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Kupffer Cells in the Liver

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Abstract

Kupffer cells are a critical component of the mononuclear phagocytic system and are central to both the hepatic and systemic response to pathogens. Kupffer cells are reemerging as critical mediators of both liver injury and repair. Kupffer cells exhibit a tremendous plasticity; depending on the local metabolic and immune environment, then can express a range of polarized phenotypes, from the proinflammatory M1 phenotype to the alternative/M2 phenotype. Multiple M2 phenotypes can be distinguished, each involved in the resolution of inflammation and wound healing. Here, we have provided an update on recent research that has contributed to the developing delineation of the contribution of Kupffer cells to different types of liver injury, with an emphasis on alcoholic and nonalcoholic liver diseases. These recent advances in our understanding of Kupffer cell function and regulation will likely provide new insights into the potential for therapeutic manipulation of Kupffer cells to promote the resolution of inflammation and enhance wound healing in liver disease.

Physiology of Kupffer Cells

Introduction

Kupffer cells, the resident macrophage in the liver, comprise the largest population of resident tissue macrophages in the body. First described by Karl Wilhelm von Kupffer in 1876 as “sternzellen” (star cells or stellate cells), Kupffer cells were first thought to be a part of the endothelium of the liver blood vessels. It was not until 1898 that Tadeusz Browiec correctly identified them as macrophages (92). Kupffer cells play a critical role in the innate immune response; their localization in the hepatic sinusoid allows them to efficiently phagocytize pathogens entering from the portal or arterial circulation. Kupffer cells also serve as a first line of defence against particulates and immunoreactive material passing from the gastrointestinal tract via the portal circulation and may be considered as a final

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component in gut barrier function. Kupffer cells thus play a major anti-inflammatory role by preventing the movement of these gut-derived immunoreactive substances from travelling past the hepatic sinusoid. Kupffer cells are also highly poised for clearance of particles, as well as dead and dying erythrocytes and cells in the hepatic parenchyma, from the systemic circulation. Kupffer cells thus comprise the major phagocytic activity of what was classically termed the reticular-endothelial system and now more properly called the mononuclear phagocytic system (139).

A change in the functional activity of Kupffer cells is associated with a variety of disease states. While Kupffer cells can be protective in a number of situations, including drug-induced liver injury (56) and toxin-induced fibrosis (112); dysregulation in the precise control of inflammatory responses in Kupffer cells can contribute to chronic inflammation in the liver, including alcoholic and nonalcoholic fatty liver diseases (NAFLDs/NASH) (17, 91). In this review, we will review the contribution of Kupffer cells and other hepatic macrophages in both health and disease.

Origin of Kupffer cells

In adult animals, monocytes in the peripheral circulation, originating from precursor cells in the bone marrow, are considered to be immature precursors for tissue macrophages (92). Peripheral blood monocytes can enter the liver and then mature into a phenotype characteristic of tissue macrophages. Differentiation of macrophages is regulated by various growth factors, but the role of macrophage colony stimulating factor appears to be the most important for the development of mature Kupffer cells (92). Control of Kupffer cell numbers in the liver is tightly maintained; however, the mechanisms for this control are not well understood. It is clear that the rate of influx of peripheral monocytes into the liver is higher than in other tissues, such as the lung; however, there is controversy over the life span of Kupffer cells in the liver. Studies done in animals depleted of Kupffer cells, either in response to clodronate or in studies of bone marrow transplants, reveal that Kupffer cell replacement to the liver occurs over 14 to 21 days (92). However, the fate of Kupffer cells under physiological circumstances is not understood; it is hypothesized that turnover of Kupffer cells may occur due to programmed cell death (apoptosis) and/or migration to other sites, such as lymph nodes. Very recent data suggest that in response to Th-2 inflammatory signals, such as increases in IL-4, resident macrophages, including Kupffer cells, can be stimulated to proliferate (55).

Localization of Kupffer cells within the hepatic architecture

The liver is a complex organ comprised of a number of highly specialized cell types that are distributed within the sinusoidal structure of the liver. Hepatocytes, which comprise the bulk of the liver, are considered the work horse of the liver and carry out a vast array of metabolic, regulatory, and toxicological functions. The hepatic sinusoid is lined with a specialized liver sinusoidal endothelial cell characterized by the presence of fenestrae. Kupffer cells, as well as other cells of the innate immune system, including natural killer, natural killer-T cells, and dendritic cells, reside within the sinusoid (Fig. 1). The close proximity of Kupffer cells to parenchymal and nonparenchymal cells within the liver supports the ability of Kupffer cells to regulate hepatic function, both in health and disease.

In a healthy liver, the Kupffer cell exhibits what has been termed a “tolerogenic” phenotype. This tolerance is necessary to prevent undesired immune responses in the face of incoming immunoreactive materials into the hepatic sinusoid, including gut-derived materials and also antigens present on dead or dying cells as they are cleared from the circulation in the liver (139). However, under certain disease conditions, the Kupffer cell shifts from this tolerogenic phenotype to a pathologically activated state that is a characteristic of chronic inflammatory diseases. Given the close proximity of the Kupffer cell to parenchymal and nonparenchymal cells within the liver, loss of the tolerogenic state can result in hepatocellular injury and damage. Thus, like many other components of the innate immune system, maintaining the appropriate functional activity of Kupffer cells is critical to maintenance of the healthy organism. Absence or lowered functional activity of Kupffer cells can contribute to pathogen invasion and/or systemic inflammation. In contrast, activation of Kupffer cells, in conditions such as NAFLD/NASH liver disease, results in uncontrolled inflammatory state in the liver. Therefore, as therapeutics are developed to treat chronic inflammatory diseases of the liver, it is critical to develop approaches that normalize or dampens, but does not completely eliminate, the functional activity of Kupffer cells in the liver.

