

Chapter 10

Kupffer Cells in Health and Disease

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10.1 Introduction

The interstitial liver cells initially discovered by von Kupffer as “star cells” constitute the largest population of mononuclear phagocytes in the body. The strategic position of Kupffer cells (KC), at the luminal side of the liver sinusoidal endothelium, places them in an ideal position for their main function in the steady state, i.e. filtering of the blood that enters the liver from both the portal vein and the hepatic artery (see Fig. 10.1a, b for a schematic representation). In this manner they play an important role in the surveillance of potentially hazardous substances entering the body via the intestine, as well as in the recycling of iron by the removal of effete erythrocytes from the circulation. In addition to this scavenger function, KC are increasingly recognized as regulatory and effector cells in innate and adaptive immune responses to infectious agents and other conditions challenging liver homeostasis. Interestingly, KC activity also affects hepatocyte function directly, thus making these cells important versatile constituents of the liver.

In this chapter we will provide an overview of the phenotypic, developmental, and functional features of KC in the steady state as well as in disease. Given the

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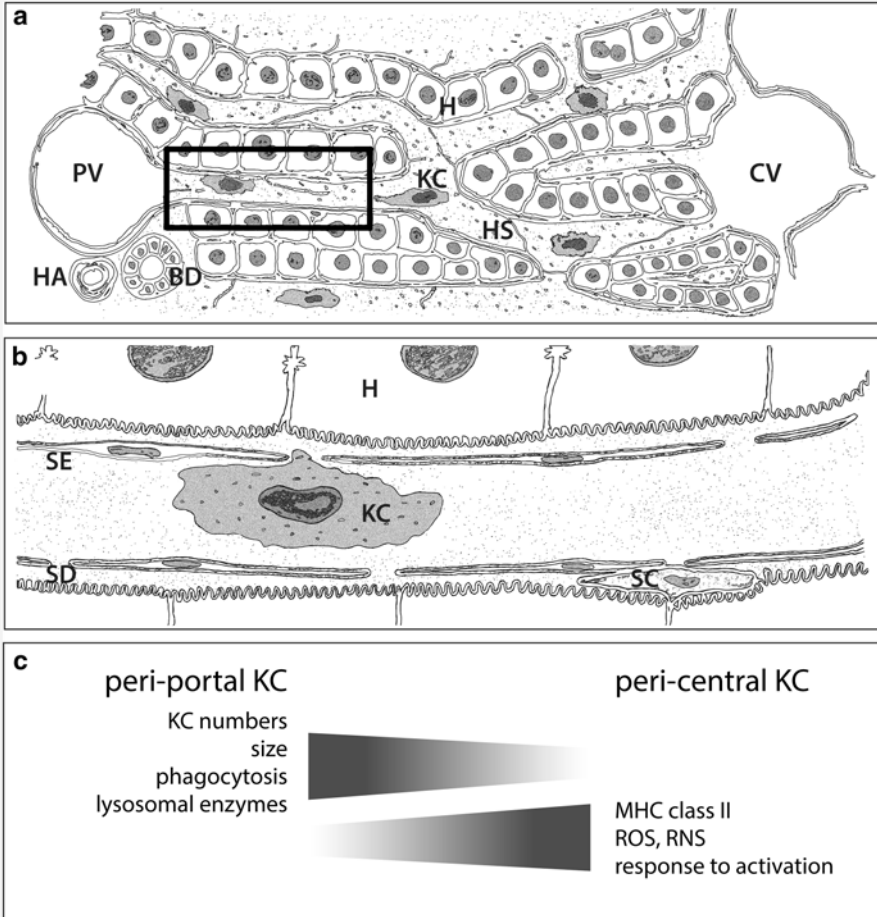


Fig. 10.1 Schematic representation of the liver micro-anatomical structure and Kupffer cell localization in lower (a) and higher magnification (b). (a). Hepatocytes (H) are arranged in plates, intermingled with hepatic sinusoids (HS) that transport blood from branches of the portal vein (PV) and hepatic artery (HA) to the central vein (CV). Together with the bile ducts (BD), portal veins and hepatic arteries constitute the portal area. Kupffer cells (KC) are found in the sinusoid lumen. (b) Between the hepatocytes and discontinuous, fenestrated sinus endothelial cells (SE), the space of Disse (SD) is located. Here, hepatic stellate cells (SC), also named Ito cells, are situated. Kupffer cells are, firmly or loosely, attached to the sinusoid wall. (c) KC in the portal areas differ in various aspects from those in the peri-central regions

ambition of this goal and the space limitations set, we necessarily restrict ourselves to the global picture as obtained from studies in human and in animal models, and refer to more specific literature where applicable. Although defining KC by their tissue localization and specific macrophage features seems clear-cut, distinguishing these cells from other myeloid cells such as circulating monocytes, perivascular macrophages, and different types of dendritic cells sometimes appears problematic. This especially concerns studying populations of cells after isolation and/or in

inflammatory conditions. In different studies, authors apply slightly different criteria for the identification of “genuine” KC vs. recent immigrants, and this easily creates confusion. Where possible, we attempt to clarify these semantic issues, but these are important considerations when interpreting experimental findings.

10.2 Kupffer Cell Phenotype

10.2.1 Tissue Localization and Morphology

Like various other tissue macrophages, KC have a characteristic amoeboid morphology with lamellipodia and an irregular surface containing many microvilli (Naito et al. 2004). They contain an ovoid or indented nucleus and their numerous cytoplasmic vesicles are reminiscent of a high level of phagocytic and pinocytic activity. Figure 10.2 provides an overview of histomorphological aspects of KC at different magnification levels. The cells adhere to the fenestrated sinusoidal

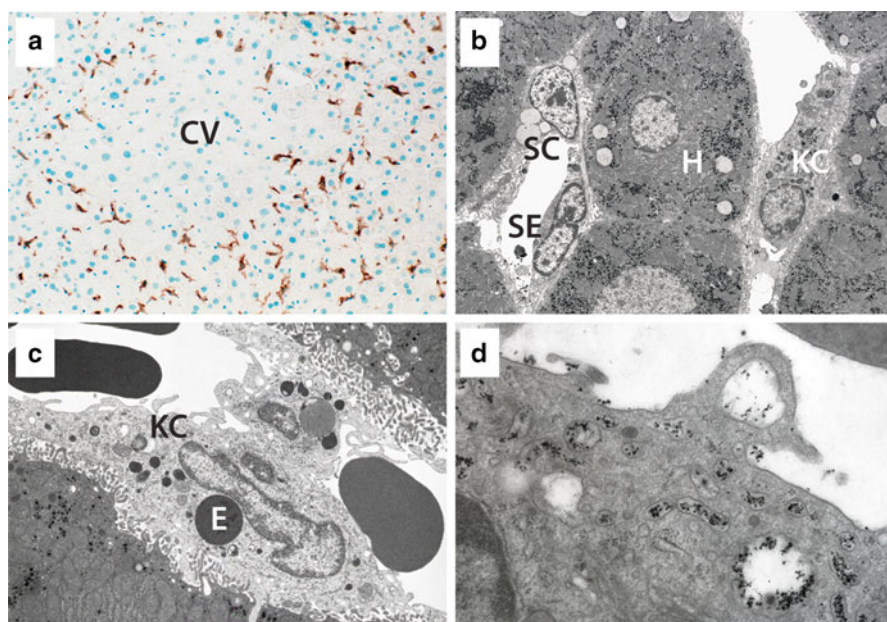


Fig. 10.2 Kupffer cell tissue localization and morphology at light microscopic (a) and electron microscopic level (b–d). (a) Mouse liver section stained with F4/80 antibody (brown) to show Kupffer cells. Nuclei are counterstained with methyl green pyronin. CV central vein. (b) Rat hepatocytes (H) and liver sinusoid with a Kupffer cell (KC), stellate cell (SC), and sinus endothelial cell (SE). (c) Rat KC with phagocytosed erythrocyte (E). (d) Surface of rat KC after 5 min of intravenous administration of thorotrast (radioactive thorium dioxide particles). Already after this short period of time particles are adhered to the cell surface and incorporated in (macro)pinocytic vesicles, and a worm-like structure

