Chapter 3

The Biological Function of Kupffer Cells in Liver Disease

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Abstract

Kupffer cells, which have a characteristic morphology and a kind of phenotype, are the resident macrophages in liver, serve as the largest population mononuclear phagocytes in the body, and are localized in the periportal zone. They have phagocytosis capacity and release all kinds of cytokines, chemokines, and soluble biological mediators. Owing to the different functions of Kupffer cells, they play an important role in liver diseases. In this chapter, we review the role of Kupffer cells in infectious disease, fatty liver disease, liver fibrosis, liver ischemia-reperfusion injury, liver transplantation immunology, as well as liver cancer and metastases.

Keywords: Kupffer cell, infectious disease, fatty liver disease, fibrosis, ischemia-reperfusion injury, liver transplantation immunology, liver cancer, metastases

1. Introduction

Kupffer cells (KCs), as the largest population mononuclear phagocytes in the body, account for 80–90% of the total number of natural macrophages and 20% of the liver nonparenchymal cells [1]. They form a self-renewing pool of organ-resident macrophages independent of the myeloid monocyte compartment and derive from resident stem cells which originate from the fetal yolk sac before [2–4]. Other studies also found that KCs derived from embryonic progenitors colonize the tissues before birth [5–11], but with the growth of mouse, bone marrow-derived monocytes will fill up additional macrophage niches that become available, competing with the resident population. This situation occurs in the liver and spleen, but not in the brain and lung [12].

KCs have a characteristic morphology with amoeboid lamellipodia and an irregular surface containing many microvilli [13], located at the luminal side of liver sinusoidal endothelium or

the lamellipodia extended into the Disse space through the fenestrae. This is an ideal position for their main function in the liver. This state can filter the blood that enters the liver from both the portal vein and the hepatic artery, which is an important part of the cellular immunity system of the mammalia (**Figure 1**). So, the structure of KCs plays a role in the mutual coordination and influence of liver parenchymal cells and other nonparenchymal cell functions and makes up these cells' important versatile constituents of the liver [14–16]. Now, according to the function of KCs, they could be distinguished as two groups: the one with higher phagocytosis capacity and the other with preference toward cytokines and chemokines production [17, 18]. Some studies found that there were large KCs in rats. They are localized in the periportal zone and have increased phagocytosis and increased production of biological mediators. These large KCs can be identified by the expression of CD163, also described as ED2 antigen, which is a scavenger receptor [19]. KCs (**Table 1**) can also be identified by the expression of CD68 (ED-1); they were called small KCs in rats. The general macrophage marker F4/80 or by ED-1 was expressed on the surface of mice KCs, which is present in all KCs regardless of their location [20]. In mice, KCs can be distinguished from monocytes among the $F4/80^{+}$ cells as Ly6C low CD11b low-cell population [21, 22]. Additionally, macrophages are functionally grouped into two classes, M1 and M2. M1 (termed classically activated) macrophages are pro-inflammatory and could produce pro-inflammatory cytokines and chemokines, while the M2 (termed alternatively activated) macrophages are suppressive and involved in cellular repair [23]. According to this situation, KCs as one kind of macrophages also have these functions and play a fundamental role in homeostasis and diseases [24]. KCs also have a unique KCs gene Clec4f to distinguish with other macrophage; Clec4F has been previously described as a KCs-specific marker [25–27].

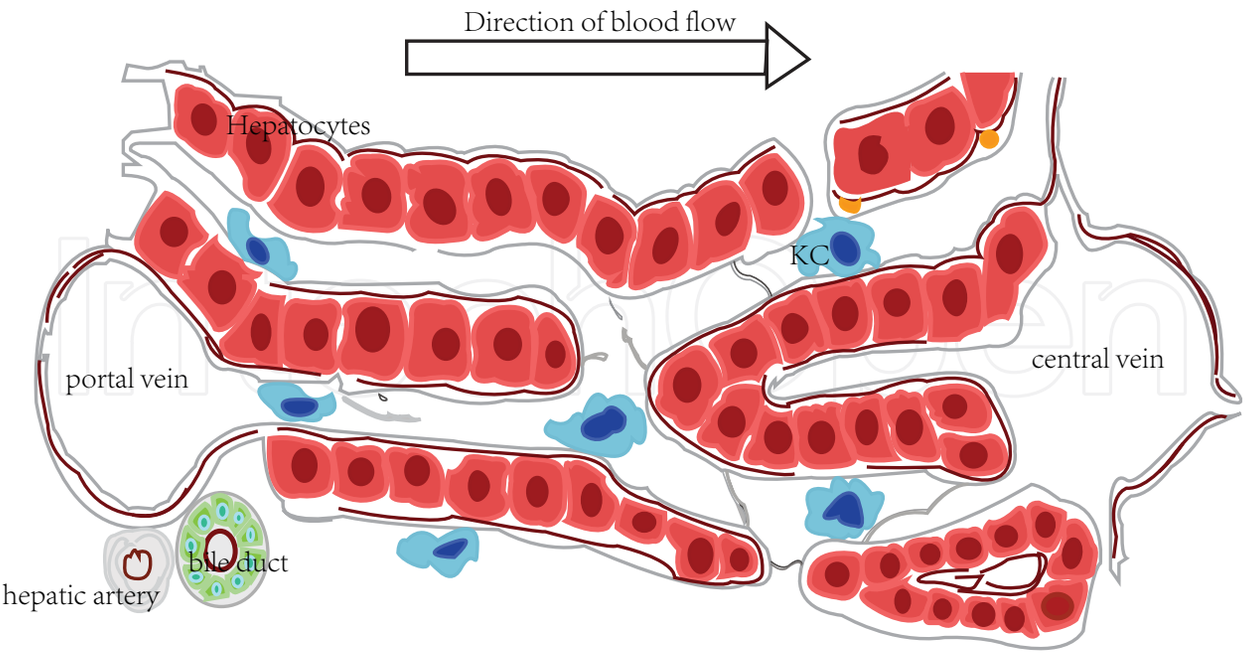

Figure 1. Schematic representation of the liver microanatomical structure and Kupffer cell localization in lower magnification.

	Origin	Marker	PRR	PAMP DAMP	Immunogenic	Polarization of macrophages	
Rat	Derived from the fetal yolk sack and embryonic progenitors colonize the tissues. Liver-resident Express Clec4F gene	CD68/ED1 CD163/ED2	Scavengers receptors (CDl3, CD14, CDl5, CD68, CD163) Mannose receptors Fc receptors (CD64, CD32, CDl6) Complement receptor (CR1, CR3, CR4) I region-associated antigen	TLR1-TLR9 NLR	MHC-II CD80 CD86 PDL-1 (CD274)	M1	Pro-inflammatory antitumoral
Mouse		F4/80 CD68 $CD11b^{low}$		TLR1-TLR9 NLR RLR		M2	Anti-inflammatory Immune suppressive protumoral
Human		CD68 CD14		TLR2 TLR3 TLR4 NLR			

Table 1. This table is used for KC identification and major surface receptors and moleculars involved in the function in human, mouse, and rat.

