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REVIEWS

Role of Hepatic Macrophages in Acute and Chronic Injury and Repair

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Under optimal physiologic conditions, liver resident macrophages, such as Kupffer cells, are abundant in maintaining homeostasis. They orchestrate postinjury inflammatory tissue remodeling, surveil malignant microbial organisms, and remove toxins. Nevertheless, during chronic inflammation and wound healing in the liver, hepatic infiltration of immune cells from the bone marrow, peritoneum, and lymph nodes can promote the maintenance of macrophages subsets that exacerbate liver injury. Depending on the span of the inflammation and the extent of the damage, the liver can undergo acute liver injury or chronic liver injury. In this review, we explain the role of macrophages in the innate immune system, specifically discussing the role of the immune innate system and the description of Kupffer cells. The review also discusses macrophage activity in acute and chronic liver diseases, such as acetaminophen-induced injury, nonalcoholic steatohepatitis/nonalcoholic fatty liver disease, and alcoholic liver disease. Finally, the review also discuss Kupffer cell mechanisms for liver repair and regeneration.

Introduction

The liver is a critical visceral organ that performs regulatory functions such as (1) maintaining chemical homeostasis in the blood; (2) producing and secreting bile; (3) metabolizing fats, proteins, and carbohydrates; (4) storing glycogen and various vitamins; and (5) synthesizing plasma proteins. The liver is an essential organ that serves, in one of its many functions, as a filter for toxins and microorganisms that enter digestive tracts. For this reason, the liver can be prone to many diseases. The underlying pathology of diseases such as is often due to complex cellular interactions of the immune system. Kupffer cells are one of the primary resident liver macrophages that provide the basis for much of these interactions. Monocyte-derived macrophages (MoMF) are the other primary group of macrophages in the liver.[1] This review discusses the interactions of Kupffer cells with other immune system cells, the characterization of macrophage populations, and the roles of macrophages in acute and chronic liver injuries such as acetaminophen (APAP)-induced injuries, alcoholic liver disease, nonalcoholic steatohepatitis

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(NASH)/nonalcoholic fatty liver disease (NAFLD), and other hepatic injuries. The work being reviewed is important to the scientific community because the role of Kupffer cells in injury and regeneration is not fully understood.

Results

Macrophage Description

MoMF and yolk sac–derived macrophages could have implications for liver regeneration parenchyma. In the study by Scott et al,[2] CLEC4F was identified as a Kupffer cell–specific gene in liver tissue of mice that were MoMF. This is described as a specific phenotype marker.[2] Perdiguero et al[3] found that CSFLR-expressing cells in mouse embryos give rise to resident macrophages in tissues in vitro. Through fate-mapping experiments, the team was able to determine a sequence of yolk sac erythromyeloid progenitors as a common origin for tissue macrophages. In both instances, the experiments conducted to show the origination of macrophages implied that macrophages can be raised from both MoMF mechanisms and yolk sac derivation mechanisms.[3]

It is important to understand hepatic macrophage descriptions because they might indicate critical hepatic mechanisms for diseased livers as well as regeneration. Kupffer cells are classified into specific cell lines such as $CD11b^{low}$, $F4/80^{hi}$, C-type lectin domain family 4 member F positive (clec4f+), $CD68^{+}$, and CX3C chemokine receptor 1 (CX_3CR1) $^{-}$. CD68+ causes more phagocytic and cytotoxic activity through the production of reactive oxygen species.[4]

According to Okamoto et al,[4] 2 populations of Kupffer cells have been described: liver resident macrophages (F4/80+CD11b–CD68+) and circulating monocytes (F4/80+CD11b+CD68– cells). The study showed that F4/80+CD11b+CD68– cells played the role of effector cells against sepsis by *Enterococcus faecalis* in normal mice when compared with irradiated gut microbiota mice. This illustrated a distinct biomarker of macrophage and its function as F4/80+CD11b+CD68– cells, which play an important role in the activation of the antibacterial response against sepsis. These cells are IL-12+IL-10–CD206–CCL1–, which is characteristic of M1 macrophage. Also, hepatic macrophages that were IL-12–IL-10+CD206+CCL1– are considered to be characteristic of M2 macrophages.[4]

Innate Immunity

Kupffer cells are the most abundant type of macrophages (80% to 90%) that reside in the liver and are responsible for activating the innate immune system and hepatic remodeling.[1] These macrophages specifically reside along the hepatic sinusoids and can interface with bacteria and other pathogens stemming from the portal circulation.[1] Kupffer cells, like many other macrophages, stem from blood monocytes.[1] These monocytes then enter the liver and, contingent on the microenvironment with specific growth factors such as macrophage-colony stimulating factors, differentiate into Kupffer cells.