endothelium and are slightly more abundant in the peri-portal than in the peri-central regions. Although KC are the specialized phagocytes of the liver, sinusoidal endothelial cells of the liver have significant endocytic capacity, and this historically gave rise to the, now deserted, concept of the reticulo-endothelial system as scavenger system. In fact, early ultrastructural and functional studies were instrumental in establishing a definitive distinction between the two cell types (Wisse 1972). Both KC and endothelial cells contain high levels of lysosomal enzymes, such as glycosidases, nucleases, lipases, and proteases, in line with their clearance function. In mouse liver, KC numbers are approximately 35–40 % of the number of hepatocytes (Baratta et al. 2009), and comparable histological pictures in other species suggest very similar frequencies.

10.2.2 Kupffer Cell Molecular Phenotype

With regard to the KC's molecular phenotype, current approaches allow the acquisition of genome-wide information on expressed genes under steady state and experimental or diseased conditions. Ideally, this would provide elaborate phenotypic information for the comparison of KC in different circumstances, or with other cell types. However, the currently available information on KC gene expression profiles is relatively limited. In part this may be related to the demanding isolation procedures, which represent a challenge to achieve reliable gene expression profiles. In the recent comparison of different tissue macrophages by the Immunological Genome Consortium, KC proved elusive for definitive identification and/or isolation through sorting by flowcytometry (Gautier et al. 2012). At the time of writing of this chapter, the publicly accessible repositories (Geo and ArrayExpress) contain only two studies describing data on KC (Gorgani et al. 2008; Roudkenar et al. 2008). Other publications describe gene expression arrays of KC in various experimental settings, but the authors have not deposited raw data, thus limiting their value for subsequent use by others (Chen et al. 2010; Gehring et al. 2009; Xu et al. 2012; Zocco et al. 2006). In general, these studies strongly confirm the macrophage identity of KC. Comparison with other resident macrophages, however, also strengthens the profound influence of unique local conditions on the expression profiles and related biological functions. In this respect, KC are characterized by the high level expression of various types of receptors involved with endocytosis (Gorgani et al. 2008). Changing environmental conditions, for instance during liver regeneration or interferon treatment also have a significant impact on global gene expression by KC, showing their general responsiveness (Xu et al. 2012; Zocco et al. 2006).

To provide the reader with a general picture of the surface molecules characterizing KC, we limit ourselves to the general markers used for identification of the cells, and further emphasize on the receptors related to the main endocytic function of KC, i.e. complement and Fc receptors, scavenger receptors and C-type lectins (Table 10.1). This table summarizes data collected from published studies on

Table 10.1 Kupffer cell phenotype: an overview^a

Marker	Gene ID	Comment	Literature
<i>Markers for KC identification</i>			
Human	CD68	See below	
	CD14	See below	
Mouse	F4/80	High level on resident KC	Hume et al. 1984. <i>Anat Rec</i> 210:503
	CD68	See below	
	CD11b	See below	
Rat	CD68/ED1	See below	
	CD163/ED2	See below	
<i>Complement receptors</i>			
	CR1g	Binds C3b and iC3b; required for phagocytosis of C'-coated pathogens; not described in rat	Helmy et al. 2006. <i>Cell</i> 124:915
	CR1/CD35	Uniform expression by human KC; only subsets in mouse; not described in rat	Hinglais et al. 1989. <i>Lab Invest</i> 61:509; Yan et al. 2000. <i>Immunopharmacol</i> 46:39
	CR3/CD11b	Low level on resident KC; high on recent immigrants	Hinglais et al. 1989. <i>Lab Invest</i> 61:509; Movita et al. (2012); Robinson et al. 1986. <i>Immunology</i> 57:239
	CR4/CD11c	Expressed by human KC; not by mouse KC; not described in rat	Hinglais et al. 1989. <i>Lab Invest</i> 61:509; Witmer-Pack et al. 1993. <i>J. Cell. Sci.</i> 105:965
<i>Fc receptors^b</i>			
	Fc gamma RI/CD64	Expressed by human and mouse KC; not described in rat	Tuijnman et al. 1993. <i>APMIS</i> 101:319; Otten et al. 2008. <i>J Immunol</i> 181:6829
	Fc gamma RIIB/CD32	Expression increased in hepatitis	Tuijnman et al. 1993. <i>APMIS</i> 101:319; Ganesan et al. 2012. <i>J Immunol</i> 189:4981; Lovdahl et al. 2001. <i>Cell Biol Int</i> 25:821
	Fc gamma RIIB/CD16	Expression increased in hepatitis	Bordessoule et al. 1993. <i>Br J Haematol</i> 83:370; Tomita et al. 1994. <i>Hepatology</i> 20:317; Lovdahl et al. 2001. <i>Cell Biol Int</i> 25:821

(continued)

Table 10.1 (continued)

	Marker	Gene ID	Comment	Literature
	Fc gamma RIV	Fcgr4	Expressed by mouse KC; gene not identified in human	Otten et al. 2008. J Immunol 181:6829
	Fc alpha RI/CD89	FCAR	Expressed by human KC; gene identified in rat, not in mouse; increased in inflammation	van Egmond et al. (2000)
	Fc epsilon RI/CD23	FCER2	Subset of mouse KC positive (20–40 %)	Eicher et al. 1995. J Leukoc Biol 58:32
	Neonatal Fc receptor	FCRN	Expressed at high level by KC	Cianga et al. 2011. Hum Immunol 72:1176; Borvak et al. 1998. Int Immunol 10:1289
<i>Scavenger receptors^s</i>				
Class A	SR-A/SCARA1/CD204	MSR1	Contributes to but not solely responsible for modified LDL clearance in liver; decreased expression upon LPS exposure in vivo	Tomokiyo et al. 2002. Atherosclerosis 161:123; Van Berkel et al. 1998. Biochem J 331 (Pt 1):29; Xie et al. 2002. Hepatobiliary Pancreat Dis Int 1:558
	MARCO/SCARA2	MARCO	Not expressed by KC in steady state; strongly induced in KC upon infection or LPS exposure; not described in rat	Elomaa et al. 1998. J Biol Chem 273:4530; van der Laan et al. 1999. J Immunol 162:939
Class B	SR-BI/CD36-like 1	SCARB1	R for HDL-cholesterol ester; high-fat diet up-regulates expression in KC, but down-regulates in hepatocytes	Nakagawa-Toyama et al. 2005. Atherosclerosis 183:75; Fluiter et al. 1998. J Biol Chem 273:8434
	SCARB3/CD36	CD36	Major R for modified LDL in rat and mouse KC; not detectable by IHC on human KC in steady state	De Rijke et al. 1994. Biochem J 304:69; Bordessoule et al. 1993. Br J Haematol 83:370; Biegans et al. 2012. PLoS One 7:e34378
Class D	Macrosialin/CD68/ED1 (rat)	CD68	Generic marker for KC identification expressed by most KC in steady state and inflammation	Tomita et al. 1994. Hepatol 20:317; Movita et al. (2012); Gomes et al. 2004. Mol Aspects Med 25:183
Class G	SR-PSOX/CXCL16	CXCL16	Chemokine with dual function as scavenger R; strongly expressed by mouse KC; only occasional staining of human KC; not described for rat KC	Wehr et al. 2013. J Immunol 190:5226; Heydtmann et al. 2005. J Immunol 174:1055

