Liver fibrosis and repair: immune regulation of wound healing in a solid organ

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Abstract | Fibrosis is a highly conserved and co-ordinated protective response to tissue injury. The interaction of multiple pathways, molecules and systems determines whether fibrosis is self-limiting and homeostatic, or whether it is uncontrolled and excessive. Immune cells have been identified as key players in this fibrotic cascade, with the capacity to exert either injury-inducing or repair-promoting effects. A multi-organ approach was recently suggested to identify the core and regulatory pathways in fibrosis, with the aim of integrating the wealth of information emerging from basic fibrosis research. In this Review, we focus on recent advances in liver fibrosis research as a paradigm for wound healing in solid organs and the role of the immune system in regulating and balancing this response.

Myofibroblast
Fibroblast-like cell of mesenchymal origin.

Idiopathic pulmonary fibrosis
(IPF). An interstitial lung disease of unknown aetiology that is characterized by a progressive fibrotic response driven by abnormally activated alveolar epithelial cells.

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The liver, more than any other solid organ, has a remarkable capacity to adapt to injury through tissue repair. In many respects, the multifaceted interactions of immune cell subsets regulate this repair process, such that fibrosis and wound healing can be considered as part of the innate immune response to tissue damage (BOX 1). Studies have mainly focused on the pathological (rather than the homeostatic) features of chronic liver injury, but a robust and self-limiting fibrotic and regenerative response is also observed in cases of acute liver failure[1]. Indeed, studies in mice show that fibrosis renders the liver more resistant to subsequent acute injury and that type I collagen, which predominates in the fibrotic scar, protects hepatocytes against various toxic stimuli[2]. Fibrosis becomes problematic, and clinically relevant, when dysregulated and excessive scarring occurs in response to persistent injury and leads to altered tissue function (FIG. 1). As our understanding of the pathogenesis of liver fibrosis has increased as a result of experimental models and the analysis of human liver tissue, it has become evident that the liver provides a useful generic model of inflammation and repair, showing the complex interplay between the epithelial, inflammatory, myofibroblast and extracellular matrix (ECM) components of the mammalian wound-healing response. In almost all situations (tendinosis of the elbow is a notable exception), fibrosis is preceded by inflammation, and elements of both the innate and adaptive immune systems are pivotal in regulating the fibrotic process.

As many fibrogenic pathways are conserved across tissues, recent findings in the liver could be extended to studies of fibrosis in the lungs, the kidneys, the heart and other organs. Indeed, the idea that there are conserved core and regulatory pathways in fibrosis has been suggested to help identify the most promising generic anti-fibrotic targets[3]. A collaborative multi-organ approach could accelerate the development of effective treatments for fibroproliferative diseases (such as liver cirrhosis, pulmonary fibroses, chronic kidney disease and cardiovascular disease), which account for ~45% of deaths in industrialized nations. A recent example of a core pathway is that involving the enzyme lysyl oxidase homologue 2 (LOXL2); the finding that LOXL2 has a crucial role in mouse lung, liver and tumour xenograft fibrosis models (a result that is supported by an increase in LOXL2 expression in human disease-associated stroma)[4] has been rapidly used as the basis for therapeutic trials of a targeted LOXL2 inhibitor simtuzumab (GS-6624; Gilead) in patients with idiopathic pulmonary fibrosis (IPF), liver fibrosis and cancer.

In this Review, we discuss how the inflammatory response leads to fibrosis and we briefly explore the diverse origins, the modes of activation and the fates of pro-fibrogenic liver myofibroblasts. We focus in detail on the immune regulation of liver fibrosis (particularly the distinct and opposing roles of macrophages) and the endogenous mechanisms that mediate the resolution of fibrosis and the restoration of tissue homeostasis. Although epithelial regeneration is an integral

component of liver repair and is required for architectural and functional recovery, it is beyond the scope of this Review and, for further information on this topic, the reader is referred to a recent expert review[5]. This Review article is timely and relevant to immunologists, as anti-fibrotic strategies using manipulation of the immune system in experimental systems are beginning to emerge in the clinical arena.

Box 1 | **The immune system in human chronic liver diseases**

HCV infection
- Attenuates both innate and adaptive immune responses
- Spontaneous clearance occurs that is associated with vigorous and multispecific T cell responses
- Once chronic infection is established, the cellular immune response is insufficient to eradicate hepatitis C virus (HCV), but triggers chronic liver inflammation and the development of progressive disease

HBV infection
- Natural immune responses control hepatitis B virus (HBV) in >90% of adult-infected patients
- Defective innate immunity and exhausted adaptive immunity characterize chronic HBV infection

Alcohol-related liver disease
- Neutrophil infiltration (mediated by Kupffer cell-derived cytokines) is a prominent feature of alcoholic hepatitis
- Activation of innate immunity involving Toll-like receptor 4 (TLR4) and complement proteins (C3 and C5) might have an important role in initiating alcoholic steatohepatitis and fibrosis
- Ethanol inhibits natural killer cell function and accelerates the progression of co-existent viral hepatitis

Non-alcoholic fatty liver disease
- There is a close link with insulin resistance and inflammation
- Kupffer cell depletion prevents the development of diet-induced steatosis and insulin resistance
- Certain cytokines exacerbate non-alcoholic fatty liver disease and insulin resistance (such as tumour necrosis factor and interleukin-6 (IL-6)), whereas others are protective (such as IL-10 and adiponectin)
- Hedgehog pathway activation leads to hepatic enrichment with natural killer T cells that contribute to fibrosis progression in non-alcoholic steatohepatitis[103]

Autoimmune hepatitis
- Genetic susceptibility, molecular mimicry and impaired immunoregulation (particularly involving regulatory T cells) contribute to the initiation and the perpetuation of autoimmune attack
- Liver damage is primarily mediated by $CD4^+$ T cells, although recent studies support the involvement of diverse populations, including T helper 17 cells[104]

Primary biliary cirrhosis
- Biliary inflammation is mediated by a distinctive loss of tolerance to a series of ubiquitous mitochondrial autoantibodies that are specific for the E2 subunits of the 2-oxo-dehydrogenase pathway
- Mouse models and human genome-wide association studies indicate a crucial role for the IL-12 signalling axis in pathogenesis[105]

Primary sclerosing cholangitis
- Gut-primed adaptive and innate immune responses contribute to chronic progressive biliary inflammation
- Approximately 75% of patients have co-existent inflammatory bowel disease
- Hepatic inflammation and activation of vascular adhesion protein 1 (VAP1) results in the aberrant expression of mucosal vascular addressin cell adhesion molecule 1 (MADCAM1)[106] and CC-chemokine ligand 25 (CCL25), and in the misdirected homing of intestinal mucosal effector T cells ($\alpha4\beta7^+$ cells) to the liver

Epithelial injury and the inflammatory response

Most types of liver 'insult' damage epithelial cells (hepatocytes and/or cholangiocytes), which leads to the release of inflammatory mediators and to the initiation of an anti-fibrinolytic coagulation cascade. Leukocytes that are recruited to the site of injury phagocytose dead or apoptotic cells and amplify the inflammatory response by generating pro-inflammatory cytokines, such as tumour necrosis factor (TNF), interleukin-6 (IL-6) and IL-1β, and by recruiting T cells[6] (FIG. 2). Pro-inflammatory mediators that are generated by cellular damage and stimulated immune cells, as well as growth factors and cytokines including platelet-derived growth factor (PDGF), connective tissue growth factor (CTGF), transforming growth factor-β (TGFβ) and IL-13, activate mesenchymal precursor cells in tissues and induce their transdifferentiation to myofibroblasts. Phagocytosis of apoptotic hepatocytes[7] or lymphocytes[8] by hepatic stellate cells also directly triggers their fibrogenic activation. TGFβ is the major pro-fibrogenic cytokine and it upregulates α-smooth muscle actin (αSMA) and type I collagen synthesis[9] by hepatic stellate cell-derived myofibroblasts, whereas PDGF induces the proliferation of myofibroblasts through extracellular signal-regulated kinase (ERK)-dependent and ERK-independent mechanisms and through changes in the intracellular pH. Altered gut permeability, particularly in alcoholic liver disease, increases bacterial translocation across the gut wall and increases lipopolysaccharide (LPS) levels in the circulation, which leads to the activation of hepatic stellate cells and liver-resident macrophages (also known as Kupffer cells) through Toll-like receptor 4 (TLR4) signalling[10].