At homeostasis, macrophages can detect pathogens using their recognition receptors (ie, toll-like receptors [TLRs] to bind various components (eg, RNA, DNA, lipopolysaccharide).[5] Turnover of Kupffer cells is not entirely characterized but may be between 2 and 3 weeks.[1]

Kupffer cells interact with other innate immune cells, such as natural killer cells, natural killer T cells, and dendritic cells within the sinusoids. Hepatocytes and Kupffer cells produce pattern recognition receptors, which help facilitate the innate immune system.[1] The pattern recognition receptors sense damage-associated molecular patterns (DAMPs) and alert the immune system by stimulating immune cell migration, therefore increasing phagocytosis by Kupffer cells. As part of the innate immune system's inflammatory response to pathogens, the liver produces active phase reactants such as hepcidin and C-reactive protein.[6] These inflammatory mediators are key in ushering the full innate immune response and its several components. Neutrophils require these mediators and upregulation of endothelial adhesion molecules to combat pathogens through a combination of phagocytosis, extracellular traps, proteases, and respiratory burst.

Kupffer cells are further characterized into 2 different groups: the M1-like (classic) and the M2-like (alternative) macrophages. Traditionally, the activation of M1 macrophages is done via lipopolysaccharide (LPS) or interferon-γ. M1 macrophages are responsible for the production of nitric oxide and reactive oxygen radicals to protect the liver from bacteria and viruses.[7] The activation of M2 macrophages is done via specific cytokines, such as IL-4, IL-10, or IL-13. These macrophages produce polyamines to stimulate cellular proliferation and produce proline to induce collagen production. This allows M2 macrophages to specialize in wound healing and tissue remodeling.[8]

The liver connects to its lymph nodes and represents a bridge to adaptive immunity. Within the liver parenchyma, there are fenestrated sinusoids that provide access to T cells and antigen-presenting cells.[9] About 25% to 50% of lymph stems from the liver and enters the thoracic duct. The lymphatic structures are located in the portal triad and carry secretions from hepatocytes and Kupffer cells, which flow into the interstitium and ultimately become part of the lymph.[10] The portal and celiac lymph nodes are where close to 80% of hepatic lymph drains. The remaining 20% is split into sublobular (along IVC) and superficial (along liver peripheral that drains to other closer lymph nodes).[10]

Overactivity of Kupffer cells has been linked to NAFLD/NASH.[11] Underactivity of Kupffer cells has been linked to infections such as hepatitis A, B, and C.[11] Overacticvity and underactivity of Kupffer cells can contribute to dysregulation of the liver and contribute to NAFLD/NASH or hepatic viruses.

Therefore, Kupffer cells are in a unique position to drive the overall ability of the liver to filter out pathogens but also be immunotolerant enough to avoid metabolic diseases such as NAFLD/NASH.

Acute Injury

ACETAMINOPHEN-INDUCED INJURY

APAP-induced liver injury is the most common cause of acute liver failure in the United States.[12] At elevated doses, cytochrome P450 enzymes react with APAP to create N-acetyl-p-benzoquinone imine.[12] This compound depletes glutathione stores and can thereafter freely react with key target proteins such as disulfide isomerase or glutaredoxin 2 that lead to liver injury.[12] Glutathione primarily plays a role in protecting cells by being an antioxidant in the body and protecting from toxicity as well as maintaining redox homeostasis. The formed protein adducts induce oxidative stress and increased mitochondrial permeability that leads to decreased ATP production and hepatocyte necrosis.[13] The dysfunctional mitochondria cause the secretion of cytokines that ultimately cause Kupffer cell activation.[14] The key activating cytokines are IL-1, tumor necrosis factor α (TNF-α), and CC-chemokine ligand 2.[13] Interestingly, without activated Kupffer cells, studies have shown that there would not be as significant of APAP-induced hepatotoxicity. In a mouse study using gadolinium chloride (macrophage inhibitor), there was significantly less production of reactive oxygen species and peroxynitrite after APAP overdose compared with mice without the use of gadolinium chloride.[13] However, as previously mentioned, Kupffer cells have both destructive and restorative functions within the liver and are largely contingent on the polarities of M1 vs M2.[12] When working optimally, Kupffer cells can promote repair in response to injury and are continually replenished. In a mouse study, 24 hours after APAP overdose, Kupffer cell levels were substantially reduced, but within 72 hours levels were back to baseline. To reinforce the point about macrophage polarization significance, IL-10 knockout mice showed increased liver toxicity and mortality post-APAP overdose.[13]