<i>Non-classified scavenger receptors</i>		
CD14	CD14	Only marginally expressed by KC in steady state; induced after LPS or ethanol in vivo
CD163	CD163	Increasingly expressed by human KC in viral hepatitis; major subset of rat KC (ED2); uniform expression peri-portal, less peri-central
<i>C-type lectin receptors^d</i>		
Mannose R/CD206	MRC1	Expressed in steady state; up-regulated by dexaamethasone
Dectin-1/beta-glucan R	CLEC7A	Expression reported in mouse KC; note that also CR3 binds beta-glucan
Asialoglycoprotein R	ASGRI, -2	R for de-sialylated proteins
DC-SIGN/CLEC4L	CD209	Expression reported in human KC
Kupffer cell R/CLECSF13	CLEC4F	Unique KC lectin in mouse and rat; R for Fuc and Gal, involved with glycolipid presentation to NKT cells; human ortholog is not expressed in liver and not a full-length protein
LSECtin	CLEC4G	Expression reported in human and mouse KC; negatively regulates activated T cells

^aThis table is restricted to markers used for KC identification and major surface receptors involved in endocytic function in human, mouse, and rat. Important species-specific or activation-related differences are indicated

^bRecent reappraisal of Fc receptor diversity in different species (Bruhns 2012, Blood 119:5640) is not recognized in older literature

^cFor a recent overview of different scavenger R families in macrophages see Kzhyshkowska et al. 2012, Immunobiology 217:492

^dSee van den Berg et al. 2012, Ann NY Acad Sci 1253:149 for a general overview

Tomita et al. 1994, Hepatol 20:317;
Enomoto et al. 1998, Gastroenterology 115:443; Xie et al. 2002, Hepatobiliary Pancreat Dis Int 1:558

Hiraoka et al. 2005, Pathol Res Pract 201:379; Polfriet et al. 2006, Immunobiology 211:419; He et al. (2009)

Haltiwanger et al. 1986, J Biol Chem 261:15696; Zhu et al. 2004, J Pharmacol Exp Ther 308:705; Noorman et al. 1997, Hepatol 26:1303

Reid et al. 2004, J Leukoc Biol 76:86; Thornton et al. 1996, J Immunol 156:1235

Coombs et al. 2006, Glycobiology 16:1C
Lai et al. 2006, Am J Pathol 169:200

Linehan et al. 2000, Microbes Infect 2:279; Fadden et al. 2003, Glycobiology 13:529; Yang et al. 2013, PLoS One 8:e65070

Dominguez-Soto et al. 2009, Hepatol 49:287; Tang et al. 2009, Gastroenterology 137:1498-508 e1

human, mouse, and rat KC. When applicable, species-specific differences are indicated. In addition to the indicated literature, readers are referred to reviews on KC (Huang et al. 2012; Crispe 2011; Naito et al. 2004).

10.2.3 *Kupffer Cell Heterogeneity*

Populating a large organ, with gradients of nutrients and oxygen from the portal to the central areas of the liver lobules, it is comprehensible that KC constitute a heterogeneous population of cells (Fig. 10.1c; discussed in Laskin et al. 2001; Naito et al. 2004). In general, KC in peri-portal regions are larger, more phagocytic, and contain higher levels of lysosomal enzyme activity, while being less responsive to inflammatory triggers. Conversely, the smaller KC in the peri-central areas express higher levels of MHC class II and produce more nitric oxide (NO) and superoxide anion upon stimulation. This diversity is also reflected in the differential expression of phenotypic and functional features of subpopulations isolated via counterflow centrifugation elutriation (e.g. ten Hagen et al. 1998).

The method of study has a significant impact on the observed heterogeneity among KC, which is caused by the differential representation of different subsets. In situ labeling of cells, mostly in tissue sections, obviously provides a picture of the full spectrum of KC. Upon isolation, however, Crispe and collaborators noted a striking discrepancy between the KC subsets represented in isolated populations characterized by flowcytometry, and those identified in situ (Klein et al. 2007). They identified a subpopulation of so-called “sessile” KC that was highly underrepresented among the cells isolated using enzymatic digestion. Since sessile KC are uniquely radio-resistant and have distinct developmental and functional features (see below), it is important to realize the impact of technical approach when interpreting experimental findings and comparing results from different studies.

Although characterization in situ is most reliable, identification of distinct subsets at this level is challenging due to the difficulty of quantification of marker expression. Kinoshita and collaborators identified by flowcytometry two major KC subsets in mouse liver, which both are F4/80⁺ but differentially express CD68 and CD11b (Kinoshita et al. 2010). In our own recent histological analysis of mouse KC, we and others observed differential expression of CD68 and F4/80 by KC with only partial overlap (Movita et al. 2012; Lloyd et al. 2008). In addition, only a minority of F4/80⁺ KC showed histologically detectable CD11b expression. Careful evaluation let us conclude that F4/80-low CD11b⁺ cells in the liver resemble monocyte-related cells, and differ from tissue-resident KC, with the latter population being more difficult to isolate from the liver by perfusion or collagenase treatment (Movita et al. 2012; Kinoshita et al. 2010). This subset distinction was recently confirmed, showing that CD68⁺ cells, but not CD11b⁺ cells, expressed CR1g, MerTK, and CD64, and were involved with systemic bactericidal activities (Ikarashi et al. 2013). In contrast, CD11b⁺ cells were pivotal in immunity against tumors. Clearly, scoring cells positive or negative in immunofluorescence or immunohistochemistry depends on mul-

multiple technical parameters, but the findings at this level illustrate the heterogeneity of KC in the steady state. Whether this represents the existence of distinct subsets or a spectrum of cells with different expression levels remains to be determined.

10.3 Kupffer Cell Origin and Growth Factor Dependence

The origin of KC in the adult steady state has long been debated, with different experimental approaches leading to different interpretations. These varied from the classic mononuclear phagocyte system view of an exclusive bone marrow (BM) origin, where circulating monocytes differentiate into KC, to an embryonic origin of the KC population, which is maintained via local proliferation. The proliferating cells could be either immature local precursor cells or mature KC themselves. Early studies, for instance, showed extensive mitosis of KC during the regeneration phase after partial hepatectomy (Widmann and Fahimi 1975). However, this could also represent proliferation of immigrants from the circulation. Functional depletion of BM cells by radioactive ^{89}Sr incorporation into bone strongly suggested the independent maintenance of KC (Yamada et al. 1990), but disparate opinions on KC origin existed for a long time in the scientific community.

Increasing light was shed on this matter by more recent BM transplantation experiments (Klein et al. 2007). In these, Crispe and colleagues identified in mice the radio-resistant “sessile” KC mentioned above. Four weeks after BM transplantation, approximately half of the KC population appeared to be of donor origin as determined at the histological level. In seeming contrast, flowcytometric analysis showed 99 % of F4/80-high, CD11b-low KC were donor-derived at this time point. This discrepancy could be explained by the presence of sessile KC, which are barely replaced and escape enzymatic isolation, and therefore flowcytometric identification. The sessile population was, however, completely depleted by *in vivo* treatment with liposome-encapsulated clodronate, and then replaced by donor-type cells. Thus, sessile KC could be either self-maintained or have acquired radioresistance and longevity, and still be hematogenous in origin. The experimental conditions, however, significantly challenge the resident KC population, and thus the obtained results leave room for diverse interpretations concerning the origin of KC in the steady state. In any case, the liposome-mediated depletion experiments gave no indication for the existence of an immature, non-phagocytic local precursor, which would have generated host-type KC. Rather, these experiments showed the potential of BM-derived cells to differentiate into genuine KC.