Plasticity of hepatic macrophages

There is a growing appreciation that the mature phenotype of resident tissue macrophages is very plastic, with the functional activity of the macrophage developing based on inputs from both the local metabolic and immune environment, superimposed on the intrinsic differentiation program of the macrophage. In the liver, plasticity can result from a change in the activation state of resident Kupffer cells and/or a recruitment of new monocytes/macrophages to the liver. Much of our understanding of macrophage plasticity comes from resident macrophages in adipose tissue and nontissue macrophages (16, 132). However, recent data in liver confirms that regulation of the functional phenotype of hepatic macrophages is associated with the progression of various liver diseases, including ALD, NASH, fibrosis, and hepatocellular carcinoma (32, 42, 71, 74, 76, 100, 126).

A number of lines of evidence now demonstrate a significant heterogeneity in macrophage activation states (35). In an attempt to classify these activation states, two extremes of macrophage polarization have been designated as M1 and M2. M1 or classically activated macrophages are characterized by increased expression of proinflammatory cytokines, including TNF- α , IL-6, IL-12, and inducible NO synthase (iNOS), while M2 or alternatively activated macrophages, exhibit low expression of proinflammatory cytokines, but increased expression of anti-inflammatory mediators, such as IL10 and IL1 decoy receptor expression (36). The M1/M2 classification is clearly oversimplified, with data demonstrating that even within each of these groupings there is significant heterogeneity. Indeed, the M2 grouping has been further subdivided into M2a, M2b, and M2c. These subclasses are induced by different regulators and exhibit distinct marker proteins on their cell surface, as well as distinct functional activity (88). Of particular interest to studies in liver, additional phenotypes for resident macrophages in the liver have been identified. For example, macrophages associated with the spontaneous resolution of hepatic fibrosis and have been termed scar-associated macrophages (SAMs) by the Iredale group (28). These SAMs are

Gr-1^{hi} and are associated with increased expression of profibrotic cytokines, transforming growth factor β (TGF- β) and platelet-derived growth factor (112). Another specific macrophage phenotype is associated with hepatocarcinoma. These tumor-associated macrophages, while predominantly expressing M2 phenotype, have a distinct transcriptional profile that can contribute to enhanced tumor angiogenesis, due to increased expression of VEGF, which stimulates angiogenesis, and matrix metalloproteases, that facilitate angiogenic remodeling (68, 126).

Our understanding of the precise mechanisms for regulating macrophage polarization is still very rudimentary, although recent studies have begun to elucidate the specific transcription factors that regulate the switch, including signal transducers and activators of transcriptions (STATs), peroxisome proliferator-activated receptor (PPAR) family members, hypoxia-inducible factor 2 α and Kruppel-like factor 4 (68,70,76). Strong M1 polarizing factors include STAT1 and interferon-regulatory factor 5 (IRF), while STAT6, IRF-4, and PPAR γ are important transcription factors regulating M2 polarization (68). MicroRNAs also contribute to the regulation of macrophage polarization in cultured macrophage cell lines (37); however, data for an *in vivo* role of microRNA in regulating Kupffer cell phenotype is still lacking. Proliferation of M2 macrophages, in response to IL-4, has also been reported (55).

There is also a growing appreciation that the metabolic state of the macrophage, in particular whether it utilizes glucose or fatty acids as a primary fuel source, influences the phenotypic activity of macrophage (16). Indeed, regulators of lipid metabolism, such as the PPAR family members (98, 99) and adiponectin (76), are important moderators macrophage polarization. Multiple PPAR family members make differential contributions to M2 polarization, with PPAR γ increasing oxidative phosphorylation and PPAR δ regulating the expression of pattern recognition receptors and costimulatory molecules (99). Similarly, full-length adiponectin coordinately regulates expression of both PPAR γ and PPAR δ , genes regulating oxidative phosphorylation, and M2 polarization (76). This dual regulation of lipid metabolism and macrophage polarization is particularly interesting in light of the contributions of Kupffer cells and other hepatic macrophages to the progression of metabolic liver diseases, such as ALD and NASH, which are initially characterized by dysregulation in hepatic lipid metabolism (see below).

Kupffer Cells in Liver Diseases

Overview

Kupffer cell activation is essential to the response of the liver to infection or injury; the ensuing inflammatory response protects from infection, as well as limits cellular and organ damage to the host organism (40). Thus, in many conditions, such as acetaminophen toxicity and in hepatocellular repair, the Kupffer cell plays an important protective, healing function. However, in other types of insults to the liver, the Kupffer cell is unable to appropriately control or resolve its state of activation. The controlled and appropriate resolution of inflammation is an essential feature of the innate immune response. This failure to resolve Kupffer cell activation contributes to a number of chronic inflammatory diseases in the liver. Given the dual protective and potentially harmful role of Kupffer cell activation,

development of therapeutic strategies to modulate Kupffer cell activity during liver disease must be critically timed, so as to appropriately regulate Kupffer cell function during the dynamic stages of liver injury.