Biomarkers for APAP overdose are not widely used. In some research, CCL5 has emerged as a potential biomarker. In a limited 15-person post-APAP overdose study, patients had higher serum CCL5 levels compared with healthy controls.[14] This correlation is supported by more in-depth mouse models that showed higher CCL5 transcription levels in both hepatocytes and nonparenchymal cells post-APAP overdose. CCL5 activates both MAPK and NF-κB pathways that promote inflammation. Knockout CCL5 mice showed faster liver recovery compared with wild-type mice 36 to 48 hours post-APAP overdose based on aspartate aminotransferase (AST)/alanine aminotransferase (ALT) levels and direct hepatocyte necrosis.[14] Enzyme-linked immunosorbent assays showed lower levels of serum TNF-α and IL-6 in the CCL5 knockout mice.[14] Glutathione and cytochrome P450 enzyme levels, as well as mitochondrial membrane potential, were comparable in both of these mice

groups, suggesting that CCL5 does not have a direct role in APAP metabolism or mitochondrial dysfunction.[14] Therefore, CCL5's role in promoting more hepatocyte destruction post-APAP overdose may be in its direct promotion of increased inflammatory activation via the MAPK and NF-κB pathways. Biomarkers can be related to hepatic macrophages by screening, evaluating, and predicting the prognosis of liver disease conditions. Specific biomarkers for the liver are difficult to reveal due to common mechanisms and pathways for a variety of liver diseases. However, it is important to identify these biomarkers for treatment purposes of acute and chronic liver diseases.

Chronic Injury

NONALCOHOLIC STEATOHEPATITIS

NASH is a liver metabolic disorder and is the most severe form of NAFLD. The role of Kupffer cells during NASH remains relatively unknown; however, Kupffer cells play a critical role in self-renewal via proliferation as well as crosstalk with surrounding cells. According to Xiong et al,[15] macrophage clusters that were isolated from single-cell RNA transcriptome in NASH-induced mouse models showed Kupffer cells with high expression of F4/80 and CLEC4F, and MoMF showed high expression of CD11b and CCR2. It is important to know expression levels of biomarkers for Kupffer cells in NASH to understand their mechanism in liver destruction and renewal. Kupffer cells from NASH livers showed high levels of expression of TREM2, GPNMB, and CD9 compared with livers without NASH.[15] In mice with choline-deficient, L-amino acid, and high fatty diet, there were substantially higher TREM2, GPNMB, and CD9 expression.[15] Plasma GPNMB levels were higher in both amylin liver NASH model and choline-deficient, L-amino acid defined, high fat diets (CDAFHD), which could serve as a biomarker in NASH.[15] In mice with CDAFHD, elafibranor (PPARα and PPARδ agonist) reversed NASH pathology and correlated with lower AST/ALT levels and lower NASH-associated macrophage number (corroborated with GPNMB expression). Following the dietary switch to chow diet, which is healthier and a less fatty diet, for 8 weeks, there was a reduction in TREM2, GPNMB, and CD9 expression.[15]

While not conclusive, IL-11 has been one of the cytokines implicated in liver fibrosis secondary to fat intake. Xiong et al[15] demonstrated that high-fat diets in mice increase IL-11 expression, which then induces hepatic stellate cells to secrete various additional cytokines, such as WNT4, CCL11, and XCXL1, that in turn influence macrophages and other immune cells. If further research supports these observations, the pathway leading to macrophage overactivation and subsequent hepatic destruction may be clearer with many potential therapeutic targets in the future.[15]

