Liver fibrosis: cellular mechanisms of progression and resolution

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ABSTRACT
Liver fibrosis represents a major worldwide health care burden. The last 15 years have seen a rapid growth in our understanding of the pathogenesis of this clinically relevant model of inflammation and repair. This work is likely to inform the design of effective antifibrotic therapies in the near future. In this review, we examine how the innate and adaptive immune response interacts with other key cell types in the liver, such as the myofibroblast, regulating the process of hepatic fibrosis and, where relevant, resolution of fibrosis with remodelling. Emphasis is placed on the increasing knowledge that has been generated by the use of transgenic animals and animals in which specific cell lines have been deleted. Additionally, we review the increasing evidence that, although significant numbers of wound-healing myofibroblasts are derived from the hepatic stellate cell, significant contributions may occur from other cell lineages, including those from distant sites such as bone marrow stem cells.

INTRODUCTION
Fibrosis of the liver and its end stage, cirrhosis, represents a massive health care burden worldwide. Liver disease is increasing in incidence globally. In the West, the main causes of liver fibrosis are chronic hepatitis C infection, alcohol and NASH (non-alcoholic steatohepatitis), whereas viral hepatitis (hepatitis B and C) predominate as the major causes of liver disease in the Middle East and Asia.

In the U.K., cirrhosis mortality rates increased steeply during the 1990s. Between the periods 1987 and 1991, and 1997 and 2001, cirrhosis mortality in men in Scotland more than doubled (104 % increase) and in England and Wales rose by over two-thirds (69 %). Mortality in women increased by almost half (46 % in Scotland and 44 % in England and Wales). Cirrhosis mortality rates in Scotland are now one of the highest in western Europe, in 2002 being 45.2 per 100,000 in men and 19.9 per 100,000 in women [1]. Furthermore, changing patterns of alcohol consumption in the West and the increasing incidence of the metabolic syndrome mean that advances in preventing and treating viral hepatitis may be offset by an increasing burden of fibrosis and cirrhosis related to alcohol and NASH.

Currently, our therapeutic repertoire for the treatment of liver fibrosis and cirrhosis is severely limited. Broadly, treatment falls into two categories. Removal of the...
underlying injurious stimulus (where possible), such as viral eradication in hepatitis B- and C-mediated liver disease, and liver transplantation. For severe end-stage liver disease, orthotopic liver transplantation is the only effective treatment. This procedure can be a highly successful treatment with an average 5 year survival rate of 75%. However, liver transplantation has several disadvantages, including increasingly limited donor liver availability and the commitment of recipients to lifelong toxic immunosuppression; in addition, patients may be excluded from transplantation as a result of psychiatric or medical comorbidities. Furthermore, in the specific case of hepatitis C-induced cirrhosis (now the commonest indication for liver transplantation in the U.S.), liver transplantation is far from curative, as recurrence of hepatitis C in the graft is universal. Therefore effective antifibrotic treatments are urgently required.

Central to fibrogenesis and the scarring of organs is the activation of tissue fibroblasts into extracellular-matrix-secreting myofibroblasts. Within the liver, the main effector cells of fibrosis are the HSCs (hepatic stellate cells). Classically, quiescent HSCs become activated to a contractile myofibroblast-like matrix-secreting phenotype. Activated HSCs secrete fibrillar (or scarring) collagens, resulting in the deposition of fibrotic matrix, and can express TIMPs (tissue inhibitors of metalloproteinases) with the result that matrix-degrading metalloproteinase activity is inhibited. This alters the balance of matrix secretion and degradation to favour accumulation. In this respect, the activated stellate cell/myofibroblast can be considered as a final common mediator of fibrosis. Interestingly, there is increasing evidence that liver myofibroblasts may derive from local sources other than HSCs and also from sites distant to the liver.