Hepatic myofibroblast precursor cells

The accumulation of pro-fibrogenic myofibroblasts is a central feature of tissue fibrosis. These cells are master regulators of the fibrotic response as a result of their acquisition of scar-producing, proliferative, migratory, contractile, immunomodulatory and phagocytic properties. Consequently, myofibroblasts have been a major focus of basic fibrosis research in recent years and are leading targets for anti-fibrotic therapies. Methodological advances have provided new insights into the origins, the regulation and the fate of myofibroblast populations in the liver. Recent studies have used bone marrow transplantation techniques in reporter mice to show that, regardless of the aetiology or the duration of the injury, liver myofibroblasts are almost exclusively derived from the activation of resident mesenchymal cells — that is, quiescent hepatic stellate cells[11] (and portal fibroblasts in biliary disease[12]) — whereas the contribution of bone marrow-derived mesenchymal cells or fibrocytes to the liver myofibroblast pool seems to be minimal[13]. By contrast, recruited fibrocytes are much more prominent in lung fibrosis (they constitute ~25% of collagen-producing cells), and a high level of fibrocytes in the peripheral blood is associated with a poor prognosis in patients with IPF[14].

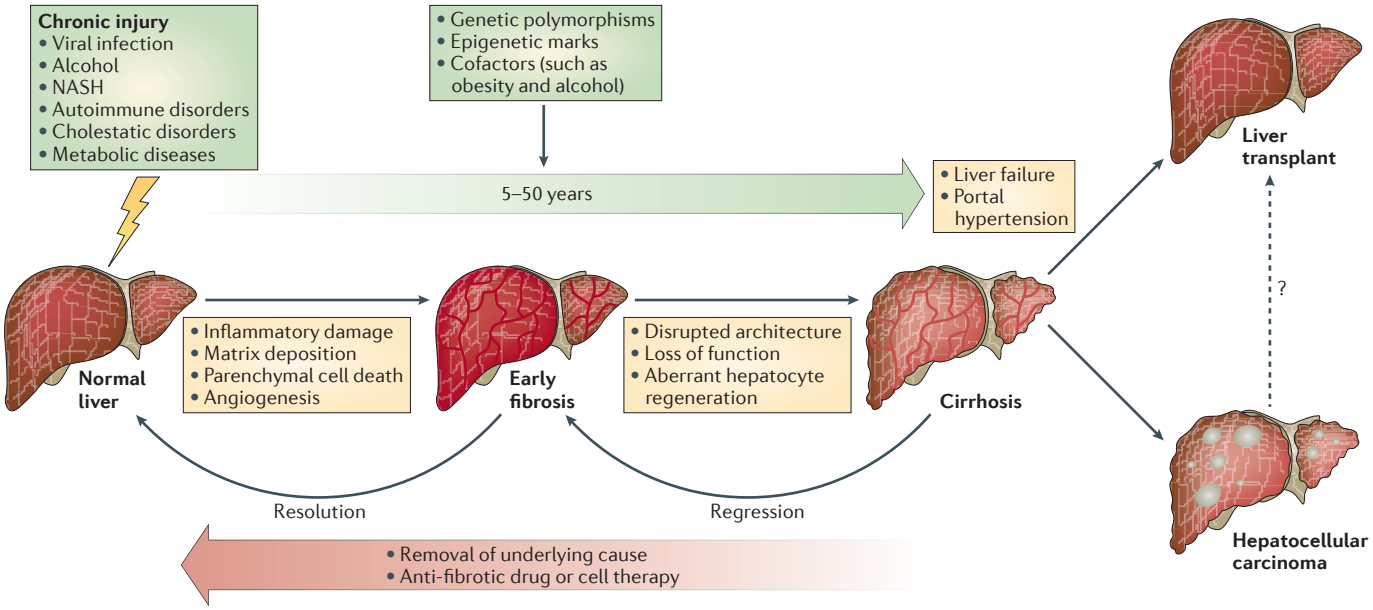


Figure 1 | **Natural history of chronic liver disease.** Hepatic fibrosis is the wound-healing response of the liver to many causes of chronic injury, of which viral infection, alcohol and non-alcoholic steatohepatitis (NASH) are the most common. Regardless of the underlying cause, iterative injury causes inflammatory damage, matrix deposition, parenchymal cell death and angiogenesis leading to progressive fibrosis. The scar matrix typically accumulates very slowly (the median time to cirrhosis in chronic hepatitis C is 30 years) but once cirrhosis is established the potential for reversing this process is decreased and complications develop. Genetic polymorphisms, epigenetic marks and cofactors (such as obesity and alcohol) can modulate the risk of fibrosis progression. If the cause of fibrosis is eliminated, resolution (that is, complete reversal to near-normal liver architecture) of early hepatic fibrosis can occur. In cirrhosis, although resolution is not possible, regression (that is, improvement but not reversal) of fibrosis improves clinical outcomes. Anti-fibrotic therapies are emerging that can slow, halt or reverse fibrosis progression. Currently, liver transplantation is the only available treatment for liver failure or for some cases of primary liver cancer. Hepatocellular carcinoma is rising in incidence worldwide and is a major cause of liver-related death in patients with cirrhosis.

Kupffer cells
Cells of the resident hepatic macrophage population. In the steady state, Kupffer cells are thought to self-renew and to originate from fetal (yolk sac) precursor cells. These cells reside in the liver sinusoids where they regulate local immune responses and remove bacteria, bacterial endotoxins and microbial debris that are derived from the gastrointestinal tract and transported to the liver via the portal vein.

Fibrocytes
Cells of haematopoietic origin (marked by CD45 expression) that can differentiate into tissue myofibroblasts.

Pericytes
Specialized mesenchymal cells that are embedded within the basement membrane of capillaries (hepatic stellate cells are considered to be the pericytes of the liver).

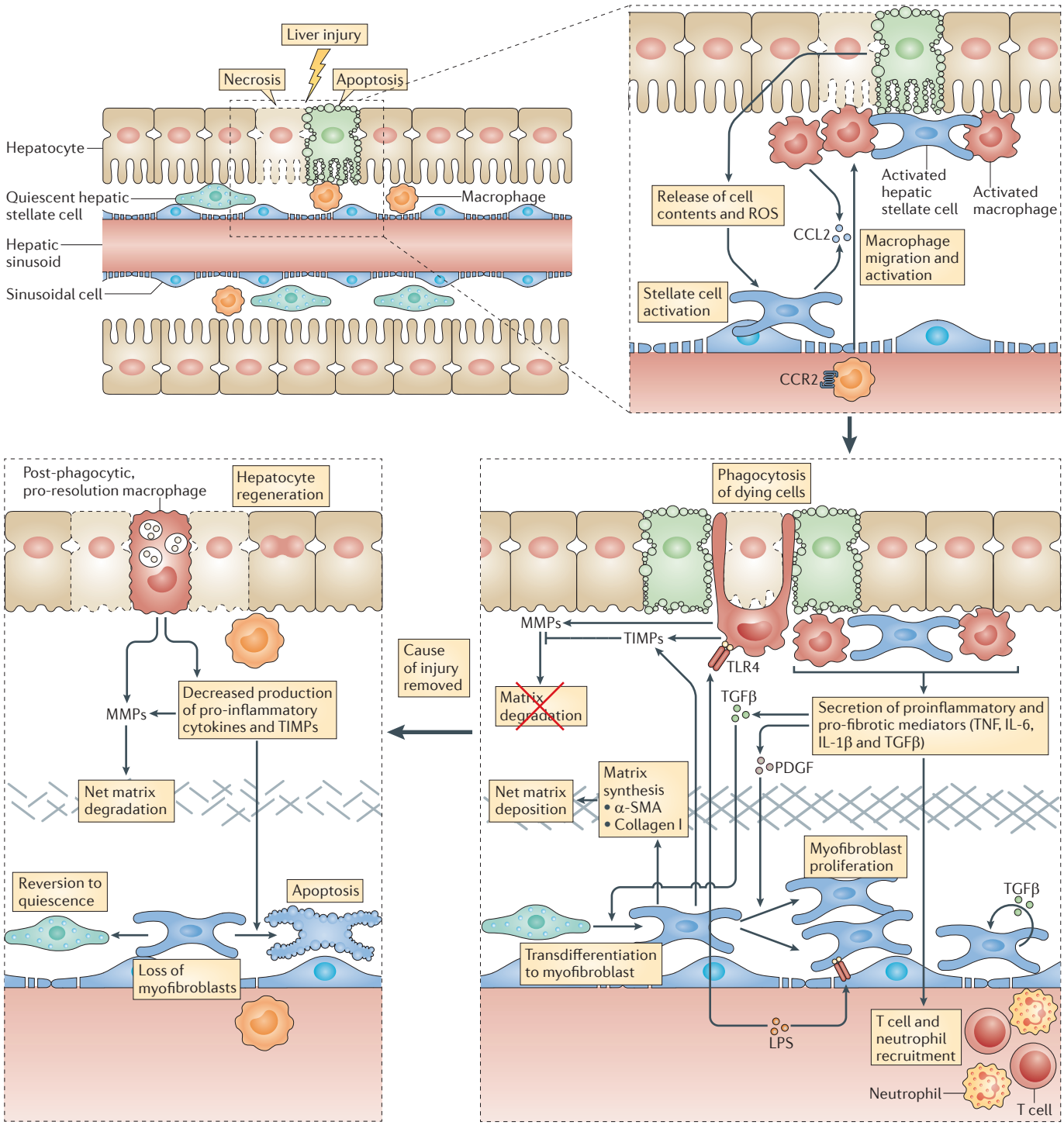
Space of Disse
The perisinusoidal space in the liver, between the endothelial cells and the hepatocytes.

