**REVIEW**

**Infiltrating monocytes in liver injury and repair**

Katherine J Brempelis and Ian N Crispe

Noninfectious liver injury causes many acute and chronic liver diseases around the globe, and particularly in developed nations. Bone marrow-derived monocytes infiltrate the damaged liver tissue and are a critical component of the innate immune response that may drive injury resolution or host death in the short term or chronic inflammation, fibrosis and hepatocellular carcinoma in the long term. Monocytes often play dual roles in liver injury—both perpetuating inflammation and promoting resolution of inflammation and fibrosis. Thus, we will address the role that monocytes play in different experimental forms of noninfectious liver injury; considering in particular the importance of the transition from inflammatory Ly6C<sup>hi</sup> monocytes to pro-resolution Ly6C<sup>lo</sup> monocyte-derived macrophages and the consequences of this transition for disease progression and resolution.

Clinical & Translational Immunology (2016) 5, e113; doi:10.1038/cti.2016.62; published online 4 November 2016

**INTRODUCTION**

Noninfectious liver injury, including the effects of drugs and diet, is the major cause of liver disease in developed nations. In Western nations, the main cause of acute liver failure is acetaminophen (APAP) overdose, accounting for nearly 50% of acute liver failure cases. Ischemia–reperfusion (IR) injury contributes to up to 10% of early organ failure following liver transplant and can ultimately lead to increased risk of short-term and long-term organ rejection. Chronic damage can also be induced by diet or alcohol. Damage induced by diet results in nonalcoholic fatty liver disease (NAFLD), the most common chronic liver disease in Western nations. Damage induced by alcohol results in alcoholic liver disease (ALD). Both NAFLD and ALD are characterized by fatty liver (steatosis), and with the development of inflammation and fibrosis, these diseases can progress to nonalcoholic steatohepatitis (NASH) and alcoholic steatohepatitis (ASH), respectively. Current studies suggest that between 20 and 30% of the population of the United States and other Western nations is afflicted by fatty liver. Chronic alcohol abuse is a global issue with an estimated 140 million alcoholics worldwide. In the United States alone, chronic alcohol consumption contributed to 13 050 deaths in 2006, ~47% of all deaths attributable to chronic liver disease and cirrhosis.

Noninfectious liver injury can induce an immune response that either resolves or results in death of the host, or a chronic immune response that produces repeating cycles of cell death and inflammation. A complex interplay of innate and adaptive cell types, inflammatory signaling molecules and molecular effectors determine these different outcomes. At steady state, the liver is comprised mainly of parenchymal cells, called hepatocytes, and several types of nonparenchymal cells: liver sinusoidal endothelial cells (LSECs), the resident macrophage population called Kupffer cells (KCs), hepatic stellate cells (HSCs), liver dendritic cells (DCs) and intrahepatic lymphocytes dominated by natural killer and natural killer T cell populations. Hepatocytes, which make up ~90% of the total liver mass, comprise only 60–80% of the total cell number in the liver. The remainder is made up of the nonparenchymal cell populations. LSECs account for ~50% of the nonparenchymal cells and KCs for ~20%, with the remainder made up by lymphocytes (~25%), hepatic DCs (~1%) and HSCs (~1%). In addition, cells of the myeloid lineage—neutrophils, monocytes, DCs and lymphocytes—are constantly passing through the liver in the bloodstream, setting them up for extravasation into the liver upon detection of inflammatory signals. Upon liver injury, innate myeloid cells—including neutrophils and monocytes—are rapidly recruited to the site of injury.

Monocytes are bone marrow-derived circulating cells that localize to injured and inflamed tissues and differentiate locally into diverse myeloid cell populations, manifesting such functions as phagocytosis, antiviral immunity, antigen presentation, immune suppression and tissue repair. Along with liver-resident cells, monocytes and monocyte-derived macrophages participate in the response to both tissue injury and infection, and in the present review we focus on the way monocytes and monocyte-derived macrophages act in the context of noninfectious liver injury. This is of particular importance as shared features of monocytes, monocyte-derived macrophages and KCs complicate the ability to define the independent roles of each of these populations in the response to injury. This review will attempt to distinguish between these populations to clarify the contribution of monocytes and monocyte-derived macrophages from that of KCs during the course of liver injury, fibrosis and repair. Thus, we will discuss how inflammatory signals are generated by noninfectious stress in the liver, how monocytes are recruited, their differentiation fate and how they impact liver injury and repair. The precise role that monocytes play in the response to insult is an area of active research involving questions such as What types of monocytes are recruited and what are the signals leading to their recruitment? Do monocytes contribute to initial injury and development of fibrosis or do monocytes contribute to the termination of injury and the regression of fibrosis?
Do Ly6C<sup>lo</sup> monocytes derive from Ly6C<sup>hi</sup> monocytes or are these independent populations? We will address these questions as they pertain to the liver while weaving in results from other systems as they help inform the role of monocytes in liver injury. The recent literature supports the hypothesis that Ly6C<sup>lo</sup> inflammatory monocytes infiltrate the site of tissue injury, contribute to inflammation and the propagation of fibrosis, and then differentiate into Ly6C<sup>lo</sup> tissue repair monocyte-derived macrophages that aid in the resolution of injury and fibrosis.

**LIVER INJURY, STERILE INFLAMMATION, AND FIBROSIS**

The innate immune system has evolved to identify and respond to pathogens through the recognition of conserved microbial motifs known as pathogen-associated molecular patterns (PAMPs); however, innate immune-mediated inflammation also occurs in the absence of pathogen, as with APAP-induced injury or IR injury during organ transplant.8,9 Termed sterile inflammation, this state is induced by the release of host-derived products, called damage-associated molecular patterns (DAMPs) during tissue damage. DAMPs, which are normally sequestered inside cells, interact with the pattern recognition receptors of the innate immune system and initiate an inflammatory response.

Liver injury is not monolithic, and there are several pathologies that straddle the border between sterile inflammation and pathogen-induced inflammation. One such example is ALD. In ALD, ethanol-induced damage results in the release of various DAMPs; however, ethanol exposure also increases intestinal permeability and results in the leakage of lipopolysaccharide, a bacterial PAMP, from the commensal intestinal flora into the blood supply to the liver. Once in the liver, lipopolysaccharide binds to and activates KCs that then produce inflammatory cytokines that promote hepatocyte damage.10–12 Although some forms of sterile liver injury may in fact respond solely to DAMPs released from dying cells, many forms of ‘sterile’ injury are complicated by a response to DAMPs. The response to PAMPs from the normal gut flora as a component of ‘sterile’ inflammation is increasingly thought to play an important role in pathologies of the liver. The unifying feature of both true sterile inflammation and inflammation involving an additional PAMP component is that they are noninfectious modes of liver injury.

A defining feature of noninfectious liver injury is the death of hepatocytes. In APAP-mediated toxicity, the main mechanism of injury is damage to the endothelial cell microvasculature followed by extensive hepatocyte death in the centrilobular regions of the liver.13 Historically, there has been controversy as to whether the mode of hepatocyte death is predominantly apoptosis or necrosis, although increasing evidence supports necrosis as the dominant pathway.13,14 Metabolic disorders such as insulin resistance and obesity also ultimately result in hepatocyte damage via an excess of free fatty acids that contribute to the development of steatosis and NAFLD.15,16 Progression to NASH occurs with the development of persistent inflammation and increased hepatocyte death, and in some cases leads to cirrhosis.16 During ASH, tumors necrosis factor-α (TNF-α) released by activated KCs mediates the death of hepatocytes.17 In both NAFLD/NASH and ALD/ASH, hepatocyte apoptosis plays a large role in the induction of inflammation and the resulting fibrosis, though it is not responsible for all liver injuries.18–21 Finally, in IR injury following liver transplant, both LSEC and hepatocyte death leads to the release of DAMPs that activate KCs, LSECs and DCs to produce reactive oxygen species and other inflammatory mediators, perpetuating inflammation, although there is disagreement to the extent and mode of death.22–24

Acute inflammation is often induced by a single insult, such as IR injury during organ transplant or by APAP overdose. In many cases, acute inflammation is not resolved, particularly in cases of repeated insult and reinjury, and a state of chronic inflammation is established in the liver, such as in NASH or ASH. With enough time, chronic inflammation can progress to fibrosis, cirrhosis and/or hepatocellular carcinoma. As a more complete analysis of liver fibrosis is beyond the scope of this review, we will introduce the main cellular mediators and players, and suggest an excellent recent review by Pellicoro et al.25 for a more in-depth analysis.

