REVIEW

Pathogenesis of Kupffer Cells in Cholestatic Liver Injury

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Kupffer cells are the resident macrophages in the liver. They are located in hepatic sinusoid, which allows them to remove foreign materials, pathogens, and apoptotic cells efficiently. Activated Kupffer cells secrete various mediators, including cytokines and chemokines, to initiate immune responses, inflammation, or recruitment of other liver cells. Bile duct ligation (BDL) surgery in rodents is often studied as an animal model of cholestatic liver disease, characterized by obstruction of bile flow. BDL mice show altered functional activities of Kupffer cells compared with sham-operated mice, including elevated cytokine secretion and impaired bacterial clearance. Various mediators produced by other liver cells can regulate Kupffer cell activation, which suggest that Kupffer cells orchestrate with other liver cells to relay inflammatory signals and to maintain liver homeostasis during BDL-induced liver injury. Blocking or depletion of Kupffer cells, an approach for the treatment of liver diseases, has shown controversial implications. Procedures in Kupffer cell research have limitations and may produce various results in Kupffer cell research. It is important, however, to reveal underlying mechanisms of activation and functions of Kupffer cells, followed by hepatic inflammation and fibrosis. This review summarizes present Kupffer cell studies in cholestatic liver injury. (*Am J Pathol* 2016, 186: 2238–2247; <http://dx.doi.org/10.1016/j.ajpath.2016.06.003>)

Kupffer cells are tissue-resident macrophages in the liver. They have high phagocytic capability, allowing them to remove foreign materials, such as viruses and bacteria, as well as apoptotic cells and cellular debris. This is an important role of Kupffer cells as a gatekeeper in the innate immune response.¹ Kupffer cells are located in the hepatic sinusoids and can migrate along hepatic sinusoidal endothelial cells,² allowing them to remove foreign pathogens entering the liver from the portal venous system efficiently. They also function as active sensory cells that detect immunoreactive materials, such as lipopolysaccharide (LPS), which is a component of the outer membrane of Gram-negative bacteria and a strong trigger for immune responses. Activated Kupffer cells secrete several mediators that regulate inflammation and homeostasis, as well as drive the inflammatory response to liver injury.³ Therefore, several studies have attempted to reveal the role and function of Kupffer cells in liver pathology to seek therapeutic treatments for chronic cholestatic liver diseases. This review

summarizes the current studies regarding the role of Kupffer cells in liver diseases, especially in cholestatic liver injury.

Cholestatic Liver Injury

Cholestatic liver diseases, including primary biliary cirrhosis and primary sclerosing cholangitis, occur in extrahepatic and

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or intrahepatic bile ducts. Cholestatic liver diseases are characterized by the accumulation of specific bile acids in the liver that initiate an inflammatory process leading to liver injury. Patients with cholestatic liver diseases may present with pruritus, fatigue, jaundice, and dark urine.⁴ Although the detailed mechanisms are still not fully understood, it is known that bile salts are toxic and induce hepatocyte apoptosis *in vitro*.⁵ Bile acids also disrupt the integrity of the biliary epithelium by affecting cholangiocyte secretion, proliferation, and survival.^{6,7} A previous study has demonstrated that bile depletion attenuates cholangiocyte proliferation,⁸ and other studies have shown that bile acids, taurocholic acid, and tauro lithocholic acid can induce ductal secretion and cholangiocyte proliferation as well as up-regulation of bile acid transporter in rats.^{9,10} Another study has also shown that taurocholic acid has protective effects against caffeic acid–induced cholangiocyte apoptosis by enhancing vascular endothelial growth factor production leading to cholangiocyte proliferation.¹¹ A recent study has shown that bile acids inhibit LPS-induced proinflammatory cytokine secretion from macrophages,¹² and it is also known that bile acids modulate innate and adaptive immunity, including inhibition of immunoglobulin production.^{13,14} These studies indicate that bile acid accumulation is associated with not only cholangiocyte proliferation but also altered immune responses via liver cells, including Kupffer cells, and this may be responsible for liver damage, inflammation, and fibrosis during cholestasis.