Hepatic stellate cells. Hepatic stellate cells are the pericytes of the liver and they reside in the space of Disse between the hepatocytes and the endothelial cells, where they encircle the liver sinusoids. They express neural markers (such as glial fibrillary acidic protein (GFAP) and synaptophysin) and desmin, and they store vitamin A in lipid droplets. Following chronic liver injury of any cause, hepatic stellate cells become activated and transdifferentiate to myofibroblast-like cells, which are characterized by a loss of lipid droplets, by increased proliferation and migration, by secretion of excessive ECM proteins, by enhanced contractility and by the release of pro-inflammatory and pro-fibrogenic factors including TGFβ. Activated hepatic stellate cells upregulate mesenchymal cell markers (such as αSMA and type I collagen) and lose their neural marker signature, which enables the cellular activation status of these cells to be discriminated *in vitro* and *in vivo*.

Perpetuation of hepatic myofibroblast activation

Perpetuation of myofibroblast activation results from several positive feedback loops involving, among other receptors, TGFβ receptors, PDGF receptor-β[15] and angiotensin II receptors[16], which are upregulated on these cells. In addition, TGFβ promotes myofibroblast survival through the activation of focal adhesion kinase (FAK) and AKT[17]. The production of soluble mediators such as CC-chemokine ligand 2 (CCL2; also known as MCP1) and macrophage colony-stimulating factor (M-CSF) augments inflammatory cell infiltration to the site of injury to initiate and to maintain myofibroblast activation. Myofibroblast differentiation and fibrosis may also be promoted by epigenetic events such as the methyl-CpG-binding protein 2 (MeCP2)-mediated silencing of the gene encoding peroxisome proliferator-activated receptor-γ (PPARγ)[18] or the transcriptional activation of pro-fibrogenic genes by specific histone modifications[19]. An inevitable consequence of fibrotic ECM accumulation (and myofibroblast contraction) is a progressive increase in tissue stiffness. There is evidence showing that biomechanical signalling, mediated through increased tissue stiffness, is a crucial mechanism to promote and to sustain the differentiated, contractile myofibroblast phenotype[20] and to stimulate the force-dependent activation of TGFβ by dissociating it from latency-associated peptide (LAP)[21].

Cell adhesion proteins such as integrins mediate complex cell–cell and cell–ECM interactions in wound-healing responses. Integrins transduce bidirectional signals that regulate cell behaviour, including proliferation, motility, differentiation, survival and apoptosis. During fibrogenesis, increased expression of αv integrins on hepatic myofibroblasts[22] and of αvβ6 integrin on activated cholangiocytes modifies the cellular response to



fibrogenic stimuli. αvβ6 integrin can bind to and can activate latent TGFβ, and expression of αvβ6 integrin is increased in human fibrotic liver and in animal models[23]. The importance of this regulatory mechanism in fibrosis has been highlighted by the development of STX-100 (Biogen Idec), a humanized monoclonal antibody specific for αvβ6 integrin, which selectively disrupts TGFβ activation in fibrotic tissue; this drug is in Phase II clinical trials in patients with IPF.

Immune properties of hepatic stellate cells

An underappreciated property of activated hepatic stellate cells and of myofibroblasts in other tissues is their role as innate immune cells. Interactions between hepatic stellate cells and different immune cell populations, as well as the activation of specific immune signalling pathways within hepatic stellate cells, function together to promote liver fibrogenesis. Hepatic stellate cells mediate a range of immunoregulatory effects

Figure 2 | **Cascade of signals following liver injury.** Liver injury causes parenchymal cell necrosis and/or apoptosis. The release of cell contents and reactive oxygen species (ROS) activates hepatic stellate cells and attracts and activates tissue macrophages through the CC-chemokine ligand 2 (CCL2)–CC-chemokine receptor 2 (CCR2) axis. Macrophages and hepatic stellate cells phagocytose necrotic and apoptotic cells. Activated macrophages and hepatic stellate cells secrete pro-inflammatory mediators, and recruit T cells and neutrophils. The secretion of transforming growth factor-β (TGFβ) in particular leads to transdifferentiation of hepatic stellate cells into myofibroblasts. Platelet-derived growth factor (PDGF) stimulates myofibroblast proliferation. Hepatic stellate cell-derived myofibroblasts express α-smooth muscle actin (αSMA) and collagen I. Macrophages have a latent capacity to degrade newly synthesised scar matrix through the secretion of matrix metalloproteinases (MMPs), but the protease activity is inhibited by concurrent production of tissue inhibitors of metalloproteinases (TIMPs) by myofibroblasts and macrophages, which results in progressive matrix deposition and scar accumulation. Increased gut permeability and hepatic lipopolysaccharide (LPS)–Toll-like receptor 4 (TLR4) signalling promotes fibrogenesis, in which TLR4-activated hepatic stellate cells produce chemokines and express adhesion molecules that recruit resident macrophages to the site of injury and, simultaneously, TLR4 signalling downregulates the TGFβ decoy receptor bone morphogenetic protein and activin membrane-bound inhibitor homologue (BAMBI) to boost TGFβ signalling (not shown). Chronic injury and inflammation and cell–matrix interactions perpetuate fibrogenesis. If the underlying irritant is removed, post-phagocytic macrophages undergo a phenotypical switch that favours the resolution of fibrosis. Levels of pro-inflammatory cytokines and TIMPs decrease, which facilitates the loss of liver myofibroblasts and enhances matrix degradation and tissue repair. Although a decrease in macrophage numbers follows the resolution of fibrosis, it is not clear how this is mediated. IL, interleukin; TNF, tumour necrosis factor.

by producing NADPH oxidase (NOX) enzymes and reactive oxygen species, and pro-inflammatory cytokines and chemokines (such as the CC-chemokine receptor 2 (CCR2) ligand CCL2, CCL5 (also known as RANTES) and the macrophage inflammatory proteins CCL3 and CCL4); by expressing chemokine receptors (including CCR5, CCR7, CXC-chemokine receptor 3 (CXCR3) and CXCR7); by responding to bacterial components through TLR4; and by functioning as non-professional antigen-presenting cells in the injured liver. Hepatic stellate cell-derived myofibroblasts[24] and the precursors of glomerular myofibroblasts in the kidney (that is, mesangial cells)[25] are also highly phagocytic. Chemokine neutralization or chemokine receptor deletion in mouse liver fibrosis models has anti-inflammatory and anti-fibrotic effects by disrupting chemokine signalling in hepatic stellate cell-derived myofibroblasts and Kupffer cells[26]. In addition, a recent study in which hepatic stellate cells were specifically depleted in mice revealed an unexpected role for these cells in amplifying hepatocellular liver damage and in decreasing the expression of protective cytokines such as IL-10 and interferon-γ (IFNγ)[27].

Immune regulation of liver fibrogenesis

There is now compelling evidence from studies examining the role of individual inflammatory cell populations in experimental models that the immune system can regulate both the progression and the regression of liver fibrosis.

Macrophages. Perhaps the most widely studied immune cell population in liver fibrosis is the macrophage population (FIG. 3). In the fibrotic liver, macrophages consistently localize in close proximity to activated myofibroblasts in areas of scar tissue[28,29]. Evidence that supports a major functional role for macrophages in fibrosis comes from studies using *in vivo* macrophage depletion or blockade strategies; for example, gadolinium chloride-mediated depletion of hepatic macrophages in rats was shown to decrease myofibroblast activation and fibrosis in response to thioacetamide injury[30]. In a landmark study, macrophages were conditionally depleted using *Cd11b*–DTR transgenic mice; macrophage depletion during ongoing hepatic injury induced by carbon tetrachloride (CCl_4) resulted in decreased numbers of hepatic stellate cell-derived myofibroblasts and attenuated liver fibrosis, which indicates that macrophages have a pro-fibrogenic role in this context[28]. Similar effects were shown using liposomal clodronate to deplete macrophages in both a transgenic model of hepatic injury and in response to chronic CCl_4 or bile duct ligation[31,32].

