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Review Article

Hepatic macrophages in drug-induced liver injury☆

Zhao Shan [a], Cynthia Ju [b, *]

[a] Center for Life Sciences, School of Life Sciences, Yunnan University, Kunming, China
[b] Department of Anesthesiology, UTHealth McGovern Medical School, Houston, TX, USA

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ABSTRACT

Drug-induced liver injury (DILI) is a major public health concern. Intrinsic DILI, for example, acetaminophen overdose accounts for half of acute liver failure in the United States. However, the most problematic type of DILI affecting drug development and health care is idiosyncratic DILI, which occurs unpredictably in a small population of patients taking the drug and the latency could be several weeks to months. Recent knowledge on the pathogenesis of DILI suggest that hepatic macrophages play a central role in the initiation, progression and restoration stages of DILI, which make hepatic macrophages attractive as therapeutic targets. Hepatic macrophages consist of liver resident macrophages (also known as Kupffer cells, KCs) and infiltrating monocyte-derived macrophages (MoMF). There is a growing appreciation that hepatic macrophage is very plastic, and assumes diverse phenotypes and functions in response to micro-environmental cues. In this review, we will summarize studies on the role of hepatic macrophages in both intrinsic DILI and idiosyncratic DILI, followed by discussing the prognostic and therapeutic potentials of targeting hepatic macrophages and the obstacles in studying hepatic macrophages.

1. Introduction

Drug-induced liver injury (DILI) is rare, but can be quite severe and potentially fatal. It can be caused by prescription medications, over-the-counter drugs, or herbal and dietary supplements (HDS).[1,2] DILI can be divided into two types: one is intrinsic DILI, which is predictable and caused by over-dose (*e.g.*, acetaminophen (APAP)); the other is idiosyncratic DILI, which is unpredictable and occurs at therapeutic doses.[3] The prevalence of DILI in general population is largely unknown, since most cases are asymptomatic and accurate diagnosis of DILI is challenging.[4,5] Although rare, DILI has become the leading cause of acute liver failure (ALF), overtaking viral hepatitis.[6] ALF is defined as a devastating disease condition that causes sudden, life-threatening liver dysfunction within 26 weeks of the onset of illness.[7] Of the 2000 cases of ALF that occur in the United States annually, medications account for >50%, with 37% due to APAP and 13% due to idiosyncratic DILI.[8,9] With increasing numbers of approved pharmaceuticals and HDS introduced into the market, DILI has become one of the major concerns in the health care community. Moreover, DILI is also the most common reason of post-marketing withdrawals.[10] From 1997 to 2016, eight drugs were withdrawn from the market due to hepatotoxicity reason, including tolcapone, troglitazone, trovafloxacin, bromfenac, nefazodone, ximelagatran, lumiracoxib and sitaxentan.[11]

Over 900 drugs, herbs and HDS have been linked to hepatotoxicity so far, but pathogenic mechanisms at molecular level remain largely unknown.[2,12] Identification of the mechanisms underlying DILI and biomarkers for the severity of liver injury, is crucial to develop both diagnostic and therapeutic strategies for DILI. Hepatic macrophages include liver resident macrophages (also known as Kupffer cells, KCs) and infiltrating monocyte-derived macrophages (MoMF). KCs are self-renewing, and they play a sentinel role in liver homeostasis. DILI induces KCs activation, which results in the release of inflammatory cytokines and chemokines. This mode of action initiates the infiltration of monocytes into the liver, which differentiated into macrophages.[13] During the pathogenesis of DILI, hepatic macrophages play a central role. This makes them an ideal target during the development of new therapies. However, hepatic macrophages are extraordinarily versatile, can assume a diversified "polarization states" under different environmental cues.[14] In this review article, we will present the

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* Corresponding author. Department of Anesthesiology, UTHealth McGovern Medical School, Houston, TX, USA.
E-mail address: changqing.ju@uth.tmc.edu (C. Ju).