In cholestatic liver disease research, bile duct ligation (BDL) is commonly used for rodent models. BDL is a surgically induced extrahepatic biliary obstruction of the common bile duct that prevents bile flow and mimics cholestasis followed by cholestatic liver injury. Rodent models show altered phagocytic, immune, and macrophage functions after BDL.^{15,16} BDL can be performed for gene knockout mice to evaluate functions of the gene of interest during cholestasis.¹⁷ It is often observed that patients with cholestatic liver diseases have a higher incidence of perioperative infectious complications. Bacterial infection and inflammation is closely associated with cholestatic liver diseases¹⁸; bacterial infection can cause cholestasis, and cholestasis increases susceptibility to bacterial infection. Nakano et al¹⁹ have demonstrated that BDL rats become more susceptible to LPS compared with the control group. Although a previous study has shown that BDL rodents may not perfectly mimic disease conditions of human cholestatic liver injury²⁰ as well as it requires technical surgery, BDL followed by LPS or bacteria injection can be a tool for studies of cholestasis and bacterial infection as well as inflammation.

Heterogeneity of Kupffer Cells

M1 and M2 Macrophages

Macrophages can be classified into two distinct subsets, M1 and M2. In response to various signals, Kupffer cells may undergo classic M1 activation or alternative M2 activation.

M1 Kupffer cells exhibit a proinflammatory phenotype and can be activated by pathogens or toxins, such as LPS, and release proinflammatory cytokines, including tumor necrosis factor (TNF)- α , IL-6 and IL-1 β , as well as transforming growth factor- β that induces fibrogenesis by activation of hepatic stellate cells (HSCs) and reactive oxygen species (ROS), leading to inflammation and liver damage.^{21,22} IL-6 also induces cell proliferation in hepatocytes and cholangiocytes and could lead to carcinogenesis via activating phosphatidylinositol 3, Janus activating kinase/STAT, and p38 mitogen activated protein kinase pathways.²³ ROS causes unfolded proteins to accumulate within the endoplasmic reticulum of hepatocytes, initiating hepatic steatosis, inflammation, and apoptosis because of endoplasmic reticulum stress.²⁴ M2 Kupffer cells display anti-inflammatory phenotypes that can be activated by IL-4 and IL-13 releasing IL-4, IL-10, IL-13, and transforming growth factor- β leading to wound repair and fibrosis.^{21,22} Table 1 summarizes mediators of Kupffer cells in liver injury, and Figure 1 shows functional differences between M1 and M2 Kupffer cells.

The balance of M1/M2 Kupffer cells may be a key in liver pathology. For example, a recent study has demonstrated that BALB/c mice, which have dominant M2 macrophages, show attenuated hepatic steatosis and inflammation compared with C57BL/6 mice (M1 dominant) induced by methionine-choline–deficient diet. Another study using BALB/c and C57BL/6 mice has shown that M2 Kupffer cells induce M1 Kupffer cell apoptosis via IL-10 secretion and regulate M1/M2 balance, leading to protective effects against alcohol or high-fat diet–induced hepatic steatosis and apoptosis.³⁶ These findings suggest that activation of M2 Kupffer cells and the regulation of M1/M2 balance could be a potential target of treatment for liver diseases.

Liver-Resident and Bone Marrow–Derived Macrophages

There are also two subsets of liver macrophages, depending on derivations: self-renewing liver-resident macrophages derived from embryonic progenitors (Kupffer cells) and bone marrow–derived macrophages circulating in blood stream as monocytes and recruited in the liver to differentiate into macrophages.⁴⁴ A study has revealed that functional properties are different between these two subsets in acute liver injury induced by *N*-acetyl-p-aminophenol.⁴⁵ Other studies, however, have shown that bone marrow–derived monocytes can be differentiated into self-renewing Kupffer cells as well as M1 and M2 subsets.^{46,47} During liver injury, it is known that bone marrow–derived monocytes are recruited in the liver to accelerate liver regeneration via communication with Kupffer cells.^{48,49} A recent study has shown that sphingosine 1-phosphate receptor 2 and 3 mediate bone marrow–derived monocyte recruitment in BDL mice.⁵⁰ These earlier findings suggest that not only Kupffer cells but also bone marrow–derived macrophages may play key roles in liver repair