In the liver, acute inflammation is characterized by the production of cytokines—interleukin (IL)-6, IL-1β and TNF-α—and chemokines—chemokine (C-C motif) ligand (CCL) and C-X-C motif chemokine (CXCL) family chemokines—in response to liver injury that is largely composed of, but not limited to, hepatocyte death. Traditionally, apoptosis has been considered an inflammatory ‘silent’ mode of cell death; however, as with necrosis and necroptosis, apoptosis also results in the release of DAMPs.15 Dying hepatocytes, DAMPs and the release of cytokines and chemokines produced in response to injury results in the activation of KCs and HSCs and their subsequent release of additional proinflammatory mediators. In addition, phagocytosis of apoptotic hepatocytes by KCs and HSCs leads to their activation.18 Activated KCs produce mediators that can directly induce hepatocyte death (TNF-α, Fas ligand and reactive oxygen species) or indirectly induce hepatocyte death through the recruitment of neutrophils via IL-1β and CXCL2.26 Production of CCL2 (also known as monocyte chemoattractant protein 1 (MCP-1)) recruits monocytes from the bone marrow to the liver. The influx of neutrophils and monocytes is a hallmark of acute liver injury, and recent literature supports the notion that infiltrating monocytes contribute to the production of inflammatory chemokines, the activation of HSCs and the promotion of fibrosis.25–27 Activated KCs and HSCs also produce transforming growth factor-β that in turn induces the transdifferentiation of HSCs into the more-activated myofibroblasts that promote deposition of extracellular matrix and collagen, leading to fibrosis of the liver. During inflammatory responses, KCs produce matrix metalloproteinases (MMPs) to degrade collagen and extracellular matrix components that have been deposited, but myofibroblasts counteract MMPs by producing tissue inhibitors of metalloproteinases that degrade MMPs.25 This cycle of matrix deposition, production of tissue inhibitors of metalloproteinases and inhibition of MMPs leads to scarring of the liver. The cycle is further perpetuated by TNF-α and IL-1β, inflammatory cytokines produced during the response to liver injury that promote myofibroblast survival.25

**ONTOGENY OF KUPFFER CELLS, MONOCYTE-DERIVED MACROPHAGES AND MONOCYTES**

The ontogeny of tissue-resident macrophages, we will consider the case of KCs specifically, has been under considerable debate for years, and remains an active area of research. Studies have shown that long-lived, self-renewing KCs are seeded from embryonic progenitors, although the specific source of these progenitors—yolk sac, fetal liver or embryo—remains controversial.28–34 In the steady state, KCs maintain without the contribution of circulating bone marrow-derived monocytes.28,31,32 Following injury, the number of KCs drastically decreases, whereas the number of monocyte-derived macrophages increases in the liver, although whether or not monocyte-derived macrophages ultimately replenish the long-lived KC population has remained an open question.27,35–39 Although it has been proposed that infiltrating monocytes do not replenish the decreased KC population,
and rather KCs recover through self-renewal, just this year, three groups have shown that infiltrating bone marrow-derived monocytes can replenish the KC population.\textsuperscript{38,40–42} Two of these groups further showed that this replacement by bone marrow-derived monocytes resulted in self-renewing macrophages with similar functional and transcriptional profiles to yolk sac-derived KCs—showing that this is yet an evolving area of research.\textsuperscript{41,42}

Although it is clear that KCs, monocyte-derived macrophages and monocytes play key roles in liver injury and the progression to fibrosis, the independent contributions of these populations is more difficult to tease apart because of overlapping phenotypic markers, inflammatory products, mechanisms, and ontology. Often, monocytes, monocyte-derived macrophages and KCs all fall within the larger umbrella of ‘liver macrophage’. Although seminal studies defined dual roles for CD11b\textsuperscript{+} and CCR2\textsuperscript{+} cells in liver injury and resolution of injury, the precise roles played by specific populations continues to be debated.\textsuperscript{43,44} KCs are well known to contribute to liver injury and, in this review, we consider the role of monocytes and monocyte-derived macrophages in injury, progression to fibrosis and repair in mouse models of sterile liver inflammation including APAP toxicity, carbon tetrachloride (CCl\textsubscript{4})-induced acute injury and NASH, as well as in models of NAFLD/NASH and ALD/ASH, and supplemented with data from human studies. We refer to monocytes as the cell population present in the bone marrow, spleen and blood that is recruited to sites of injury whereupon it enters the tissue and differentiates into monocyte-derived macrophages or DCs.\textsuperscript{45}

**INFLITRATING CELLS: MONOCYTES**

Monocytes are bone marrow-derived myeloid cells that circulate in the blood during the steady state as well as maintain a splenic reservoir.\textsuperscript{46,47} Upon injury or infection, monocytes are mobilized to the tissue site of insult where they differentiate into monocyte-derived macrophages or DCs. Although there are varying reports on the different subtypes of monocytes in mice, it is generally agreed that there are two main subsets: the classical, inflammatory monocyte and the nonclassical, patrolling (also variably called tissue repair or resident) monocyte.\textsuperscript{48–50} These subsets are defined by specific combinations of cell surface molecules and receptors including the chemokine receptors CCR2 and C\textsubscript{X}\textsubscript{C}R1, the cell surface molecule Ly6C—often referred to as GR1, although it should be noted that GR1 encompasses both the Ly6C and Ly6G epitopes, the latter expressed by neutrophils—as well as CD62L or (L-selectin). Classical monocytes express high levels of CCR2 and Ly6C but low levels of C\textsubscript{X}\textsubscript{C}R1, whereas nonclassical monocytes express high levels of C\textsubscript{X}\textsubscript{C}R1 and low levels of CCR2 and Ly6G.\textsuperscript{48–50} These monocyte subsets will be referred to as Ly6C\textsuperscript{hi} (CCR2\textsuperscript{+}CX\textsubscript{X}\textsubscript{C}R1\textsuperscript{lo}) and Ly6C\textsuperscript{lo} (CCR2\textsuperscript{−}CX\textsubscript{X}\textsubscript{C}R1\textsuperscript{hi}), respectively. In addition, CD62L is expressed on Ly6C\textsuperscript{−}CCR2\textsuperscript{-} monocytes but not on Ly6C\textsuperscript{+}CCR2\textsuperscript{+} monocytes.\textsuperscript{38,49}

Initially, human monocytes were classified into two subsets, but have since been separated into three functionally and transcriptionally unique subsets with the identification of an intermediate subset.\textsuperscript{49,51–57} Surface expression of CD14 and CD16 is used to delineate the three subsets of human monocytes: classical (CD14\textsuperscript{+}CD16\textsuperscript{−} or CD14\textsuperscript{+}CD16\textsuperscript{+}), intermediate (CD14\textsuperscript{+}CD16\textsuperscript{−} or CD14\textsuperscript{+}CD16\textsuperscript{+}) and nonclassical (CD14\textsuperscript{−}CD16\textsuperscript{+} or CD14\textsuperscript{−}CD16\textsuperscript{+}), although a consistent method of identifying the intermediate subset remains to be developed.\textsuperscript{58,59} Like mouse classical monocytes, human classical monocytes express high levels of CCR2 and CD62L but low levels of C\textsubscript{X}\textsubscript{C}R1, and like mouse nonclassical monocytes, human nonclassical monocytes express low levels of CCR2 and CD62L and high levels of C\textsubscript{X}\textsubscript{C}R1.\textsuperscript{48–50,54,60}