When investigating the role of Kupffer cells in health and disease, it is important to understand that the localized response of the innate immune system in the liver may be distinct from the systemic innate immune response and/or localized responses of other organs. For example, chronic alcohol consumption generally increases the susceptibility of individuals to infections (94,133), suggesting that, despite increased inflammatory responses observed in the liver after chronic ethanol (43), systemic immune responses are suppressed by chronic ethanol exposure. Even within the liver, there may be distinct responses of individual components of the immune response. One example is the interaction of ethanol and hepatitis C virus (HCV) infection. Chronic alcohol abuse is associated with increased incidence of HCV infection (157). This decreased ability to ward off viral infections contrasts with the increased response of the liver to endotoxin. These differential responses may be related to the highly specific tolerized status of the Kupffer cell in the liver. Understanding the localized and specific responses of the immune system in the liver will help to develop intervention strategies specifically directed at targets of the immune system in the liver that contribute to the initiation and maintenance of liver disease. Here, we will first review the deleterious role of Kupffer cells in metabolic liver diseases and then summarize data demonstrating that Kupffer cells are protective in other types of liver injury, such as drug-induced liver injury and fibrosis.

Kupffer cells contribute to metabolic liver diseases: Alcoholic and nonalcoholic liver disease

The progression of ALD and NAFLD/NASH follows a pattern characteristic to all types of liver disease, regardless of the causative agent. This progression is marked by the appearance of fatty liver, hepatocyte necrosis and apoptosis, inflammation, regenerating nodules, fibrosis, and cirrhosis (78). The development of steatosis, inflammation, fibrosis, and cirrhosis is a complex process involving both parenchymal and nonparenchymal cells resident in the liver, as well as the recruitment of other cell types to the liver in response to damage and inflammation (38). Many of the events involved in the development of ALD or NAFLD/NASH are typical of other tissue responses to injury, such as wound healing in the skin and soft tissues (110). As with all wound healing responses, the innate immune system makes a critical contribution to a timely and effective restoration of both structure and function of the injured tissue or organ. Indeed, while the development of hepatic fibrosis is initiated in response to hepatocellular damage, dysregulated inflammatory processes contribute to the progression of fibrotic disease (38).

Hepatic macrophages play a particularly important role in the development of liver disease. The role of Kupffer cells, the resident hepatic macrophages, was first identified as a key contributor to the progression of ALD (91, 140). While early work in the field of obesity characterized a critical role for resident macrophages in adipose tissue in the development of metabolic syndrome, more recent studies have also identified Kupffer cells as critical mediators of NAFLD/NASH (4,51,66,99). For example, in a high-fat diet model of hepatic

steatosis in mice, depletion of Kupffer cells with clodronate results in the reversal of hepatic steatosis (131).

Prevalence of ALD and NAFLD/NASH

Alcohol abuse is a leading cause of morbidity and mortality worldwide (111, 114). Estimates suggest that in the US 18 million Americans abuse alcohol (77) and that ALD affects over 10 million people (85). While fatty liver occurs in up to 90% of alcoholics, only a minority of heavy drinkers ever develop hepatitis, fibrosis, and cirrhosis, suggesting a role for genetic and environmental risk factors, such as factors influencing the severity of steatosis and oxidative stress, the cytokine milieu, the magnitude of the immune response, and/or epigenetic regulation (151).

NAFLD is a spectrum of disorders that include steatosis, steatosis with inflammation, nonalcoholic steatohepatitis (NASH) and NASH with fibrosis. The development of NAFLD/NASH is strongly associated with obesity and metabolic syndrome; collectively metabolic syndrome is characterized by an increased risk for chronic disease, including insulin resistance and type 2 diabetes, dyslipidemia, and cardiovascular disease, as well as NAFLD/NASH (59). Similar to the rates of progression of ALD, not all obese individuals develop metabolic syndrome and NAFLD/NASH, suggesting a role for environmental and/or genetic contributions to the progression of liver injury (20). For example, 10% to 29% of patients with NASH will progress to cirrhosis within 10 years, and, of these 4% to 27%, are expected to develop hepatocellular carcinoma (20). Thus, advanced NAFLD, particularly NASH with fibrosis, is a considerable risk for progression into cirrhosis and hepatocellular cancer. Because of its high prevalence, NAFLD may surpass chronic hepatitis C as the leading indication for liver transplant by 2020. Rates of obesity are reaching epidemic proportions worldwide and there is an urgent need to understand the pathophysiological mechanisms of obesity-induced tissue injury to identify novel therapeutic targets and risk factors for the progression from obesity to disease.

While several components of the innate immune response are involved in the initiation and progression of ALD and NAFLD/NASH, Kupffer cells, the resident macrophages in the liver, are particularly critical to the onset and chronicity of liver injury (8, 91). Ablation of Kupffer cells prevents the development of fatty liver and inflammation in rats chronically exposed to ethanol via intragastric feeding (1). Similarly, depletion of Kupffer cells protects from the development of hepatic insulin resistance in response to high-fat diets (66), as well as hepatic steatosis after longer feeding of high-fat diets (96).