Table 1 Mediators Associated with Kupffer Cells and Cholestatic Liver Injury

Mediators	Pathophysiological events induced by mediators
M1 Kupffer cells	
TNF- α	Inflammation and apoptosis ²⁵
IL-1	Inflammatory responses, ²⁶ hepatic steatosis, ²⁷ and fibrosis ²⁸
IL-6	Inflammation, ²⁹ cell proliferation, and carcinogenesis ²³
M2 Kupffer cells	
IL-4	Wound repair, ³⁰ liver regeneration, ³¹ and anti-inflammatory responses ³²
IL-10	Anti-inflammatory responses, ³³ fibrosis, ³⁴ bacteria clearance, ³⁵ apoptosis of M1 macrophages ³⁶
IL-13	Anti-inflammatory responses ³⁷ and fibrosis ³⁸
TGF- β	Fibrosis ³⁹
Others	
IL-17	Kupffer cell activation ⁴⁰
miR-124	Macrophage deactivation ⁴¹
miR-155	Inhibition of M2 macrophage polarization ⁴²
miR-223	Inhibition of M1 macrophage polarization ⁴³

TGF- β , transforming growth factor- β ; TNF- α , tumor necrosis factor- α .

and regeneration during liver injury, although detailed functional differences between these two subsets of macrophages are still unclear and further studies are required.

Functional Alterations of Kupffer Cells in Cholestatic Liver Disease

Kupffer cells play an important role in liver pathology with various functions: i) engulf and eliminate foreign pathogens, apoptotic cells, and cell debris by phagocytosis; ii) present antigens to attract and stimulate T cells; iii) recruit non-parenchymal cells in the liver, including monocytes, neutrophils, natural killer T cells for immune responses; and iv) initiate an immune response in other liver cells, such as hepatocytes, by releasing active mediators, including cytokines, chemokines, and ROS.⁵¹ During cholestatic liver injury, however, various functional alterations of Kupffer cells and Kupffer cell–related physiological events that may be associated with disease conditions have been reported.

Immunologic Abnormality

One of the functions of Kupffer cells is to present antigens as previous studies have reported that human leukocyte antigen (HLA) genes are associated with cholestatic liver diseases. It has been reported that genome-wide association studies using primary biliary cirrhosis or primary sclerosing cholangitis patients identified candidate loci on regions of HLA.⁵² A study using Japanese primary biliary cirrhosis patients has identified association between haplotypes of HLA class II and susceptibility and progression of primary

Figure 1 M1 and M2 Kupffer cells. Kupffer cells are stimulated by various triggering agents and become active as M1 or M2 Kupffer cells. Foreign pathogens or proinflammatory cytokines activate Kupffer cells as M1 Kupffer cells, leading to further proinflammatory cytokine secretion or production of reactive oxygen species (ROS). Other cytokines, such as IL-4 and IL-13, promote Kupffer cell activation into M2 subsets, leading to anti-inflammatory cytokine secretion, as well as transforming growth factor (TGF)- β secretion, leading to fibrogenesis. A later study has shown that M2 Kupffer cells promote apoptosis of M1 Kupffer cells via IL-10 secretion to maintain homeostasis and balance of M1/M2 Kupffer cell population.³⁶ Recent studies have demonstrated that some miRNAs can deactivate Kupffer cells or inhibit Kupffer cell activation, which may also be associated with liver diseases.^{41–43} LPS, lipopolysaccharide; TNF- α , tumor necrosis factor- α .

biliary cirrhosis.⁵³ Intrahepatic cholestasis of pregnancy is a liver disorder specific to pregnancy characterized by increased levels of serum bile acids, although pathogenesis of intrahepatic cholestasis of pregnancy is still unknown. Yayi et al⁵⁴ have revealed that cytokines for the M1 subset (TNF- α) are increased and those for the M2 subset (IL-4) are decreased as well as HLA-G and HLA-E expressions are significantly decreased in intrahepatic cholestasis of pregnancy patients. Another study using human patients has also shown that down-regulation of HLA-G and up-regulation of miR-148a are associated with intrahepatic cholestasis of pregnancy.⁵⁵ These studies suggest that HLA genes and antigen-presenting cells, including Kupffer cells, may be responsible for unbalanced cytokines and pathogenesis of cholestatic liver diseases.