In recent years, work has focused on understanding the molecular mechanisms that drive liver injury, inflammation and fibrosis. These mechanisms involve a complex interplay of cytokines and cells of several lineages. This review examines the key cellular interactions regulating the hepatic fibrogenic response in the context of inflammation. We will examine the role of inflammation and how this complex immune process interdigitates with other key cell types in the liver, such as the myofibroblast, culminating in scar formation and, in some situations, subsequent resolution.

THE IMPORTANCE OF INFLAMMATORY CELLS IN HEPATIC FIBROSIS

Tissue injury sets in motion a cascade of events whose ultimate aim is repair. The processes of inflammation and subsequent repair are tightly regulated and exquisitely complex. The smooth transition in normal wound healing, whereby initial acute inflammation fades and a switch occurs to healing and repair, appears seamless, but how these two fundamental phases of the liver injury response interdigitate and overlap is still largely unknown.

Chronic liver disease results from iterative hepatic injury with sustained inflammation, formation of scar tissue, loss of tissue architecture and organ failure. There is no doubt, from both human and animal studies, that too much or too protracted inflammation in the liver leads to excess scarring. Although the constituents of inflammation vary in different liver diseases, fibrosis represents the final common pathway of chronic hepatic inflammation.

WHICH ARE THE KEY INFLAMMATORY CELL TYPES IN HEPATIC FIBROGENESIS?

Although there are a significant number of leucocytes within the resting liver, liver injury results in a massive accumulation of recruited inflammatory cells, with contemporaneous activation of the resident inflammatory cell pool. The blend of the individual activated and recruited inflammatory cell populations in response to a given type of hepatic injury may govern the duration and vigour of the inflammatory response and may affect the timing of the switch to healing and resolution, if this is to occur (see below). Therefore the degree of inflammation itself may not necessarily be the sole factor dictating the hepatic wounding response, rather that constituent parts of the inflammatory response may determine the pattern of outcome.

In broad terms, inflammation in liver fibrogenesis can be subdivided into the innate and adaptive immune responses. To dissect and understand the relationship between inflammation and hepatic fibrosis further a number of studies have examined the role of individual inflammatory cell types in liver fibrogenesis using depletion and knockdown studies, which are summarized in Table 1. Clearly these studies cannot be translated directly to human disease, but they do offer very useful insights into the cellular cross-talk and complex interplay between inflammation and the fibrotic process.

THE INNATE IMMUNE RESPONSE IN HEPATIC FIBROGENESIS

Platelets
Platelets are the first cells recruited to sites of injury, limiting blood loss by forming aggregates at the end of damaged blood vessels and converting fibrinogen into fibrin. Platelets exhibit many properties which may be important in wound healing, as they initiate coagulation and can release growth factors such as PDGF (platelet-derived growth factor) and TGF-β (transforming growth factor-β), which are potent stimulators of fibroblasts and
Table 1  Studies of inflammatory cells in animal models of liver fibrosis