In mice models, there was increased hepatic infiltration of Ly-6C^{hi} monocytes in the early stages of NASH. The mice that were induced to have NASH via Western diet had high MoMF. DAMPs, extracellular vesicles, and harmful

lipids stem from hepatocytes and lead to Kupffer cell and MoMF activation. These DAMPs include proteins in the cytosol, purine nucleotides, and mitochondrial compounds. They work via transforming growth factor-β receptor, IL-1β, platelet-derived growth factor receptor, and CC-chemokine ligand 2. The lipid overload causes the release of inflammatory cytokines such as TNF-α and IL-6 due to the release of mitochondrial DNA. This DNA binds to TLR9 on Kupffer cells to cause a proinflammatory response. Cholesterol crystals from remnants of hepatocytes, when processed by Kupffer cells, activate the NLRP3 inflammasome in Kupffer cells.[16] In mice models, targeting the NLRP3 inflammasome complex improved NASH fibrosis outcomes. Caspase activation was seen via TNF-α activity and that has been linked to mitochondrial dysfunction.[16] IL-1β signaling in Kupffer cells has been linked to more lipogenesis within these macrophages plus increased lipid deposition. The phenotype of liver sinusoidal endothelial cells changed from anti-inflammatory to inflammatory as NASH severity worsened. This is orchestrated via cytokine CCL2.

Song et al[17] investigated the yes-associated protein (YAP) in Kupffer cells, which promote the production of proinflammatory cytokines in NASH. The study investigated the increased expression of Kupffer cells of wild-type mice that were fed a high-fat diet (HFD). Macrophage/monocyte-specific deletion of YAP ($YAP^{\phi KO}$) or TLR4 mice were produced and fed an HFD or treated with LPS.[17] The results showed that $YAP^{\phi KO}$ mice that were fed HFD showed lower serum ALT and aspartate aminotransferase levels, which indicates a lower hepatic inflammation when compared with controls. Furthermore, LPS treatment increased the concentration of YAP in Kupffer cells in vitro as well as in mice, which was prevented by $TLR4^{\phi KO}$.[17] LPS activates YAP via activator protein 1 in Kupffer cells, which increases the expression of inflammatory cytokines via YAP association.[17] Song et al[17] determined that the treatment of HFD-fed mice with verteporfin decreased Kupffer cell activation, reduced liver inflammation, and decreased ALT and AST levels. Finally, human liver tissues from patients with NASH determined that YAP is increased in Kupffer cells and is significantly correlated with proinflammatory cytokines.[17] Ultimately, there are multiple cytokines and inflammatory markers that showed the mechanism of NASH development.

ALCOHOLIC LIVER DISEASE

Acute alcoholic liver disease (ALD) is a term that refers to acute liver injury from excessive alcohol consumption. Macrophages have a critical role in the development of alcohol-induced inflammation of the liver. Furthermore, various studies have identified macrophage polarization, phenotypic morphology, differences between infiltrating and resident macrophages/monocytes, and macrophage actions. Fisher et al[18] found that concentrations of chemokines in peripheral circulation reflect disease activity. Serum MCP-1 concentrations were increased in patients with ALD when compared with healthy controls. Furthermore, macrophage inflammatory protein (MIP-1α)

is secreted by macrophages and recruits inflammatory responses, which was found to be higher in patients with ALD along with MCP-1 levels when compared with healthy controls.[18] Also, the study showed that proinflammatory cytokines, such as TNF-α and IL-1, were increased in the circulation of patients with ALD when compared with healthy controls. A higher concentration of TNF-α levels in the blood circulation correlates with negative outcomes with acute alcoholic hepatitis patients.[18]

Mandal et al[19] concluded that IL-10 is involved in preventing overproduction of TNF-α. IL-10 is known to be an immunomodulatory cytokine with adequate anti-inflammatory properties. The research showed that acute exposure to alcohol or ethanol decreases the concentration of IL-10 in humans as well as mice.[19] Additionally, patients with ALD produce increased levels of IL-6, IL-8, and IL-18, as well as other cytokines for which monocytes and liver resident macrophages are the source cell. It is also known that IL-17 induces liver fibrosis in metabolic pathways in mice.[20] Ultimately, the consumption of alcohol can lead to disregulation of cytokines such as IL-1, IL-6, IL-8, and IL-18, which can lead to acute ALD.

Scar-Associated Macrophages

Selective macrophages exist during different time points of the injury and recovery phases of inflammatory scarring. Scar-associated macrophages play an imperative role in normal injury and recovery. Ablating macrophages during the injury phase significantly ameliorates fibrosis. In contrast, depleting during the recovery results in significant fibrosis and persistence of extracellular matrix contents.[21] During the injury phase, injury-associated macrophages promote apoptosis and fibroblast proliferation. The phenotype most closely associated with this phenomenon is the alternatively activated macrophages type of macrophage.[21] Alternatively activated macrophages are activated by T_H2 lymphokines, IL-4, apoptotic cells, and corticosteroids, and they produce anti-inflammatory cytokines such as IL-10 and TNF-β.[21] These macrophages are distinct from the scar-associated macrophages during recovery because they do not have a high expression of transforming growth factor-β or TNF-α like their counterpart macrophages responding during injury. Duffield et al[21] (2004) showed that distinct populations of macrophages are present during the recovery and injury phases of liver damage.