Danger signaling in Kupffer cells: Role in metabolic liver disease

Multiple pathogen-recognition receptors (PRRs) are involved in the control of the innate immune response. Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) are the two major classes of PRRs that recognize both pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs). PAMPs represent exogenous stimuli, while DAMPs are endogenous indicators of stress. Lipopolysaccharide (LPS), a component of the cell wall of gram-negative bacteria, is a ligand for the TLR4. The role for LPS-dependent TLR4 activation has been well studied in

the context of ALD and NAFLD/NASH; however, much less is known about the role of other PRRs in the progression of liver injury. In recent work, both TLR2 and TLR9 have also been identified as specific contributors to NAFLD/NASH in mouse models (86, 116). Gustot and colleagues (39) found that chronic ethanol feeding to mice increased expression of mRNA for TLR-2, 4, 6, 7, 8, and 9, as well as enhanced the sensitivity of the mice to multiple bacterial ligands. Expression of mRNA for TLR-7 and 9 are increased in liver biopsies from patients with ALD (129). Based on these few reports, there is a clear need at this time to expand our paradigm for PRRs in progression of liver injury beyond a specific interaction with TLR4 and incorporate the likely complex interactions between Kupffer cells and multiple PAMPs and DAMPs in the progression of chronic liver injury.

Kupffer cells and the LPS-TLR4 axis in ALD and NAFLD/NASH

LPS is a potent activator of Kupffer cells, stimulating the production of inflammatory and fibrogenic cytokines, as well as reactive oxygen species (ROS). LPS concentration is increased in the blood of alcoholics (12, 31) and rats exposed to ethanol via gastric infusion (93), probably due to impaired barrier function of the intestinal mucosa (113). In a series of elegant experiments from the laboratory of Ron Thurman using genetically manipulated mice, a working model for the development of ALD was developed which proposes that increased exposure of Kupffer cells to LPS during chronic ethanol consumption results in activation of TLR-4 and increased production of inflammatory mediators (140). Studies over the last 10 years have expanded on this working model, further elucidating the effects of ethanol on intestinal barrier function and documenting the development of intestinal dysbiosis (58, 90, 113, 155), both of which are associated with increased inflammatory responses in the liver, as well as characterizing an ethanol-induced sensitization of Kupffer cells to activation by LPS (21).

Following the paradigm elucidated for the role of LPS-TLR4 in the development of ALD, investigators have also identified a critical role for this LPS-TLR4 axis in the development of NAFLD/NASH (83,119). Just as in mouse models of ALD, mice deficient in TLR4 are protected from high-fat-diet-induced liver injury (115,134). TLR2 and TLR9 have also been identified as specific contributors to NAFLD/NASH in mouse models (86, 116).

Importantly, TLR4 activation and subsequent TNF- α expression have recently been identified as critical contributors to the development of hepatocellular carcinoma in mouse models of both ALD and NAFLD/NASH (73, 102).

Gut-liver interaction in Kupffer cell activation

A critical interaction between gut microbiota and ethanol-induced liver injury has been demonstrated in a number of animal models. Ethanol feeding to mice results in both quantitative and qualitative changes in gut microbiota; recent studies using massively parallel pyrosequencing have identified specific populations of gut bacteria that are impacted by ethanol feeding (155). Further, when mice or rats are treated with antibiotics to reduce intestinal microbial populations, ethanol-induced liver injury is attenuated (140). Further studies indicate that modulation of gut bacterial populations with probiotics, such as lactobacillus GG also attenuates ethanol-induced liver injury in rats (58). However, data on

the effectiveness of pro- and/or prebiotics in treatment of ALD in human populations is still lacking.

Similar associations between changes in the gut microbiome in mouse models of obesity and fatty liver have also been identified (26,142). As with ALD, the promise of probiotic treatment has not yet been validated in human populations of NAFLD/NASH patients (65).

Activation of TLR-4-mediated signaling

Activation of TLR-4-dependent signaling initiates a complex series of events, leading to increased expression of a number of inflammatory cytokines, chemokines, and mediators. TNF- α is thought to play a particularly critical role in the pathogenesis of ALD and NAFLD/NASH. TNF- α is one of the principal mediators of the inflammatory response in mammals, transducing differential signals that regulate cellular activation and proliferation, cytotoxicity, and apoptosis (11, 53). In addition to its role in acute septic shock, TNF- α is implicated in the pathogenesis of a wide variety of inflammatory diseases (11), as well as in the progression of ALD (140, 141). TNF- α is primarily produced by monocytes and macrophages; increased TNF- α production is one of the earliest responses of the liver to injury (141). Circulating TNF- α is increased in the blood of alcoholics and in animals chronically exposed to ethanol (60,80). When intestinal flora is decreased with antibiotics, both chronic ethanol-induced TNF- α expression and liver injury are suppressed in rats (140), suggesting that increased TNF- α after ethanol exposure is due, at least in part, to increased exposure to LPS derived from intestinal microbes.

Mice with global knockouts for TLR4, CD14 (the TLR4 coreceptor), and TNF- α have revealed a required role for this PAMP-dependent signaling pathway in ethanol-induced and high-fat diet induced liver injury in mice. Signaling via TLR4-MyD88-independent pathway (TRIF dependent) is a critical contributor to increased steatosis and inflammation during metabolic liver disease (50, 159). Interestingly, a recent publication suggests that there is a differential importance of the TRIF-dependent pathway between ALD and NAFLD/NASH, since MyD88-deficient mice are protected from steatosis in the high-fat-diet model of NAFLD/NASH (9).