The alignment of mouse and human subsets remains a topic of debate, particularly when attempting to compare the human intermediate subset with the mouse subsets. Much of this discord is caused by earlier comparisons of only two monocyte subsets (CD14\textsuperscript{+}CD16\textsuperscript{−} and CD14\textsuperscript{+}CD16\textsuperscript{+}), effectively either excluding or condensing into one of the two populations, the intermediate monocyte population. In addition, differences in gating for the intermediate population add to the variability.\textsuperscript{58} On the basis of surface markers as described above, mouse Ly6C\textsuperscript{hi} monocytes align with human CD14\textsuperscript{+}CD16\textsuperscript{-} monocytes, whereas mouse Ly6C\textsuperscript{lo} monocytes align with human CD14\textsuperscript{+}CD16\textsuperscript{+} monocytes. Although there is quite a bit of variation in the cytokine production following lipopolysaccharide stimulation, the literature firmly suggests that CD14\textsuperscript{+}CD16\textsuperscript{-} monocytes are highly phagocytic and that CD14\textsuperscript{+}CD16\textsuperscript{+} monocytes are weakly phagocytic, further supporting alignment of mouse and human subsets as suggested by surface markers.\textsuperscript{51,55–57,61–64} Perhaps most suggestive is that like Ly6C\textsuperscript{lo} mouse monocytes, CD14\textsuperscript{+}CD16\textsuperscript{-} human monocytes exhibit extensive patrolling behavior.\textsuperscript{55}

Two transcriptional studies support the notion that CD14\textsuperscript{+}CD16\textsuperscript{-} monocytes are the human counterparts to the mouse Ly6C\textsuperscript{hi} inflammatory monocytes, whereas the CD14\textsuperscript{+}CD16\textsuperscript{-} nonclassical monocytes are the human counterparts of the mouse Ly6C\textsuperscript{lo} nonclassical monocytes; however, exact grouping of the intermediate CD14\textsuperscript{+}CD16\textsuperscript{-} subset is controversial.\textsuperscript{55,65} Ingersoll et al. grouped monocytes as CD16\textsuperscript{-} or CD16\textsuperscript{+}, suggesting that both CD16\textsuperscript{-} human monocyte populations (intermediate and nonclassical) group with Ly6C\textsuperscript{lo} monocytes. A study done in the same year by Cros et al. grouped monocytes based on expression levels of CD14, suggesting that both CD14\textsuperscript{-} monocytes (classical and intermediate) group with the Ly6C\textsuperscript{hi} monocytes, whereas the CD14\textsuperscript{+}CD16\textsuperscript{-} nonclassical population groups with the Ly6C\textsuperscript{lo} mouse population. The relation of the human intermediate subset is further complicated by an additional two transcriptional studies that suggest that the intermediate and nonclassical human subsets are more closely related to each other than either are to the classical human subset.\textsuperscript{56,57}

Mouse studies have been crucial for elucidating the recruitment patterns of monocytes and the relationship between monocyte subsets. During steady state, Ly6C\textsuperscript{lo} monocytes, or patrolling monocytes, patrol the vasculature via interactions controlled by C\textsubscript{X}\textsubscript{C}R1 and the integrin LFA-1.\textsuperscript{66} Patrolling of the mouse endothelium by human nonclassical monocytes in an LFA-1-dependent manner has also been observed, with C\textsubscript{X}\textsubscript{C}R1 playing an important role in their arrest and migration.\textsuperscript{54,55} Upon injury, predominantly Ly6C\textsuperscript{hi} monocytes are recruited from the bone marrow and spleen to the sites of injury in a CCR2- and CCL2-dependent manner.\textsuperscript{27,47,67,68} Human classical monocytes have also been observed to rely on CCL2, as well as CCL3, for migration.\textsuperscript{54,60} In steady-state conditions, Ly6C\textsuperscript{hi} monocytes are the precursors for Ly6C\textsuperscript{lo} monocytes, differentiating into the more mature Ly6C\textsuperscript{lo} monocyte subset in both the bone marrow and the blood.\textsuperscript{31,46,50} Human studies suggest that classical monocytes differentiate into the intermediate subset before finally differentiating into the nonclassical subset, although this needs to be addressed in further detail before solid conclusions can be drawn.\textsuperscript{56,57,65} In contrast to development from Ly6C\textsuperscript{lo} monocytes, it is also possible that Ly6C\textsuperscript{lo} monocytes develop from a distinct precursor in the bone marrow, although transition from Ly6C\textsuperscript{hi} to Ly6C\textsuperscript{lo} within the bone marrow cannot be ruled out.\textsuperscript{70} This distinction between classical and nonclassical monocytes is critical when assessing the roles that monocytes play in the inflammatory response to liver injury.
Infiltrating monocytes in liver injury and repair

KJ Brempels and IN Crispe

RECRUITMENT OF MONOCYTES TO THE INJURED LIVER

The CCL2/CCR2 axis is important for recruiting Ly6C\(^hi\) monocytes to the liver following insult. Early studies by Dambach et al.\(^{35}\) show that CCL2 is produced in the liver following APAP-induced liver injury, whereas studies using CCR2\(^{-/-}\) and CCR2\(^{-/-}\)CCR6\(^{-/-}\) mice demonstrated the importance of CCR2 for monocyte/macrophage recruitment to the liver.\(^{27,35,44}\) In humans, CCL2 serum levels are elevated in patients with NAFLD and NASH, as well as with acute liver failure.\(^{72-74}\) Furthermore, patients with APAP-induced acute liver failure (AALF) show increased expression of CCR2 on their interstitial, but not classical or nonclassical, monocyte subset.\(^{24}\)

Although in vitro studies suggest that the CX3CL1/CX3CR1 axis recruits human monocytes, the literature in murine liver injury is sparse.\(^{75,76}\) In mouse models of myocardial infarct and \textit{Listeria monocytogenes} infection of the peritoneum, CX3CR1 is required for Ly6C\(^hi\) monocyte recruitment to the injured or infected tissues.\(^{56,77}\)

The seemingly strong dependence on the CCL2/CCR2 axis compared with the CX3CL1/CX3CR1 axis for monocyte recruitment to sites of injury may be explained by studies that suggest that Ly6C\(^hi\) monocytes are initially recruited and then differentiate into Ly6C\(^lo\) monocytes at the site of injury.\(^{37,38,50,78}\) In contrast, studies in heart, lung and peritoneum suggest that Ly6C\(^hi\) and Ly6C\(^lo\) monocytes are recruited independently and in successive waves to the site of injury, although this does not rule out a role for Ly6C\(^hi\) differentiation into Ly6C\(^lo\) in the blood or bone marrow before recruitment to inflamed tissues.\(^{66,77,79}\)

In addition to the CCL2/CCR2 axis, other liver-specific pathways of monocyte recruitment have been recently elucidated. Recruitment of inflammatory Ly6C\(^hi\) monocytes to the liver has been found to occur via CXCL1/CXCR8 interactions in chronic injury.\(^{80}\) In a mouse model of \textit{L. monocytogenes} infection, intercellular adhesion molecule-1 and CD44 interactions were critical for CCR2\(^+\)Ly6C\(^hi\) monocyte recruitment to the liver, whereas CCR2 was only required for emigration out of the bone marrow.\(^{81}\) Recently, in a mouse model of cholestatic liver injury, the use of agonists against the sphingolipid metabolite S1PR2 (sphingosine 1-phosphate receptor 2) and S1PR3 receptors resulted in reduced recruitment of bone marrow-derived monocytes/macrophages to the liver.\(^{82}\) The role for S1PR receptors in monocyte trafficking is further supported by a study that showed that S1PR5 was critical for regulating Ly6C\(^lo\) monocyte egress from the bone marrow.\(^{83}\) Finally, recent studies have identified the CCR6 and paired immunoglobulin-like type 2 receptor-α (PILRα) receptors as negative regulators of monocyte recruitment to the liver during inflammation.\(^{84,85}\)

MONOCYTES AS DRIVERS OF INJURY?