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updated knowledge on the role of hepatic macrophages in DILI, including intrinsic DILI and idiosyncratic DILI. We will discuss the heterogeneity and functional diversity of these cells, their crosstalk with other cells and the therapeutic potential of targeting macrophages.

2. Hepatic macrophages in intrinsic DILI

Intrinsic DILI is caused by over-dose and predictably occurs in most individuals exposed to certain drugs and onset is rapid within a couple of hours to days.[15] Overdose APAP-induced liver injury (AILI) is a prototypical example of intrinsic DILI. APAP overdose is the most common cause of severe intrinsic DILI in the United States, accounting for about half of all ALF cases. The recommended daily dose of APAP is 4 g per day, which can arise the serum alanine aminotransferase (ALT) up to 3 times the upper limit of normal in 44% of healthy people. However, long-term administration of low-dose APAP doesn't induce liver damage clinically.[16]

APAP is metabolized by cytochrome p450 (CYP)-2E1 in hepatocytes to form a reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI). NAPQI is detoxified by intracellular glutathione. However, excessive accumulation of NAPQI during APAP overdose will cause covalent modification of intracellular proteins, especially mitochondrial proteins, and lead to hepatocyte necrosis. Emerging evidence suggests that the host immune response might also be involved in the pathogenesis of AILI.[16,17] Mouse models of AILI are probably the most studied example, clearly showing typical steps in response to acute liver damage (Fig. 1).[18–25] First, KCs sense "danger signals" and become activated, resulting in the release of cytokines and chemokines.[26,27] Second, released chemokines recruit a number of leukocytes including monocytes from circulation, which differentiate into macrophages.[21,23,28] Third, the death of KCs orchestrate tissue repair through promoting phenotype switch of infiltrating macrophages from pro-inflammatory to anti-inflammatory states.[29] These steps have also been observed in other types of injury, including sterile heat injury, carbon tetrachloride (CCl_4) injection, viral infection and ischemia-reperfusion liver injury.[30–33]

KCs constitute the main macrophage population in the liver at steady state. Upon APAP challenge, hepatocytes are damaged and release various danger-associated molecular patterns (DAMPs), such as high mobility group protein B1 (HMGB-1) and heat-shock protein-70 (HSP-70), which activate KCs.[34] Activated KCs release cytokines and chemokines that recruit neutrophils and a large number of Ly6C^{hi} monocytes.[21,23,35,36] The number of KCs significantly reduced by 24 h post-APAP and the population is replenished by 72 h.[21,23,28] The phenomenon of KC death during liver injury is also reported in hepatic ischemia/reperfusion injury.[37] It was believed that maintenance of the KC population was through self-renewal, tightly controlled by specific repressive macrophage activation factor (Maf) transcription factors and enhancers.[38] However, circulating monocytes have also been shown to contribute to the KC pool during adulthood, suggesting the circulating monocytes have the potential to develop into KCs, as their embryonic counterparts.[39] An interesting "niche competition" model has been proposed regarding the maintenance of tissue-resident macrophages including KCs, in which circulating bone marrow-derived monocytes and fetal liver-derived erythro-myeloid progenitors (EMPs) have an almost identical potential to develop into KCs; however they compete for a restricted number of niches. The majority of the liver niches are taken by EMPs and few by bone marrow-derived monocytes during liver development. The niches in the liver are self-maintained with limited input from circulating monocytes at steady state but circulating monocytes contribute to KC replenishment if significant numbers of KCs die during liver injury or after depletion experimentally.[40]

Starting approximately 12 h after APAP challenge, Ly6C^{hi} monocytes are recruited in a C–C chemokine receptor (CCR) 2- and macrophage colony-stimulating factor (M-CSF)-dependent manner.[21,23,41] They rapidly populate the injured liver and become the dominant subset at 24 h post-APAP.[23,41] The infiltrating Ly6C^{hi} monocytes spatially and temporally overlap with neutrophils in the centrilobular necrotic areas during the necro-inflammatory and