In vivo, under steady condition, the KCs are resting situation; they play a role in eliminating macromolecules, immune complexes, toxins, and degenerated cells from circulation. Pattern recognition receptors (PRRs) on the KCs are the main factor to eliminate the debris, toxins, and insoluble macromolecules, such as scavengers receptors (CDl3, CD14, CDl5, and CD68, CD163), mannose receptors, Fc receptors (including CD64, CD32, and CDl6), complement receptor (including the complement receptor L, complement receptor 3, and complement receptor 4), I region-associated antigen, which are able to bind to toxins lipopolysaccharide (LPS), immune complexes, or opsonized cells [28]. Since KCs reside in the liver sinusoids in large numbers and are adherent to the endothelial cells, they are able to sample the blood entering the liver from the gut as well as from the main circulation. KCs also could remove the senescent or damaged erythrocytes. In this process, following phagocytosis and hemolysis, KCs could express HO (including HO-1, HO-2, and HO-3) to degrade hemoglobin, which is part of erythrocytes component. HO-1 catalyzes the degradation of heme into iron, biliverdin, and carbon monoxide, which are all considered to be hepatoprotective at low quantities under steady-state conditions [29, 30].

Pathogen- and damage-associated molecular patterns (PAMPs and DAMPs, respectively) were two kinds of PRRs to express on the surface of KCs. They included multiple families, such as Toll-like, RIG-like, and NOD-like receptors (TLR, RLR, and NLR, respectively), and C-type lectin receptors (CLR) [31]. Mouse KCs can express TLR1-TLR9, all of which appear to be functional [32]. Human KCs, so far, have only been described to express TLR2, TLR3, and TLR4 [33, 34]. Furthermore, in the *Listeria monocytogenes* (Lm) infection model, mouse KCs are shown to express RIG-like receptor I [35]. Hepatocytes and $CD68^{+}$ liver mononuclear cells (presumably KCs) express NLRC2 (NOD2) [36]. When the KCs were activated by the emergence of endotoxins and harmful exogenous particles from the portal vein and circulation, their functions were enhanced. They could produce all kinds of cytokines and chemokines significantly. In the presence of TLR ligands, such as LPS and CpG,

the CD14-positive KCs were stimulated by TLR4, which activates the intracellular signal pathway via myeloid differentiation factor 88 (MyD 88), resulting in NF-κB activation to produce the pro-inflammation cytokines IL-6, TNF-α, IL-1β, ICAM-1, VCAM-1, and VAP-1 [37], and the CD14 expression on KCs is increased [31]. CD14-transgenic mice that overexpress CD14 on monocytes have increased sensitivity to LPS [38]. As a receptor of dsRNA, TLR3 on KCs is one of the primary triggers in the defense of viral diseases. TLR3 activation induces the strongest IFN-γ response. KCs were activated presumably due to the induction of IL-12 in the absence of IL-10 coproduction, which was observed upon TLR2 and TLR4 ligation [39]. Activation of TLR7 triggers the secretion of type I interferons and activation of subsequent genes encoding CXCL10, CXCL11, Mx1 (antiviral G-Protein), CCL2 (also known as MCP-1), also secretion of IL-10, leading to enhanced viral clearance [40]. TLR9 activation on KCs attenuates inflammation by the secretion of IL-10, suppressing the activation of infiltrating monocyte-derived macrophages in mice. This finding supports a dual role of TLR9 engagement, which depends on the target T-cell type [41]. LPS, DNA, SFA, amyloid cholesterol, cathepsin κ, and reactive oxygen species (ROS) and so on have been suggested as NLPR3 activators, which comprise the NOD-like receptor NLRP3, the apoptosis-associated speck-like protein containing a caspase recruitment domain, and the effector molecule procaspase inflammation [42].

KCs likely derived from infiltrating monocytes express MHC-II antigens and costimulatory molecules (CD80 and CD86), which can present foreign antigens to the reactive T cells, induced T cell responses, and thus conferred tolerance to induce regulatory T cells in immune response [28]. IL-10 and PDL-1 (also known as CD274) participated in the immune tolerance, which reduce the antigen-presenting capacity of KCs by downregulating the expression of MHC molecules and costimulators, but without strongly affecting the scavenger function of KCs.

KCs not only can interact with T cells but can also interact with many cellular components in the liver. For instance, KCs can initiate the recruitment of other monocytes to the liver in case of injuries, which is important for liver regeneration, and they also interact with hepatic stellate cells (HSCs) to play a role in liver diseases and repair [43, 44]. TLR4 signal on KCs indirectly silences patrolling NK cells by MYD88-dependent IL-10 secretion, whereas TLR2 or TLR3 induces IL-18 and IL-1β, leading to NK-cell activation in liver inflammation [45]. Traditionally, M1 macrophage phenotype is marked by the release of pro-inflammatory cytokines like TNF-κ, IL-1, and IL-12. Alternative activation of M2 phenotype is more heterogeneous, as different stimuli are main to release anti-inflammation cytokines (such as IL-10). Typically, the increased expression of arginase 1, the secretion of immune-modulatory cytokines (such as IL-10 and TGF-κ), and the involvement in tissue repair phase are considered as indicators of M2 macrophage differentiation. Different origin of the cells together with the functional plasticity of macrophages can explain the phenotypic and functional heterogeneity of KCs observed upon different triggers of liver pathology [46, 47]. On the basis of these concepts, in the next sections, we summarize the role of KCs to various diseases involving the liver, in particular infectious disease, fatty liver disease, liver fibrosis and cirrhosis, ischemia and reperfusion (I/R) injury, liver cancer as well as liver transplantation immunology (**Figure 2**).