(a) Innate immunity

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Model of liver fibrosis</th>
<th>Manipulation</th>
<th>Summary</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Neutrophils</td>
<td>ANIT</td>
<td>2. Mice with an underlying defect in neutrophil function due to transgenic expression of IL-8.</td>
<td>No difference in hepatic fibrosis compared with wild type.</td>
<td>Xu et al. (2004) [3]</td>
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<tr>
<td>Macrophages</td>
<td>CCl₃</td>
<td>Selective depletion of macrophages (using a transgenic mouse CD11b-DTR) during liver injury and repair.</td>
<td>Functionally distinct subpopulations of macrophages exist in the same tissue, and play critical roles in both the injury and recovery phases of inflammatory scarring.</td>
<td>Duffield et al. (2005) [4]</td>
</tr>
<tr>
<td>Macrophages</td>
<td>Dimethylnitrosamine</td>
<td>Inhibited monocyte/macrophage infiltration into the liver with adenoviral overexpression (Ad7ND) of a dominant-negative mutant form of MCP-1.</td>
<td>Suppression of macrophage infiltration, inhibited activation of HSCs and liver fibrogenesis.</td>
<td>Imamura et al. (2005) [5]</td>
</tr>
<tr>
<td>Macrophages</td>
<td>TAA</td>
<td>TAA-induced hepatic fibrosis without or with pre-treatment with GdCl₃ (a Kupffer cell inhibitor).</td>
<td>Decreased numbers of macrophages may contribute to improvement of hepatic fibrosis.</td>
<td>Ide et al. (2005) [6]</td>
</tr>
<tr>
<td>NK cells</td>
<td>DDC diet or CCl₄</td>
<td>Activation and depletion of NK cells</td>
<td>NK cells ameliorate liver fibrosis by killing activated stellate cells.</td>
<td>Radaeva et al. 2005 [7]</td>
</tr>
<tr>
<td>Mast cells</td>
<td>Rats: bile-duct resection, CCl₄ or porcine serum. Mice: bile-duct resection or CCL.</td>
<td>Mast-cell-deficient mutant Ws/Ws rats and W/Wv mice.</td>
<td>Mast cells play no role in the development of liver fibrosis in rats and mice.</td>
<td>Sugihara et al. (1999) [8]</td>
</tr>
<tr>
<td>Mast cells</td>
<td>Pig serum</td>
<td>Ws/Ws mast-cell-deficient rats.</td>
<td>Mast cells did not appear to play important roles in the induction of fibrosis.</td>
<td>Okazaki et al. (1998) [9]</td>
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(b) Adaptive immunity

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Model</th>
<th>Manipulation</th>
<th>Summary</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-cells</td>
<td>CCl₃ or TAA</td>
<td>Transgenic mice with hepatocyte expression of rIL-10. Adaptive transfer of various lymphocyte subsets to SCID recipients.</td>
<td>Fibrosis may be a CD8⁺ T-cell-mediated disease that is attenuated by rIL-10.</td>
<td>Safadi et al. (2004) [10]</td>
</tr>
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<td>T-cells</td>
<td>CCl₃</td>
<td>Series of CCl₃-induced liver injury experiments with mice that lack CD4⁺ or T-cells (Aβ⁻/⁻/⁻), CD8⁺ T-cells (B2m⁻/⁻/⁻), γδ T-cells (TCRδ⁻/⁻/⁻), and both B- and T-cells (RAG2⁻/⁻/⁻).</td>
<td>CD4⁺, CD8⁺ and γδ T-cells do not influence hepatic fibrosis to a significant degree.</td>
<td>Novobrantseva et al. (2005) [11]</td>
</tr>
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<td>T-cells</td>
<td>S. mansoni</td>
<td>S. mansoni-infected mice were treated with sIL-13Fc (IL-13 inhibitor)</td>
<td>IL-13 inhibitor blocked the development of hepatic fibrosis during a T12-dominated inflammatory response.</td>
<td>Chiaramonte et al. (1999) [12]</td>
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<td>B-cells</td>
<td>S. mansoni</td>
<td>B-cell-deficient mice</td>
<td>B-cell-deficient mice had increased hepatic fibrosis.</td>
<td>Ferru et al. (1998) [13]</td>
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other mesenchymal cells important in tissue healing. A previous study [14] rendered mice thrombocytopenic by intraperitoneal administration of a rabbit anti-(mouse platelet) serum and examined skin wound healing. Wounds of thrombocytopenic mice contained significantly more macrophages and T-cells, but the rate of wound re-epithelialization, angiogenesis and collagen synthesis was nearly identical for thrombocytopenic and control mice. To date, there have been no similar studies examining the role of platelets in hepatic fibrogenesis, despite a recent study [15] highlighting the importance of platelet-derived serotonin in hepatic regeneration.