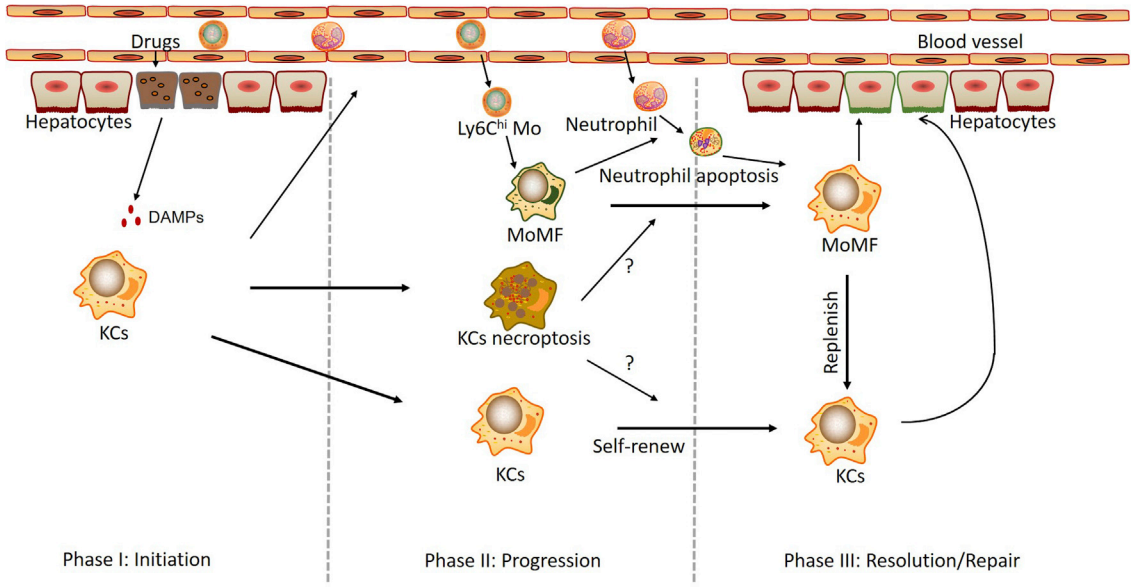

Fig. 1. Schematic graph of DILI. Phase I: Initiation. Upon drug challenge, hepatocytes are damaged and release DAMPs, which activate KCs. Activated KCs release cytokines and chemokines that recruit neutrophils and a large number of Ly6C^{hi} monocytes (Ly6C^{hi} Mo). Phase II: Progression. KCs rapidly undergo necroptosis. Recruited Ly6C^{hi} Mo differentiate into MoMF, which secrete pro-inflammatory cytokines and thus promote liver injury. Phase III: Resolution. MoMF release mediators that induce apoptosis of neutrophils. MoMF switch phenotype from pro-inflammatory to anti-inflammatory state. MoMF and newly self-renewed KCs promote resolution of inflammation by clearance of apoptotic neutrophils, angiogenesis and liver repair together. Abbreviations: DAMPs, danger-associated molecular patterns; KCs, Kupffer cells; MoMF, monocyte-derived macrophages.

resolution phases of AILI. The MoMF release mediators that promote resolution of the inflammation by inducing apoptosis of neutrophils.[21] During the early resolution phase, Ly6C^{hi} monocytes differentiated into MoMF, absence of which results in a significant accumulation of late apoptotic neutrophils.[28] Hepatic macrophages are involved in the disease development through cross-talk interactions with other cells in the liver. For example, neutrophils have been reported to be responsible for converting the pro-inflammatory Ly6C^{hi}CX3CR1^{lo} MoMF to pro-resolving Ly6C^{lo}CX3CR1^{hi} MoMF, likely through the generation of reactive oxygen species (ROS).[42] Hepatocytes release carbamoyl phosphate synthetase-1 (CPS1) during AILI, which promotes monocyte infiltration into the liver and their differentiation into resolving MoMF. Recombinant CPS1 increases the numbers of hepatic macrophages and phagocytic activity.[43]