Recent lineage tracing studies provided more definitive answers on the KC origin in the steady state (Schulz et al. 2012; Yona et al. 2013). These findings have shown that KC, as well as other F4/80-high macrophage populations like brain microglia, derive from yolk sac macrophages, and are maintained in adult mice independently from BM hematopoietic stem cell-derived monocytes. Development of these F4/80-high macrophages does not depend on the transcription factor *c-Myb*, in contrast to the differentiation of adult blood monocytes and their progeny. The

F4/80-high/CD11b-low KC are embedded in the parenchyma, while BM-HSC-dependent macrophages with the reverse phenotype are mostly located around the larger vessels in the liver (Yona et al. 2013).

Together, the picture emerges that in steady state at least three populations of macrophages can be discerned in the mouse liver. The similarly sized sessile F4/80-high/CD11b-low KC and the enzymatically isolatable F4/80-high/CD11b-low KC both have a yolk sac origin and are self-maintained in the steady state. Of these subpopulations, the latter exclusively takes part in local inflammatory responses (Klein et al. 2007). Only upon serious challenge, when resident KC are affected, these will be replaced by BM-derived cells. In contrast, the third population of F4/80-low/CD11b-high cells is monocytic in origin, and may develop into perivascular liver tissue macrophages.

For their development KC depend strongly, but not absolutely, on CSF-1/M-CSF, as different studies observed a reduction of the KC population in CSF-1-deficient *op/op* mice to approximately 30–50 % of the cells compared to controls (summarized in Wiktor-Jedrzejczak and Gordon 1996). Activity of the alternative ligand for CSF-1 receptor, IL-34, probably explains the generation of these residual KC. Absence of only IL-34, in turn, does not affect KC development, in contrast to generation of epidermal Langerhans cells and microglia (Wang et al. 2012). The notion that CSF-1 is an important growth factor for KC is supported by the finding that KC fail to repopulate in adult CSF-1-deficient but IL-34-sufficient *op/op* mice after liposomal clodronate-mediated depletion, while KC reach normal numbers after 14 days in CSF-1-sufficient mice (Yamamoto et al. 2008). Exogenous CSF-1 supplementation restores KC development in *op/op* mice. Interestingly, it was shown recently that resident macrophage populations, including KC, are stimulated under type inflammatory type 2 conditions to proliferate in an IL-4-mediated, CSF-1-independent fashion (Jenkins et al. 2013).

KC not only depend on CSF-1 as growth factor, being the largest macrophage population in the body they are also important regulators of macrophage homeostasis in general. They do so, on the one hand, by functioning as a sink of circulating CSF-1 (Bartocci et al. 1987), and, on the other hand, by producing CSF-1 at significant levels upon demand (Moriyama et al. 1997).

10.4 Kupffer Cell Functions in Steady State

Under steady state conditions, in the absence of triggers by pathogens, disease or physical stress, KC fulfill an important role to eliminate insoluble macromolecules, immune complexes, toxins, and degenerated cells from the circulation. Since KC 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. Elimination of debris and insoluble macromolecules is via pattern recognition receptors (PRR), such as scavenger receptors, mannose receptors, and Fc receptors that are able to bind immune complexes or opsonized cells.

Senescent or damaged erythrocytes are removed in the liver by KC, but also by macrophages in spleen and bone marrow. Furthermore, during the lifespan of erythrocytes, part of their hemoglobin content as well as of their membrane is shed as vesicles. These hemoglobin-containing vesicles are rapidly removed from the circulation by liver KC and, to a lesser extent, macrophages from other tissues mainly by scavenger receptors (Willekens et al. 2005). Following phagocytosis and hemolysis, the different components are degraded and recycled. Hemoglobin is degraded by heme-oxygenases (HO), of which HO-1 is expressed in KC. HO-1 is induced by diverse stimuli including heme, heat stress, LPS, and various cytokines. 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. Iron is then either stored intracellularly as ferritin or conveyed to circulating transferrin via membrane-bound ferroportin. Interestingly, it was also shown that heme-degrading HO-1 acts as a downstream effector molecule of IL-10 that mediates its immunosuppressive activities (Lee and Chau 2002).

Removal of leukocytes like T cells or neutrophils from the circulation also takes place in the liver by KC. The importance of the liver in this respect is indicated by the finding that apoptotic neutrophils appeared in the lungs and spleen only after inactivation of KC by gadolinium chloride (Shi et al. 2001). Phagocytosis of apoptotic cells by KC is mediated by recognition of surface phosphatidylserine and, importantly, enhances the production of IL-10, and may favor a state of unresponsiveness. The induction of IL-10 may prevent development of sterile inflammation, activation of macrophages, and release of activating signals. Ongoing phagocytosis of apoptotic cells as part of liver homeostasis, in combination with low levels of LPS that are continuously released from the gut are likely responsible for constant low levels of anti-inflammatory mediators, such as IL-10, TGF- β and prostaglandins that restrict inflammation under steady state conditions. In addition, continuous exposure to serotonin probably contributes to the induction and maintenance of a non-inflammatory state (de las Casas-Engel et al. 2013).

10.5 Kupffer Cell Responsiveness

Macrophages are abundantly equipped with PRR to identify pathogen- and damage-associated molecular patterns (PAMPs and DAMPs, respectively). These PRR encompass multiple families, including Toll-, RIG-, and NOD-like receptors (TLR, RLR, NLR, respectively), and C-type lectins receptors (CLR). While macrophages in general express a range of these receptors, related to their sentinel function, not all of these have been described in KC. Mouse KC express TLR1–TLR9, all of which appear to be functional (Wu et al. 2009). Human KC so far have only been described to express TLR2, TLR3, TLR4 (Takii et al. 2005; Visvanathan et al. 2007). Furthermore, in the *Listeria monocytogenes* infection model, mouse KC are shown to express RIG-I (Imaizumi et al. 2006). Hepatocytes and CD68⁺ liver mononuclear cells (presumably KC) express NLRC2 (NOD2) (Body-Malapel et al. 2008).

The ability of KC to produce various cytokines in significant amounts is still debated, mainly due to differences in KC definition and the used purification techniques (see discussion above). Previous studies on human and rat KC, using counterflow centrifugal elutriation, show that they are able to produce IL-10, TNF- α , and IL-6 upon LPS stimulation (Knolle et al. 1995; Kono et al. 2002). In addition, using flowcytometrically sorted cells and in the setting of bile duct ligation-induced liver inflammation, He et al demonstrated that ED1⁺ED2⁺ KC have a high mRNA level of IL-1 β (He et al. 2009). In a mouse model of ischemia reperfusion, TLR9-mediated activation of the NLRP3 inflammasome by extracellular histones appeared to play an important role in IL-1 activation and liver damage (Huang et al. 2013). However, several other studies show that steady state KC are relatively weak in producing cytokines. In vitro stimulation with LPS, R848, or CpG resulted in very low levels of IL-10, TNF- α , and IL-12p40 produced by KC (Movita et al. 2012). Similarly, Kinoshita et al. (2010) showed that the F4/80⁺CD68⁺ KC produce only low levels of TNF- α and IL-12 upon in vitro LPS stimulation.