**Neutrophils**

Neutrophils arrive early following liver injury and constitute one of the ‘rapid-response’ members of the innate immune system. Following the fibrogenic liver injury BDL (bile-duct ligation) in rats, neutrophils rapidly infiltrate the liver. Saito et al. [2] depleted neutrophils in BDL by means of an anti-neutrophil antiserum. Despite this, there was no difference in either bile-duct proliferation or hepatic fibrogenesis after BDL compared with control rats. In a second set of experiments, BDL was performed on mice with an underlying defect in neutrophil function. These mice exhibited less hepatic collagen following BDL than wild-type mice, but the difference was not statistically significant. A further study examining the role of CXC chemokines and neutrophils in hepatic fibrogenesis utilized a mouse model of ANIT (α-naphthylisothiocyanate) hepatotoxicity [3]. ANIT is a hepatotoxin that causes severe neutrophilic inflammation around the portal tracts and bile ducts, which coincides with rapid hepatic induction of the CXC chemokine MIP-2 (macrophage inflammatory protein-2). Neutrophil recruitment was suppressed by 50% in mice lacking the receptor for MIP-2 (CXCR2<sup>-/-</sup>). Despite this marked reduction in neutrophil recruitment, CXCR2<sup>-/-</sup> mice displayed equivalent hepatocellular injury and cholestasis after ANIT treatment compared with wild-type mice. Furthermore, following chronic exposure to ANIT, CXCR2<sup>-/-</sup> mice developed a similar degree of liver fibrosis compared with wild-type mice. Therefore these studies would suggest that the rapid and early infiltration of neutrophils during repetitive liver injury does not significantly impact on subsequent hepatic fibrogenesis.

**Macrophages**

The importance of macrophages in the wound-healing response has been known for some time. Seminal studies on skin wound healing in the 1970s by Leibovich and Ross [16,17] demonstrated that macrophage depletion (with hydrocortisone and anti-macrophage serum) resulted in elevated wound fibrin levels and clearance of fibrin, neutrophils, erythrocytes and other miscellaneous debris from these wounds was delayed. Furthermore, there was delayed appearance of fibroblasts, and their subsequent rate of proliferation was lower than that of controls.

Current evidence indicates that macrophages perform a wide repertoire of functions in inflammation and repair. These include cell killing, the regulation of inflammatory cells, recruiting and activating myofibroblasts, and regulating spontaneous recovery of fibrosis in physiological or aberrant wound healing [18–22].

The factors determining the behaviour of macrophages at sites of inflammation is complex and contextual, dependent among other variables on the organ and mode of tissue injury [23,24]. Cell-surface phenotyping studies suggest that distinct macrophage populations exist. For example, the haemoglobin scavenger receptor CD163 is expressed by a subpopulation of macrophages at inflamed sites [25]. Likewise, pattern-recognition receptors, such as dectin-1 and mannose receptors, are highly expressed only in subsets of macrophages in inflamed tissue [26]. To make sense of this diversity, two forms of inflammatory macrophages have been proposed: the classically activated macrophage and the aa-M (alternatively activated macrophage) [27]. The former, activated by T<sub>H</sub>1 (T-helper type 1) lymphokines, bacterial and fungal cell wall components or degraded matrix (e.g. hyaluronic acid), liberates pro-inflammatory cytokines and chemokines and is predominantly lytic to matrix components [28,29]. The aa-M is activated by T<sub>H</sub>2 (T-helper type 2) lymphokines, including IL (interleukin)-4, as well as apoptotic cells and corticosteroids, and produces anti-inflammatory cytokines, including IL-10 and TGF-β. When cultured with myofibroblasts, aa-Ms promote complex matrix deposition [27,30–33]. Furthermore, in vitro experiments have highlighted the ability of macrophages to generate a diverse array of agents that regulate the degradation of matrix [24,34–36].

However, these in vitro experiments do not reflect the complexity of the macrophage phenotype in vivo. Macrophages may perform both injury-inducing and repair-promoting tasks synchronously in an injured organ. Moreover, in an injured tissue, the macrophage population appears to display features of both the pro-inflammatory and alternative activation programmes [31,33,37–39], but the mechanisms which programme the macrophage phenotype down either route are still under investigation. Furthermore, whether macrophages can ‘re-align’ once committed to either the pro-inflammatory or alternative activation programmes is also unknown.