Monocytes play a contradictory role in acute liver injury. Studies in both MCP1\(^{-/-}\) and CCR2\(^{-/-}\) mice suggest that CCL2-recruited CCR2\(^+\) monocytes participate in the induction of early injury, showing reduced injury at 24 h but equivalent injury by 48 h, in single-dose CCL2 models of acute liver injury.\(^{44,86}\) Most recently, two studies have shown that CCR2\(^+\) monocytes infiltrate the site of liver injury as early as 8 h following insult and traffic to the site of injury.\(^{79,78}\) In a model using APAP-induced injury, Tacke and colleagues\(^{39}\) found that CCR2\(^{-/-}\) mice had reduced levels of alanine aminotransferase (ALT), a measure of liver injury, and necrosis 12 h following injury. Furthermore, blockade of CCR2 by a pharmacological inhibitor resulted in reduced levels of ALT and necrosis 12 h following injury, but equivalent injury at 24 and 48 h, supporting the data from the CCL2 acute liver injury models in which CCR2\(^+\) monocytes enhance the early phase of liver injury.\(^{39}\) In contrast, using a model of heat-induced focal necrotic injury, Kubis and colleagues\(^{78}\) show that lack of CCR2\(^{-/-}\)CX3CR1\(^{+\+}\) monocyte recruitment results in the persistence of dead cells out to 48 h, whereas dead cells are cleared by 24 h when CCR2\(^{-/-}\)CX3CR1\(^{+\+}\) cells are present. In combination with the finding that the transition of CCR2\(^{-/-}\)CX3CR1\(^{+\+}\) to CCR2\(^{-/-}\)CX3CR1\(^{+\+}\) cells within the liver is critical for tissue repair, this study indicates that recruitment of CCR2\(^{-/-}\)CX3CR1\(^{+\+}\) monocytes is more important for repair as opposed to initial injury.\(^{79}\) In support of this notion, depletion of all blood monocytes by clodronate liposomes resulted in no difference in ALT levels 24 h following CCL2 injection, suggesting that recruited monocytes do not contribute to the initial stages of liver injury.\(^{77}\) Although it is possible that the Ly6C\(^hi\) inflammatory monocytes were recruited directly from the bone marrow, which is partially resistant to the effects of clodronate depletion, this group further showed that CCR2\(^{-/-}\) and CCR2\(^{-/-}\) CX3CR1\(^{+\+}\) mice exhibited no difference in liver injury at 24 and 48 h following CCL2 administration, even though infiltrating monocyte levels were decreased.\(^{23,87}\) Further supporting the lack of a role for monocytes in initial injury are studies using APAP-induced liver injury in which both CCR2\(^{-/-}\) and MCP1\(^{-/-}\) mice showed no significant differences in ALT levels compared with wild-type mice following APAP administration through 72 h, even though they observed a significant decrease in CD68\(^+\) (defined as a marker of activation) macrophages at 72 h in CCR2\(^{-/-}\) mice.\(^{35}\)

In humans, serum and liver tissue CCL2 levels are increased in patients with AALF in parallel with an increase in MAC387\(^+\) infiltrating monocytes/macrophages and Ki67\(^+\)CD68\(^+\) resident macrophages in the necrotic areas of the liver.\(^{74}\) The authors postulate that the anti-inflammatory environment of the liver in AALF patients (increased CCL2, CCL3, IL-6, IL-10 and transforming growth factor-β) suggests that these cells are in fact participating in the resolution of injury; however, the severity of AALF inversely correlated with the number of monocytes in the blood and directly correlated with serum CCL2 levels, suggesting that those patients with poorer outcomes recruited more monocytes to the liver.\(^{74}\) In line with this conclusion, Mossanen et al.\(^{39}\) show that CCR2\(^+\) cells are increased in the livers of patients with AALF compared with controls and, furthermore, show that they express the inflammatory marker S100A9, indicating a role in injury.

Collectively, these studies do not clearly define whether or not infiltrating monocytes are involved in promoting acute injury (0–48 h), potentially as a result of variations in the implementation of sterile injury models and as a result of the difficulty of assessing monocytes in human liver samples. For example, in 2012, Galasti et al.\(^{88}\) demonstrated that although CCL2\(^{-/-}\) mice on the BALB/c background were protected from chronic damage in a mouse model of NASH, CCL2\(^{-/-}\) mice on the C57BL/6J background were not. Clearly, further assessment of infiltrating monocytes, monocyte-derived macrophages, and KCs will be required to conclusively distinguish whether infiltrating monocytes are initially contributing to or inhibiting acute liver injury.

MONOCYTES AS DRIVERS OF FIBROSIS OR RESOLUTION OF FIBROSIS?

Repeated injection of CCL2 or feeding of specialized diets—high in fat or carbohydrates—are two common models used to induce chronic inflammation and fibrosis within the murine liver. In such models of murine fibrosis, inhibition of the CCL2/CCR2 axis often results in protection from liver inflammation and subsequent fibrosis. In a mouse model of NASH, CCR2\(^{-/-}\) mice exhibited less steatosis, infiltrating cells, fibrosis and fewer CD68\(^+\)Ly6C\(^{+\+}\) monocyte-derived
Infiltrating monocytes in liver injury and repair

In contrast, a recent study from MacDonald and colleagues, using a thioacetamide-induced chronic fibrosis model showed that blockade of macrophage colony-stimulating factor receptor (CSF1R, also known as CD115), the receptor for CSF-1, resulted in decreased Ly6C<sup>lo</sup> monocyte-derived macrophages in parallel with decreased fibrosis, suggesting that Ly6C<sup>lo</sup> monocyte-derived macrophages are critical for driving fibrosis. Blockade of CSF1R, however, also results in the depletion of resident KCs, and hence the effects of depleted KCs would need to be disentangled before firm conclusions about the causation of reduced fibrosis can be gleaned from this model. In humans, the intermediate CD14<sup>++</sup>CD16<sup>+</sup> monocyte subset may contribute to fibrosis in chronic liver disease, as it is increased in the livers of patients with ALD and NASH, and produces proinflammatory mediators including TNF-α, IL-6, IL-1β, CCL2, and CCL3.

Finally, a potential confounding factor to consider is the recent description of invading peritoneal macrophages into the liver upon injury. These cells, described as F4/80<sup>−</sup>CD11b<sup>+</sup>CD102<sup>−</sup> and GATA6<sup>+</sup>, were found to infiltrate the liver within 1 h following liver injury in an ATP- and CD44-dependent manner, express markers and genes associated with tissue repair and be absolutely critical for the survival of mice following CCL2-induced acute liver injury. Furthermore, use of a CCR2<sup>RFP</sup>+/CX<sub>3</sub>CR1<sup>GFP</sup> reporter mouse showed that these peritoneal macrophages are indeed distinct from infiltrating monocytes, representing another infiltrating monocyte/macrophage population. Although further studies will need to be conducted to assess how infiltrating peritoneal macrophages and monocytes interface, if at all, as the literature stands, it is quite possible that infiltrating peritoneal macrophages enter the liver at early stages, within 1 h following injury, acting as initial damage control and setting the stage for the subsequent infiltrating monocytes. This is supported by the observations that peritoneal macrophages localize to the center of injury, whereas the later-infiltrating CCR2<sup>hi</sup>CX<sub>3</sub>CR1<sup>hi</sup> monocytes form a ring around the area of injury, indicating distinct functions for each wave of infiltrating cell populations.