The reduced capacity of KC to produce pro-inflammatory cytokines has been related to their continuous exposure to bacterial products, such as LPS from Gram-negative bacteria in the gut, supplied via the portal vein (Knolle and Gerken 2000). This so-called endotoxin tolerance to repetitive antigenic stimulation prevents excessive activation of KC and inflammation of the liver. The state of unresponsiveness is mediated via immunosuppressive cytokines, such as IL-10 and TGF- β , and prostaglandins, and further supported by down-regulation of TLR expression levels and negative regulation of TLR signaling, for instance by IRAK-M (Liu et al. 2008). Active suppression by IL-10 and TGF- β reduces the antigen-presenting capacity of KC by down-regulating the expression of MHC molecules and co-stimulators, without strongly affecting the scavenger function of KC. The consequence of the immunosuppressive milieu on KC, but also on other potential antigen-presenting cells including dendritic cells and sinus endothelial cells, is allograft tolerance following liver transplantation, but also a limited ability to eliminate intrahepatic pathogens. It has been suggested that, depending on the microenvironment and the quality and strength of signals received by KC, they can become immune-stimulatory cells, as has been demonstrated in co-culture experiments with human NK cells (Tu et al. 2008).

10.6 Kupffer Cells in Disturbed Homeostasis

Under steady state conditions the distinction between tissue-resident KC and monocytes can be made on the basis of surface markers, but under inflammatory conditions this is more difficult due to changes in the expression of identifying markers such as F4/80, CD11b, and CD68, in the case of mouse KC (Beschin et al. 2013). In humans, this situation is even more complex. Studies on healthy KC generally use cells collected upon perfusion of donor livers prior to transplantation. These cells likely represent a selected subset as they are only loosely attached to the sinusoids. Phenotypic examination of these cells indicated that they are KC, although

contamination with peripheral monocytes cannot be excluded (Tu et al. 2008). Other studies on human KC use surplus liver material from diagnostic liver biopsies, which is mostly obtained from patients with chronic autoimmune diseases or chronic viral hepatitis to determine the severity of liver disease. However, in these chronic diseases the distinction between tissue-resident KC and inflammatory monocytes may be even more difficult, since the latter cells may have differentiated and established a long-lasting equilibrium during disease. Also, no distinctive surface markers are available, and the identification is generally performed using antibodies against CD14. In mice, the models used to study disturbed homeostasis are generally more acute, and chronic disease models have received less attention. Bearing these conceptual considerations in mind, we attempt to summarize in the next sections the contribution of KC to various diseases involving the liver, in particular metabolic disease, different types of infection, and liver injury.

10.6.1 Kupffer Cells in Metabolic Disease

KC 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. Non-alcoholic fatty liver disease (NAFLD) has become the most common liver disease in developed countries, and most patients carry the hallmarks of obesity and metabolic syndrome (Baffy 2009). NAFLD starts with steatosis, the accumulation of hepatic fat, which is generally followed by the increased production of ROS and secretion of pro-inflammatory mediators that may induce liver inflammation and injury. This inflammatory condition is recognized as non-alcoholic steatohepatitis (NASH).

The involvement of KC in NAFLD has long been recognized. In humans, increased numbers of CD68-expressing KC have been shown to correlate with histological severity of NAFLD (Park et al. 2007). In a mouse model of NAFLD, in which ob/ob mice are fed a high-fat diet, the total number of KC is not affected (Leroux et al. 2012). However, the function of KC is altered, since KC in NAFLD secrete higher levels of pro-inflammatory cytokines and express a so-called “M1-phenotype,” which has also been shown for the increased numbers of macrophages present in obese adipose tissue (Lumeng et al. 2007). Enhanced secretion of pro-inflammatory cytokines such as IL-6, IL-1 β , and TNF- α by activated macrophages may lead to more tissue damage. The crucial role of KC in fatty liver disease has been indicated by experiments in mice where KC were eliminated by liposomal clodronate or by gadolinium chloride, which significantly decreased hepatic steatosis and insulin resistance (Huang et al. 2010; Rivera et al. 2007) and was accompanied by lower TNF- α and IL-6 mRNA expression.

The mechanisms driving KC activation in NAFLD and leading to hepatitis are beginning to be elucidated. Exposure of KC to elevated leptin levels, as present in metabolic syndrome, induce iNOS- and NADPH oxidase-mediated oxidative stress in KC, causing their activation (Chatterjee et al. 2013). Moreover, oxidized LDL

particles, but not unmodified or acetylated LDL, appear to accumulate in KC lysosomes and cause increased expression of inflammatory genes in the liver (Bieghs et al. 2013). Thus, the type of lipids to which KC are exposed play an important role in their polarization and activation, as was also indicated by a previous study by Papackova et al. (2012), in which high-fat diet rich in mono-unsaturated lipids stimulated an alternative M2-like program in KC, and not a classical M1 activation program characterized by pro-inflammatory cytokine production.

Recently, it has been shown that TNF- α -producing KC are crucial during the early stages of NAFLD to promote blood monocyte infiltration of the liver. Importantly, targeted knockdown of TNF- α expression by siRNA in myeloid cells decreases the incidence of NAFLD development by decreasing steatosis, liver damage, monocyte infiltration, and the production of inflammatory chemokines (Tosello-Tramont et al. 2012). In line with the observation that enhanced and altered activation of KC promotes pathogenesis of NAFLD, the well-known suppressive effects of IL-10 and IL-1 receptor antagonist ameliorate steatohepatitis in mice (Byun et al. 2013; Petrasek et al. 2012). Interestingly, it was recently shown that IL-10 released by M2-like alternatively activated KC stimulated apoptotic death of pro-inflammatory M1-like cells (Wan et al. 2014). This mechanism mediated resistance to hepatocyte steatosis and subsequent death.

Triggering of TLR4 by gut-derived LPS, which leads to activation of KC, is considered a key event in the pathogenesis of NAFLD (Baffy 2009). Remarkably, administration of LPS exacerbates liver injury in a model for NAFLD, whereas peptidoglycan administration, triggering TLR2/6, does not. One of the initiation events of NAFLD is likely an alteration in the intestinal microbiota due to over-nutrition or metabolic disturbances. These changes in intestinal microbial profiles affect fat accumulation in the liver. In this respect, it has been shown that ob/ob mice, which are a well-known model for obesity, display a different microbial profile compared to control mice (Tilg 2010). As a consequence of altered gut microbiota the local as well as systemic levels of endotoxins, such as LPS, may increase and affect innate immune cells, including KC. Furthermore, and this is therapeutically extremely interesting, probiotics have been shown to have a beneficial effect on NAFLD in mouse models by reducing hepatic injury and inflammation, which further supports the crucial role of gut-derived LPS in initiating and maintaining NAFLD (Farrell et al. 2012).

Besides gut microbiota also other parameters involving KC influence NAFLD. Activity of distinct PPAR members have an effect on NAFLD pathogenesis, since PPAR- γ signaling improves insulin sensitivity and may be beneficial for NAFLD treatment, whereas lower levels of PPAR- α and PPAR- δ lead to increased fat storage and consequently more severe disease (Stienstra et al. 2010). In this respect, macrophage-specific induction of PPAR- δ signaling stimulates alternative or M2-like activation of KC, which is thought to mediate insulin-sensitizing effects (Odegaard et al. 2008). In addition, mediators produced by adipose tissue, such as leptins and other cytokines, may modulate KC function. In this regard, adiponectin may inhibit NK- κ B and Erk1/2 signaling and thereby inhibit cytokine induction, whereas leptins induce the expression of pro-inflammatory cytokines.

