specifically in macrophages.[132] Thus, the selective targeting of macrophages and the specific interference with a detrimental activation pathway may shift the immune response toward being beneficial. As such, it is tempting to believe that *in situ* macrophage reprogramming will become a major area of intervention in the future. These adaptable and flexible alterations of gene expression offer tremendous possibilities in macrophage polarization modulation. It is thus conceivable to alter macrophage activation from a pathogenic to a regenerative phenotype and to direct immune cells to a response "à la carte," by

orientating epigenetic modifications specifically in targeted macrophage populations (Fig. 1).

Translational Research: From Mouse Models to Human Diseases

A vast majority of the data referred to in this review is derived from animal models. While animal models are necessary to dissect molecular mechanisms leading to pathology, it is important to evaluate the human relevance of recent findings.

Substantial differences were identified in terms of KC- and MoMF-specific identifiers between humans and mice (reviewed in Heymann and Tacke[41]) as well as polarization markers.[115] At present, comparative studies on human versus mouse liver macrophage subsets are limited, although many key aspects of resident KC versus recruited MoMF, activation and recruitment signals, and metabolic activities appear to be conserved.[39,60,133,134] The liver microenvironment is also different between mouse models and human disease, and several studies have attempted to tackle species differences by using humanized livers. However, such studies currently require the mice to be immune deficient (e.g., severe combined immunodeficient [SCID] mice), thus limiting the ability to study macrophages in the context of a normal immune environment.

Much comparative work from mouse models to human diseases remains to be done. On the other hand, initial findings from macrophage-directed therapies in early phase clinical studies, such as the CCR2/CCR5 inhibitor cenicriviroc in patients with NASH and fibrosis, indicate that principal observations can be successfully translated from bench to bedside.[135]

Prognostic Value of Macrophage-Related Markers in Human Liver Disease

One particularly exciting area of translational research uses liver macrophage heterogeneity as the starting point for exploring novel biomarkers reflecting characteristics of human disease. For instance, staining liver samples with macrophage-related markers, assessing hepatic or circulating monocyte/macrophage populations (by flow cytometry), and measuring macrophage-related surface proteins in circulating blood have been suggested as novel biomarkers in hepatology.[17,22]

One prototypical situation in which macrophage marker expression has been studied is liver cancer. It is generally believed that tumor-associated macrophages mainly promote cancer progression by maintaining an immunotolerant tumor microenvironment. As such, CCL2 expression has a prognostic value in patients with hepatocellular carcinoma, and CCR2 blockade has been shown to inhibit cancer growth by favoring an antitumoral immune response[136] and inhibiting angiogenesis.[86] Recently, CD163, a macrophage-specific marker with anti-inflammatory functions, has been used to evidence the presence of tumor-associated macrophages in cholangiocarcinoma. The authors concluded that CD163+ macrophage number correlated with cholangiocarcinoma stage.[137] The presence of soluble CD163, released by activated macrophages in the blood, has also been correlated to hepatocellular carcinoma progression.[138] However, the correlation between CD163 and hepatocellular carcinoma prognosis remains debatable.[139]

Soluble CD163 has, however, been widely described as a macrophage activation marker in patients with different liver diseases. Despite its anti-inflammatory function as a scavenger receptor on macrophages, circulating CD163 seems to be associated with advanced disease states, disturbed intestinal barrier, and adverse prognosis. This has been convincingly demonstrated for NASH,[140] viral hepatitis,[141] autoimmune hepatitis,[142] and decompensated cirrhosis.[143] Interestingly, this marker dynamically responds to efficient treatment, such as for autoimmune disorders[142] or viral hepatitis,[144,145] suggesting that it could be helpful monitoring treatment responses.

Similarly, CD206+ macrophages are classically defined as harboring an anti-inflammatory phenotype, and a retrospective study identified high CD206+ macrophage infiltration in the liver to be a poor prognostic indicator in patients with hepatocellular carcinoma.[146] CD206+ macrophages have, however, also been described as proinflammatory in

patients with viral hepatitis.[147] Moreover, monocytes are regarded as major contributors to the initiation and perpetuation of tissue damage, providing the soil for chronic liver disease, including fibrosis and cancer.[148,149] In line with these, it has been reported that soluble CD163 can be used in combination with the Model for End-Stage Liver Disease score and other clinical scores to obtain a better prognostic for acute-on-chronic liver failure and cirrhosis.[143,150] Similarly, the expansion of myeloid cells (mainly monocytes) with an immune-suppressive phenotype in peripheral blood has been linked to impaired antimicrobial responses in patients with acute-on-chronic liver failure.[151]

While these markers indicate adverse prognosis, there might be markers capturing the beneficial repair functions of macrophages as well. In this regard, MER receptor tyrosine kinase (MERTK) is expressed by monocytes and macrophages that have features of restorative phagocytes.[152] Although these MERTK + macrophages seem to be associated with disease severity in acute-on-chronic liver failure,[153] they apparently indicate proper resolution from acute liver failure in patients.[154]

Conclusions

Expanding knowledge on liver macrophages has certainly modified (or replaced) old dogmas in the field. For instance, it has become clear over the past years that liver macrophages cannot be sufficiently described as M1 or M2 cells. As recently suggested, all studies should precisely describe macrophage populations based on their origins, the activation signals, and a relevant choice of markers.[115] The ongoing thorough macrophage characterization in a variety of liver diseases as well as the rapidly increasing knowledge of the biomolecular mechanisms implicated in gene expression regulation or cell communication associated with technical advances in targeted drug delivery may one day allow clinicians to provide their patients with personalized treatments instructing liver macrophages on how to properly orchestrate the liver response to a defined type of injury.

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