The split nature of the monocyte response—both promoting fibrosis and resolution of fibrosis—may be because of the different functions of Ly6C<sup>hi</sup> and Ly6C<sup>lo</sup> monocytes/macrophage populations. Gaining traction in the field is the hypothesis that Ly6C<sup>hi</sup> monocytes are recruited to the site of insult and drive the initial fibrotic response, but as they differentiate into Ly6C<sup>lo</sup> monocyte-derived macrophages, their role switches to driving tissue repair and resolution of fibrosis. Such an explanation may explain why CCR2<sup>−/−</sup> mice have been shown to both promote and resolve fibrosis—the initial influx of CCR2<sup>+</sup> (Ly6C<sup>hi</sup>) monocytes promotes the initial stages of fibrosis, but their differentiation into CCR2<sup>−</sup> (Ly6C<sup>lo</sup>) monocyte-derived macrophages is critical for the resolution of fibrosis.

**LY6C<sup>hi</sup> VERSUS LY6C<sup>lo</sup> MONOCYTES: DISTINCT POPULATIONS?**

The plasticity of inflammatory and tissue repair monocytes remains an area of active research, although many models of liver injury suggest that the tissue repair Ly6C<sup>lo</sup> monocyte-derived macrophage differentiates from the inflammatory Ly6C<sup>hi</sup> monocyte/macrophage-derived macrophage. Thus, Sunderkotter et al. used clodronate liposomes to deplete blood monocytes and observed that Ly6C<sup>hi</sup> monocytes repopulated the blood within 2–4 days following depletion, whereas Ly6C<sup>lo</sup> monocytes did not appear until 7 days following depletion, consistent with the idea that Ly6C<sup>lo</sup> monocytes derive from Ly6C<sup>hi</sup> monocytes.

More recently, a series of elegant studies has supported the hypothesis that inflammatory Ly6C<sup>lo</sup> monocytes transition into tissue...
repair Ly6C^hi monocyte-derived macrophages at the site of injury. In 2012, Ramachandran et al.\(^{37}\) used a CCL2-induced model of liver fibrosis and adoptive transfer of CD45.1^+Ly6C^hi monocytes into wild-type CD45.2^− mice to show that Ly6C^hi monocytes recruited by 24 h during fibrogenesis differentiated into Ly6C^lo monocyte-derived macrophages, which dominated at 72 h, during maximal scar resolution. Furthermore, elimination of Ly6C^lo monocyte-derived macrophages during maximal scar resolution resulted in a persistence of fibrosis, indicating that the Ly6C^lo subset were active in tissue repair (they term these ‘restorative’).\(^{37}\) In a model of APAP-induced liver injury, adoptive transfer of CD45.1^+CX3CR1^+ Ly6C^lo monocytes into CD45.2^− mice recipients demonstrated that Ly6C^lo monocytes differentiated into a Ly6C^lo subset by 72 h that were cleared by 96 h following liver injury.\(^{38}\) Finally, an elegant study by Dal-Secco et al.\(^{78}\) used CCR2-RFP and CX3CR1-GFP single and double reporter mice to demonstrate that CCR2^hiCX3CR1^hi (Ly6C^hi) monocytes were initially recruited to the site of liver injury where they encircled the site of injury by 24 h and transitioned into a CCR2^loCX3CR1^hi (Ly6C^lo) subset that were prevalent at 48 and 72 h. Importantly, this transition relied on IL-10 and IL-4 and inhibition of this transition resulted in reduced clearance of necrotic cells and reduced deposition of collagen within the site of injury.\(^{78}\) In vitro studies of cultured CD14^+CD16 human monocytes showed that they upregulate expression of CD16 following treatment with either IL-10 or transforming growth factor-β, further supporting the idea that classical monocytes can differentiate into more mature subsets.\(^{95}\) Taken together, these studies strongly suggest that inflammatory Ly6C^hi (CCR2^hiCX3CR1^hi) monocytes infiltrate the liver by 24 h and participate in fibrosis progression until they transition to Ly6C^lo (CCR2^loCX3CR1^hi) monocyte-derived macrophages that are active in fibrosis resolution.

In contrast to the notion that the Ly6C^lo subset only derives from recruited Ly6C^hi monocytes at the site of injury is the idea that these monocyte populations are recruited in successive waves. Although this is not supported by studies in the liver, it has been described to occur in the peritoneum, heart, and lung.\(^{66,77,79}\) The paper that defined the patrolling function of GR1^−GFP^hi (CX3CR1^hi) monocytes showed that they were the first responders to L. monocytogenes infection of the peritoneum, extravasating from patrolling the endothelium to the site of infection within the first 1–2 h, before GR1^+GFP^lo (CX3CR1^lo) monocytes entered the tissue.\(^{66}\) In a mouse model of myocardial infarct, inflammatory and phagocytic Ly6C^hi monocytes were recruited to injured tissue initially and were followed by the recruitment of Ly6C^lo monocytes primed to promote tissue repair.\(^{77}\) Intriguingly, in both inflamed and noninflamed conditions, GR1^hi and GR1^lo monocytes were capable of trafficking to the lung tissue independently, but GR1^hi monocytes were required to transition to GR1^lo monocytes before they could differentiate into lung macrophage.\(^{79}\) These examples are all unified by recruitment of distinct populations of Ly6C^lo and Ly6C^hi monocyte populations, but it is apparent that even with distinct recruitment of subsets that there may be significant overlap and plasticity that exists among Ly6C^lo and Ly6C^hi monocytes. Although successive recruitment of distinct populations of monocytes cannot be ruled out in cases of sterile liver injury, the majority of evidence from the liver supports the hypothesis of the Ly6C^lo to Ly6C^hi transition at the site of injury.

**WHAT FACTORS DRIVE THE LY6C^HI TO LY6C^LO PHENOTYPIC TRANSITION?**

Although the CX3CL1/CX3CR1 axis seems to minimally contribute to monocyte recruitment to injured liver in mice, it may play an important role in controlling the transition from Ly6C^hi to Ly6C^lo. In a model of CCL2-induced injury, mice lacking CX3CR1 showed increased levels of ALT, transcripts for inflammatory genes and recruitment of inflammatory F4/80^+CD68^ macrophages, indicating a protective role for CX3CR1.\(^{80}\) Intriguingly, the CX3CL1/CX3CR1 axis appeared to promote anti-inflammatory properties in KCs that then suppressed HSC activation, leading to reduced injury and fibrosis.\(^{80}\) The hepatoprotective role for CX3CL1/CX3CR1 was further delineated by studies from Tacke and colleagues\(^{39,80}\) showing that CX3CR1^−/− mice developed enhanced fibrosis in models of CCL2- and bile duct ligation-induced injury. In comparison with control mice, CX3CR1^−/− mice had increased numbers of CD11b^+F4/80^+ monocyte-derived macrophages in the liver and exhibited a more inflammatory phenotype (Tnf and iNOS), indicating that CX3CR1 is important in driving the transition from Ly6C^lo to Ly6C^hi phenotypes.\(^{80}\) Finally, they found that CX3CR1 was also critical for regulating Bcl2 expression and, therefore, survival of monocytes.\(^{80}\) In summary, CX3CR1 appears to be required for this transition from Ly6C^loCCR2^+CX3CR1^lo to Ly6C^hiCCR2^−CX3CR1^hi, and hence without it inflammatory monocytes/macrophages cannot differentiate into the tissue repair phenotype, resulting in the perpetuation of inflammation and fibrosis.

Alternatively, or in addition, CCR8 may also control differentiation of infiltrating monocytes. CCR8^−/− mice had fewer CD45^+CD11b^+ F4/80^−CD11c^− cells, termed inflammatory macrophages by Heymann et al.,\(^{80}\) than wild-type mice in a model of CCL2-induced chronic fibrosis. Intriguingly, CD11b^+F4/80^+ hepatic macrophages isolated from CCR8^−/− mice and stimulated with lipopolysaccharide in vitro expressed a more DC-like phenotype (higher expression of major histocompatibility complex class II, CD86 and IL-12) when compared with wild-type macrophages.\(^{80}\) Although the impact of CCR8 may be indirect, reducing the number of recruited Ly6C^hi monocytes and therefore reducing the number of monocytes available to differentiate through different mechanisms, these results indicate that CCR8 may also be directly controlling the differentiation of infiltrating monocytes into inflammatory macrophages and/or inhibiting their differentiation into more DC-like cells.