Similar as in NAFLD, fatty liver disease caused by excessive alcohol consumption also leads to higher endotoxin levels in serum, which is primarily due to changes in the microbial gut flora and enhanced intestinal permeability (Thurman et al. 1998). Although the initiating trigger that causes alcoholic liver disease is different from NAFLD, many of the clinical observations and their underlying mechanisms are similar. Ethanol may directly affect the transcriptional regulation of genes involved in lipid metabolism, such as PPAR- α . Similar as in NAFLD, depletion of KC in mice also attenuates alcohol-induced disease, demonstrating a central role for KC (Nath and Szabo 2009). In addition, abrogation of the TNF- α pathway in vivo using antibodies against TNF- α or TNF- α RI gene-targeted mice strongly reduces alcohol-induced liver pathology, as demonstrated by reduced serum ALT levels and pathology scores (Iimuro et al. 1997; Yin et al. 1999). Interestingly, signaling via TLR4 activates KC in these mouse models, with the TRIF pathway—but not the MyD88 pathway—being responsible for TNF- α induction (Hritz et al. 2008).

10.6.2 *Kupffer Cells in Infection*

KC form, together with the sinusoidal endothelial cells, the first barrier for pathogens to enter the liver via the portal vein (Vollmar and Menger 2009). Their endocytic capacity, expression of different PRR, MHC and co-stimulatory molecules, and ability to produce cytokines upon stimulation, renders them potent immune cells contributing to either pathogen clearance or persistence. Pathogen recognition may activate KC leading to the production of pro-inflammatory mediators important for inhibition of pathogen replication, the induction of resistance to infection of neighboring cells, and attraction and activation of other immune cells. KC are the prime cells in the liver to present lipid antigens in a CD1-restricted manner to NKT cells. In conjunction, KC activate NK cells (also called Pit cells, representing the large intrahepatic NK cell population) and NKT cells via the production of pro-inflammatory cytokines, which on their turn produce activating cytokines such as IFN- γ and provide cytotoxic activity (Tu et al. 2008; Dao et al. 1998). Although liver macrophages are probably not involved in priming of naïve T cells, mouse KC have been shown to present antigen to CD4⁺ and CD8⁺ T cells, inducing these to proliferate and produce IFN- γ (Ebrahimkhani et al. 2011; You et al. 2008). The interaction of KC with membrane-bound as well as soluble mediators expressed by infiltrating immune cells probably leads to further regulation of KC function and of the intrahepatic inflammatory response. In contrast to their role as effective host defense cells favoring the survival of the infected host, evidence is accumulating that some pathogens use the transport properties of sinusoidal cells, including KC, to increase the efficiency of hepatocyte infection. Furthermore, pathogens may exploit the tolerogenic capacities of KC to evade immunity and/or may have evolved to inhibit the immunogenic functions of KC. In the following paragraphs we will provide examples of the various roles of KC in viral, bacterial, and parasitic infection, without the ambition, however, to elaborate in the discussion on the different pathogens that challenge hepatic homeostasis.

10.6.2.1 Liver Infection by Viruses: Mouse Models

The defensive role of KC, as the first line barrier, to take up viral particles and thereby limit infection, has been shown in lymphocytic choriomeningitis virus (LCMV), murine hepatitis virus (MHV), and adenovirus infection (Lang et al. 2010; Pereira et al. 1984; Smith et al. 2008). Failure in direct clearing of viruses resulted in “spill-over” infection to hepatocytes, which prolonged the duration of infection and exacerbated immunopathology. Furthermore, the number of intrahepatic F4/80⁺ cells increased during LCMV infection, suggesting the involvement of KC and/or recruited inflammatory monocytes in the immune response to LCMV (Dixon et al. 1986) and in the regulation of immunopathology (Lang et al. 2010). In mouse cytomegalovirus (MCMV) and adenovirus infection, KC were found to produce pro-inflammatory cytokines and chemokines, which were directly or indirectly responsible for monocyte, NK cell, and T cell infiltration in the liver (Salazar-Mather and Hokeness 2006; Lieber et al. 1997; Liu et al. 2003; Zaiss et al. 2002). Instead of promoting the expression of pro-inflammatory signals, MHV infection was found to down-regulate the production of IL-10 and PGE₂ by mouse KC (Jacques et al. 2008).

In general, the mouse models of viral hepatitis have generated much insight in disease pathogenesis, but fall short in important aspects, unfortunately. In contrast to the most common human hepatitis viruses, i.e., hepatitis A, B, and C viruses (HAV, HBV, and HCV, respectively) the mouse viruses also infect cells other than hepatocytes, including KC, and even other organs. Due to the narrow host specificity, studies on the anti-viral immune functions of KC in human hepatitis viruses are limited.

10.6.2.2 Liver Infection by Viruses: Human Hepatotropic Viruses

Both HBV and HCV are blood-borne viruses that can cause chronic liver disease and may elicit progressive liver injury leading to increased risk of developing liver cirrhosis, liver failure, and liver cancer. A very low number of HBV particles (<10) is sufficient to establish hepatocyte infection in vivo (Jilbert et al. 1996; Asabe et al. 2009), indicating that liver targeting by HBV is extremely efficient. This may be enabled by initial scavenging of the virus by endothelial cells, as described for duck HBV (Breiner et al. 2001), or by other sinusoidal cells.

Productive infection of KC by HBV has been suggested on the basis of viral HBsAg-positivity of KC using suboptimal detection methods (Deodhar et al. 1975), but detailed information on the presence of HBV (proteins) in KC in vivo or the uptake of HBV or its proteins by human KC ex vivo has not been reported. So far, it is mostly unclear which receptors KC may use to recognize and take up HBV. Possible candidate receptors for direct binding of HBV to KC are heparan sulfate proteoglycan (HSPG), CD14, and the mannose receptor. Another molecule that binds HBsAg avidly is albumin, which is efficiently taken up by KC as well as hepatocytes, and may offer the virus a physiological transport pathway to parenchymal cells (Wright et al. 1988). Similarly, KC also express several receptors known

to be involved in hepatocyte infection by HCV (Ploss and Evans 2012), including HSPG (Pradel et al. 2002), LDL-receptor (Kleinherenbrink-Stins et al. 1991; Kamps et al. 1991), SR-B1 (Terpstra and van Berkel 2000), CD81 (Petracca et al. 2000), and DC-SIGN (Tu et al. 2008), a lectin known to bind the HCV envelope protein E2 (Pohlmann et al. 2003). Liver biopsy-derived cells exposed to E2 demonstrated binding to liver cells including KC (Petracca et al. 2000), but so far, there is no evidence for the occurrence of HCV *trans*-infection *in vivo*.

Exposure of KC to HCV-derived proteins leads to activation and the expression of pro-inflammatory factors, including IL-1 β and TNF- α (Tu et al. 2010). Similarly, stimulation of human non-parenchymal cells, presumably KC, with HBV leads to the activation of NF- κ B and production of IL-1 β , IL-6, and TNF- α , cytokines known to inhibit HBV replication in hepatocytes (Hosel et al. 2009). However, when incubating rat KC with HBV virions, they hardly expressed IL-1 β , IL-6, or TNF- α , but instead preferably produced TGF- β (Li et al. 2012). Likewise, also HCV has been shown to induce the production of immune regulatory factors including IL-10 by monocyte-derived macrophages, which may promote viral persistence (Chang et al. 2007). A recent study, investigating the difference between responders and non-responders to IFN- α therapy against HCV, showed that non-responders had relatively high expression levels before treatment of IFN-stimulated genes in the liver (Lau et al. 2013). In these patients, KC appeared to be a local source of type 1 IFN that promoted basal expression of IFN-stimulated genes in hepatocytes, and thus negatively influenced the outcome of IFN-based therapy.