Impaired Bacterial Clearance

A previous study using dogs has shown that BDL dogs fail rapid bacterial clearance from blood and bile after bacteria injection.⁵⁶ Abe et al³⁵ have found that BDL mice showed delayed clearance of bacteria and higher levels of IL-10 after bacterial injection compared with the sham-operated group. This study has also shown that *in vivo* administration of anti-IL-10 antibody improves bacterial clearance in BDL mice. Another study using IL-10 knockout mice, however,

has demonstrated that IL-10 deficiency does not attenuate bacterial proliferation and even increases late mortality after BDL and *Escherichia coli* injection compared with wild type.⁵⁷ Although IL-10 released from Kupffer cells may play an important role in bacterial clearance from the liver as well as anti-inflammatory response, only limited studies are available to date and detailed functions of IL-10 in cholestatic liver disease are still unknown.

Cytokine Production

Activated Kupffer cells secrete various mediators to recruit, activate, or initiate differentiation of other liver cells (Figure 2). BDL mice show elevated secretion of TNF- α and IL-6 induced by LPS challenge.⁵⁸ TNF- α , IL-1, and IL-6 are major proinflammatory cytokines and are associated with cholestatic liver injury. A previous study has shown that TNF- α knockout mice show improved survival rate and attenuated BDL-induced liver damage and fibrosis.⁵⁹ Another study using IL-1 receptor knockout mice has demonstrated that these mice show insusceptibility against LPS challenge after BDL, secreting fewer cytokines and showing lower mortality compared with wild-type mice.⁶⁰ Deletion of IL-1 α also showed attenuated high-fat diet–induced inflammation as well as TNF- α , IL-1 β , and IL-6 secretion.⁶¹ Deletion of IL-6, however, has shown significantly elevated cytokine secretion, including TNF- α and IL-1 β , as well as increased mortality against LPS challenge after BDL compared with wild-type mice.⁶² Another study has shown impaired immune responses of IL-6 knockout mice against LPS injection.⁶³ These findings suggest that cytokines secreted from Kupffer cells are associated with liver conditions and mortality in cholestatic liver injury, although detailed mechanisms for each cytokine are unclear and functions may vary depending on cytokines.

Activation of Kupffer Cells

It is known that hypoxia-inducible factors are critical regulators of mediator production, such as monocyte chemoattractant protein-1 by hypoxic Kupffer cells.⁶⁴ A study using mouse models has demonstrated that selective knockout of hypoxia-inducible factors in bone marrow–derived liver macrophages attenuates liver fibrosis induced by BDL.⁶⁵ Another study has shown that hypoxia-inducible factor-1 α knockout in HSCs decreases proinflammatory cytokine secretion and M1 macrophage differentiation, leading to impaired necrotic cell clearance caused by carbon tetrachloride injection in mice.⁶⁶

IL-17 is secreted from neutrophils and T helper 17 cells, and recent studies have revealed that Kupffer cells can be regulated by IL-17, leading to cytokine secretion during cholestatic liver injury. A study using IL-17A knockout mice has shown that IL-17A deficiency attenuates BDL-induced liver damage and expression levels of TNF- α and transforming growth factor- β .⁴⁰ This study has also shown that Kupffer cells treated with IL-17A secrete significantly higher levels of TNF- α and transforming growth factor- β induced by LPS. Another study has shown that neutralization of IL-17A by anti-IL-17A antibody attenuates BDL-induced fibrosis and proinflammatory cytokine expression, including TNF- α and IL-6.⁶⁷ Furthermore, IL-17 has been shown to promote proinflammatory and fibrogenic cytokines from Kupffer cells and enhances production of collagen type I in HSCs, leading to liver damage and fibrosis.⁶⁸

Small noncoding RNAs, miRNAs, have also been suggested as Kupffer cell mediators. Xiao et al⁶⁹ have demonstrated that BDL rats show elevated IL-6 expression and decreased miR-124 expression in the liver, and miR-124 inhibits IL-6–mediated cholangiocyte proliferation by suppressing the expression levels of IL-6 receptor. miR-124 is also known to promote deactivation of macrophages in