Given the heterogeneity of tissue macrophages, work has subsequently focused on characterizing the pro-fibrotic macrophage population. In the liver, macrophages can be broadly defined as either resident Kupffer cells or monocyte-derived macrophages[33]. In the uninjured liver, Kupffer cells predominate and are the largest population of resident macrophages in the body. Recent fate-mapping studies have shown that these resident hepatic macrophages are established before birth and are maintained in adult life through self-renewal and longevity, with no contribution from the monocyte pool[34]. Importantly, Kupffer cells are located in the hepatic sinusoids, which enables them to directly sample the antigens that are transported from the gastrointestinal tract through the portal vein, ensuring early exposure to pathogenic bacteria, and to be in close contact with other circulating immune cells. Therefore, Kupffer cells have a crucial homeostatic role in protecting the host and are capable of inducing both immunogenic and tolerogenic immune responses[35–37]. In steady-state conditions, monocyte-derived hepatic macrophages can also be identified in the liver, but they are a minor population[38] and localize to the perivascular zone[34].

In response to hepatic injury, liver macrophage populations markedly change. Resident Kupffer cells probably have a role in the early response to injury, by secreting pro-inflammatory cytokines and chemokines, such as CCL2 and CCL5 (REF. 39). However, the number of Kupffer cells decreases during hepatic inflammation and fibrogenesis; these cells are gradually replenished as the inflammation and fibrosis resolves[33,38]. Both the fate of this population (whether it is cell death or migration from the liver to present antigens) and the source of the cell replenishment (be it local expansion of the cell population or recruitment of monocytes) remain unclear. Conversely, the number of monocyte-derived hepatic macrophages markedly increases in response to tissue injury, which suggests that pro-fibrogenic macrophages must be derived from this population[33,38]. In a crucial study, a $LY6C^{hi}CD11b^{+}F4/80^{+}$ inducible nitric oxide sythase (iNOS)-producing hepatic macrophage population that was derived from the CCR2-dependent recruitment of $LY6C^{hi}$ inflammatory monocytes was identified as the main pro-fibrogenic population in a mouse model of CCl_4-induced fibrosis[40]. In addition, macrophages have now been identified

Integrins
A family of transmembrane receptors composed of non-covalently linked heterodimers of α-subunits and β-subunits that form at least 24 combinations in mammalian tissues. Several integrins, in particular β3 integrins, recognize a tripeptide arginine–glycine–aspartic acid (RGD) sequence in specific ECM ligands.

Cd11b–DTR transgenic mice
Transgenic mice that express the human diphtheria toxin receptor (DTR) under the control of the *Cd11b* promoter, which facilitates the selective depletion of monocytes and macrophages by the administration of diphtheria toxin.

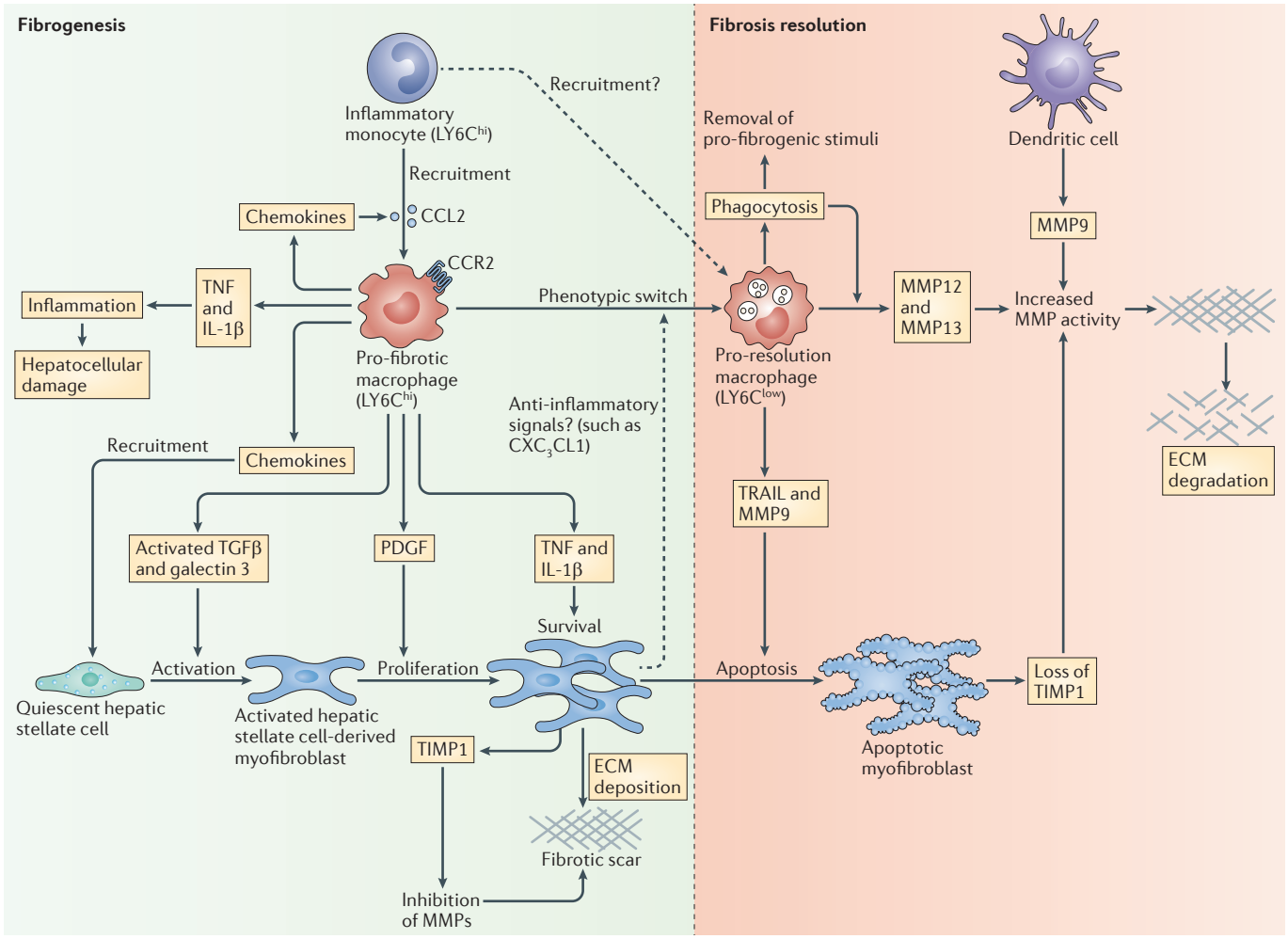


Figure 3 | **The duality of macrophages in liver fibrosis.** During fibrogenesis, LY6C^{hi} monocytes are recruited to the inflamed liver via the CC-chemokine ligand 2 (CCL2)–CC-chemokine receptor 2 (CCR2) axis, forming a pro-fibrotic LY6C^{hi} (also known as GR1^{hi}) macrophage population. These cells express pro-inflammatory cytokines such as tumour necrosis factor (TNF) and interleukin-1β (IL-1β), which perpetuate hepatocellular injury and enhance the survival of hepatic myofibroblasts. Chemokine expression by these macrophages promotes the recruitment of monocytes, other inflammatory cells and hepatic stellate cells. The direct effects of pro-fibrotic macrophages on hepatic myofibroblasts may be mediated by transforming growth factor-β (TGFβ) or galectin 3 expression to promote myofibroblast activation, or by platelet-derived growth factor (PDGF) production to stimulate myofibroblast proliferation. Hepatic myofibroblasts express tissue inhibitor of metalloproteinase 1 (TIMP1), which inhibits matrix metalloproteinase (MMP) activity and augments the accumulation of extracellular matrix (ECM) in the scar tissue. A pro-resolution (also known as restorative) macrophage population derives from a switch in macrophage phenotype, potentially via hepatic expression of anti-inflammatory signals such as CX$_3$C-chemokine ligand 1 (CX$_3$CL1). Pro-resolution macrophages remove cellular debris and express TNF-related apoptosis-inducing ligand (TRAIL) and MMP9, which promote myofibroblast apoptosis. This removes the source of scar production and TIMP1 expression, which enables endogenous MMPs to degrade the scar ECM. Finally, pro-resolution macrophages are a rich source of fibrolytic proteases including MMP12 and MMP13. Hepatic dendritic cells may also express MMP9 and may promote the resolution of fibrosis. Figure is modified, with permission, from REF. 113 © (2012) Elsevier.

as potential pro-fibrotic effectors in other fibrotic tissues including the kidneys[41], heart[42], lungs[43] and skin[44]. As in the liver, LY6C^{hi} monocyte-derived macrophages have been shown to be the predominant pro-fibrogenic macrophage population in these tissues[41,43].