Similar to other acute and chronic liver injury situations, MoMF have dual functions. They are pro-inflammatory at early stage, promoting inflammation by the expression of inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin (IL)-1β.[21,23,41,44] However, blocking the recruitment of MoMF using anti-CCR2 antibody or genetic deletion of CCR2 significantly delays tissue recovery from AILI, suggesting that MoMF contribute to the liver repair.[21,23,41] It is reported that the MoMF switch their phenotypes from pro-inflammatory to anti-inflammatory cells producing IL-10, IL-4 and IL-13 by 24 h.[45–47] MoMF, derived from circulating monocytes, transition from CCR2^{+}CX3CR1^{-} to CCR2^{-}CX3CR1^{+} cells surrounding necrotic areas in the liver.[30] KCs are also involved in tissue repair, as depletion of both KCs and MoMF results in a marked delay in liver repair.[22] Interestingly, it is reported that the death of KCs plays a critical role in orchestrating liver repair from injury. In a murine model of *Listeria monocytogenes* infection, it is reported that KCs are the first cells in the liver to be infected and that they undergo necroptosis rapidly upon infection.[48] KCs necroptosis triggers the recruitment of circulating monocytes, which differentiate into MoMF that produce high amounts of interferon-γ (IFN-γ) to control bacterial growth. Interestingly, KCs necroptosis also leads to caspase-1-dependent IL-1β secretion, which further induces IL-33 production by hepatocytes. IL-33 stimulates basophils to secret IL-4, which induces local proliferation of MoMF and MoMF to acquire a KCs-like phenotype that contributes to the restoration of tissue integrity after bacterial clearance.[48] Several molecular pathways may be involved in macrophage-mediated liver repair. Hypoxia-inducible factor (HIF) expressed in both KCs and MoMF could induce liver sinusoidal endothelial cell (LSEC) proliferation and migration by regulation of expression of angiogenic factors and thus facilitate liver blood vessel repair and tissue recovery from acute injury.[22] The macrophages also synthesize prostaglandin (PGE2) and phagocytose dead cells to reduce neutrophil-mediated inflammation. Moreover, macrophage colony-stimulating factor (CSF1) and secretory leukocyte protease inhibitor (SLPI) appear to play important roles in promoting the transition of macrophages from a pro-inflammatory towards an anti-inflammatory state through induction of a Mer tyrosine kinase (MerTK)+HLA-DR (high) phenotype, which promotes neutrophil apoptosis and their subsequent clearance.[29,49] In patients with AILI, CSF1 and SLPI have been indicated to cause a significant expansion of resolution-like MerTK + HLA-DR (high) cells in the circulation and liver tissues.[29]

The involvement of hepatic macrophages in AILI has been a topic of debate and requires further investigation.[18,19,50] Several lines of evidence suggest that KCs may contribute to the initial exacerbation of AILI. Treatment of hepatocytes *in vitro* with APAP causes direct toxicity as it does *in vivo* but with much less extent, suggesting that other cells in the liver play a role in the progression of AILI *in vivo*. Platelets usually accumulate rapidly at the initiation of tissue injury. It is reported that depleting platelets markedly attenuates AILI in mice.[51] Emerging evidence also suggests that KCs play an important role in platelet recruitment in the liver during acute infection as well as in chronic liver diseases.[52–54] These findings point toward a role of KCs in the initiation and progression of liver injury. However, KC depletion studies in mice have yielded opposing results.[18,19,50] The timing liposome-entrapped chlodronate treatment, commonly used to deplete KCs, may be the key to reconcile the discrepancies. In the majority of the reported studies, mice were treated with APAP at 48 h after chlodronate administration. At this time point, although KCs are depleted, massive influx of MoMF has also occurred. Thus, the phenotype observed in these studies reflects the function of MoMF, not just a result of KC depletion. To avoid the confounding factor of MoMF, future experiments are warranted to investigate AILI at a time point at which KCs are depleted but yet MoMF are absent. It has been reported that KCs are a source of both pro-inflammatory and anti-inflammatory cytokines.[22,26,45–47] Further insights are provided by more recent studies demonstrating that the MoMF play an important role in liver repair and that KCs actually undergo cell death approximately the same time MoMF are recruited.[21,23,25,48,55] Taken together, the current literature suggests that KCs may contribute to the initiation and progression of liver injury; however, their death triggers an alternative activation of the MoMF, thereby transitioning from injury to repair/restoration of the tissue.