Figure 2 Functions of Kupffer cells in liver diseases. Kupffer cells are localized in hepatic sinusoid and can be mobile along with hepatic sinusoidal endothelial cells (HSECs) to encounter foreign materials effectively. Once Kupffer cells are activated, they secrete various mediators, including cytokines and chemokines, to stimulate other liver cells and initiate inflammatory responses. Cytokines, such as IL-6, induce inflammation and proliferation of hepatocytes and cholangiocytes. Transforming growth factor (TGF)- β activates hepatic stellate cells (HSCs), leading to fibrosis, and reactive oxygen species (ROS) cause accumulation of unfolded proteins in the endoplasmic reticulum of hepatocytes, leading to hepatic steatosis and inflammation. Kupffer cells also secrete adhesion molecules to enhance interaction with neutrophils, and neutrophil accumulation helps pathogen clearance from blood stream. LPS, lipopolysaccharide.

the brain.⁴¹ miR-155 is another candidate miRNA that may be associated with Kupffer cell activation. A previous study has reported that miR-155 enhances immune responses against LPS, and miR-155 deficiency attenuates hepatic steatosis and fibrosis induced by methionine-choline–deficient diet.⁷⁰ Arranz et al⁷¹ have demonstrated that reduced levels of miR-155 leads to M2 Kupffer cell polarization and attenuates cytokine expression induced by LPS. Another study has also shown that miR-155 regulates IL-13 receptor $\alpha 1$ and inhibits polarization of M2 Kupffer cells via IL-13.⁴² It has also been reported that polarization of M1 macrophages can be regulated by miR-223. A study using the miR-223 knockout mouse model has demonstrated that miR-223 suppresses M1 macrophage polarization, and miR-223 deficiency exacerbates inflammation induced by high-fat diet.⁴³ Although detailed mechanisms are still unclear and further studies are required, these previous studies suggest that transcription factors, cytokines, and miRNAs can regulate Kupffer cells and they could be a target for novel treatments of chronic cholestatic liver diseases.

Orchestration of Kupffer Cells with Other Liver Cells during Liver Injury

As described above, hypoxia-inducible factors and IL-17 can regulate Kupffer cell activation, which indicates that other liver cells, such as HSCs and neutrophils, may be able to regulate Kupffer cells via secretion of those mediators, and Kupffer cells may orchestrate with other liver cells to maintain liver homeostasis during liver injury. In fact, a previous study has demonstrated that ROS and IL-6 activate Kupffer cells and these activated Kupffer cells modulate fibrogenic responses in HSCs.⁷² A latest study has also shown that HSCs receive an inflammation signal from sinusoids and relay it to parenchyma during concanavalin A–induced hepatitis in mice.⁷³ Another study has shown that HSC depletion attenuates liver damage and TNF- α production caused by ischemia/reperfusion or LPS.⁷⁴ There is also an implication that 42% of HSCs are vicinal to Kupffer cells in the liver, and these two cells may interact or communicate with each other for responses against liver injury, although further studies are required for conclusion.⁷⁵ These earlier findings suggest that Kupffer cells and HSCs may orchestrate in signaling and cytokine secretion during liver diseases.

Kupffer cells also orchestrate with neutrophils in liver injury. BDL-induced cholestatic liver injury is associated with larger populations of Kupffer cells and neutrophils,⁷⁶ and neutrophils are essential for collagenase activity and liver repair in BDL rats.⁷⁷ Kupffer cells produce adhesion molecules that induce neutrophil interactions, promote neutrophil attachment to Kupffer cells, and promote clearance of bacteria in the liver.⁷⁸ A recent study has shown that Kupffer cells coordinate with hepatic sinusoidal endothelial

cells for neutrophil adhesion and recruitment during LPS-induced liver injury, and activation of Toll-like receptor 4, which is a receptor for LPS, in hepatic sinusoidal endothelial cells is essential.⁷⁹

In cholestatic liver injury, portal fibroblasts may also be important for fibrosis.⁸⁰ HSCs and portal fibroblasts are the major cellular sources of collagens in carbon tetrachloride or BDL-induced fibrogenesis, respectively.⁸¹ Another study has revealed that >70% of myofibroblasts are derived from portal fibroblasts in BDL-induced cholestatic liver injury.⁸² Although only limited studies are available to date and further investigations are required, not only Kupffer cells but also other liver cells are associated with liver homeostasis, and these cells cooperate for the responses to liver injury. This suggests that these liver cells can be targets for the development of novel treatments.