In an attempt to determine the role of macrophages in hepatic fibrogenesis in vivo, recent studies have employed a variety of techniques to manipulate macrophages in animal models of liver fibrosis. Ide et al. [6] used GdCl<sub>3</sub> (gadolinium chloride) in a rat model of TAA (thioacetamide)-induced fibrosis. GdCl<sub>3</sub> treatment decreased the amount of macrophages appearing in the fibrotic areas, reduced the numbers of α-SMA (α-smooth muscle actin)-positive myofibroblasts and inhibited fibrosis. However,
there are concerns over the use of GdCl₃, including toxicity and non-specific depletion of other cell types. Another recent study [5] inhibited monocyte/macrophage infiltration into the liver during fibrogenesis using adenoviral overexpression (Ad7ND) of a dominant-negative mutant form of MCP-1 (monocyte chemoattractant protein-1). Using dimethylnitrosamine to induce hepatic fibrosis, Imamura et al. [5] demonstrated that blockade of macrophage infiltration inhibited activation of HSCs and led to suppression of liver fibrogenesis.

However, recent work using a novel conditional ablation system has allowed non-toxic, cell-type-specific, time-point-selective depletion of macrophages in a reversible model of liver fibrosis. Duffield et al. [4] investigated macrophage function mechanistically in a reversible model of liver injury in which the injury and recovery phases are distinct. Using a transgenic mouse (CD11b-DTR line) in which macrophages can be effectively deleted, the authors demonstrated that depletion of macrophages when liver fibrosis was advanced resulted in reduced scarring and fewer myofibroblasts. By contrast, macrophage depletion during recovery led to a failure of matrix degradation. This work demonstrated that functionally distinct subpopulations of macrophages exist (with opposite but complementary functions) in the same tissue and that these macrophages play critical roles in both the injury and recovery phases of inflammatory scarring. Further work in this area may delineate whether these functionally distinct macrophages are separately recruited subpopulations or highly plastic macrophages able to switch phenotype from matrix promoters to recovery enhancers when the surrounding milieu of injury and inflammation changes.

**Macrophage interaction with HSCs and/or myofibroblasts**

Tissue culture experiments have demonstrated that macrophages are likely to promote activation of HSCs during fibrosis progression via release of paracrine factors, including TGF-β1 [40,41]. However, the simultaneous loss of macrophages and activated HSCs during the recovery phase suggests that macrophages may promote apoptosis of HSCs by the expression of TRAIL [TNF (tumour necrosis factor)-related apoptosis-inducing ligand] and other apoptotic stimuli [42,43].

**Do macrophages enhance matrix degradation during fibrosis regression directly or indirectly?**

Macrophage promotion of matrix regression may be direct with macrophage release of matrix-degrading proteases, or by stimulating either the release or activation of proteases by other cell types, including HSCs. Macrophages could be the source of enzymes that degrade the fibrillar interstitial matrix (i.e. scar) during fibrosis resolution, including MMP (matrix metalloproteinase)-13 (collagenase 3) [44] and MMP-9 (gelatinase B) [42,45].

Macrophages may also profoundly affect scar resolution indirectly, as well as via direct matrix breakdown. It has been shown previously that interstitial collagen represents an important survival signal for HSC [46]. Furthermore, failure to degrade collagen-I critically impairs HSC apoptosis and may prevent the effective restoration of hepatocyte mass in liver fibrosis. Therefore macrophage-mediated alterations in the extracellular milieu may make the local environment more hostile to HSCs, encouraging their apoptosis and hence fibrosis resolution.