3. Hepatic macrophages in idiosyncratic DILI

The relatively predictable course of intrinsic DILI contrasts dramatically with idiosyncratic DILI, which is characterized by hepatotoxicity that unpredictably occurs in some exposed individuals at therapeutic doses of a drug for an extended latency.[15] Despite that idiosyncratic DILI occurs in a very small proportion of individuals, there are a large number of drugs known to cause idiosyncratic DILI.[56,57] In the United States, antimicrobials (amoxicillin-clavulanate, nitrofurantoin, sul-famethoxazole-trimethoprim, ciprofloxacin, isoniazid) are the most common agents causing idiosyncratic DILI. Besides, anti-cancer drugs, such as tyrosine kinase inhibitors (TKIs) and drugs to treat anti-autoimmune diseases, such as TNF-α antagonists can also cause idiosyncratic drug reactions.[58] However, the molecular mechanism underlying idiosyncratic DILI is largely unknown.

There is a growing evidence suggesting that hepatic macrophages play a role in the development of idiosyncratic DILI. MoMF has been suggested to contribute to amodiaquine-induced liver injury through recruiting natural killer (NK) and cytotoxic T cells.[59] An interesting *in vitro* cell culture study showed that the supernatant from FLC-4 cells (a human hepatocarcinoma cell line) incubated with amodiaquine or nevirapine for 7 days led to increased caspase-1 activity and the production of IL-1β by THP-1 cells (a human macrophage cell line). This result suggests that reactive metabolites of drugs can cause the release of DAMPs from hepatocytes, which in turn can activate macrophages.[60] Trovafloxacin delays the recruitment of neutrophils and monocytes into the liver, thereby hampering the resolution of the initial trovafloxacin-induced hepatotoxicity and resulting in liver injury.[61] The immunotherapeutic catumaxomab is approved for the treatment of peritoneal carcinomatosis. Treatment with catumaxomab causes hepatitis partly through binding to FcγR-positive KCs via its Fc-fragment and inducing the production of acute phase C-reactive protein, chemokines and cytokines, which further exacerbate T-cell mediated cytotoxicity.[62] Penicillamine, a medication primarily used for the treatment of Wilson's disease, has been shown to cause autoimmune syndrome in some patients. Mechanistic studies showed that penicillamine binds to aldehydes on the surface of

hepatic macrophages and activates these cells. Activated hepatic macrophages produce IL-15 and IL-1β to further activate NK cells. Activated NK cells express macrophage activating cytokines, IFN-γ and granulocyte-macrophage colony-stimulating factor (GM-CSF) to maintain macrophages activation.[63]

Both TNF-α antagonists and TKIs are new additions in the list of drugs that cause idiosyncratic DILI. TNF-α antagonists are being used to treat a variety of inflammatory and autoimmune disease and have been associated with idiosyncratic DILI. A recent research identified 34 cases from 2003 to 2011, likely to have DILI associated with use of TNF-α antagonists. The drugs associated with DILI are infliximab ($N = 26$), etanercept ($N = 4$), and adalimumab ($N = 4$).[64] However, the mechanism of hepatotoxicity is still unclear. Many TKIs, developed as anti-tumor therapies, possess risk factors for developing DILI such as their high daily dose and being involved in significant hepatic metabolism. There is a variable incidence of liver adverse events due to TKIs treatment, from 5 to 25%. If TKIs treatment is maintained despite ongoing liver injury, mild liver injury can progress to severe liver injury in a minority of patients. However, the mechanism of TKIs-mediated hepatotoxicity still needs to be better understood in order to avoid hepatotoxicity in patients.[65] Together, the molecular and cellular mechanism behind idiosyncratic DILI is still at infancy, which needs more investigations to know the role of hepatic macrophages in process of DILI.