Protection or Promotion by Kupffer Cells in Cholestatic Liver Injury

As Kupffer cells play a key role in signaling of liver inflammation and fibrosis, a potential approach for treatments is to block or deplete Kupffer cells. The function of Kupffer cells in liver pathology, however, is still controversial because depletion of Kupffer cells attenuates hepatic inflammation and liver damage in previous studies,^{83–86} although some recent studies claim that Kupffer cell depletion exacerbates liver damage.^{87,88}

Kupffer Cells Promote Liver Injury

Gadolinium chloride (GdCl₃) is the most widely used agent for macrophage blocking in immunology research. Administration of 5 mg/kg GdCl₃ blocks phagocytosis of Kupffer cells, and it reduces lethality against LPS challenge in rats.⁸⁹ It has also been reported that 10 mg/kg GdCl₃ pretreatment inhibits Kupffer cell activity during BDL and LPS challenge, and as a result, suppresses TNF- α secretion in rats.⁸³ Another study has shown that daily 20 mg/kg GdCl₃ administration after BDL operation for 28 days attenuates liver damage and fibrosis in rats.⁸⁴ Jones et al⁸⁵ administered 10 mg/kg GdCl₃ to BDL rats 6 days after the surgery for 24 hours and found that GdCl₃-treated rats showed fewer Kupffer cells, less liver damage, and lower plasma TNF- α levels. A study using liposomal clodronate for Kupffer cell depletion has shown that Kupffer cell–depleted rats show reduced TNF- α and IL-1 β production.⁸⁶ These studies suggest that Kupffer cells are responsible for promotion of cholestatic liver injury and hence depletion of Kupffer cells can be a tool for treatments.

Kupffer Cells Protect Liver Injury

Although some studies have shown that Kupffer cell depletion attenuates cholestatic liver injury, a few studies

have suggested opposite implications. For example, Gehring et al⁸⁷ have treated mice with liposome-encapsulated dichloromethylene diphosphonate before BDL to deplete Kupffer cells, and found that Kupffer cell–depleted mice show significantly more severe liver damage compared with control mice. Another study using alendronate for Kupffer cell depletion has revealed that Kupffer cell–depleted mice show more severe liver damage and decreased hepatocyte regeneration but also reduced fibrosis after BDL.⁸⁸ These studies indicate that Kupffer cells protect the liver and are required for cell survival and regeneration in cholestatic liver injury.

Limitations and Controversies in Kupffer Cell Research

There are two hypotheses for functions of Kupffer cells and immune responses in liver pathology (Figure 3): Kupffer cell–induced responses, such as inflammation and fibrosis, are responsible for liver diseases, and hence depletion of Kupffer cells, followed by reduced immune responses, is a potential cure leading to better cell survival and liver conditions. Several studies have reported that depletion or blocking of Kupffer cells has protective effects against liver diseases, and this is still a common approach to seek a therapeutic target in live pathology. Alternatively, Kupffer cell–induced responses are a counteraction against triggers, such as pathogens and accumulated bile acids, and these

responses help cells to survive and maintain homeostasis, and hence depletion of Kupffer cells exacerbates liver diseases. These hypotheses are still controversial, and no conclusive studies have been established. There are some limitations and problems, however, in studies of Kupffer cells.

Agents for Kupffer Cell Blocking or Depletion

Previous studies use various agents for blocking or depletion of Kupffer cells, including GdCl_3 , liposomes containing clodronate, dichloromethylene diphosphonate, or alendronate. Selection of agents for Kupffer cell depletion may be critical, and different agents could produce different results. In addition, other factors induced by the agent itself should be considered. GdCl_3 , for example, is a blocker of L-type calcium channel located on the cell membrane and inhibits calcium influx into the cytosol through that channel.⁹⁰ A previous study has shown that calcium signaling may play an important role in inflammatory responses and cytokine production, such as $\text{TNF-}\alpha$.⁹¹ Calcium flash is also required for hydrogen peroxide production and wound inflammatory response.⁹² These studies suggest that Kupffer cell activation, followed by cytokine secretion and ROS production, may be an important signal to initiate inflammatory responses and could be regulated by free calcium in cytosol. It is also unknown if Kupffer cell blocking or depletion alters physiological events in the liver or other cells, such as bone marrow–derived macrophages can compensate functions of depleted Kupffer cells. Results may differ between short-time Kupffer cell blocking or depletion and long-term chronic treatment. Careful attention should be paid on agent selection and treatment procedures.