While it has been assumed that the expression of TLR4/MyD88/TRIF on macrophages and other cells of the innate immune system are the critical sites of expression, studies in conditional knockouts where expression of PAMPs on specific cell types is ablated are required to validate this assumption. While cells of the innate immune system are the principal respondents to PAMP signaling, recent studies highlight the ability of hepatocytes to respond to PAMP-mediated signals. For example, in models of high-fat-diet-induced NAFLD/NASH, hepatocytes exhibit activation of inflammasomes (26), as well as activation via HMGB-1, an important endogenous danger signal (69). Similarly, high-fat diets also increase free fatty acid (FFA)-mediated activation of TLR4 signaling (124) and contribute to the insulin resistance associated with obesity. Recent studies suggest an interaction between FFA-mediated signals and the release of HMGB-1 (69) and there is also a likely interaction between FFA and the activation of inflammasomes in both hepatocytes (26) and Kupffer cells (25).

Sensitization of Kupffer cells to TLR4-mediated signaling after chronic ethanol

In addition to increasing LPS exposure, chronic ethanol also increases sensitivity of Kupffer cells to activation by LPS. For example, long-term ethanol consumption increases the susceptibility of rats to endotoxin-induced liver injury (49, 79). Moreover, we have shown that TLR4-mediated signaling via both MyD88-dependent and independent arms is increased in Kupffer cells isolated from rats fed ethanol in their diet for 4 weeks compared to pair-fed controls (2, 62, 63). After chronic ethanol exposure, Kupffer cells are also sensitized to the proinflammatory effects of cellular fibronectin (7) and apoptotic cells (82). Furthermore, LPS can act synergistically with other DAMPs generated during chronic ethanol feeding, such as malondialdehyde-acetaldehyde adducts, to increase cytokine and chemokine expression (27).

Production of inflammatory cytokines is a highly regulated process; regulation occurs at the level of transcription, translation, and secretion (101, 148). Using the expression of TNF α as a “reporter” for the impact of chronic ethanol on Kupffer cell signaling, studies from my laboratory and others have found that ethanol exposure impacts the molecular regulation of TNF- α expression at each level of control, resulting in an enhanced initiation of inflammation in the liver (91). Ethanol mediates these changes in the activation of Kupffer cells by a profound dysregulation in TLR-4-initiated signal transduction (91). Interestingly, many of the same signaling pathways targeted by ethanol in neurons, resulting in the complex behavioral effects of ethanol, are also involved in TLR-4-mediated signal transduction in macrophages.

Aberrant regulation of proximal TLR-4-dependent signaling after chronic ethanol leads to increased transcription of TNF- α and other inflammatory mediators, as well as a stabilization of TNF- α mRNA (63). Increased transcription of TNF- α and other inflammatory cytokines is associated with increased binding of nuclear factor κ B and early growth response-1 (107, 125) to the TNF- α promoter, although these responses may be both gender and species dependent (127, 158). Increased p38 mitogen-activated protein kinase (MAPK) activity (63), upregulation of miRNA-155 (26) and decreased retinoic acid receptor element binding activity (89) in response to chronic ethanol have all been implicated in the mechanisms for stabilization of TNF- α mRNA in macrophages. Finally, acetate, a metabolite of ethanol, may contribute to dysregulation of SIRT-1 activity and alter the regulation of DNA methylation, an important regulatory mechanism in the control of inflammatory cytokine expression (123).

While these changes in LPS-stimulated signaling via TLR-4 have been well described, the mechanisms for the sensitization of signaling are still not understood. Recent data suggest that an increased production of ROS during chronic ethanol exposure may contribute to this sensitization in LPS-dependent signal transduction (154). Increased iron in hepatic macrophages may serve to exacerbate the impact of increased ROS (153).

Chronic ethanol exposure causes oxidative stress in the liver and enhances the formation of free radicals; it is widely accepted that ROS play a critical role in the development of alcoholic liver injury (5, 47, 150). While ethanol metabolism by hepatocytes is an important source of ROS (5), Kupffer cells also generate excess ROS during ethanol exposure (150)

and in response to LPS (128). Indeed, chronic ethanol feeding increases the expression of CYP2E1 in Kupffer cells (15, 138). Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-dependent production of ROS is implicated in ethanol-induced liver injury since *p47^{phox}-/-* mice are resistant to chronic ethanol-induced injury (64) and the expression of mRNA for multiple NADPH oxidase subunits are increased in the liver after chronic ethanol feeding to mice (50). Recent gene array studies of expression of candidate genes in the livers of patients with alcoholic hepatitis found that several components of the NADPH oxidase complex were upregulated, including rac-1, p22^{phox}, and gp91^{phox} (23). Our work in isolated Kupffer cells indicates that increased ROS production via NADPH oxidase after chronic ethanol feeding contributes to increased activation of MAPK family members and increased TNF- α production (138).

Sensitivity of Kupffer cells to activation in NAFLD/NASH

Despite our complex understanding of the effects of chronic alcohol exposure on the functional activity of Kupffer cells (91), much less is known about the impact of high-fat diets/obesity on signal transduction in Kupffer cells. Recent investigations have begun to explore these issues. For example, recent studies have found that high-fat diets increase Kupffer cell activation in a time-dependent mechanism, leading to increased expression of inflammatory cytokines and iNOS expression, due, at least in part, to reduced endothelial NO signalling in the liver (136).