Ramachandran and colleagues[22] investigated transcriptomes that were profiled from approximately 100000 single human cells and observed for nonparenchymal cell types that were predominantly found in healthy as well as cirrhotic human livers. The study investigated and observed a scar-associated $TREM2^+CD9^+$ subpopulation of macrophages. This subpopulation of macrophages contributes to liver fibrosis that is derived from circulating monocytes that also promote fibrogenesis.[22] The study also investigated $ACKR1^+$ and $PLVAP^+$ endothelial cells that contribute to liver cirrhosis as well due to their roles in leukocyte migration.[22] Furthermore, PDGFRα+

collagen-producing myofibroblasts were also identified due to their pathogenic properties as a subpopulation macrophage. Being able to identify liver organ fibrosis in human samples via spatial map using scRNA-seq made it theoretically accessible to identify relevant fibrotic pathways that can potentially be druggable.[22]

Identifying single-cell subpopulations and key ligand-receptor interactions between scar-related non-parenchymal cells underlines how pathway signaling is important for human liver fibrosis. Ultimately, performing cell biomarkers can lead to macrophage therapy to resolve fibrosis in the liver by identifying macrophages that contribute to liver cirrhosis.

Regeneration

Liver regeneration is determined by a number of factors and cell types, including growth factors, inflammatory mediators, mature hepatocytes, and various liver-resident immune cell populations. This section will discuss the important role Kupffer cells play in the regenerative process. IL-6 is an important cytokine secreted by Kupffer cells that is present during the regeneration process. This was studied in patients with chronic alcoholism with a targeted disruption of the inflammatory mediators.[23] This caused reduced hepatocyte proliferation, which can be restored with a preoperative dose of IL-6.[23] Furthermore, diseased liver regeneration was observed in mice that were treated with antibodies that targeted TNF-α. The regeneration of the liver that uses TNF-α blocks the possibility of a proapoptotic effect of TNF-α signaling. It also outlines the complexity of the inflammatory signal that is required to regulate the regeneration of the diseased liver.[23] Kupffer cells also play an important role in liver regeneration by releasing IL-6 and TNF-α because these cytokines promote hepatocyte proliferation.[24] Furthermore, Kupffer cells are activated through neutrophil recruitment to the liver because of the inflammatory signals. This is dependent on ICAM-1 as well as proteins C3 and C5 that contribute to developing Kupffer cells and promoting hepatocyte proliferation.[25] Finally, Dong et al[26] found that depleted natural killer cells and Kupffer cells in transgenic mice with hepatitis B virus enhanced liver regeneration due to decreased interferon-γ production, which inhibits hepatocyte proliferation.[26] Ultimately, this shows how the immune system of the liver mitigates liver homeostasis and regeneration when there is wound healing.

Conclusion

Based on this research, we identified specific functions of Kupffer cells in acute and chronic liver diseases and the mechanisms that contributed to them. We also identified knowledge gaps that are important to further build on, such as biomarkers of liver diseases and identification of cytokine with specific liver diseases. We recommend further research into identifying common biomarkers for liver diseases and targeting them for drug validation and development. Due to the limited human studies for liver diseases, it is important to model

mouse experimentations that can be translated to clinical trials. Furthermore, this review helped decipher between human and mouse studies regarding liver diseases and differentiating modalities for various acute and chronic liver diseases.

This review highlighted the role of Kupffer cells in the hepatic immune system and how they are incorporated in the management of injuries caused by acute and chronic diseases, as well as repair. Kupffer cells can be both beneficial and harmful depending on their level of activity and their immunomodulatory mechanism in certain conditions. Kupffer cells can be beneficial by providing host defense and removing apoptotic cells from circulation. Nonetheless, the overproduction and utilization of Kupffer cells can be harmful due to the dysregulation of cytokines and reactive oxygen species that can worsen acute and chronic liver injuries. The literature reviewed provides invaluable information on the role of Kupffer cells as well other macrophage subsets in injury and repair.

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