Therefore, it seems that monocyte recruitment is essential for hepatic fibrogenesis and, indeed, studies have shown that inhibition of the main monocyte chemoattractant CCL2 in rats or genetic deletion of its receptor CCR2 in mice decreased macrophage infiltration in response to injury and markedly inhibited liver fibrosis[45,46]. A role for local macrophage proliferation in the innate immune response to chronic inflammation has also been defined. In particular, T helper 2 (T_H2) cell-related pathologies, such as parasitic lung infection, result in the IL-4-induced proliferation of resident tissue macrophages[47]. Furthermore, studies have shown the capacity of monocyte-derived macrophages to

proliferate *in situ* in the context of atherosclerosis[48] and peritoneal inflammation[49]. It is therefore not surprising that, in the context of chronic hepatic inflammation induced by CCl_4, proliferative activity, as assessed by KI67 staining, can be detected in both resident Kupffer cells and monocyte-derived macrophages[38]. The pro-fibrogenic LY6C^{hi}CD11b^{+}F4/80^{+} hepatic macrophage population is the most proliferative macrophage population in this setting, which emphasizes the potential importance of this population in the macrophage-dependent wound-healing response.

Focus has now turned to the mechanisms by which macrophages can promote hepatic fibrotic responses. Macrophages can produce a range of cytokines, chemokines and other soluble mediators that directly influence the behaviour of hepatic stellate cells and other myofibroblasts. Of particular interest, macrophages express TGFβ which drives myofibroblast activation and ECM synthesis[40,50]. In addition, pro-fibrogenic LY6C^{hi} hepatic macrophages express high levels of the TGFβ-activating protein thrombospondin 1 (REF. 38). Macrophages can also express the potent mitogen PDGF and the T_H2 cell cytokines IL-4 and IL-13, which directly stimulate collagen synthesis in myofibroblasts. Macrophages produce chemokines, including CCL8 (also known as MCP2) and CCL7 (also known as MCP3)[50], which can recruit myofibroblasts, and CCL2, CCL3 and CCL5, which facilitate leukocyte recruitment[51] to the site of inflammation, thereby perpetuating the fibrotic response. Galectin 3 is a macrophage-derived lectin that has been shown to promote myofibroblast activation in both liver and kidney fibrosis models[52]. The hepatic macrophage-derived pro-inflammatory cytokines TNF and IL-1β have more recently been shown to affect activated hepatic stellate cells and, through nuclear factor-κB (NF-κB) activation, to promote the survival of hepatic stellate cell-derived myofibroblasts and the development of liver fibrogenesis[32]. Indeed, macrophage subsets that have been isolated from the cirrhotic human liver also express high levels of pro-inflammatory mediators[53].

Adaptive immune cells. Indirect evidence indicates that the relative balance of T_H1 and T_H2 cells might influence the outcome of the fibrotic response. Indeed, C57BL/6 mice (in which a T_H1 cell response predominates) have a weaker fibrotic reaction than BALB/c mice (which have an adaptive response that is skewed towards a T_H2-type response)[54]. Furthermore, the co-administration of the T_H1 cell cytokine IL-12 with *Schistosoma* spp. decreased the granuloma formation and markedly reduced the fibrosis that are associated with this infection[55]. Moreover, T_H2 cells are strongly pro-fibrogenic, as shown in experimental models involving parasitic infection with eggs from *Schistosoma* spp. In this setting (and in other tissues such as the lungs, the skin and the gut), IL-13 seems to be an important pro-fibrogenic mediator, as it stimulates TGFβ1 synthesis and upregulates matrix metalloproteinase 9 (MMP9) expression, which in turn activates TGFβ1. IL-13 can also promote fibrosis independently of TGFβ, through a mechanism that is controlled by the relative expression of IL-13 receptor-α1 (IL-13Rα1; the signalling receptor) versus IL-13Rα2 (the decoy receptor) on myofibroblasts[56]. Indeed, antagonism of IL-13 signalling ameliorates fibrosis[57]. Conversely, a T_H1 cell-dominant immune response is associated with IFNγ and IL-12 production, which have been shown to be anti-fibrotic[8]. IFNγ suppresses collagen deposition by regulating the balance of MMP and tissue inhibitor of metalloproteinases (TIMP) expression. IFNγ and/or IL-12 might also decrease the production of pro-fibrotic cytokines by T_H2 cells[56]. However, with the discovery of new T_H cell subsets, this classical view of fibrosis as an imbalance of T_H1 and T_H2 cell responses has been challenged.

T_H17 cells are characterized by the secretion of IL-17 and IL-22 (REF. 58). The number of T_H17 cells is increased in the liver and the serum from patients with various forms of acute and chronic liver injury. The IL-17 receptor is expressed on many different cell types including monocytes, Kupffer cells, cholangiocytes and hepatic stellate cells, and receptor activation induces the secretion of pro-inflammatory cytokines such as IL-1β, IL-6, TNF and TGFβ. IL-17 also directly induces type I collagen production in hepatic stellate cells through activation of the signal transducer and activator of transcription 3 (STAT3) signalling pathway[59]. Expression of the IL-22 receptor in the liver is restricted to hepatocytes and signalling through this receptor promotes hepatocyte survival and proliferation. However, the effect of IL-22 was shown to be pro-inflammatory in hepatitis B virus infection[60].

Regulatory T (T_{Reg}) cells are a subset of CD4^{+} T_H cells that regulate other immune cells in a dominant-negative manner. T_{Reg} cells are characterized by the expression of the transcription factors forkhead box P3 (FOXP3) and STAT5, the marker CD25 on their surface and the production of immunosuppressive cytokines such as IL-10. In contrast to in the healthy liver, T_{Reg} cells are more abundant in the livers of patients with chronic viral hepatitis, autoimmune liver disease and primary biliary cirrhosis. In a bile duct ligation rat model, the depletion of T_{Reg} cells exacerbated fibrosis[61], whereas in *Schistosoma* spp. infection, T_{Reg} cells were shown to attenuate the T_H2 cell response[61,62]. By contrast, intrahepatic IL-8-producing T_{Reg} cells were found to be pro-fibrogenic in chronic hepatitis C virus (HCV) infection[63].

Adoptive transfer experiments have suggested that cytotoxic T cells (that is, CD8^{+} T cells) can have a pro-fibrogenic role in the liver[64] and that CD8^{+} T cell-mediated liver fibrosis can be ameliorated by the expression of IL-10 (REF. 64). However, in other studies, mice that are deficient in CD4^{+} or CD8^{+} T cells showed no difference in the development of liver fibrosis in response to CCl_4 compared with control animals[65]. Consequently, the precise role of different T cell populations in the development of hepatic fibrosis remains unclear and it is likely to be dependent on the underlying aetiology that drives the fibrotic process. Intriguingly, a recent study showed that fusion of T cell microparticles with the cell membrane of hepatic

stellate cells upregulated the expression of fibrolytic genes in hepatic stellate cells, which led to the down-regulation of expression of pro-collagen α1 mRNA and to the suppression of TGFβ1 activity[66].

In addition, the role of non-conventional T cell subsets in fibrosis has become increasingly appreciated. Although natural killer T cells (NKT cells) constitute approximately 30% of the total number of hepatic lymphocytes, their functional role in liver fibrogenesis has remained unclear. A subset of innate-like invariant NKT cells (iNKT cells) has been shown to patrol the liver sinusoids in search of pathogens in steady-state conditions[67]; however, following acute or chronic liver injury, these cells are activated to a pro-inflammatory, pro-fibrogenic state. This activation is driven by the CXCR6–CXC-chemokine ligand 16 (CXCL16) axis and results in the secretion of various cytokines, including IFNγ and IL-4. Indeed, the CXCR6–CXCL16 chemokine axis is upregulated in patients with chronic liver disease, such as that induced by HCV infection. *Cxcr6*$^{-/-}$ mice have decreased macrophage accumulation and pro-inflammatory cytokine production following liver injury and are protected from developing liver fibrosis. Furthermore, the adoptive transfer of hepatic NKT cells from wild-type animals was shown to restore the fibrotic phenotype in *Cxcr6*$^{-/-}$ mice, which indicates that iNKT cells have a role in initiating and perpetuating liver fibrogenesis[68].

γδ T cells are enriched in epithelial tissue, and they constitute up to 25% of T cells in the liver. Following liver injury, an IL-17^{+}IL-22^{+} γδ T cell subset is recruited to the liver by the CCR6–CCL20 chemokine axis, where these cells have an anti-fibrotic effect by promoting hepatic stellate cell apoptosis[69].

The development of group 2 innate lymphoid cells (also known as nuocytes) was shown to be induced by IL-33 expression in adenovirus-induced hepatitis, and these cells were shown to attenuate liver injury by decreasing the levels of hepatic TNF[70]. However, in liver fibrosis models, group 2 ILCs were shown to be pro-fibrogenic, functioning through an IL-33-dependent IL-13-mediated mechanism[71]. Similar results that showed a pro-fibrogenic role for group 2 ILCs were also observed in lung fibrosis[72].