Recruitment of myeloid cells to the liver and macrophage polarization in ALD and NAFLD/NASH

While Kupffer cells, the resident macrophage in the liver, makes critical contributions to the status of innate immune activity in the liver, recruitment of nonresident myeloid cells to the liver in times of stress also makes a significant contribution to innate immune activity in the liver. Indeed, both in mouse models of ALD and NAFLD/NASH, CCR2/CCL2/MCP-1-mediated recruitment of myeloid cells to the liver contributes to steatosis (9, 97). This CCR2/CCL2/MCP-1 axis is also involved in adipose tissue inflammation in models of diet-induced obesity (72, 149), further illustrating the important parallels in innate immune activity in adipose and liver in the progression of metabolic liver injury.

Interestingly, recent studies making use of clodronate to deplete hepatic macrophages over short periods of high-fat-diet feeding versus continuous depletion over many weeks of high-fat-diet feeding reported differential contributions of hepatic macrophages to high-fat-diet-induced insulin resistance (19, 66). While hepatic macrophages had a detrimental effect in the early stages of high-fat-diet feeding after many weeks of high-fat diets, hepatic macrophage depletion no longer protected from the development of insulin resistance or hepatic steatosis (19, 66). These difference in short-term and long-term depletion of hepatic macrophages on the development of high-fat-diet-induced insulin resistance may reflect changes in the phenotypic profile of hepatic macrophages at different stages in the progression of metabolic syndrome and NAFLD/NASH.

Macrophage polarization in ALD and NAFLD/NASH

In mouse and rat models of ALD in mice, there is an increase in M1 polarization (71, 76). Similar shifts in macrophage polarization are also observed in mouse models of NAFLD/NASH (32, 74). Interestingly, in mice, there is also an increase in the numbers of M2 polarized macrophages after alcohol feeding (71). The polarization state of macrophages after alcohol feeding can be restored either by treatment with adiponectin, an anti-inflammatory adipokine (76), or by activating the cannabinoid CB2 receptor (71). Both adiponectin and CB2 receptor agonists can polarize macrophages in cell culture, suggesting that there may be a direct effect of these agents on macrophage polarization in the liver (71, 76). The functional impact of these two mediators on macrophage polarization is of interest, given their dual role in regulation of lipid metabolism in hepatocytes (117, 135). Indeed, studies have suggested that production of endogenous endocannabinoids by Kupffer cells impacts both hepatic lipid accumulation and fibrogenesis in mouse models of NAFLD/NASH (135). These data suggest an important mechanistic link between activation of Kupffer cells in metabolic disease and the development of hepatocellular steatosis and injury.

Additional receptor-dependent innate immune pathways contributing to activation of Kupffer cells

NOD-like receptors—Virtually no information is available in the literature related to the interactions of ethanol with the second major class of receptors for PAMPs, the family of NLRs. The Nod proteins, Nod1 and Nod2, are mainly expressed by antigen-presenting cells and epithelial cells, functioning as cytosolic sensors for innate recognition of microorganisms and regulation of inflammatory responses, as well as for the induction of apoptosis (52). LPS was initially proposed as a Nod2 ligand; however, it is now well established that Nod2 ligand is muramyl dipeptide, a component from both Gram-positive and Gram-negative bacteria (34). Upon activation, Nod-2 can signal independently or in conjunction with multiple TLRs, including TLR-2, 3, 4, 5, 7, and 9. The interaction can either be synergistic or inhibitory (95, 143, 144, 152). Thus, either alone or in conjunction with a TLR family member, ethanol-induced changes in NOD2 signaling would likely impact on the activation of Kupffer cells and the progression of ethanol-induced liver injury. Further studies on this potentially important interaction are clearly required at this point.

In response to different stimuli, including exogenous PAMPs, like flagellin, or endogenous signals, including free fatty acids, or uric acid crystals, other NLRs such as NLRC4, NLRP1, and NLRP3 (also called NALP3) and apoptosis inhibitor proteins form a complex with caspase-1 and apoptosis-associated speck-like protein. This complex is responsible for caspase-1 activation and is named the inflammasome (29). Caspases are intracellular cysteine proteases that cleave substrates after aspartate residues and play integral roles in apoptosis (29). Caspases can be further classified as proinflammatory or proapoptotic, dependent upon cellular processes. The proinflammatory caspases include caspase 1, 11, and 12 in mouse and caspase 1, 4, and 5 in human (29). Caspase-1, also known as the interleukin cleavage enzyme is responsible for the cleavage of prointerleukin-1 (IL-1), 18 (IL-18), and 33 (IL-33) into their mature forms, IL-1 β , IL-18, and IL-33. While recent studies in mouse models of NASH implicate caspase-1 and the inflammasome in inflammatory responses

associated with metabolic syndrome (26, 130, 145), it is not known if there is a role for these NLRs in ALD.

Danger-Associated Molecular Patterns

Endogenous DAMPs, including high-mobility group box-1 (HMGB1), heat shock proteins, hyaluronic acid and uric acid, are released from cells under stress. These endogenous DAMPs interact with their cognate receptors, such as the interactions between HMGB1 and receptor for advanced glycation endproducts (RAGE), as well as interacting with TLRs. A few reports suggest that high-fat-diet-induced NAFLD/NASH is associated with the release of HMGB-1, an important endogenous danger signal (69). While the release of hyaluronic acid during the progression of liver injury has been commercialized as a biomarker for the progression of liver injury, we know very little about the effect of chronic ethanol on either the production and/or sensitivity to different DAMPs. Because DAMPs can interact with the TLR family, it is very likely that there will be as yet unknown interactions between PAMPs and DAMPs in the activation of Kupffer cells during the progression of both ALD and NAFLD/NASH.