In addition to chemokine receptors, other potential pathways may contribute to the Ly6C^lo to Ly6C^hi transition. Using a variety of in vitro and in vivo methods, Ramachandran et al.\(^{37}\) show that the process of phagocytosis is a key factor in the transition from inflammatory Ly6C^hi to restorative Ly6C^lo monocyte-derived macrophages. Specifically, phagocytosis of liposomes during the fibrosis resolution phase in CCL2-injured mice resulted in a reduced amount of Ly6C^hi monocyte-derived macrophages and an increased number of Ly6C^lo monocyte-derived macrophages that were predominantly liposome positive and correlated with enhanced regression of fibrosis.\(^{37}\) Concurrent antibody inhibition of IL-10 and IL-4 in a model of acute liver injury resulted in both delayed differentiation of CCR2^hiCX3CR1^hi to CX3CR1^hiCCR2^lo cells and removal of necrotic cells, indicating that this transition is important for the resolution of injury.\(^{78}\) Rantakari et al.\(^{80}\) propose that expression of the scavenger receptor stabilin-1 by monocytes and monocyte-derived macrophages is critical for the resolution of fibrosis, showing that fibrosis is enhanced in stabilin-1^−/− mice and mice lacking stabilin-1 in monocytes following repeated CCL2 injection. Monocytes and macrophages in stabilin-1^−/− mice had a more proinflammatory phenotype (G-CSF, TNF) and they further show that during the fibrosis resolution phase, stabilin-1^−/− mice exhibited an increase in Ly6C^hi and a decrease in Ly6C^lo monocyte-derived macrophages, indicating that stabilin-1^−/− may be involved in this transition required for fibrosis resolution.\(^{84}\) Blockade of CSF1R resulted in the reduction of...
Ly6\textsuperscript{Ch}i monocytes contribute to both the progression and regression of fibrosis, likely through the differentiation into tissue repair/restorative Ly6\textsuperscript{Cl}o monocyte-derived macrophages within the injured liver (Figure 1). Specifically, the recruitment of Ly6\textsuperscript{Ch}i monocytes initially contributes to driving the initiation of fibrosis, but as the phenotypic switch to the Ly6\textsuperscript{Cl}o subset occurs, the functional output transitions to one of repair and resolution of fibrosis. Intriguingly, monocytes do not always appear to contribute to fibrosis resolution, and particularly in models of chronic fibrosis induced by diets high in fats and carbohydrates, monocytes appear to contribute to continuing inflammation and fibrosis. This posits the question of what factors signal the differentiation of Ly6\textsuperscript{Ch}i monocytes into the tissue repair Ly6\textsuperscript{Cl}o monocytes versus their switch into other inflammatory cell types, such as inflammatory macrophages or DCs. CX\textsubscript{2}CR1, IL-10, IL-4 and the process of phagocytosis have all been described to participate in this transition of Ly6\textsuperscript{Ch}i to Ly6\textsuperscript{Cl}o. It is currently unknown whether the absence of differentiation signals or the presence of additional signals drives the transition of Ly6\textsuperscript{Ch}i monocytes/monocyte-derived macrophages away from the Ly6\textsuperscript{Cl}o subset and toward a more inflammatory cell type. An alternate hypothesis is that continual recruitment of Ly6\textsuperscript{Ch}i monocytes from the bone marrow and blood continually propagates inflammation. In tissues, the balance between the Ly6\textsuperscript{Ch}i and Ly6\textsuperscript{Cl}o subsets could be governed by the rate of the Ly6\textsuperscript{Ch}i to Ly6\textsuperscript{Cl}o transition, or by de novo recruitment of Ly6\textsuperscript{Cl}o monocytes or both.

**CONFLICT OF INTEREST**
The authors declare no conflict of interest.

---

**Figure 1** The differentiation of Ly6\textsuperscript{Ch}i monocytes in liver injury. Following liver injury, monocytes traffic to the tissue. In the liver, Ly6\textsuperscript{Ch}i monocytes (blue arrow) are recruited by CCL2- and CCR2-dependent mechanisms and extravasate from the circulation into the tissue where they surround the site of injury. The Ly6\textsuperscript{Ch}iCCR2\textsuperscript{−}CX\textsubscript{2}CR1\textsuperscript{hi} monocyte-derived macrophages then differentiate into Ly6\textsuperscript{Ch}iCCR2\textsuperscript{−}CX\textsubscript{2}CR1\textsuperscript{hi} monocyte-derived macrophages. This transition relies on a number of mechanisms and interactions (blue to green arrow) including phagocytosis, IL-10 and IL-4, and CX\textsubscript{2}CR1. The CCR8, CSF1R and stabilin-1 receptors and the transcription factor Nur77 may also play a role in this process. Infiltrating Ly6\textsuperscript{Ch}i monocytes and the Ly6\textsuperscript{Cl}o monocyte-derived macrophages are inflammatory and contribute to inflammation and the progression of fibrosis, whereas Ly6\textsuperscript{Ch}i monocytes and monocyte-derived macrophages exhibit tissue repair and restorative phenotypes, leading to the resolution of fibrosis. Alternate pathways observed in other tissues that may also occur in the liver (gray arrows) are the extravasation of the Ly6\textsuperscript{Cl}o monocyte subset into the injured tissue within 1–2 h following injury, and successive recruitment of Ly6\textsuperscript{Ch}i and Ly6\textsuperscript{Cl}o monocytes that then differentiate into their respective inflammatory and pro-resolution macrophage populations.

GR1\textsuperscript{+} monocytes and an increase in GR1\textsuperscript{+} monocytes in peripheral blood, suggesting that the CSF-1/CSF1R interaction plays a role in the Ly6\textsuperscript{Ch}i to Ly6\textsuperscript{Cl}o phenotypic switch.\textsuperscript{99} In a mouse model, CSF-1 blood, suggesting that the CSF-1/CSF1R interaction plays a role in the differentiation of monocytes in the liver.\textsuperscript{100} In addition, Melino et al.\textsuperscript{40} show that CSF1R blockade reduced injury in a mouse model of chronic fibrosis in parallel with a reduction in the numbers of Ly6\textsuperscript{Ch}i and Ly6\textsuperscript{Cl}o monocytes that then differentiate into their respective inflammatory and pro-resolution macrophage populations.

CONCLUSION
Monocytes and monocyte-derived macrophages appear to simultaneously play many distinct roles during tissue injury as drivers of injury, fibrosis and resolution. The plasticity of the monocyte compartment may provide an explanation for these seemingly conflicting roles. Although the data are contradictory on the role that Ly6\textsuperscript{Ch}i monocytes play in amplifying the initial insult, infiltrating Ly6\textsuperscript{Ch}i monocytes contribute to both the progression and regression of fibrosis, likely through the differentiation into tissue repair/restorative Ly6\textsuperscript{Cl}o monocyte-derived macrophages within the injured liver (Figure 1). Specifically, the recruitment of Ly6\textsuperscript{Ch}i monocytes initially contributes to driving the initiation of fibrosis, but as the phenotypic switch to the Ly6\textsuperscript{Cl}o subset occurs, the functional output transitions to one of repair and resolution of fibrosis. Intriguingly, monocytes do not always appear to contribute to fibrosis resolution, and particularly in models of chronic fibrosis induced by diets high in fats and carbohydrates, monocytes appear to contribute to continuing inflammation and fibrosis. This posits the question of what factors signal the differentiation of Ly6\textsuperscript{Ch}i monocytes into the tissue repair Ly6\textsuperscript{Cl}o monocytes versus their switch into other inflammatory cell types, such as inflammatory macrophages or DCs. CX\textsubscript{2}CR1, IL-10, IL-4 and the process of phagocytosis have all been described to participate in this transition of Ly6\textsuperscript{Ch}i to Ly6\textsuperscript{Cl}o. It is currently unknown whether the absence of differentiation signals or the presence of additional signals drives the transition of Ly6\textsuperscript{Ch}i monocytes/monocyte-derived macrophages away from the Ly6\textsuperscript{Cl}o subset and toward a more inflammatory cell type. An alternate hypothesis is that continual recruitment of Ly6\textsuperscript{Ch}i monocytes from the bone marrow and blood continually propagates inflammation. In tissues, the balance between the Ly6\textsuperscript{Ch}i and Ly6\textsuperscript{Cl}o subsets could be governed by the rate of the Ly6\textsuperscript{Ch}i to Ly6\textsuperscript{Cl}o transition, or by de novo recruitment of Ly6\textsuperscript{Cl}o monocytes or both.