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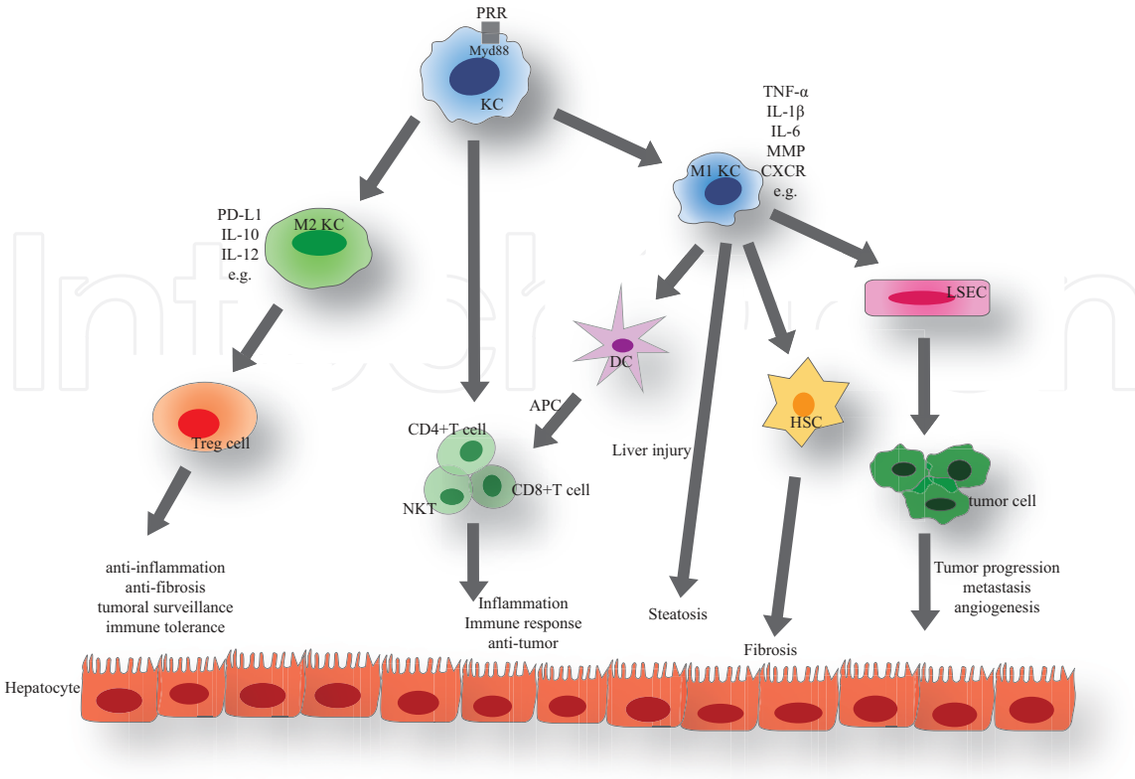

Figure 2. KCs interact with other cells in liver diseases and have the bidirectional function.

2. Kupffer cells in infectious disease

KCs and the sinusoidal endothelial cells are the first barriers for pathogens to enter the liver via the portal vein [48]. Their endocytic capacity, the expression of different PRRs, MHC, and costimulatory molecules, and the ability to produce a variety of physiologically active substances (mediators of the inflammatory process) when they were stimulated make them as the potent immune cells that aim to either pathogen clearance or persistence. The liver is constantly exposed to non–self-protein which is derived from nutrients or microbiota, and bacterial endotoxins would trigger immune response to induce inflammation. These pathogens may activate KCs that lead to produce anti-inflammation cytokines and chemokines for the inhibition of pathogen replication, or recruit and activate other immune cells to liver to participate in the inflammation reaction. So the inflammation process is a multifactor and multicell interaction to participate in. In this process, KCs can recruit other immune cells such as monocytes into the liver, which are then polarized into regulatory IL-10^{+}IL-12^{-}DCs by hepatocyte growth factor [49], macrophage colony-stimulating factor (M-CSF) [50], through inducing activation of the signal of STAT3 and SMAD, then blocking NF-κB [51], and then producing anti-inflammation cytokines. At the same time, stimulation of the body-wide DCs response by the administration of Fms-related tyrosine kinase 3 ligand (Flt3L), granulocyte colony-stimulating factor (G-CSF), or granulocyte-macrophage colony-stimulating factor (GM-CSF) reverses endotoxin-related immunoparalysis that probably over produces

unprimed myeloid cells, which in turn are capable of developing into TNF-IL-12-DCs after stimulation with LPS and other pathogens [52]. This approach may effect on patients with acute-on-chronic liver failure to overcome immunoparalysis [53].

NK cells are important during liver inflammation, TLR2 or TLR3 signal on KCs are activated to induce cytokines IL-18 and IL-1β production, then lead to NK cells activation to immune responses [54]. The chemokine CXCL16 secretion from KCs could guide the $CXCR6^+$ NKT-cell trafficking in the liver to regulate immune responses during microbial infection, and KCs might interact with patrolling NKT cells via glycolipid receptors such as CD1d to produce pro-inflammation cytokines IL-4, IFN-γ, and then provide cytotoxic activity [55–57]. When KCs were activated, they become immunogenic to induce CD8 T cells activation, and the generation of efficient CLT response [58, 59]. Thus, during liver infection, KCs support the development of antimicrobial T cell responses. Besides CD8 T cells responses, recent studies describe that naive CD4 T cells also could be activated in the murine liver disease [60].

The interaction of KCs with membrane-bound as well as soluble mediators expressed by infiltrating immune cells probably leads to further regulation of KCs function. Several studies have reported the involvement of adhesion molecule vascular endothelial growth factor-1 (VEGF-1), which is expressed by KCs, in liver inflammation. In common with endothelial cells which express both VCAM-1 and VEGF-Rs, KCs also could express several antigens that functionally regulate the bioactivities of KCs, including cytokine activation and production, cytoskeleton rearrangement, survival, and chemotaxis in liver inflammation [61–68]. The infiltration of neutrophils is commonly seen in all types of liver disease, especially in liver inflammation [69]. Neutrophils also could activate KCs and endothelial cells, leading to upregulation of cellular adhesion molecules such as ICAM-1, VCAM-1, or VAP-1 to induce neutrophils infiltration and endocytose the microbe [70]. Furthermore, KCs might play a dual effect in liver inflammation, and pathogens may exploit the tolerogenic capacities of KCs to evade immunity and may have evolved to inhibit the immunogenic functions of KCs. Then, we provide examples of the various roles of KCs in bacterial, viral, and parasitic infection.