Complement

The complement cascade is a phylogenetically ancient part of our immune system critical to an organism's ability to ward off infection (33). Activation of the complement pathway can occur via the classical, lectin, or alternative pathways (147). All three pathways of complement activation converge at the point of C3 cleavage. The cleavage products C3a and C5a, termed the anaphylatoxins, stimulate the production of cytokines in a number of cell types (87, 120), either alone or in the presence of other inflammatory mediators, such as LPS (120) (Fig. 2). C5a is also a potent chemokine, recruiting neutrophils to the site of infection/injury by regulating the expression of chemokines and adhesion molecules (24, 54).

Kupffer cells express multiple receptors for complement, including the anaphylatoxin receptors C3a receptor and C5a receptor (103, 121) and complement receptors 1, 3, and 4 (CR1, CR3, and CR4) (46). CR3, also termed CD11b/CD18, serves as a receptor for IgM opsonized red blood cells and facilitates their clearance from the circulation (156). More recently, Helmy et al. described CR1g as a macrophage complement receptor that is required for the clearance of circulating pathogens (41). Thus, Kupffer cells are very responsive to complement activation products; complement receptors on the cell surface facilitate both the proinflammatory role of Kupffer cells, as well as their critical role in the clearance of pathogens and dead or dying erythrocytes (Fig. 2).

Both C3 and C5 are critical to the development of ethanol-induced liver injury in mice and absence of CD55/DAF exacerbates injury (14, 106). Complement activation in response to ethanol occurs early in the progression of injury; markers of complement activation are colocalized with F4/80 positive macrophages within 4 days of ethanol feeding to mice (118). Complement activation during ethanol exposure is mediated via C1q, the key protein in the classical pathway of activation (22) and leads to an increase in inflammatory cytokine expression in the liver that is dependent both on the presence of the anaphylatoxin receptors,

C3aR and C5aR, and hepatic macrophages (22). Taken together, these data suggest that ethanol exposure activates complement and that complement activation products interact with receptors on Kupffer cells, contributing to the production of proinflammatory cytokines in the liver.

Complement activation has also been associated with the progression of NAFLD/NASH in mouse models: mice deficient in C3aR are protected from high-fat-diet-induced steatosis (75). Importantly, complement factor 5 is a quantitative trait for the development of hepatic fibrosis in both mice and humans (44). While the mechanisms for the activation of complement in NAFLD/NASH are not yet completely understood, C1q has been implicated in the early phases of hepatic insulin resistance in response to high fat diets (45).

Resident macrophages in adipose tissue in ALD and NAFLD/NASH

While studies of Kupffer cell function in liver have led insights into the role of hepatic macrophages in the development of ALD and NAFLD/NASH, it is also clear that tissue macrophages in adipose tissue are causative contributors to NAFLD/NASH (16). Adipose tissue plays an important role in the regulation of metabolism and innate immunity; changes in the phenotypic characteristics of resident macrophages in adipose tissue in obesity and metabolic syndrome clearly contribute to the progression of NAFLD/NASH (16,99). Following on this paradigm, investigators have now identified critical roles for adipose tissue and macrophages resident in adipose tissue as likely contributors to the development of ALD (57, 122). The parallel roles of adipose and adipose-resident macrophages with Kupffer cells in the development of both ALD and NAFLD/NASH clearly suggest that tissue resident macrophages respond in similar ways to the metabolic stress evoked in metabolic liver disease. Thus, despite the differential initial signals activating tissue macrophages in ALD and NAFLD/NASH, these two chronic inflammatory diseases share common intermediary mechanisms leading to the activation of tissue macrophages in both liver and adipose tissue.

Protective Effects of Kupffer Cells in Liver Injury: Link between Inflammation and Hepatocyte Proliferation/Fibrotic Responses

As detailed above, activation of Kupffer cells is an important contributor to hepatocyte injury in conditions of chronic inflammation. However, emerging evidence suggests that Kupffer cells also play important protective functions in initiating hepatocyte proliferation in response to hepatotoxic injury, as well as in the resolution of fibrotic scarring.

Kupffer cells and the initiation of hepatocyte proliferation/fibrosis in response to injury

The association between enhanced expression of inflammatory cytokines and the initiation of cell cycle progression by hepatocytes has been well described in response to partial hepatectomy, where the rapid and transient expression of cytokines programs hepatocytes for rapid proliferation (137).

More recent studies making use of knockout mouse models have illustrated this dichotomy between the possible injurious versus protective effects of proinflammatory mediators in

different stages of liver injury. For example, mice deficient in early growth response-1 (Egr-1) are protected from both chronic ethanol-induced liver injury and galactose amine/LPS-induced inflammation (81, 109). In contrast, Egr-1^{-/-} mice are more sensitive to carbon tetrachloride-or bile-duct ligation-induced fibrosis and exhibit reduced hepatocyte cell-cycle progression in response to injury (61,104,105,108). This impaired regenerative response is associated with a dysregulation in the precisely time expression of inflammatory and hepatoprotective mediators by Kupffer cells (Fig. 3). Plasminogen activator inhibitor-1 (PAI-1), an acute phase protein that plays an important role in acute and chronic liver inflammation, also has differential effects in response to inflammatory injury compared with carbon tetrachloride-induced hepatotoxicity; PAI-1-deficient mice are protected from ethanol-induced inflammation, but have impaired hepatocyte proliferation after carbon tetrachloride-mediated hepatotoxicity (6,146). In contrast to the role of Egr-1, PAI-1 deficient mice are protected from fibrosis in response to bile duct ligation or exposure to angiotensin II (6, 10).