**Mast cells**

Mast cells, derived from circulating basophils, are another leucocyte lineage described in the innate immune response to liver injury. Mast cell numbers increase with hepatic fibrosis in CCl₄ (carbon tetrachloride)-induced rat liver cirrhosis [47] and are a source of pro-fibrogenic cytokines, including TGF-β, PDGF and bFGF (basic fibroblast growth factor) [48]. Two different studies have utilized mast-cell-deficient mutant Ws/Ws rats and W/Wv mice to examine mast-cell depletion in the context of liver fibrosis [8,9]. Neither study found a significant role for mast cells in the pathogenesis of liver fibrosis; however, systemic mastocytosis in humans can be associated with liver fibrosis and portal hypertension [49,50].

**NK (natural killer) cells**

NK cells are large granular lymphocytes that do not express TCRs (T-cell antigen receptors) and constitute a major component of the innate immune system. NK cells are one of the first lines of defence against invading pathogens and usually become activated in the early phases of a viral infection. The liver has abundant NK cells, which are activated by hepatotropic viruses such as HCV (hepatitis C virus). The activated NK cells play an essential role in recruiting virus-specific T-cells and in inducing antiviral immunity in liver. They also eliminate virus-infected hepatocytes directly by cytolytic mechanisms and indirectly by secreting cytokines, which induce an antiviral state in host cells [51]. As viral hepatitis is a major cause of liver fibrosis and is associated with activation of NK cells and the innate immune system, Radaeva et al. [7] examined the role of NK cells in two animal models of liver fibrosis. Liver fibrosis was induced either by feeding mice with the DDC (3,5-diethoxycarbonyl-1,4-dihydrocollidine) diet or by CCl₄ injection. The TLR-3 (Toll-like receptor 3) ligand, poly I:C (polynosinic-polycytidylic acid), was used to activate cells and mediators of the innate immune system, including NK cells and IFN (interferon)-γ. In both models, NK cell activation by poly I:C induced death of activated HSCs and attenuated the severity of liver fibrosis. The protective effect of poly I:C on liver fibrosis was diminished...
through either depletion of NK cells or by disruption of the IFN-γ gene.

**THE ADAPTIVE IMMUNE RESPONSE IN HEPATIC FIBROGENESIS**

**T-cells**

Several studies using different models of fibrosis induction have examined the role of T-cells in hepatic fibrogenesis. Transgenic mice with hepatocyte expression of rIL-10 (rat IL-10) are less susceptible to hepatic fibrosis following liver injury from CCl₄ or TAA [10]. Subtotal irradiation diminished fibrosis equally in both the wild-type and transgenic groups, suggesting that the anti-fibrotic effect of rIL-10 was lymphocyte-mediated. Adoptive transfer of CD8⁺ T-cells from wild-type animals with CCl₄-induced fibrosis to SCID (severe combined immunodeficiency) recipients led to significantly higher serum aminotransferase levels and HSC activation compared with transfer of splenic lymphocytes or CD4⁺ T-cells, thus suggesting an important role for CD8⁺ T-cells in hepatic fibrogenesis.

CD4⁺ T-cells have also been heavily implicated in the pathogenesis of hepatic fibrosis. Akin to human disease, mice infected with *Schistosoma mansoni* develop liver fibrosis dominated by a T₂ (T-helper type 2) cytokine response. Chiaramonte et al. [12] demonstrated that mice treated with sIL-13Rα2-Fc (an IL-13 inhibitor) developed less hepatic fibrosis during *S. mansoni* infection, suggesting that IL-13 inhibition may be useful therapeutically in preventing liver fibrosis secondary to T₂-dominated inflammatory responses.

However, a recent study [11] utilizing combinations of mice lacking CD4⁺ T-cells (Aβ⁻/⁻), CD8⁺ T-cells (β2m⁻/⁻), γδ T-cells (TCRδ⁻/⁻) and both B- and T-cells (RAG2⁻/⁻) in a CCl₄-induced model of hepatic fibrosis did not demonstrate any protection from liver fibrosis in the T-cell-deficient mice. Only RAG2⁻/⁻ mice (lacking both B- and T-cells) had a reduction in fibrosis.