2.1. Liver infection by bacteria

Kupffer cells act as sentinels capturing antigens and pathogens and are key contributors of host defense against enteroinvasive bacteria [5]. *L. monocytogenes* (Lm) is a very well-characterized facultative intracellular model microorganism [71]. Lm, which could be captured by KCs, triggers a massive recruitment of monocytes leading to the formation of liver Lm-containing microabscesses 2–3 days post inoculation [72]. These microabscesses contain M1 macrophages, TNF/iNOS-producing dendritic cells (Tip-DCs), and neutrophils to play a critical role in the rapid control of the infection [73, 74]. Infected KCs secrete inflammation mediators such as IL-1β and IL-4 to inhibit proliferation of the microorganism [75]. At the same time, infected KCs could secret chemokines such as MIP-1α (CCL3), MIP-1β (CCL4), MCP-1 (CCL2), and MIP-2 (CXCL2/-3), leading to "pro-inflammatory" M1 macrophages that express the chemokine receptor CCR2 recruitment to the liver, which egress from the bone marrow, then control the infection [76, 77]. But some studies indicate that KCs undergo a rapid necroptotic death upon the first hours of their infection by Lm. KCs necroptosis triggers

hepatocytes to release the alarmin interleukin-33 (IL-33), which triggers basophil IL-4 production [78], then in turn causes recruited monocyte-derived macrophages to proliferate and shift from an M1 to M2 phenotype. This allows ultimately the replacement of dead KCs by M2 macrophages after Lm infection. During infection with Lm, tissue-resident KCs are quantitatively replaced by monocytes, which develop into tissue-resident macrophages. The lethal irradiation also led to the replacement of embryo-derived KCs by bone-marrow-derived macrophages, which acquired a highly similar cell identity as indicated by the adoption of a KCs characteristic global enhancer landscape. Initially, these cells contribute to antibacterial immunity in a typical IFN-γ-driven inflammatory response. In the second phase, KCs necroptosis also initiates a cascade of IL-4-driven events inducing proliferative expansion and phenotypic changes of monocyte-derived macrophages that promote restoration of tissue integrity after bacterial clearance. Similar results were obtained with the enteroinvasive bacterium of *Salmonella enterica* [79]. This is a new field of investigations for infection control and tissue return to homeostasis.

When liver infection with *Francisella tularensis* occurs, it is able to infect and replicate within Kupffer cells which release pro-inflammatory cytokines TNFα, IL-1β, and IL-6, leading to sepsis [80]. But hepatocytes as well as dendritic cells may support the intracellular replication of *F. tularensis* without undergoing proptosis or apoptosis, because the hepatocytes could release chemokines FKN to reverse this process [81, 82]. So, KCs inactivation or depletion results in impaired bacterial clearance. Although KCs play a critical role in infection, various studies indicate that the actual elimination of the bacteria taken up by the liver depends on a complex interaction of KC and other inflammation cells.

2.2. Liver infection by viruses

Both hepatitis B virus (HBV) and hepatitis C virus (HCV) are blood-borne viruses, when infected by them can result in chronic liver disease with an increased risk for liver fibrosis/cirrhosis, hepatic failure, and liver cancer [83, 84]. Studies suggested that hepatic macrophages played an important role in viral hepatitis. KCs have a beneficial antiviral effect on the early phase after infection. During systemic viral infection, liver resident KCs are essential for the efficient capture of the virus and preventing viral replication. The next involves fast induction of an antiviral status in KCs by producing IFN-γ and prevents viral spread to neighboring hepatocytes [85, 86]. Activated KCs express high levels of immunogenic MHC II and can thereby activate virus-specific $CD4^+$ T cells in liver; $CD4^+$ T cells also can produce IFN-γ in response to antigen exposure. At the same time, under an antiviral status, this might enhance the phagocytic capacity of KCs, which might additionally contribute to control virus replication [87, 88]. Some studies make use of a short-term LCMV-Cl13 infection in mice to examine phenotypic and functional changes in inflammatory monocytes and F4/80-high-Kupffer cells instead of virus infection animal models; these cells are the first innate immune cells to encounter a viral pathogen in liver. They observed F4/80-high-Kupffer cells, which maintain their endocytic activity and increase the expression of several pro- and anti-inflammatory cytokines and chemokines after LCMV infection. KCs from LCMV-infected mice clearly show the induction of pro- and anti-inflammatory cytokines and chemokines,

including TNF, IL-6, IL-10, MCP, CXCL-10, and others. The active uptake of LCMV by KCs limits viral spread and immunopathology [89, 90].

In human body, when they are infected by HBV particles and HBs, the virus induces IL-1β, IL-6, IL-18, CXCL8, and TNF production by human $CD68^+$ KCs via NF-κB activation leading to NK cell activity and then NK cells produce IFN-γ, which plays an important role in anti-viral immunity.

KCs have two functional AIM2 and NLRP3 inflammasomes, and that AIM2 production of IL-1β and IL-18 is essential for IL-8 transcription as well as activating liver and peripheral blood NK cells, respectively [91, 92]. Some studies demonstrated that rat $ED1^+$-adherent KCs exposed to HBV virus hardly expressed IL-1β, IL-6, or TNF, but produced the immuno-regulatory cytokine TGF-β, because hepatitis B surface Ag blocks IRF7 binding to the AIM2 promoter. Targeting AIM2 prevents the recognition of dsDNA expressed by the HBV, and that the limited innate response observed upon HBV infection may be due to viral-mediated immune evasion [93, 94]. Another link between hepatic inflammation and disease in patients with chronic HCV was attributed to IL-1β secretion following the activation of the NLRP3 inflammasome in liver macrophages ($CD68^+$/$CD14^+$) [95].

Chronic infection associated with hepatitis B virus (HBV) is a major cause of liver fibrosis and cirrhosis. The activation of NADPH oxidase during the phagocytosis of HBV particles, and signal transducers and activators of transcription-3 (STAT-3) binding to elements in the TGF-β promoter may also be involved to increase TGF-β production. So KCs could produce the pro-fibrogenic/anti-inflammatory cytokine TGF-β rather than the pro-inflammatory cytokines IL-6, IL-1, and TNF-α. This may partly explain why overt liver fibrosis is still present when chronic hepatitis B virus infection occurs with minimal (or no) necroinflammation [93, 96, 97]. KCs in the HBs-Tg mice expressed higher level of CD205 and produced greater amounts of interleukin (IL)-12 than did those in the WT mice. Depletion of KCs, neutralization of IL-12, or specific silencing of CD205 on KCs significantly inhibited CpG-oligodeoxynucleotides (CpG-ODN)-induced liver injury and NKT cells activation in the HBs-Tg mice. These data CD205-expressing KCs respond to CpG-ODNs and subsequently release IL-12 to promote NKT cell activation. Activated NKT cells induce liver damage through the Fas-signaling pathway in HBs-Tg mice [98].

HCV infection also could make KCs and liver-infiltrating lymphocytes the major sources of TGF-protein, leading to liver fibrosis [99]. The cellular protein, glucose-regulated protein 94 (GRP94), which is directly mediated by NF-κB activation to interact with HCV E2, plays an important role in TGF-protein induction, suggesting that GRP94 is a potential target for the development of drugs that prevent hepatic fibrosis caused by HCV infection. Moreover, TGF plays a pivotal role in the generation of Treg cells from precursor cells, such that a GRP94-inhibiting drug would also likely boost immunity against HCV infection by blocking the induction of Treg cells, which direct the immune tolerance against HCV [100, 101].