The PRR, either extracellularly or intracellularly expressed, needed for these responses to viral proteins remain to be identified. TLR2 has been put forward as a possible specific recognition receptor for the nucleocapsid protein of HBV, i.e. HBcAg, and the non-particulate version of this protein, HBeAg. Moreover, HBeAg present in the cytosol seems to interfere actively with TLR signaling (Lang et al. 2011). TLR2 has also been suggested to play a role in HCV-core and nonstructural-3 protein (NS3) recognition by macrophages leading to MyD88-dependent activation, including increased IRAK activity, p38 phosphorylation, ERK and JNK activity, and AP-1 activation (Imran et al. 2012). Furthermore, it was shown that NS3-induced TNF- α production by KC is partially TLR4-dependent (Hosomura et al. 2011).

Due to high-level expression of adhesion molecules, KC can trap activated T cells and other cells in the liver sinusoids (John and Crispe 2004) leading to either immune activation or tolerance, depending on the status of the KC. It was reported that KC from chronic hepatitis C patients display an activated phenotype and form clusters with T lymphocytes, suggesting an interaction between KC and intrahepatic T cells (Burgio et al. 1998). However, KC derived from hepatitis B or C patients with chronic disease or KC exposed to these viruses *in vitro* were found to express also enhanced levels of immune regulatory molecules including PD-L1 and galectin-9, the ligands for PD-1 and TIM3 on T cells, respectively, which may suppress the activity of intrahepatic virus-specific T cells (Nebbia et al. 2012; Mengshol et al. 2010; Tu et al. 2010).

In contrast to HBV and HCV, HAV infection is self-limiting and does not induce chronic infectious disease. Although *in vivo* evidence is lacking, it has been

proposed that HAV reaches hepatocytes via KC that bind complexes of HAV and HAV-specific IgA antibodies via the Fc α receptor (van Egmond et al. 2000), and subsequently transfer the virus to hepatocytes. In contrast to HBV and HCV, HAV requires the disruption of host cell membranes to release its progeny. These dying hepatocytes may provide DAMP, such as ATP and nucleic acids, which can be recognized by KC and other intrahepatic immune cells, leading to activation of these cells that can overcome viral immune escape and liver-intrinsic tolerogenic mechanisms (Canbay et al. 2003).

Recently a direct contribution has been reported of KC to the pathogenesis of hepatitis in response to infection with viruses with tropism for other organs and not detected in the liver (Polakos et al. 2006). In influenza infection, KC were indicated as the effector cells killing hepatocytes in an as yet unidentified manner, leading to damage-associated hepatitis. KC can kill hepatocytes either directly via CD95-dependent apoptotic pathways or indirectly by interacting with CD8⁺ (and possibly CD4⁺) lymphocytes through stimulation of cytokine secretion and other mediators like phospholipases and NO (Polakos et al. 2006; Kolios et al. 2006). Although such a mechanism might explain the hepatitis observed in influenza, measles, SARS, and CMV infection, where the virus is not identified in the liver, a similar mechanism could well operate in the pathogenesis of hepatitis induced by hepatotropic viruses like HBV and HCV.

10.6.2.3 Liver Infection by Bacteria

Exclusive infection of the liver by bacteria has not been described, and most bacteria that reach the liver through the blood are efficiently cleared by immune cells. A major role for KC in host defense against bacterial infection is indicated by several studies in experimental models. Infection of mice with *Listeria monocytogenes* is a well-studied liver infection model. On the one hand, *Listeria* infection is dependent on KC function, as accumulation of bacteria in the liver depends on recognition of bacterial surface molecules by cognate receptors on KC (Ofek and Sharon 1988). On the other hand, production of inflammatory mediators such as IL-6, IL-12, IL-1 β , TNF- α , and NO by infected KC inhibits proliferation of the microorganism (Ehlers et al. 1992; Ofek and Sharon 1988). At the same time KC-derived chemokines such as MIP-1 α (CCL3), MIP-1 β (CCL4), MCP-1 (CCL2), and MIP-2 (CXCL2 /-3), drive monocyte and neutrophil recruitment into the liver in order to control infection (Salkowski et al. 1998; Barsig et al. 1998; Ebe et al. 1999). In line with this, LPS treatment prior to infection has been shown to increase KC numbers leading to a reduction of bacterial load and improvement of prognosis in a *Salmonella* septicemia model (Lehner et al. 2001). Thus, as expected, KC inactivation or depletion results in impaired bacterial clearance (Cousens and Wing 2000; Tomioka et al. 2000; Pinto et al. 1991). In humans, the increased frequency of septicemia and septic shock involving Gram-negative bacteria in patients with hepatic failure have been attributed to the inability of KC to clear the portal circulation of microorganisms and endotoxin (Wyke 1987; Triger and Wright 1973).

Although KC play a critical role in blood clearance, various studies indicate that the actual elimination of the bulk of bacteria taken up by the liver depends on a complex interaction of KC and neutrophils that immigrate rapidly into the liver in response to infection (Gregory et al. 2002; Shi et al. 1996, 2001). While neutrophils are bactericidal cells par excellence, KC have a limited capacity to kill *Listeria*, for instance (Tomioka et al. 2000). Several mouse and rat studies demonstrated the presence of apoptotic neutrophils in KC upon bacterial infection or endotoxin exposure (Shi et al. 1996, 2001; Gregory et al. 2002). These findings suggest that KC play a critical role in eliminating neutrophils that accumulate in the liver sinusoids subsequent to clearance of bacteria, bacterial endotoxin, and microbial debris from the blood. Furthermore, it was found that neutrophils, accumulated in the liver sinusoids, suppress cytokine and chemokine production by KC, thereby showing an important role for neutrophil-KC interaction in moderating the pro-inflammatory response to bacteria taken up by the liver (Holub et al. 2009).

10.6.2.4 Liver Infection by Parasites

KC also represent the port of liver entry for parasites such as *Plasmodium* and *Leishmania*, which parasitize KC and then infect other liver cells (Tavares et al. 2013). Following the delivery of malaria parasites into the skin by a mosquito bite, 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 KC (Pradel and Frevert 2001). Interaction with and passage through KC is important for hepatocyte infection (Ishino et al. 2004; Baer et al. 2007), indicating that these parasites use KC to overcome the sinusoidal barrier and, ultimately, to infect hepatocytes (Mota et al. 2002). TREM2 expression by KC appears to be an important determinant in resistance to liver stage infection against *Plasmodium* parasites (Goncalves et al. 2013). Once inside a hepatocyte, the parasites develop into merozoites, which will be released from the liver to infect erythrocytes (Sturm et al. 2006). Taken together, these data show that sporozoites not only use their migratory capacity to escape elimination by phagocytic cells, but also use KC to increase their efficiency at infecting hepatocytes.

10.6.3 Kupffer Cells in Liver Injury

Liver inflammation is initiated in response to a variety of signals to protect hepatocytes against damage and to favor tissue repair. KC, as prime sentinel cells, play an important role in initiating inflammation, while both KC and inflammatory macrophages contribute significantly to repair once the triggering factor has been eliminated (You et al. 2013). However, persistent or too intense inflammatory responses

will induce massive death of hepatocytes and hence cause irreversible tissue damage. Chronic inflammation is the basis of liver fibrosis and cirrhosis and also significantly increases the risk for hepatocellular carcinoma. Since KC, as major sentinel cells, contribute significantly to inflammation and its sequels, they play an important role in the induction and regulation of liver fibrosis and hepatocellular carcinoma. The knowledge on the role of KC in these processes is mainly obtained from experimental models in rodents.