Population of M1/M2 Kupffer Cells

There are two subsets of Kupffer cells, M1 and M2, and their population may be critical for protection or promotion of cholestatic liver diseases.⁹³ Populations or ratios of M1/M2 Kupffer cells may change liver conditions or responses against triggering agents, although it is not easily feasible to discriminate and isolate M1 and M2 Kupffer cells. In addition, a study has shown that there are two subsets of M2 Kupffer cells showing different expression levels of marker proteins.⁹⁴ It is known that Kupffer cells are positive for F4/80 and CD68, and M2 but not M1 Kupffer cells are positive for CD163, which is often used for discrimination between M1 and M2 Kupffer cells. Kinoshita et al,⁹⁵ however, have demonstrated that within F4/80^+ Kupffer cells, 46% are $\text{CD11b}^-\text{CD68}^+$, 22% are $\text{CD11b}^+\text{CD68}^-$, and 6% are $\text{CD11b}^+\text{CD68}^+$, and CD68^+ subsets are functionally different from CD11b^+ subsets. Another study has shown that CD11b^+ subsets may have higher secretion capacity of $\text{TNF-}\alpha$ compared with other subsets.⁹⁶ Functional differences of Kupffer cell subsets are still largely

Figure 3 Promotion or protection of liver diseases by Kupffer cells. Various triggering agents, such as pathogens and lipopolysaccharide, activate Kupffer cells. Functions of activated Kupffer cells, however, are still controversial. Some previous studies have claimed that Kupffer cells initiate disease conditions, including inflammation and fibrosis, and hence Kupffer cell blocking or depletion can be a candidate approach for the treatment of liver diseases, which suggests that Kupffer cells are responsible for liver damage.^{83–86} In contrast, other studies have demonstrated that Kupffer cell depletion exacerbates liver damage, which implies that Kupffer cells protect the liver and activated Kupffer cells orchestrate with other cells for a counteraction against pathogens.^{87,88} This suggests that Kupffer cell–mediated responses are necessary for cell survival and Kupffer cell depletion cannot be a treatment of liver diseases.

unknown. In addition, it is also unknown which subsets can be blocked or depleted by the agents administered. Further studies are required to understand the association between M1/M2 Kupffer cells and liver conditions.

Isolation of Kupffer Cells

It is critical for Kupffer cell research to isolate primary Kupffer cells from liver specimens with good purity and cell viability. There are various isolation techniques available, although none of them are perfect and no golden standard method is established to date. Different isolation methods could produce different results because of varied cell viability or purity. Common methods include centrifugal elutriation and selective adherence.^{97,98} Density gradient centrifugation followed by magnetic-activated cell sorting gives high purity and cell viability, maintaining phagocytic activity of Kupffer cells after isolation, although it is still not feasible to eliminate contamination of other nonparenchymal cells.⁹⁹ Laser-capture microdissection allows selective isolation of stained cells from tissues, and Gehring et al¹⁰⁰ have demonstrated Kupffer cell isolation using laser capture microdissection and India ink injection for Kupffer cell staining. This technique can produce selective and high-quality RNA isolation of Kupffer cells from mouse liver sections, although this technique allows only RNA or DNA isolation and cell cultivation cannot be performed. Isolation of Kupffer cells is still challenging and, therefore, careful attention on experimental procedures and evidence for Kupffer cell viability and purity is required in Kupffer cell research.

Conclusion

Present studies have demonstrated that Kupffer cells are associated with cholestatic liver diseases, their functions are altered during liver injury and those physiological events are performed not only by Kupffer cells but also other liver cells communicating or regulating each other to orchestrate for liver homeostasis. There are controversial implications about Kupffer cell functions during liver injury, and further studies are required to confirm whether Kupffer cells are responsible for promotion or protection of liver damage and whether Kupffer cell depletion can be a treatment of liver diseases or exacerbate liver conditions. Although there are limitations in Kupffer cell research, it is important to understand functions and regulations of Kupffer cells and underlying mechanisms of liver damage with which Kupffer cells are associated to develop a novel treatment in cholestatic liver diseases.

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