KCs with heme are metabolized and detoxified by heme oxygenase-1 (HMOX1) to carbon monoxide (CO), biliverdin, and free iron (which induces ferritin). The HMOX1 and metabolites of heme besides possessing anti-inflammatory and antioxidant properties have been

noted to have antiviral effects in hepatitis C-infected cell lines. Additionally, these substances have been shown to enhance the response to IFN-α by restoring interferon-stimulated genes (ISGs) [102].

Only few studies on HEV-infected animals and humans have been published. But through immunohistochemistry, HEV antigens were detected mainly in KCs and liver sinusoidal endothelial cells, partially associated with hepatic lesions and infiltrates of CD3-positive cells. Since KCs and liver sinusoidal endothelial cells have antigen-presenting functions, they may also play a role in the host defense mechanisms and immunopathogenesis [103, 104].

In contrast to HBV and HCV, infection of HAV is self-limiting and does not induce chronic infectious disease. HAV reaches hepatocytes via KCs that bind complexes of HAV- and HAV-specific IgA antibodies via the Fcα receptor [105], and subsequently transfer the virus to hepatocytes. Different from HBV and HCV, HAV requires the disruption of host cell membranes to release its progeny. These dying hepatocytes may provide DAMP, which can be recognized by KCs and other intrahepatic immune cells, leading to the activation of these cells that can overcome viral immune escape and liver-intrinsic tolerogenic mechanisms [106].

2.3. Liver infection by parasites

Infection by the Echinococcus larval stages (larval echinococcoses) can affect humans [107], which are thus accidental to be intermediate hosts. Intermediate host infection occurs after the ingestion of eggs (passed out with the definitive host feces), which hatch releasing oncospheres that penetrate the intestinal wall, and then are carried by blood or lymph to organs. Lectins are central players in innate immune to pathogens. A screen among lectins known to be expressed in mammalian macrophages identified only the mouse Kupffer cells receptor (KCR; CLEC4F) as a lectin able to bind the *Echinococcus granulosus* LL [108]. KCs in particular are known to be tolerogenic, as opposed to conventional priming in the lymph nodes draining the organ [52]. Thus, the new data are consistent with the hypothesis that the LL carbohydrates are evolutionarily optimized for ensuring the clearance of shed LL particles by KCs. This hypothesis includes the possibility that KCR engagement favors the KCs release of anti-inflammatory mediators to participate in the infectious process to alleviate the liver injury [109–111].

Infection by the protozoan parasite *Entamoeba histolytica* causes hepatocyte damage in focal areas leading to amebic liver abscess (ALA). Selective depletion of KCs using liposome-entrapped clodronate or the inhibition of monocytes infiltration using CCR22/2 mice revealed that KCs and inflammatory Ly-6Chi monocytes, through producing TNF-α, are the main effector cells responsible for liver destruction during ALA [112].

KCs also represent the port of liver entry for Plasmodium and Leishmania, which parasitize KCs and then infect other liver cells [113]. Parasites enter into the skin after a mosquito bite, and the rapid migration of sporozoites allows them to escape clearance by local tissue phagocytic cells and to enter lymphatics and blood vessels. Via the blood, sporozoites rapidly reach the liver and, after gliding on HSPG in liver sinusoids, they use circumsporozoite protein (CSP) and thrombospondin-related anonymous protein (TRAP) to bind to KCs.

KCs are the potent target of *Leishmania donovani* amastigotes; early studies identified this on the basis of KCs characteristic morphology and anatomical position within the sinusoids [114, 115]. In these processes, hepatocytes infection is through KCs [116], indicating that these parasites use KCs to overcome the sinusoidal barrier and, ultimately, to infect hepatocytes. TREM2 expression by KCs appears to be an important determinant in resistance to liver-stage infection against Plasmodium parasites [117]. Once invading a hepatocyte, the parasites develop into merozoites, which will be released from the liver to infect erythrocytes [118]. Taken together, these data show that sporozoites not only use their migratory capacity to escape elimination by phagocytic and immune cells but also interact with and use KCs to increase their efficiency at infecting hepatocytes.

3. Kupffer cells in fatty liver disease

KCs have been implicated in various liver diseases with different etiologies that are associated with metabolic complications, such as over-nutrition, and may lead to fatty liver disease. Nonalcoholic fatty liver diseases (NAFLDs) are a series of disorders that include nonalcoholic fatty liver (NAFL), steatosis with inflammation, and nonalcoholic steatohepatitis. NAFLD could cause insulin resistance and is known to increase morbidity and mortality, particularly due to an increased cardiovascular risk [119–121]. KCs, liver-resident macrophages, display a critical mediator in the development of NAFLD. PAMPs and DAMPs are well known to be able to activate various Toll-like receptors (TLR) such as TLR2, 4, and 9 present on KCs, by recruiting MyD88 and engaging MAP kinases and activating NF-κB signaling, and could be responsible for the inflammatory reaction at different disease stages. Obese and steatotic patients corroborate the observation highlighting an increased CD68 mRNA of KCs with obesity, and upregulation of many other genes such as chemoattractant protein-1 (MCP-1), which is also named chemokine ligand 2 (CCL2). So CCR2-deficient animals show decreased steatosis. Soluble CD163 would also correlate with nonalcoholic fatty liver disease activity and fibrosis. Deletion of ED2-positive KCs by GdCl3 or clodronate attenuates pro-inflammatory and profibrogenic cytokines release, thereby protecting fatty livers from progression to NAFLD [122–125].

More recently, it was shown that over-expression of CD14, a coreceptor of TLR4, in KCs of mice with high-fat diet (HFD)-induced steatosis increased the hypersensitivity to low-dose LPS [126]. TLR4 in KCs mediates the progression of simple steatosis to NAFLD, by inducing ROS-dependent activation of X-box–binding protein-1 [127]. When KCs are activated by LPS through TLR4, they display an M1 TNF-expressing pro-inflammatory phenotype and increase triglyceride accumulation, decrease fatty acid oxidation and insulin responsiveness of hepatocytes. KC-derived TNF production seems to be central in NAFLD development, when silencing liver TNF or using TNFR1/2-deficient mice attenuating liver steatosis compared with wild-type mice [128, 129].

NOD-like receptors of KCs (NLRs) are intracellular PRRs that are part of the inflammasomes briefly mentioned above. Inflammasomes are multiprotein complexes that through NLRs sense intracellular danger signals and initiate an activation cascade of events that culminate

with autoactivation of caspase 1 and cleavage of promoting IL-1κ and IL-18 production. By controlling the release of these important inflammatory cytokines, inflammasomes play an important role in the inflammatory process underlying NAFLD [130].