NK cells seem to have a negative regulatory effect on fibrosis and to mediate the direct killing of senescent activated hepatic stellate cells[73]. NK cells also produce cytokines such as IFNγ that induce hepatic stellate cell apoptosis and cell cycle arrest.

Other immune cells. Evidence to support a role for neutrophils in fibrogenesis is limited. Indeed, the administration of either neutrophil anti-serum to rats in which bile duct ligation has been carried out[74] or α-naphthylisothiocyanate (ANIT) to CXCR2-deficient mice[75] had no discernible effect on fibrosis, despite there being a decrease in the number of hepatic neutrophils. Conversely, neutrophil-derived MMP expression might contribute to collagen degradation during the resolution of fibrosis[76], although it is debatable whether neutrophils are present in the liver in sufficient numbers during the resolution phase to have a meaningful effect.

Numbers of mast cells are increased in the liver in animal models of fibrosis and in human disease[77] and these cells are a rich potential source of pro-fibrotic mediators such as TGFβ and PDGF. However, studies in mast cell-deficient rats and mice do not indicate a major role for mast cells in hepatic fibrogenesis[78].

Taken together, there is compelling evidence that tissue injury and, subsequently, wound healing are dynamically regulated by different facets of the immune system. Initially, pro-inflammatory mediators derived from infiltrating inflammatory cells and from damaged epithelial cells converge to activate myofibroblast precursors, mainly hepatic stellate cells. Differentiated myofibroblasts regulate the fibrotic response and function as innate immune cells. Perpetuation of myofibroblast fibrogenic activity is mediated by several positive feedback loops that involve the autocrine and paracrine effects of cytokines and growth factors, and cell–cell and cell–matrix interactions. It is likely that the balance of T_H1 cell- and T_H2 cell-mediated adaptive immune responses, the influence of non-conventional T cell subsets and the equilibrium between pro-inflammatory (that is, pro-fibrotic) and pro-resolution macrophage populations determine whether the outcome of tissue injury is homeostatic and self-limited or whether it results in pathogenic scarring.

Fibrosis regression: a return to homeostasis

Seminal studies in the 1970s documented the regression of both liver fibrosis and cirrhosis in animal and human models and considered the pathological and the temporal factors that might determine susceptibility to ECM degradation. Reversal of fibrosis is now a reality in the clinic and the serial assessment of biopsy samples from patients with chronic liver disease of diverse aetiology who have been successfully treated indicates that liver fibrosis is a dynamic, bidirectional process that has an inherent capacity for recovery and remodelling[79]. Perhaps more surprising are the clinical studies showing that even cirrhosis can regress, for example, following 5 years of treatment with the antiviral drug tenofovir in chronic hepatitis B virus infection[80]: of those patients with cirrhosis at the start of the study, 74% showed extensive histological regression such that they were no longer considered to be cirrhotic by year 5 of tenofovir treatment.

The induction and the spontaneous resolution of fibrosis have also been observed in rodent models[81,82], which have been invaluable in defining the underlying biological mechanisms. However, there are marked differences compared with the human condition. The fibrotic response to CCl_4 in mice is weak and rapidly resolves, although there is variation between inbred strains. Outbred rats have a more robust fibrotic reaction and cirrhosis can be generated. However, advanced liver fibrosis is much less reversible in humans than in rodents, probably as a result of the densely crosslinked collagen that typically develops over decades (rather than weeks in rodents). Such discrepancies limit the predictivity of pre-clinical models, particularly regarding

Natural killer T cells
(NKT cells). A subset of T cells that have characteristics of both T cells and NK cells, expressing both a T cell receptor and NK lineage markers.

Innate-like invariant NKT cells
(iNKT cells). A subset of natural killer T cells that express a T cell receptor (TCR) with an invariant Vα14-Jα18 TCRα chain paired with a restricted subset of TCR Vβ chains. iNKT cells exclusively recognize glycolipid antigens that are presented on CD1d molecules.

γδ T cells
A subset of T cells that are defined by the genetic composition of their T cell receptor; the TCR is made up of one γ-chain and one δ-chain, rather than the one α-chain and one β-chain that are found in classical αβ T cells.

Innate lymphoid cells
Innate immune cells that produce many T helper cell-associated cytokines but do not express cell surface markers that are associated with other immune cell lineages. In addition, these cells do not express a T cell receptor and do not respond in an antigen-specific manner.

Senescent
A cellular state in which a growth arrest programme has been initiated that limits the lifespan of the cell and prevents unlimited cell proliferation.

Glitazones
Drugs used for the treatment of type 2 diabetes mellitus that function as agonists of peroxisome proliferator-activated receptor-γ (PPARγ) and that increase insulin sensitivity.

M1 macrophage
A macrophage that is activated by interferon-γ or by lipopolysaccharide; also known as a classically activated macrophage. These cells express pro-inflammatory cytokines and inducible nitric oxide synthase (among other things), and they inhibit cell proliferation and cause tissue damage.

M2 macrophage
A macrophage that is activated by the T helper 2 cell cytokines interleukin-4 (IL-4) and IL-13; also known as an alternatively activated macrophage. These cells express arginase 1, the mannose receptor CD206 and the IL-4 receptor α-chain (among other things), and they promote cell proliferation and tissue repair.

proof-of-concept studies that evaluate anti-fibrotic compounds (such as angiotensin II type-1 receptor blockers and glitazones), for which efficacy in rodents has not yet been translated to humans[83,84].

ECM degradation and pro-resolution macrophages. A constant turnover of the matrix occurs during liver fibrogenesis and the increased expression of a range of MMPs, including collagenases[85], has been shown to occur in the fibrotic liver, which indicates that the liver has considerable inherent protease activity. Fibrotic ECM continues to accumulate in chronic liver injury because MMP activity is inhibited by high levels of TIMPs (particularly myofibroblast-derived TIMP1)[86] (FIGS 2,3). In addition, several features of mature scar ECM render it less susceptible to degradation over time (BOX 2). Seminal studies of the resolution of liver fibrosis in rats showed that levels of TIMP1 decreased after the cessation of injury[81,87]. In association with this decrease, hepatic collagenase activity increased and net ECM degradation occurred. Subsequent mechanistic studies to alter the TIMP to MMP balance *in situ* have confirmed the powerful influence of this ratio on the development and the resolution of fibrosis in the liver[88,89] and in other tissues such as the lungs.

In the liver, macrophages have also been shown to be crucial for the resolution of fibrosis[28], which emphasizes their role as regulators of effective wound healing and organ homeostasis. Similar observations have been made in the lungs, in which macrophage depletion using liposomal clodronate during the recovery phase following bleomycin injury prevented the resolution of fibrosis[43]. Located in close proximity to fibrotic tissue in the liver, pro-resolution macrophages are ideally placed to mediate ECM degradation and are a rich source of fibrolytic MMPs, including MMP12 (REF. 90) and MMP13 (REF. 29) (FIG. 3). Macrophages also express gene products such as MMP9 and TNF-related apoptosis-inducing ligand (TRAIL; also known as TNFSF10) that promote myofibroblast apoptosis. Furthermore, phagocytosis of apoptotic cells by macrophages induces MMP expression and augments ECM degradation in rodent models of resolving hepatocellular[38] and biliary[91] fibrosis.

Box 2 | **Factors that limit the reversibility of liver fibrosis**

Although extracellular matrix (ECM) deposition by myofibroblasts following parenchymal damage is part of a homeostatic wound-healing response, persistent tissue injury leads to pathological alterations in the quantity and the composition of the matrix (particularly to an increase in fibrillar collagens) as well as leading to biochemical modifications that render the fibrotic liver less susceptible to remodelling and repair. Mature scar ECM, which is composed of crosslinked collagen and elastin, is more resistant to proteases[87]. In addition, fibrils that are sequestered in deeper portions of a scar are inaccessible to degrading enzymes. The pattern and the distribution of fibrosis differ depending on the aetiology and are associated with varying degrees of angiogenesis and potential for reversibility[107]. In addition, the persistent expression of tissue inhibitors of metalloproteinases (TIMPs) regulates membrane metalloproteinases (MMPs), but acellular scars lack endogenous sources of MMPs (that is, inflammatory cells and myofibroblasts)[87]. The angio-architectural changes, such as vascularized septa, that accompany cirrhosis are probably irreversible and therefore represent a point-of-no-return.