**B-cells**

The recent study by Novobrantseva et al. [11] provided the first direct evidence that B-cells are an important inflammatory cell type in the pathogenesis of liver fibrosis. Using B-cell-deficient (J_{H₁⁻/⁻}) mice in a CCl₄-induced model of liver fibrosis, they demonstrated reduced collagen deposition compared with wild-type mice. The authors also examined whether B-cell regulation of liver fibrosis required Ig production. Mice with normal numbers of B-cells that lacked Ig in their serum or had low Ig levels developed the same amount of fibrosis compared with wild-type mice, indicating that B-cells influence the development of liver fibrosis in an antibody-independent manner; however, a pro-fibrogenic role for B-cells following hepatic injury is not universal [13].

**ORIGIN OF HEPATIC MYOFIBROBLASTS**

A simple philosophy in the design of an effective anti-fibrotic therapy would be that the more information we can accrue regarding the key cell types involved in secreting the scar the better. This would allow informed decisions regarding the targeting of fibrosis inhibitors to one or even more cell types. However, with the identification and characterization of an increasing number of cell types implicated in hepatic fibrogenesis, the story has become increasingly complex. A growing body of evidence suggests that the HSC is not the only cell type within the liver with fibrogenic potential. The anatomic location, source and lineage of myofibroblasts in the liver has been hotly debated in recent years. In addition to HSCs, there are now a number of different postulated subclasses of hepatic fibrogenic cells. The potential origins of the hepatic myofibroblast pool are summarized diagrammatically in Figure 1.

**Heterogeneity of hepatic fibrogenic cell populations: is there more than one target cell type in the fight against fibrosis?**

**HSCs**

HSCs (Ito cells, fat-storing cells, interstitial cells and lipocytes) reside in the space of Disse and were first described by von Kupffer in 1876 as liver sternzellen (star-shaped cells). No comment on the function of these cells was made for a further 76 years until Ito and Nemoto [52] observed fat-storing cells in the capillary wall of human liver. The unique ability of these non-parenchymal perisinusoidal cells to store vitamin A led to their identification in tissue [53] and their isolation using density gradient methods. This facilitated a major advance in the understanding of HSC function in the 1980s, with the discovery that HSC were the principal collagen-producing cells of normal rat liver [54,55]. This reproducible technique for the isolation of HSCs based on buoyancy in density gradients allowed further characterization of the intracellular filaments in HSCs. The expression of the contractile filaments α-SMA (Figure 2) and myosin were identified in HSCs activated both in vivo and in vitro when activated on tissue culture plastic [56] in addition to other cytoskeletal proteins, such as desmin [57] and GFAP (glial fibrillary acidic protein) [58].

These studies resulted in the genesis of the now classical paradigm of HSCs undergoing unidirectional activation, a major transdifferentiation in response to hepatic injury, with development of a proliferative, contractile and matrix-secreting phenotype. Activated HSCs proliferate, with the result that increases in numbers of HSCs, in addition to increases in secretion of the fibrillar (or scarring) collagens, result in the deposition of excess fibrotic matrix. Simultaneously activated HSCs express TIMPs with...
the result that metalloproteinase activity is inhibited in the extracellular milieu and the pattern of matrix degradation is altered to favour accumulation. The characteristic scarring of the liver seen in fibrosis and cirrhosis has important implications for hepatic function beyond architectural disruption and may result in dysregulated hepatocyte function and proliferation.

**Portal myofibroblasts**

Previous work demonstrated that, in the early phase of BDL in the rat, there is a marked and transient proliferation of bile duct epithelial cells associated with activation and proliferation of portal periductular fibroblasts. This results in the accumulation of peribiliary myofibroblasts which rapidly express α-SMA. Bile duct epithelial cells of the ductular reaction may actively contribute to myofibroblastic transdifferentiation and the promotion of biliary type liver fibrogenesis by the release of a number of paracrine mediators, such as TGF-β, CTGF (connective tissue growth factor), PDGF-BB, endothelin-1 and MCP-1, that target different liver cell types, including HSCs and portal fibroblasts. For example, recent work [59] demonstrated that bile duct epithelial cell secretions were able to induce α-SMA expression and regulate portal fibroblast proliferation via secretion of MCP-1. It has been postulated that this population of peribiliary myofibroblasts may play a dominant role in the early portal fibrosis after BDL [60,61].