Interestingly, it was recently shown that IL-10 released by activated KCs stimulated apoptotic death of pro-inflammatory cells [131]. This mechanism mediated resistance to hepatocyte steatosis and subsequently death. Fatty liver disease mechanism caused by excessive alcohol consumption is similar as NAFLD. In the same way, the depletion of KCs in mice also attenuates alcohol-induced diseases. Then it demonstrated a central role of KCs in fatty liver diseases [132].

4. Kupffer cells in liver fibrosis

Fibrogenesis development has many pathological factors, such as inflammation derived from Kupffer cells, angiogenesis, and hepatic stellate cell (HSC) activation, and interacts with each other, leading to collagen deposition. Cirrhosis is the most advanced stage of fibrosis, with septa and nodule formation being the most notable features [133]. KCs or resident hepatic macrophages carry out an important role in modulating inflammation in liver fibrosis development. KCs produce reactive oxygen species, a variety of pro-inflammatory cytokines, such as TNF-α, IL-1β, and macrophage inflammatory protein (MIP)-1, which could provoke HSC activation to produce pro-fibrotic cytokines TGF-β and platelet-derived growth factor (PDGF) and subsequently contribute to hepatic injury [134, 135].

The accumulation of circulating Ly6Chi monocytes within the liver is greatly dependent on CCR2/CCL2 and CCL1/CCR8 axis, in the pathogenesis process, KCs also express multiple chemokines and matrix metalloproteinases (MMP-9, -12, and -13) that recruit immune cells and promote extracellular matrix degradation, thus favoring the resolution of fibrosis [136]. Then, senescent hepatocytes and NF-κB-inducing kinase (NIK) activation in hepatocytes lead to the release of numerous chemokines. These chemokines can influence the migration or activation state of macrophages that in turn induce hepatocyte apoptosis. Accordingly, the NIK in vivo triggers massive liver inflammation and hepatocyte apoptosis leading to liver fibrosis. The fact that on the basis of above experiments KCs depletion using clodronate reversed NIK-induced damage [137, 138].

Some studies indicate that activating CX3CR1 on KCs increases their IL-10 expression and reduces their TNF and TGF-β [139], IL-10 is a potent anti-inflammatory mediator that has been shown to inhibit the production of TNF-α and IL-1 and to suppress the activation of NF-κB . IL-10 reduces macrophage production of nitric oxide (NO) and reactive oxygen intermediates, and also reduces the expression of adhesion molecules and chemokines [140, 141]. Thus, fractalkine (the ligand of CX3CR1) represents a negative feedback on the extension of liver inflammation through affecting KCs.

An antifibrotic effect of liver macrophages was also demonstrated when macrophage infiltration was blocked during the induction of fibrogenesis in rats. Delta-like ligand 4 (Dll4) is a kind of antifibrotic factor. It was expressed in patients' KCs and liver sinusoidal endothelial cells.

In vitro, rDll4 significantly decreased lipopolysaccharide-dependent chemokine expression in both KCs and HSCs. Then the inflammatory cell infiltration and their chemokine ligand 2 (CCL2) expression were significantly reduced in rDll4-receiving bile duct ligation mice. Dll4 expression was inversely associated with CCL2 abundance. Mechanistically, Dll4 regulated CCL2 expression via NF-κB. Taken together, Dll4 modulates liver inflammatory response by downregulating chemokine expression and then participates in the role of antifibrosis of liver [142, 143]. With regard to recovery from fibrosis, KCs and macrophages secrete proteinases that promote the degradation of scarring extracellular matrix proteins.

5. Kupffer cells in liver ischemia-reperfusion injury

Liver ischemia reperfusion (I/R) injury refers to the paradoxic aggravation of ischemic liver resulting from the return of blood flow and oxygen delivery, which is encountered frequently in a variety of clinical situations, including liver transplantation, trauma, hepatic resection, or hypovolemic shock. If hepatic I/R injury progresses out of control, it can lead to liver failure, systemic inflammatory response syndrome, and multiple organ failure, and lastly leading to death [144, 145]. Oxidative stress is the major contributor for I/R-induced injury, so the therapeutic strategies to antioxidants have gained interest. In I/R injury, KC activation is presumed to occur first, resulting in generation of reactive oxygen species (ROS) and preinflammatory cytokines such as TNF-α, IL-1β, nitric oxide, and chemokines, which contribute to hepatocyte death, endothelial damage and recruitment, and activation of leukocytes [146].

KCs secrete CCL2 to promote CCR2-expressing neutrophil recruitment from the bone marrow and subsequent infiltration into the liver during I/R [147], and secrete matrix metalloproteinases (MMPs) to increase graft dysfunction [148]. In this process, platelets could be adherent to the KCs, which reflect the activation of KCs and lead to leukocyte accumulation affecting sinusoidal perfusion, causing liver failure [149].

Large amounts of endotoxin contact KCs through the portal circulation following IR after liver transplantation. The LPS first binds to CD14, triggering KCs activation, then integrates with TLR4, and further increases the expression of CD14, the activation signals are transduced into cytoplasm, resulting in NF-κB nuclear translocation and cytokines such as TNF-α and IL-6 release, harming the liver graft. TLR4 knockout mice are protected from endothelial overactivation in the absence of KCs after IR injury [150]. At the same time, endoplasmic reticulum (ER) stress of KCs in evoking liver inflammation following reperfusion contributed to the conversion of natural Tregs to Th17 cells due to IL-6 release, resulting in liver injury [151]. Whereas the inhibition of high-mobility group box 1 production by KCs after I/R in rats could prevent liver injury [152], suppression of TNF-α–mediated apoptotic signaling by glutathione (GSH) pretreatment can attenuate hepatic I/R injury in young and aged rats [153].

In IR injury, activated KCs could produce pro-inflammation cytokine IL-18, blocking of IL-18 by IL-18-binding protein may inhibit KCs activation, resulting in a reduction of KC-derived harmful stimuli, then ameliorates I/R injury [154]. KCs also could protect liver grafts against liver-transplant–induced I/R injury. The protection appears to be mediated by the release

of anti-inflammatory IL-10 and the production of antioxidant heme oxygenase by KCs [41, 155]. The IL-10 secreted by KCs controls pro-inflammatory mediators released from LSEC in response to LPS challenge, KCs depletion has also been shown to impair hepatic IL-10 production after partial hepatectomy. Pretreatment with IL-10 protects steatotic livers undergoing I/R, and that active KCs retain a hepatoprotective role in the steatotic environment [156, 157].