The emerging understanding of hepatic macrophage heterogeneity has enabled our group to identify a restorative hepatic macrophage subpopulation (characterized as $CD11b^{hi}F4/80^{int}LY6C^{low}$) as being crucial for the remodelling of liver fibrosis[38]. The fact that these cells derive from a phenotypical switch of the pro-inflammatory $LY6C^{hi}$ subset, which is potentially induced by phagocytosis, highlights the fundamental importance of monocyte-derived hepatic macrophages in both the response to hepatic injury and the restoration of normal tissue architecture. This restorative population, in addition to having an increased production of MMPs, showed decreased expression of pro-inflammatory cytokines and chemokines, as well as an upregulation of genes encoding factors such as CX_3C-chemokine receptor 1 (CX_3CR1) and arginase 1, which have been shown to have anti-fibrotic effects[50,92,93]. Importantly, both the pro-inflammatory $LY6C^{hi}$ hepatic macrophages and the restorative $LY6C^{low}$ hepatic macrophages had markers of M1 macrophage and M2 macrophage phenotypes, and therefore represent intermediate subpopulations. This highlights the limitations of the M1 versus M2 classification in an *in vivo* setting.

In addition to the effects of pro-resolution macrophages, the disappearance of the pro-inflammatory and pro-fibrotic macrophages may also modulate the local microenvironment to favour fibrosis regression. Another mechanism by which macrophages may indirectly be involved in the resolution of fibrosis is through the recruitment of other immune cells, such as neutrophils. The injection of autologous bone marrow-derived macrophages in mice during CCl_4-mediated liver injury was shown to lead to the recruitment of neutrophils into the liver, to the upregulation of MMPs and to an anti-fibrotic effect[94].

Dendritic cells (DCs) have also been investigated in the context of the resolution of liver fibrosis. Using *Cd11c*–DTR transgenic mice to deplete hepatic DCs during the recovery phase following CCl_4-mediated injury, as well as using adoptive transfer protocols and using FLT3 ligand to expand the DC population *in vivo*, DCs were shown to mediate ECM degradation, probably through enhanced MMP9 expression[95]. The interplay between this population of DCs and the restorative hepatic macrophages merits further study.

It is not yet clear how relevant these observations from mouse models are to human disease. Similarly to mice, in the human cirrhotic liver, a population of $CD14^{low}CD16^{-}$ resident Kupffer cells was identified, whereas monocyte-derived macrophages were defined as $CD14^{hi}CD16^{-}$ and $CD14^{+}CD16^{+}$; the number of these $CD14^{+}CD16^{+}$ cells was highest in the diseased liver[51,53]. Simlarly to mouse $LY6C^{low}$ restorative hepatic macrophages, the $CD14^{+}CD16^{+}$ human macrophages might derive from the differentiation of $CD14^{hi}CD16^{-}$ cells and these cells have considerable phagocytic capacity[51]. However, in humans, this population also expresses high levels of pro-inflammatory and pro-fibrogenic cytokines and chemokines[51] and can directly activate hepatic stellate cells[53], which is a phenotype that is more similar to that of mouse $LY6C^{hi}$ macrophages.

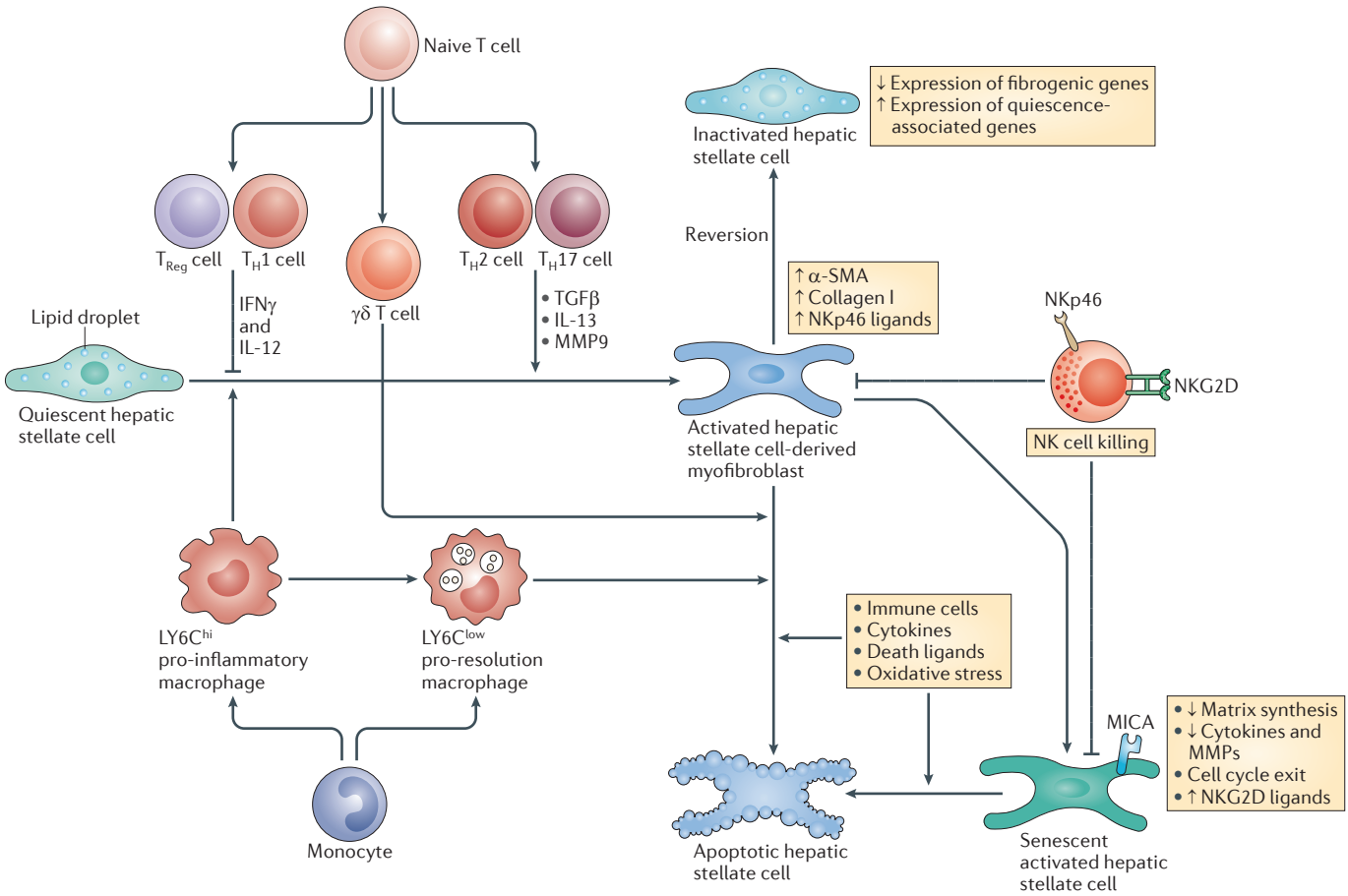


Figure 4 | **Hepatic stellate cell-derived myofibroblast fate in liver fibrosis.** Liver injury induces the production of pro-inflammatory cytokines, growth factors and reactive oxygen species, which leads to the transdifferentiation of quiescent hepatic stellate cells into myofibroblast-like cells, which are characterized by a loss of lipid droplets and an upregulation of α-smooth muscle actin (αSMA) and collagen I expression. LY6C^{hi} macrophages are crucial in driving this process. However, pro-inflammatory T cells (T helper 2 (T_H2) and T_H17 cells) are also pro-fibrogenic, either directly or by inducing transforming growth factor-β (TGFβ) secretion. The regression of fibrosis after the cessation of injury is associated with a loss of hepatic stellate cell-derived myofibroblasts from the receding hepatic scar. Immune cells, such as γδ T cells, cytokines, death ligands and oxidative stress may promote hepatic stellate cell apoptosis. A phenotypical switch of post-phagocytic macrophages to a LY6C^{low} pro-resolution phenotype is essential for extracellular matrix (ECM) resorption. T_H1 cells and regulatory T (T_{Reg}) cells limit T_H2 cell responses and have an anti-fibrotic effect. Some hepatic stellate cell-derived myofibroblasts become senescent — a phenotype that is associated with decreased matrix and cytokine synthesis, with decreased matrix metalloproteinase (MMP) secretion and with a gene expression profile that is consistent with cell cycle exit. Senescent cells are subsequently deleted by natural killer (NK) cells. Approximately 50% of hepatic stellate cell-derived myofibroblasts revert to an inactivated intermediate phenotype, downregulate pro-fibrogenic genes and re-acquire features of quiescence. Reverted cells remain in a 'primed' state, with a higher level of responsiveness to fibrogenic stimuli. IFNγ, interferon-γ; IL, interleukin; MICA, MHC class I polypeptide-related sequence A; NKG2D, NK group 2 member D; NKp46, NK cell protein 46.

Therefore, more in-depth analysis is required to attribute specific functions to human hepatic macrophage subsets in liver injury and homeostasis. Comparative gene expression profiling between mouse and human cells could be particularly informative.