**CONFLICT OF INTEREST**
The authors declare no conflict of interest.

---

1. Lee WM. Acute liver failure. *Semin Respir Crit Care Med* 2012; 33: 36-45.
2. Shen XD, Ke B, Zhai Y, Gao F, Tsuchishashi S, Lassman CR et al. Absence of toll-like receptor 4 (TLR4) signaling in the donor organ reduces ischemia and reperfusion injury in a murine liver transplantation model. *Liver Transpl* 2007; 13: 1435-1443.
3. Neuschwander-Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. *Hepatology* 2003; 37: 1202-1219.
4. Szabo G, Petrasek J, Bala S. Innate immunity and alcoholic liver disease. *Dig Dis* 2012; 30 (Suppl 1): 55-60.
5. Heron M, Hoyert DL, Murphy SL, Xu J, Kochanek KD, Tejada-Vera B. Deaths: final data for 2006. *Nat Vital Stat Rep* 2009; 57: 1-134.
6. Chen Y, Sun R. Toll-like receptors in acute liver injury and regeneration. *Int Immunopharmacol* 2011; 11: 1433-1441.
7. Arumugam TV, Okun E, Tang GC, Thundil J, Taylor SM, Woodruff TM. Toll-like receptors in ischemia-reperfusion injury. *Shock* 2009; 32: 4–16.
8. Kumar H, Kawai T, Akira S. Toll-like receptors and innate immunity. *Biochem Biophys Res Commun* 2009; 388: 621–625.
9. Chen GY, Nunez G. Sterile inflammation: sensing and reacting to damage. *Nat Rev Immunol* 2010; 10: 826-837.
10. Enomoto N, Ikejima K, Bradford B, Rivera C, Kono H, Brenner DA et al. Alcohol causes both tolerance and sensitization of rat Kupffer cells via mechanisms dependent on endothoxin. *Gastroenterology* 1998; 115: 443-451.
11. Nanji AA, Khettry U, Sadzadzem SM, Yamazaki T. Severity of liver injury in experimental alcoholic liver disease. Correlation with plasma endotoxin, prostaglandin E2, leukotriene B4, and thromboxane B2. *Am J Pathol* 1993; 142: 367-373.
12. Uesugi T, Furh M, Arteel GE, Bradford BU, Thurman RG. Toll-like receptor 4 is involved in the mechanism of early alcohol-induced liver injury in mice. *Hepatology* 2001; 34: 101–108.
13. Hinson JA, Roberts DW, James LP. Mechanisms of acetaminophen-induced liver necrosis. *Hand Exp Pharmacol* 2010; 196: 369-405.
14. Wang K. Molecular mechanisms of liver injury: apoptosis or necrosis. *Exp Toxicol Pathol* 2014; 66: 351-356.
15. Brenner C, Galluzzo L, Kepp O, Kromer G. Decoding cell death signals in liver inflammation. *J Hepatol* 2013; 59: 583-594.
16. Malhi H, Gores GJ. Molecular mechanisms of lipotoxicity in nonalcoholic fatty liver disease. *Semin Liver Dis* 2008; 28: 360-369.
17. Yin M, Wheeler MD, Kono H, Bradford BU, Galliucci RM, Lustor MI et al. Essential role of tumor necrosis factor alpha in alcohol-induced liver injury in mice. *Gastroenterology* 1999; 117: 942-952.
18. Malhi H, Guinea-Ilardi ME, Gores GJ. Hepatocyte death: a clear and present danger. *Physiol Rev* 2010; 90: 1165-1194.
Swinski FK, Nahrendorf M, Ettredt M, Wildgruber M, Cortez-Retamozo V, Panizzi P et al. Identification of splenic monocytes and their deployment to inflammatory sites. Science 2009; 325: 612–616.

Pfalzmann RT, Jung S, Cheng G, Weninger W, Luo Y, Dorf M et al. Inflammatory chemokine transport and presentation in HEV, a remote control mechanism for monocyte recruitment to lymph nodes in inflamed tissues. J Exp Med 2001; 194: 1361–1373.

Geissmann F, Jung S, Littman DR. Blood monocytes consist of two principal subsets with distinct migratory properties. Immunity 2003; 19: 71–82.

Sunsketter C, Nikolic T, Dillon MJ, Van Rooijen N, Stehling M, Drevets DA et al. Subpopulations of mouse blood monocytes differ in maturation stage and inflammatory response. J Immunol 2004; 172: 4410–4417.

Passlick S, Flierger D, Ziegler-Heitbrock HW. Identification and characterization of a novel monocyte subpopulation in human peripheral blood. Blood 1989; 74: 2527–2534.

Rothe G, Gabriel H, Kovacs E, Klucken J, Stohr J, Kindermann W et al. Peripheral blood mononuclear phagocyte subpopulations as cellular markers in nonalcoholic steatohepatitis. Gastroenterology 2006; 130: 1437–1447.

Tanaka M, Honda J, Imamura Y, Shiraishi K, Tanaka K, Ozumi K. Surface phenotype analysis of CD16+ monocytes from leukapheresis collections for peripheral blood mononuclear cell products. Clin Immunol 2011; 139: 57–61.

Ancuta P, Rao R, Moses A, Mehe A, Shaw SK, Lusczinskas FW et al. Fractalkine preferentially mediates arrest and migration of CD16+ monocytes. J Clin Invest 2002; 109: 1701–1707.

Cros J, Cagnard N, Woolard K, Paley N, Zhang SY, Senechal B et al. Human CD14dim monocytes patrol liver sinusoids. Chemokine transport and presentation in HEV: a remote control mechanism for monocyte recruitment to lymph nodes in inflamed tissues. J Exp Med 2001; 194: 1361–1373.

Geissmann F, Jung S, Littman DR. Blood monocytes consist of two principal subsets with distinct migratory properties. Immunity 2003; 19: 71–82.

Sunsketter C, Nikolic T, Dillon MJ, Van Rooijen N, Stehling M, Drevets DA et al. Subpopulations of mouse blood monocytes differ in maturation stage and inflammatory response. J Immunol 2004; 172: 4410–4417.

Passlick S, Flierger D, Ziegler-Heitbrock HW. Identification and characterization of a novel monocyte subpopulation in human peripheral blood. Blood 1989; 74: 2527–2534.

Rothe G, Gabriel H, Kovacs E, Klucken J, Stohr J, Kindermann W et al. Peripheral blood mononuclear phagocyte subpopulations as cellular markers in nonalcoholic steatohepatitis. Gastroenterology 2006; 130: 1437–1447.

Tanaka M, Honda J, Imamura Y, Shiraishi K, Tanaka K, Ozumi K. Surface phenotype analysis of CD16+ monocytes from leukapheresis collections for peripheral blood mononuclear cell products. Clin Immunol 2011; 139: 57–61.