Heme oxygenase-1 (HO-1) is a rate-limiting enzyme of heme degradation, exerts antioxidative, antiapoptotic, anti-inflammatory, and vasoactive effects through its byproducts or itself. HO-1 and its byproducts (CO, biliverdin, and iron ion) induction could protect the graft from IR injury after liver transplantation in several experimental studies [158]. Our study also has the same results. Our results of immunofluorescence also demonstrated that preconditioning with Nodosin perfusion induced HO-1 expression mainly in KCs at 24 h after transplantation [159] (**Figure 3**). HO-1 upregulation in KCs plays a protective role in modulating immune responses of I/R-injured tissues, or reducing apoptosis induced directly by TNF-α [160]. Preincubation of KCs with CO upregulated heat-shock protein 70 (HSP70) and inhibited ROS generation. CO-pretreated liver grafts showed less upregulation of TNF-α and inducible nitric oxide synthase messenger RNA (mRNA), reduced expression of pro-apoptotic B cell lymphoma 2-associated X protein mRNA, cleaved caspase-3, and poly(adenosine diphosphate ribose) polymerase. So, pretreatment of donors with CO ameliorates LT-associated I/R injury with increased hepatic HSP70 expression, particularly in the KCs population [161].

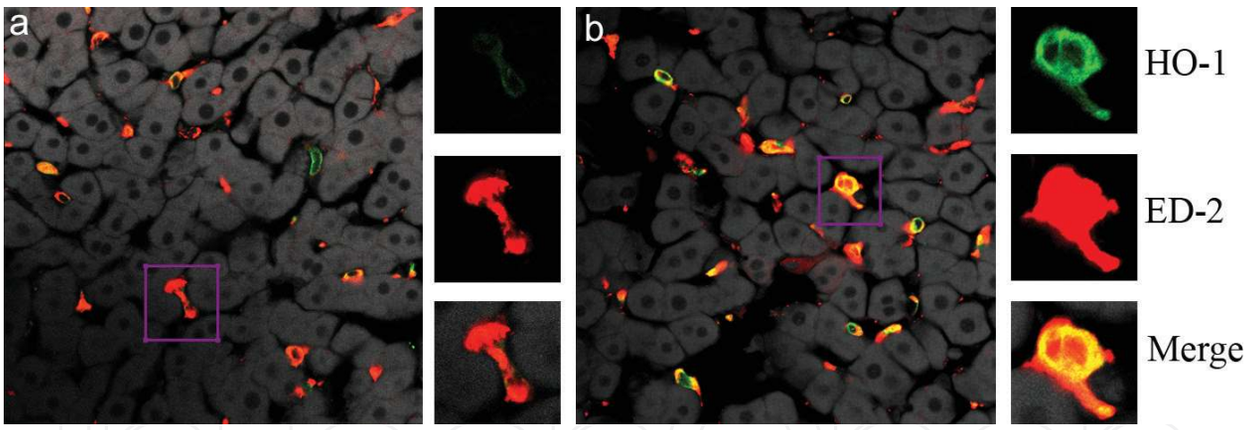

Figure 3. Immunofluorescence double staining for cellular localization of heme oxygenase 1 (HO-1) expression in the rat liver after nodosin perfusion. Liver sections are stained for HO-1 (green) and the Kupffer cell marker ED2 (red). Colocalization of these two colors can be recognized by the yellow color. (a) Control group; (b) *Nodosin* group; (x40) [165].

6. Kupffer cells in liver transplantation immunology

Liver transplantation is an effective treatment for advanced liver diseases, but immune rejection is a major obstacle after transplantation. KCs not only can engulf and kill pathogenic microorganisms, rid of endotoxin, but also have effects of antigen presentation, secretion of cytokines, and immune regulation. They can express high levels of MHC and costimulatory molecules and are capable of activating naive T cells [17]. At the same time, they could be

activated by antigen to produce T1 cytokines IL-2, IL-1, IL-6, TNF-α, and IFN-γ. They interact with the recipient T cells that migrate into the graft and play an important role in immune response [162]. Furthermore, the replacement of KCs by recipient bone marrow-derived cells (BMDCs) was observed in the liver graft, and functional inhibition of KCs by GdCl3 abrogated prolonged survival. Analysis of mRNA expression levels in liver grafts showed a shift of the Th1/Th2 balance toward reducing rejection in the BMC groups. So replacement of KCs by recipient BMDCs may play an important role in this mechanism of inhibiting rejection [163].

After liver transplantation, the reduction of B7 expression in donor KCs could suppress the activation of recipient T lymphocytes and secretion of IL-2 via the CD28/B7 costimulatory pathway and may induce immune tolerance [164]. The cytokines TNF-α expression in KCs is a marker of activated KCs after transplantation and it may be a good target for reversing acute rejection post transplantation [165]. GdCl3 depletion of KCs also plays a protective role in liver transplantation through suppressing bile duct cell apoptosis, including decreasing expression of ALT, ALP, TBIL, and TNF-α, and suppressing Fas-FasL-Caspase signal transduction [166].

KCs not only play a role in immune response directly but also activate the immature DCs or recruit immature DCs to liver to mature DCs to take part in immune response, by producing pro-inflammation cytokines and chemokines .Then, mDCs could express costimulatory molecules highly and present antigen to T cells [167, 168].

Recently, it has found that KCs can induce T lymphocyte apoptosis and play an important role in the regulation of liver transplantation tolerance. They also could produce high levels of Th2 cytokines IL-10 and TGF-β and low levels of IL-12 to protect the graft [169]. Although KCs can promote immature DCs to mature DCs as immunogenic APCs, they are frequently accompanied by an upregulation of PD-L1 [170], release of IL-10 and TGF-β [171], prostaglandin E2 (PGE2) [172], IDO [173, 174] and/or arginase [175], which inhibit DC-mediated T cell activation within the sinusoids, and the presentation of high-affinity peptide by KCs results in the deletion of $CD8^+$ T cell tolerance. Furthermore, they promote the suppressive capacity of Tregs ($CD4^+CD25^+FoxP3^+$ T cells) toward hepatic antigens to induce tolerance [176]. KCs could also recruit TH17 cells and also $\gamma\delta$ T cells are facilitated by CCR6 and possibly also CCR4 via CCL17, CCL22, and CCL20. A broad variety of chemokine receptors have been linked to Treg cell migration (e.g., CCR1, CCR4, CCR5, and CCR6) showing a functional tolerance [28]. KCs mediate $CD8^+$ T cells apoptosis by expressing Fas ligand (FasL), which can ligate Fas on $CD8^+$ T cells [177]. V-set and Ig domain-containing 4 (VSIG4, CRIg, or Z39Ig), a newly identified B7-related cosignaling molecule, exclusive expression on liver KCs is a complement receptor for C3b and iC3b and a coinhibitory ligand that negatively regulates T-cell immunity, $VSIG4^+$ KCs play a critical role in the induction and maintenance of liver T- and NKT-cell tolerance [178]. So, KCs have a dual effect after liver transplantation immunology