4. Diagnostic and therapeutic potential of targeting hepatic macrophages in DILI

Due to the significant role of hepatic macrophages in DILI, the diagnostic and therapeutic potential of targeting hepatic macrophages have been explored. It is reported that the levels of macrophage activation markers, soluble CD163 (sCD163) and soluble mannose receptor (sMR) are associated with the severity and prognosis of AILI in patients.[66] The levels of sCD163 and sMR were measured in two independent prospective cohorts of APAP overdosed patients (mild APAP overdose cohort, 49 patients; severe acute APAP overdose cohort, 30 patients) and one cohort of 14 healthy controls during N-acetylcysteine (NAC) treatment. Within the mild cohort, patients with elevated alanine transaminase on admission had significantly higher levels of sCD163 compared with patients with normal alanine transaminase, whereas sMR showed no significant difference. In patients with severe acute liver injury, both markers were markedly higher compared to the mild cohort. NAC treatment significantly reduced sCD163 levels in both APAP overdosed patients and healthy controls.[66] Macrophage colony-stimulating factor (CSF1), which promotes hepatic macrophage accumulation via proliferation of resident macrophages and recruitment of monocytes, also seems to be a prognostic marker for patients with DILI. In a study of 78 patients with APAP-induced ALF, a low serum level of CSF1 was associated with increased mortality.[49] Osteopontin (OPN) is a phosphoglycoprotein expressed by KCs and plays a pivotal role in activating NK cells, neutrophils and macrophages. OPN levels appeared to correlate with degree of liver necrosis in patients with AILI and very high levels were associated with hyperacute injury.[67]

Lactoferin, a glycoprotein found in milk, has been demonstrated to activate KCs to reduce APAP-induced liver sinusoidal endothelial cell dysfunction and hepatic congestion, thereby protecting against AILI.[68] Berberine (BBR) is a natural alkaloid derived from traditional medicine Rhizoma coptidis. It has been shown that BBR dramatically eliminated APAP-induced hepatotoxicity by inhibiting oxidative stress, hepatocyte necrosis and inflammatory responses including reducing the expression of pro-inflammatory cytokines, and inhibited the infiltration of macrophages.[69] CPS1, the major mitochondrial urea cycle enzyme, protects against AILI even when given therapeutically after injury induction, which is hepatic macrophages-dependent.[43] Osthole, a natural coumarin found in traditional Chinese medicine, can prevent AILI in mice partly by suppressing APAP-caused elevation of inflammatory cytokines in macorphages.[70] Metformin is reported to bind high mobility group box 1 (HMGB1), which is an alarmin released from necrotic hepatocytes and can activate KCs in the initiation of AILI, and thus effectively reduce AILI.[71] Manganese porphyrin (MnP)-loaded poly (vanillyl alcohol-co-oxalate) (PVAX) particles (MnP-loaded PVAX particles) significantly reduced the serum ALT level and protected liver damages during AILI through inhibiting oxidant and pro-inflammatory activities.[72]

Moreover, it has been shown that N-myc and STAT interactor (NMI) and interferon-induced protein 35 (IFP35) act as damage-associated molecular patterns (DAMPs) to promote inflammation by activating macrophages via the toll-like receptor 4 and NF-κB pathways during AILI.[73] CCR5 is related to macrophage infiltration into the liver and subsequent hepatotoxicity upon challenge of APAP.[74] It is worthwhile exploring the therapeutic potential of targeting NMI, IFP35 and CCR5 in DILI. Looking ahead, continued advances in biomarkers identification, preclinical models and mechanism studies will provide further understanding of the pathogenesis of DILI. These advances will lead to improved diagnosis and therapy strategies for DILI. Ultimately, the knowledge gained will lead to more confident assessment of DILI and the identification of new drug candidates thus saving more lives.