10.6.3.1 Kupffer Cells and Liver Fibrosis

Liver fibrosis occurs as a wound-healing scar response following chronic liver inflammation and is characterized by excess collagen deposition and accumulation of extracellular matrix in response to chronic hepatocellular damage. Macrophage numbers increase in damaged liver and they are principally located around the regions of damage and fibrosis (Wallace et al. 2008). Inhibition of KC function using gadolinium chloride was found to reduce liver fibrosis in experimental models (Rivera et al. 2001). KC are thought to be involved in fibrogenesis via the production of ROS, cytokines, and growth factors that induce hepatic stellate cell myofibroblastic transformation (Wallace et al. 2008). In addition, they not only produce metalloproteinases and their inhibitors but also regulate the production of these factors by other cells, and stimulate their survival, leading to disturbance of the homeostatic mechanisms involved in extracellular matrix deposition (Xidakis et al. 2005; Pradere et al. 2013). Recent studies demonstrate that these actions are only partially conducted by liver-resident macrophages, but largely depend on recruitment of Ly6C-expressing monocytes as precursors of tissue macrophages into the inflamed and damaged liver (Imamura et al. 2005; Karlmark et al. 2009).

TGF- β is considered the main cytokine that drives fibrosis upon hepatic damage (Czaja et al. 1989; Castilla et al. 1991). KC-derived TGF- β has been suggested to drive hepatic stellate cell transformation and to induce production of collagen and proteoglycans by these cells (Meyer et al. 1990). In this context, IL-17A, which has been found elevated in fibrosis associated with alcoholic liver disease, appears to stimulate both KC and stellate cell activation (Hara et al. 2013). In vitro studies have shown that KC also can induce expression of platelet-derived growth factor (PDGF) receptors on hepatic stellate cells, thus enhancing stellate cell proliferation in response to PDGF produced by activated macrophages and stellate cells (Friedman and Arthur 1989). TNF- α , IL-1, and MCP-1 (CCL2), that are produced by activated KC, are also mitogenic and chemoattractant for hepatic stellate cells (Marra et al. 1999; Matsuoaka et al. 1989). In addition, TGF- β and IL-6 induce mRNA expression of metalloproteinases and also their specific inhibitors in hepatocytes, KC, and hepatic stellate cells in rat liver (Knittel et al. 1999).

Experimental animal models indicate that monocytes/macrophages are not only critical for fibrosis progression, but also for fibrosis regression (reviewed in Wynn and Barron 2010). In the CCl₄ model of liver fibrosis, Duffield et al. (2005) demonstrated

that macrophage depletion during the fibrosis resolution phase impeded matrix degradation. An anti-fibrotic effect of liver macrophages was also demonstrated when macrophage infiltration was blocked during the induction of fibrogenesis in rats (Imamura et al. 2005). With regard to recovery from fibrosis, macrophages secrete proteinases that promote the degradation of scarring extracellular matrix proteins.

10.6.3.2 Kupffer Cells in Hepatocellular Carcinoma and Liver Metastases

Chronic hepatitis and cirrhosis are major risk factors for the development of hepatocellular carcinoma. In these conditions, hepatocytes are killed, and KC as well as other cells are activated to produce cytokines, such as hepatocyte growth factor (HGF), IL-6, and TNF- α , that drive the compensatory proliferation of surviving hepatocytes (Maeda et al. 2005). Dying hepatocytes trigger the inflammatory response by activation of KC. Recently, it was demonstrated that the expression of TREM-1 by mouse KC plays a crucial role in their activation upon recognition of necrotic hepatocytes (Wu et al. 2012). The augmented proliferation rate of hepatocytes increases the probability of genomic mutation in these cells. In addition, there are several possible mechanisms by which inflammation and inflammatory mediators may lead to genetic alterations.

Besides primary liver cancer, liver metastases are frequently observed, especially in gastro-intestinal malignancies. Hepatic metastases result from initial detachment of tumor cells from the primary site, entry into the portal circulation, and entrapment of metastatic cells in the liver sinusoids (Van den Eynden et al. 2013). In vivo microscopy has shown that KC are attracted to tumor cells in the hepatic circulation and have the ability to phagocytose these cells (Kan et al. 1995). NO, produced by KC after stimulation with TNF- α , PGE₂ (Valatas et al. 2004; Gaillard et al. 1991), and endotoxin derived from the portal circulation, is thought to be an effective weapon of the KC machinery against tumor cells, since NO is able to inhibit proliferation and induce apoptosis in cancer cells (Aono et al. 1994; Hussain and Harris 2007). However, NO also mediates tumor-promoting effects that include the ability to induce DNA damage, to increase angiogenesis by inducing VEGF production, to stimulate tumor cell proliferation and invasion, and to suppress anti-tumor immunity (Hussain and Harris 2007; Koblish et al. 1998). Furthermore, KC are able to produce HGF, which has been shown to contribute to tumor cell proliferation, and KC might facilitate tumor angiogenesis and invasion by secreting proteases, which alter the extracellular matrix in favor of tumor progression (Knittel et al. 1999). Moreover, KC may contribute to an immunosuppressive microenvironment by expressing immune regulatory factors, such as IL-10, PD-L1 in addition to NO, that could prevent an effective immune response towards the tumor (Trinchieri 2012).

Although KC may have ambivalent roles in interaction with tumor cells, the protective role of KC against hepatic metastases has been emphasized by the finding that KC depletion prior to tumor cell challenge resulted in a drastic increase in tumor development in the liver (Paschos et al. 2010; Kruse et al. 2013). In support

of this protective role of KC, isolated KC were found to be cytotoxic against human colon adenocarcinoma cells and this cytotoxicity was increased significantly when KC were stimulated with IFN- γ and endotoxin (Roh et al. 1990; Heuff et al. 1995). Other studies have demonstrated that KC induce Fas expression in colon cancer cells (Song et al. 2001) and malignant glioma cells (Lau et al. 2001) leading to Fas-mediated apoptosis and death in the presence of tumor-infiltrating lymphocytes or TNF- α . The opposing tumor-restricting and -promoting role of KC was elegantly modeled recently by depleting KC at different time points during tumor induction (Wen et al. 2013). Depletion prior to tumor transfer facilitated tumor growth, while late stage KC depletion led to decreased tumor mass.

10.7 Concluding Remarks

Studies in experimental animal models in particular have indicated that KC and liver-infiltrating macrophages contribute significantly to homeostasis in health and disease. They perform important tasks in protecting the host against invading pathogens and are thought to play a major role in induction and maintenance of immunological tolerance. Furthermore, evidence is accumulating that liver macrophages are essential for the resolution of fibrosis and display anti-tumor activities. However, they are also at least partly responsible for the development of liver diseases, including fatty liver disease, the initiation of various forms of hepatitis and sequelae, like fibrosis. Several microbial pathogens seem to use KC as transport cells to infect the liver. Once liver infection is established and/or liver cell damage has occurred, liver macrophages contribute importantly to the inflammatory response and further tissue damage by producing pro-inflammatory and pro-fibrotic mediators, by killing hepatocytes and even by supporting carcinogenesis.

The exact contributions of liver-resident KC vs. liver-infiltrating monocyte-derived macrophages to various processes of homeostasis and disease pathogenesis are difficult to determine, because of the highly overlapping characteristics of these cells. Moreover, the notion that infiltrating monocytes readily develop into KC in non-steady state conditions seemingly makes the distinction between “true” KC and recent immigrants also a semantic issue. However, resident KC and liver-infiltrating macrophages probably respond differently to various environmental conditions, given their distinct developmental histories, thus leading to diverse phenotypic and functional states of these cells. It is tempting to speculate that these differences may eventually be essential in the contribution of different liver macrophages to various homeostatic processes. With our growing appreciation of the complex roles of liver macrophages in both protective and harmful responses, these cells form an interesting but difficult cellular target for treatment options in liver diseases. Future efforts should therefore focus on identifying the characteristics of the specific macrophage subpopulations that exert the distinctive functions of interest and on identifying the underlying mechanisms of actions.

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