7. Kupffer cells in liver cancer and metastases

Persistent hepatic inflammation resulting from hepatitis B or C virus infections (HBV or HCV, respectively), NAFLD, or alcohol abuse is a hallmark feature of chronic liver diseases

and appears to be an essential prerequisite of hepatocarcinogenesis. The results of this activation involve the production of multiple inflammatory cytokines, ROS, growth control mediators, various chemokines, which orchestrate the interaction between parenchymal and nonparenchymal liver cells, especially KCs to be activated in the process of hepatic carcinogenesis. They are also involved in the enhancement of clonal expansion of preneoplastic cells, then leading to neoplasia [179]. In diethylnitrosamine-induced HCC in mice, pro-inflammatory activation of KCs during the early stages of chemical-induced carcinogenesis is important in tumor development. Then, the antitumor effects of KCs are widely studied, such as to release TNF-α and iNO to recruit cytotoxic T cells and NK cells, to induce apoptosis of cancer cells and phagocytose cancer cells [180]. And some studies demonstrated that the expression of TREM-1 by mouse KCs plays a crucial role in their activation upon the recognition of necrotic hepatocytes and tumor cells [181]. Activated KCs suppress tumor cells through the ADCC pathway via FcγRIII (CD16) and directly or indirectly by cytokines. The existence of CD16a in KCs and that the activation of KCs, which mainly resulted in CD16a expression, then via NK cells, mediated ADCC reactions to induce NK cell cytotoxicity to tumor cells.

The activated KCs kill target cells directly by swallowing and releasing lysosomal enzyme, NO, and peroxidase; they also cooperate to resist tumor cells by secreting cytokines including TNF-α, IL-1, IL-6, and granulocyte-macrophage colony-stimulating factor (GM-CSF) [182, 183]. IL-6 is highly produced by KCs, it has been related with tumor progression and angiogenesis in several tumors, and it is overproduced in HCC. So decreasing the IL-6 production by KCs inhibits hepatocellular carcinoma growths [184]. KCs derived from male but not female SART1+/− mice produced increased levels of the hypoxia inducible factor (HIF-1)-dependent chemokine (RANTES) and cytokine promoting oxidative damage and inflammation, driving progression to hepatocellular carcinoma. Reventing inappropriate HIF-1 activation in male mice, as a novel therapeutic target for hepatocellular carcinoma [185, 186].

KCs play an essential function in the host tumoral surveillance system. Their strategic position in liver allows them to discriminate and remove neoplastic cells that develop in liver. Besides primary liver cancer, liver metastases are frequently observed, especially in gastrointestinal malignancies. The metastatic cells migrate via the bloodstream into the portal circulation, and they are entrapped in the liver sinusoids [187]. KCs play an important role in tumor growth, angiogenesis, and metastasis through the production of a number of growth factors (PDGF-β, vascular endothelial growth factor (VEGF), TGF-β, and EGFR ligands), cytokines (IL-6, TNFα, and IL-10), chemokines (CCL17, CCL22, CCL24, CXCL12, and IL-8), as well as other soluble factors (MMPs, osteopontin, and cyclooxyganse-2). In the liver, CEA binds with heterogeneous nuclear RNA-binding protein M (hnRNP M) receptor on KCs and causes activation and production of pro- and anti-inflammatory cytokines including IL-1, IL-10, IL-6, and TNF-α. These cytokines affect the upregulation of adhesion molecules on the hepatic sinusoidal endothelium and protect the tumor cells against cytotoxicity by nitric oxide (NO) and other reactive oxygen radicals. This activation is the key to the role of CEA in liver metastasis. A large number of clinical studies have shown correlations between serum CEA levels and advanced colorectal cancer, in particular, in the presence of liver metastasis [188].

KCs were found to have promoted tumor invasion and exacerbated the metastasis and they are responsible for the accumulation of liposomes. In the metastatic hepatic cancer, KCs taking up liposomes were significantly increased, and PEGylated can reverse this result through a reduction in tumor-supportive KCs [189]. Primary pancreatic tumor cells release exosomes that contain migration inhibitory factor (MIF) into the blood circulation. These PDAC-derived exosomes are selectively taken up by liver KCs, leading to the MIF-dependent production of fibrotic cytokines by KCs. These fibrotic cytokines, particularly TGF-β, activate liver HSCs to produce fibronectin. Deposition of fibronectin in the liver leads to the formation of a fibrotic microenvironment that promotes the recruitment of bone marrow-derived cells. These sequential events establish a premetastatic niche, which permits the survival and proliferation of disseminated PDAC cells and the formation of metastases in the liver [190]. Some studies demonstrated that KCs could help metastatic cancer cells extravasate from vessel via CXCL12/CXCR4 pathway.

KCs in liver can interact with myeloid-derived suppressor cells (MDSCs) and cause their upregulation of PD-L1, a negative T cell costimulatory molecule, and ultimately lead to tumor immunosuppression in accordance with further tumor progression and metastasis. They can suppress $CD8^+$ T cells function via B7-H1/programmed death-1 interactions, which diminishes antitumor effect of $CD8^+$ T cells. The metastatic tumor cells entering the liver from portal vein triggered KCs and mediated also upregulation of vascular endothelial cell adhesion receptors, such as E-selectin to help metastatic tumor cells arrest and extravasate [191–193]. KCs themselves are controversial, in metastatic colon tumors, the cytokines produced by KCs (IL-12 and IFN-α) are indeed important for the activation of NK cells and NKT cells and for preventing tumor liver metastases, depletion of KCs by gadolinium chloride or clodronate liposomes increased the number of liver metastasis in some reports [194]. Other studies have demonstrated that KCs induce Fas expression in colon cancer cells and malignant glioma cells leading to Fas-mediated apoptosis and death in the presence of tumor-infiltrating lymphocytes or TNF-α [195].

8. Conclusion

Kupffer cells have various functions in liver injury and repair. KCs, as liver-resident macrophages, localize within the lumen of the liver sinusoids and are adherent to the endothelial cells that compose the blood vessel walls. They are the first immune cells in the liver that come in contact with the gut bacteria, gut bacterial endotoxins, and microbial debris derived from the gastrointestinal tract that have been transported to the liver via the portal vein. They also interact with other hepatic cells to play an essential role in the host defense. They are responsible for the development of liver diseases including infectious disease, fatty liver disease, liver fibrosis and cirrhosis, ischemia and reperfusion injury, liver transplantation immunology as well as liver cancer. But KCs express various phenotypes to have various functions. Because of the highly overlapping characteristics of these cells, their functions are controversial. The complex roles of KCs in both protective and harmful responses make the liver diseases treatment interesting but difficult. So, further efforts should therefore focus on regulatory mechanisms in specific subpopulations of KCs differentiation and function.

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