Loss of liver myofibroblasts. Regression of liver fibrosis is also characterized by the loss of myofibroblasts from the receding hepatic scar[81,87] (FIG. 4). Myofibroblast fate could therefore be a key determinant between a normal and an aberrant wound-healing response. Multiple mechanisms may regulate the switch from survival to apoptosis of myofibroblasts. The persistence of myofibroblasts in liver fibrosis may be due to the influence of anti-apoptotic factors such as TGFβ and TIMP1 (REF. 96) or to survival signals that are mediated by the NF-κB cascade and kinase activities[97]. Activated hepatic stellate cells also express death receptors (such as CD95 (also known as FAS), TNF receptor 1 (TNFR1), p75 and TRAIL receptors), and the withdrawal of fibrogenic and anti-apoptotic signalling and/or the stimulation of death receptors by their cognate ligands (CD95 ligand (CD95L; also known as FASL) for FAS, TNF for TNFR1, nerve growth factor (NGF) for p75 and

Box 3 | **Examples of potential anti-fibrotic approaches in human fibrotic liver disease**

Eliminate primary disease

- Anti-viral drug therapy in chronic viral hepatitis[80]

Downregulate early hepatic myofibroblast activation

- Antioxidants (such as resveratrol in non-alcoholic steatohepatitis (NASH) (ClinicalTrials.gov, number: NCT02030977))
- Farnesoid X receptor ligands (such as obeticholic acid in NASH (ClinicalTrials.gov, number: NCT01265498))
- Hepatocyte growth factor (HGF) mimetics (such as refanalin (Angion Biomedica), which is a small-molecule activator of the HGF receptor MET)
- Caspase inhibitors (such as pancaspase inhibitor emricasan (IDN-6556; Conatus Pharmaceuticals) in hepatitis C virus (HCV) infection or alcoholic liver disease (ClinicalTrials.gov, numbers: NCT00088140 and NCT01912404))
- Cytokine antagonists (such as SAR156597 (Sanofi), which is a bispecific antibody targeting interleukin-13 (IL-13) and IL-4 that is under evaluation in patients with idiopathic pulmonary fibrosis (IPF) (ClinicalTrials.gov, number: NCT01529853))
- Recombinant forms of pentraxin 2 (also known as serum amyloid P)[108] such as PRM-151 (Promedior), which is under evaluation in patients with IPF (ClinicalTrials.gov, number: NCT01254409) and in the prevention of scarring after trabeculectomy for glaucoma (ClinicalTrials.gov, number: NCT01064817)

Disrupt chemokine pathways

- Disrupt the CXC-chemokine ligand 9 (CXCL9)–CXC-chemokine receptor 3 (CXCR3) axis[109]

Inhibit specific properties of hepatic myofibroblasts

- Proliferation (for example, multikinase inhibitors such as nintedanib (BIBF 1120; Boehringer Ingelheim), which is under evaluation in patients with IPF (ClinicalTrials.gov, number: NCT01170065)
- Fibrogenesis (for example, angiotensin II receptor antagonists (such as irbesartan in HCV infection (ClinicalTrials.gov, number NCT00265642)); relaxin[110]; TGFβ inhibitors (such as STX-100 (Biogen Idec), which is under evaluation in patients with IPF (ClinicalTrials.gov, number NCT01371305)); and pirfenidone (Esbriet; InterMune), a small molecule with anti-inflammatory and anti-TGFβ activity in patients with IPF (ClinicalTrials.gov, number NCT01366209))

Promote apoptosis or quiescence of hepatic myofibroblasts

- Cannabinoid receptor (CB1) antagonists[111]
- Apoptotic ligands (such as TNF-related apoptosis-inducing ligand (TRAIL))
- Tissue inhibitor of metalloproteinase 1 (TIMP1) inhibitors

Stimulate the degradation of accumulated scar extracellular matrix

- TIMP1 inhibitors[112] or matrix metalloproteinase (MMP) gene therapy
- Relaxin
- Lysyl oxidase homologue 2 (LOXL2) inhibitors[4] (such as simtuzumab (GS-6624; Gilead) in patients with NASH fibrosis or cirrhosis (ClinicalTrials.gov, numbers: NCT01672866 and NCT01672879) or with primary sclerosing cholangitis (ClinicalTrials.gov, number: NCT01672853)
- Cell therapies (for example, repeated autologous infusions of granulocyte colony-stimulating factor (G-CSF)-mobilized $CD133^+$ bone marrow stem cells in patients with cirrhosis (International Standard Randomised Controlled Trial Number Register, number: ISRCTN91288089). Cell-based approaches may also promote hepatic regeneration

Modify macrophage phenotype *in vivo*

- Promote matrix degradation; for example, by inducing a pro-resolution phenotype through phagocytosis of administered liposomes

TRAIL for the TRAIL receptors) may stimulate apoptosis. Alternatively, the overexpression of pro-apoptotic proteins such as BCL-2-associated X protein (BAX) and B cell lymphoma 2 (BCL-2) leads to caspase 9-mediated programmed cell death. The induction of hepatic stellate cell-derived myofibroblast apoptosis (for example, using gliotoxin) enhances the resolution of fibrosis[98]. NK cells and liver-specific γδ T cells are also involved in the resolution of liver fibrosis. After activation by IFNγ, they can induce rapid killing of hepatic stellate cells. In addition, some hepatic stellate cell-derived myofibroblasts may become senescent — a phenotype that is associated with decreased matrix and cytokine synthesis, decreased MMP secretion and a gene expression profile that is consistent with cell cycle exit[99]. Senescent activated hepatic stellate cells are subsequently deleted by NK cells. Several mechanisms have been suggested to induce hepatic stellate cell senescence, including replicative exhaustion, overstimulation and oxidative stress.

Recent studies using genetic tracking techniques have challenged our previous understanding of hepatic stellate cell fate. In mouse models of hepatocellular injury, two independent groups reported that reversion of activated hepatic stellate cell-derived myofibroblasts (towards a more quiescent phenotype) contributed to the termination of fibrogenesis during the resolution of fibrosis[100,101]. In resolving fibrosis, approximately 50% of hepatic stellate cell-derived myofibroblasts were shown to adopt an intermediate phenotype, to downregulate fibrogenic genes and to reacquire features of quiescence, although

they were not identical to cells that had never been activated[100]. This inactivation of hepatic stellate cell-derived myofibroblasts was associated with an upregulation of the anti-apoptotic genes heat shock protein 70kDa 1A (*Hspa1a*) and *Hspa1b*, which participate in the survival of hepatic stellate cells in culture and *in vivo*. Importantly, there was no evidence of a role for mesenchymal–epithelial transition in the resolution of fibrosis[101]. In both studies, reverted hepatic stellate cells seemed to remain in a 'primed' state, with a higher level of responsiveness to fibrogenic stimuli. However, the relevance of these findings in human liver fibrosis has not yet been confirmed. In addition, the determinants of hepatic stellate cell fate during the resolution of liver injury are unknown, but could involve a crucial balance of pro- and anti-apoptotic signals (such as TIMP1 (REF. 96)), epigenetic changes (such as methylation of PPARγ[18]) or extracellular cues (such as changes in tissue stiffness[20]).

In summary, liver fibrosis almost always has some potential for regression. Early liver fibrosis, which lacks ECM crosslinking and marked angiogenesis, can even reverse to near-normal architecture if the underlying cause is successfully treated. This remains the best form of anti-fibrotic therapy and facilitates the subsequent endogenous (that is, homeostatic) regulation of wound healing. Failing this, our increased understanding of the mechanisms that regulate liver fibrosis and repair has provided a logical framework to develop novel therapeutic approaches (BOX 3).

Future perspectives

Studies of the past 30 years have shown that the liver has provided the most cogent evidence for the bi-directionality of organ fibrosis. This has enabled the key mediators and the cellular players in the wound-healing response to be identified, including the role of the immune system in maintaining tissue homeostasis. Comparative mechanistic insights in different organs have identified tractable targets for generic anti-fibrotic therapy, including immunological approaches (BOX 3). In liver fibrosis, studies indicate that a cell therapy approach (for example, the delivery of bone marrow-derived macrophages) could potentially induce ECM-degrading and/or pro-regenerative effects[94,102]. Several early-phase clinical trials using infusions of autologous bone marrow-derived cell fractions have shown encouraging effects on liver function and a good safety profile in short-term follow up. However, most studies were neither blinded nor randomized and were carried out in small numbers of heterogeneous patients. Therefore, it is too early to judge the potential effect of these approaches and it is still unclear which cells (from bone marrow or blood and of mesenchymal or haematopoietic origin) or which routes of delivery are optimal. Nevertheless, this represents remarkable progress for a condition that, until relatively recently, was considered to be irreversible and relentlessly progressive, with liver transplantation being the only potentially effective treatment.

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Competing interests statement
The authors declare competing interests: see Web version for details.