Ancuta P, Rao R, Moses A, Mehe A, Shaw SK, Lusczinskas FW et al. Fractalkine preferentially mediates arrest and migration of CD16+ monocytes. J Clin Invest 2002; 109: 1701–1707.

Cros J, Cagnard N, Woolard K, Paley N, Zhang SY, Senechal B et al. Human CD14dim monocytes patrol liver sinusoids. Chemokine transport and presentation in HEV: a remote control mechanism for monocyte recruitment to lymph nodes in inflamed tissues. J Exp Med 2001; 194: 1361–1373.
74 Antoniades CG, Quaglia A, Taams LS, Milty RR, Hussein M, Abeles R et al. Source and characterization of hepatic macrophages in acetaminophen-induced acute liver failure in humans. Hepatology 2012; 56: 735–746.

75 Boud-Baillot K, Basset L, Bonnier D, L’Helgoualc’h A, Samson M, Theret N. CX3CL1/fractalkine shedding by human hepatic stellate cells: contribution to chronic inflammation in the liver. J Cell Mol Med 2009; 13: 1526–1535.

76 Aspinall AI, Curbishtley SM, Lalor PF, Weston CJ, Blahova M, Liaskou E et al. The healing myocardium sequentially mobilizes two monocyte subsets with divergent and complementary functions. J Exp Med 2007; 204: 3037–3047.

77 Nakrendorf M, Swinski FK, Akawa E, Stangenbarg L, Wurdinger T, Figueiredo JL et al. The healing myocardium sequentially mobilizes two monocyte subsets with divergent and complementary functions. J Exp Med 2007; 204: 3037–3047.

78 Dal-Secco D, Wang J, Zeng Z, Kolaczkowska E, Wong CH, Petri B et al. Distinct differentiation potential of blood monocyte subsets in the lung. J Exp Med 2015; 212: 447–456.

79 Landsman L, Varol C, Jung S. Distinct differentiation potential of blood monocyte subsets in the lung. J Immunol 2007; 178: 2000–2007.

80 Heymann F, Hammerich L, Storch D, Bartneck M, Huss S, Russeler V et al. Hepatic macrophage migration and differentiation critical for liver fibrosis is mediated by the chemokine receptor C-C motif chemokine receptor 8 in mice. Hepatology 2012; 55: 898–909.

81 Shi C, Velazquez P, Hohl TM, Leiner I, Dustin ML, Pamer EG. Monocyte trafficking to hepatic sites of bacterial infection is chemokine independent and directed by focal intercellular adhesion molecule-1 expression. J Immunol 2010; 184: 6266–6274.

82 Yang L, Han Z, Tian L, Mai P, Zhang Y, Wang L et al. Sphingosine 1-phosphate receptor 2 and 3 mediate bone marrow-derived monocyte/macrophage motility in cholestatic liver injury in mice. Sci Rep 2015; 5: 13423.

83 Debiens E, Mayol K, Biajoux V, Dauvey C, De Aguirre MG, Taillardet M et al. S1PR5 is pivotal for the homeostasis of patrolling monocytes. Eur J Immunol 2013; 43: 1667–1675.

84 Affo S, Rodrigo-Torres D, Blaya D, Morales-Ibanez O, Coll M, Millan C et al. Chemokine receptor Ccr6 deficiency alters hepatic inflammatory cell recruitment and promotes liver inflammation and fibrosis. PLoS ONE 2015; 10: e0145147.

85 Kohyama M, Matsuoka S, Shida K, Sugihara F, Aoshi T, Kishida K et al. Monocyte infiltration into obese and fibrosed tissues is regulated by PILRalpha. J Cell Mol Med 2016; 20: 1214–1223.

86 Zamara E, Galusti S, Allefi S, Petrai I, Aragno M, Mastrocola R et al. Prevention of severe toxic liver injury and oxidative stress in MCP-1-deficient mice. J Hepatol 2007; 46: 230–238.

87 Tacke F, Girhoux F, Jakubzick C, van Rosjienn N, Mersad M, Randolph GJ. Immature monocytes acquire antigens from other cells in the bone marrow and present them to T cells after maturing in the periphery. J Exp Med 2006; 203: 583–597.

88 Galastri S, Zamara E, Milani S, Novo E, Provenzano A, Delugi W et al. Lack of CC chemokine ligand 2 differentially affects inflammation and fibrosis according to the genetic background in a murine model of steatohepatitis. Clin Sci (Lond) 2012; 123: 459–471.

89 Elsegood CL, Chan CW, DeGli-Esposti MA, Wikstrom ME, Domenichini A, Lazarus K et al. Kupffer cell monocyte communication is essential for initiating murine liver progenitor cell-mediated liver regeneration. Hepatology 2015; 62: 1272–1284.

90 Baeck C, Wehr A, Karlmark KR, Heymann F, Vucur M, Gassler N et al. Pharmacological inhibition of the chemokine CCL2 (MCP-1) diminishes liver macrophage infiltration and steatohepatitis in chronic hepatic injury. Gut 2012; 61: 416–426.

91 Baeck C, Wei X, Bartneck M, Fech V, Heymann F, Gassler N et al. Pharmacological inhibition of the chemokine C-C motif chemokine ligand 2 (monocyte chemotactant protein 1) accelerates liver fibrosis regression by suppressing Ly-6C(+) macrophage infiltration in mice. Hepatology 2014; 59: 1060–1072.

92 Eliing J, Bartneck M, Wei X, Gremse F, Fech V, Mockel D et al. CCL2-dependent infiltrating macrophages promote angiogenesis in progressive liver fibrosis. Gut 2014; 63: 1960–1971.

93 Seki E, de Minicis S, Inokuchi S, Taura K, Miyai K, van Rooijen N et al. CCR2 promotes hepatic fibrosis in mice. Hepatology 2009; 50: 185–197.

94 Rantakari P, Patten DA, Valtonen J, Karikoski M, Gerke H, Dawes H et al. Stabilin-1 expression defines a subset of macrophages that mediate tissue homeostasis and prevent fibrosis in chronic liver injury. Proc Natl Acad Sci USA 2016; 113: 9298–9303.

95 Liaskou E, Zimmermann HW, Li KK, Oo YH, Suresh S, Stamatakis Z et al. Monocyte subsets in human liver disease show distinct phenotypic and functional characteristics. Hepatology 2013; 57: 385–398.

96 Wang J, Kubes P. A reservoir of mature cavity macrophages that can rapidly invade visceral organs to affect tissue repair. Cell 2016; 165: 668–678.

97 Aoyama T, Inokuchi S, Brenner DA, Seki E. CX3CL1-CX3CR1 interaction prevents carbon tetrachloride-induced liver inflammation and fibrosis in mice. Hepatology 2010; 52: 1390–1400.

98 Karlmark KR, Zimmermann HW, Roderburg C, Gassler N, Wasmuth HE, Luedde T et al. The fractalkine receptor CX3CR1 protects against liver fibrosis by controlling differentiation and survival of infiltrating hepatic monocytes. Hepatology 2010; 52: 1769–1782.

99 MacDonald KP, Palmer JS, Cronau S, Seppanen E, Oliver S, Raffel NC et al. An antibody against the colony-stimulating factor 1 receptor depletes the resident subset of monocytes and tissue- and tumor-macrophages but does not inhibit inflammation. Blood 2010; 116: 3955–3963.

100 Hutchins B, Antone DJ, Mackinnon AC, Gow DJ, Bain CC, Hawley CA et al. CSF1 restores innate immunity after liver injury in mice and serum levels indicate outcomes of patients with acute liver failure. Gastroenterology 2015; 149: 1896–1909 e1814.

101 Hanna RN, Shaked I, Hubbeling HG, Punta JA, Wu R, Herrly E et al. NR4A1 (Nur77) deletion polarizes macrophages toward an inflammatory phenotype and increases atherosclerosis. Circ Res 2012; 110: 416–427.

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.