Interestingly, Kupffer cells are also critical to the appropriate speciation of hepatic progenitor cells in the liver during hepatocyte regeneration; when Kupffer cells engulf debris in the damaged liver, they increase expression of Wnt3, a ligand in the Wnt/Notch signaling pathways (13). Activation of Kupffer cells by dead/dying hepatocytes after injury also results in an increase in the expression of TGF- β , a cytokine involved in the activation of hepatic stellate cells from their resting/quiescent state to their profibrotic state (30). Thus, activation of Kupffer cells contributes to the progression of fibrosis. However, Kupffer cells/resident hepatic macrophages are also an important source of matrix metalloproteases (MMPs). While MMPs can actually contribute to fibrosis during the progression stage, they can also promote the removal of extracellular matrix during the resolution of fibrosis (30). Hepatic macrophages associated with the spontaneous resolution of hepatic fibrosis are identified as SAMs (28) and are associated with a high expression of MMP-13 (28).

These data thus place Kupffer cells as an important nexus in the sensing of danger within the localized environment of the liver and initiating critical signals to stimulate repair. Taken together, these studies illustrate the complex interactions between the activation of inflammatory responses in the liver and the ability of the liver to maintain function and repair. The precise, timely, and localized activation of hepatic macrophages is required in the response to different types of stress and injury.

Kupffer cells in acetaminophen-induced liver injury

The role of the Kupffer cell in acetaminophen (APAP)-induced and other drug-induced (DILI) liver injury is the subject of controversy. While Kupffer cells can contribute to the injury in DILI due to increased expression of inflammatory cytokines and chemokines and reactive oxygen and nitrogen species (48, 67, 84). However, as discussed above regarding the role of the Kupffer cell in response to partial hepatectomy and carbon tetrachloride, the Kupffer cell plays a bifunctional role in DILI. For example, the Kupffer cell produces hepato-protective cytokines, such as IL-6, as well as cytokines critical to the resolution of inflammation, such as IL-10 (48,56). The protective role for a number of Kupffer cell-derived cytokines is evident from data indicating that mice lacking TNF-receptor 1 have

impaired hepatocyte regeneration after APAP-induced liver injury (18). Further, IL6^{-/-} and IL-10^{-/-} mice are also more sensitive to APAP-induced hepatotoxicity (3).

Summary

Kupffer cells are reemerging as a critical mediator of liver injury and repair. While the role of Kupffer cells in ALD has been well studied, there is a growing appreciation that Kupffer cells are also critical to the development of hepatic insulin resistance, as well NAFLD in obesity and metabolic syndrome. Kupffer cells exhibit a tremendous plasticity: depending on the local metabolic and immune environment, Kupffer cells can exhibit a range of polarized phenotypes, ranging from a proinflammatory M1 phenotype to an alternative/M2 phenotype. Multiple M2 phenotypes can be distinguished, each involved in the resolution of inflammation and wound healing. Now that we have a more complete understanding of the complex phenotypes displayed by resident hepatic macrophages, future studies will need to focus on regulating the specific phenotype of Kupffer cells at different stages of progression of liver diseases to maximize possible therapeutic outcomes. Here, we have provided an update on research related to our current broadening perspectives on contribution of Kupffer cells to different types of liver injury, with an emphasis on ALD and NAFLD/NASH. These recent advances will likely provide new insights into the potential for therapeutic manipulation of Kupffer cells to promote the resolution of inflammation and enhance wound healing in liver disease.

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Figure 1.

Localization of Kupffer cells within the hepatic sinusoid in healthy and diseased liver. The Kupffer cell is located to the hepatic sinusoid and is therefore in close proximity to other cells in the sinusoid, including natural killer (NK) and natural killer T cells (NKT), as well as the liver sinusoidal endothelial cells (LSEC). Despite the barrier of the LSEC, Kupffer cell products, such as cytokines, chemokines, reactive nitrogen, and oxygen species, influence the activity of both stellate cells and hepatocytes.

Figure 2.

The complement anaphylatoxins activate Kupffer cells to express inflammatory cytokines and chemokines. Complement activation via the classical, lectin or alternative pathways culminates in the cleavage of C3. C3a and C5a, termed the anaphylatoxins, then interact with cognate receptors on the surface of macrophages. Interaction between Toll-like receptor 4 (TLR4) and the anaphylatoxin receptors can exacerbate cytokine/chemokine production.

Figure 3.

Interactions between Kupffer cells and the regulation of hepatocyte proliferation in response to tissue injury: role of early growth response-1. In acute carbon tetrachloride-induced hepatotoxicity, production of cytokines and other hepatoprotective factors is precisely controlled, both spatially and temporally. Absence of individual transcription factors, such as early growth response-1 (Egr-1), controlling these responses to injury results in an impaired hepatoproliferative response and an increased susceptibility to hepatotoxicity (104, 105, 108).