5. Current challenges in study of hepatic macrophages in DILI

Most of the experimental findings discussed in this review were obtained in various strains of mice. Different strains of mice used might cause differences in the results and potential mechanistic conclusions. Mouse models are also dependent on genetic background, sex, age, diet and microbiota.[75] For example, in halothane-induced hepatitis mouse model, BALB/c mice were found to be the most susceptible strain whereas there is no significant hepatotoxicity observed in C57BL/6J mice.[76] Male mice are also more susceptible to APAP-induced liver injury compared to female mice.[77–79] Although the different susceptibility to APAP overdose among various mouse strains are difficult to understand, basal gene expression in drug metabolism, stress responses and innate immune responses should be taken into consideration. Moreover, mouse models of DILI usually do not recapitulate the full spectrum of human pathology, therefore it is necessary to evaluate the human relevance of findings observed in animal models carefully. There is a large difference between humans and mice in terms of definition of KCs and MoMF.[23,80] Much comparative work between human versus mouse hepatic macrophage subsets remains to be done.

Several tools have been widely used to analyze the function of hepatic macrophages in both homeostasis and disease. Hepatic macrophages are extremely versatile and replenish each other to certain extent upon selective depletion. They adapt their phenotype (either pro-inflammatory or anti-inflammatory) according to micro-environmental cues in healthy or injured livers.[13] Clodronate-encapsuled in liposomes (which are phagocytosed by KCs and deplete KCs transiently) has been widely used to deplete KCs in rodents.[81] Gadolinium chloride treatment (which deactivates KCs for unknown mechanism) was also used to study KCs in earlier studies.[18,19,50] Sublethal irradiation, bone marrow transplantation and parabiosis are used to understand the replacement of KCs by MoMF.[82] All of these methods could have undesired side effects, such as inducing immune responses that confound the interpretation of experimental results. Therefore, multiple approaches are necessary when designing an experiment.

The lack of experimental animal models largely limits our understanding about the mechanism underlying DILI, particularly idiosyncratic DILI.[83] A more recent breakthrough is the development of a mouse model of idiosyncratic DILI resembling to human idiosyncratic DILI by inhibiting immune tolerance. Amodiaquine (AQ) has been withdrawn from the market due to severe hepatotoxicity and agranulocytosis.[84] Female C57BL/6 mice treated with AQ will develop mild liver injury with a delayed initiation and repair regardless of consistent treatment.[85] The AQ-induced liver injury mouse model shares similarities with the mild idiosyncratic DILI in humans, including a delayed onset and the infiltration of macrophages and CD8 T cells.[86] However, treating Casitas-B-lineage lymphoma protein-B (Cbl-b)$^{-/-}$ and programmed cell death 1 (PD-1)$^{-/-}$ mice with AQ can result in a slightly greater injury. Furthermore, co-treatment of PD-1$^{-/-}$ mice with anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA4) and AQ produces a much more severe liver injury characterized by portal infiltration of T and B lymphocytes with interface hepatitis. The infiltrated CD8^{+} T cells produce perforin and granzyme, suggesting an immune-mediated underlying mechanism. This murine model of AQ-induced liver injury was the first model to provide insights into the immune mechanism of idiosyncratic DILI. Additional animal models that can recapitulate the characteristics of idiosyncratic DILI in humans will greatly advance research in this field and help identify strategies to treat the disease.

6. Future directions in studies of hepatic macrophages in DILI

First, the role of hepatic macrophages in DILI is most characterized in AILI and the contribution of hepatic macrophages to other DILI is largely unknown. It is interesting to identify the role of hepatic macrophages in different kinds of DILI besides AILI. Second, developing an approach to track either KCs or MoMF will be helpful in uncovering the role of hepatic macrophages in the progression of DILI dynamically. Third, DILI is a complex process involving multiple cell types. While studying the role of hepatic macrophages, it is necessary to consider the cross-talk of hepatic macrophages with other resident and infiltrating cells in the liver. Fourth, C-type lectin domain family 4, member f (Clec4f) has been reported to be specifically expressed on hepatic macrophages. Thus, Clec4f-Cre mouse would be a good model to study the role of hepatic macrophages, as well as genes specifically expressed by these cells in DILI.[87]

Authors' contributions

Both authors wrote, critically reviewed, and edited the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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