Other studies have examined further the existence of a myofibroblast population distinct from HSCs. Having established a method for the isolation of rat liver myofibroblasts, Ramadori and co-workers [62,63] demonstrated that, unlike HSCs, rat liver myofibroblasts express fibulin-2, produce large amounts of IL-6, do not undergo spontaneous apoptosis in vitro and do not express Fas ligand.

Cassiman et al. [64], examining both human tissue and livers from two different rat models of liver fibrosis, suggested that in advanced fibrosis and in cirrhosis, regardless of cause or species, three distinct mesenchymal myofibroblast-like liver cell subpopulations can be discerned immunophenotypically: portal/septal myofibroblasts, interface myofibroblasts and perisinusoidally...
located HSCs. Whether these all contribute to fibrosis in a similar manner is an unresolved issue. Moreover, recent data on both the potential for BM (bone marrow) derivation of hepatic myofibroblasts (whether periportal or central) and the increasing evidence for the plasticity of myofibroblasts suggests that definitions based on cell function rather than cell lineage may be more valuable.

**Plasticity of hepatic myofibroblasts**

A recent and elegant study by Magness et al. [65] has helped shed further light on the heterogeneity of fibrogenic cell populations during liver fibrosis. As discussed above, activation of HSCs and potentially the trans-differentiation of other resident mesenchymal cells into myofibroblasts expressing α-SMA and collagen-I is a key event in hepatic fibrogenesis. Using transgenic technology, Magness et al. [65] examined α-SMA and collagen α1(I) transcriptional patterns in primary cultures of HSCs and, additionally, in an in vivo model of secondary biliary fibrosis using double transgenic mice that expressed the Discosoma sp. RFP (red fluorescent protein) and the EGFP [enhanced GFP (green fluorescent protein)] reporter genes under direction of the mouse α-SMA and collagen α1(I) promoter/enhancers respectively.

Reporter gene expression in cultured HSCs demonstrated that both transgenes were induced at day 3 with continued expression through to day 14, i.e. during activation to a myofibroblast-like phenotype. However, α-SMA and collagen α1(I) transgenes were not co-expressed in all cells. Flow cytometry analysis showed three different patterns of gene expression: α-SMA–RFP-positive cells, collagen–EGFP-positive cells and cells expressing both transgenes. There was also significant plasticity of cell phenotype with cells initially expressing collagen–GFP re-acquiring expression of all three phenotypes (α-SMA–RFP only, collagen–GFP only, or both) following long-term tissue culture. However, cells initially expressing α-SMA–RFP were less plastic [65,66].

In vivo, following BDL, α-SMA and collagen α1(I) transgenes were differentially expressed by peribiliary, parenchymal and vascular fibrogenic cells in these mice. Peribiliary cells preferentially expressed collagen α1(I), whereas parenchymal myofibroblasts expressed both α-SMA and collagen α1(I). Therefore these data demonstrate in vitro and in vivo that there is heterogeneity in HSCs/myofibroblasts with regard to gene expression. The fact that α-SMA and collagen α1(I) are not always co-expressed in fibrogenic cell types in vitro or in vivo also has relevance to the way we interpret the degree of active fibrogenesis in liver tissue. α-SMA immunohistochemistry and quantification has been the accepted method for the estimation of activity of fibrogenic cells in the liver. However, the authors [65] stressed that concomitant estimation of collagen expression should also always be sought to give a true reflection of the degree of hepatic fibrogenesis.

**BM-derived myofibroblasts**

Studies exploiting cross-sex (BM) transplantation have highlighted the contribution of the BM to tissue fibrosis. Myofibroblasts of BM origin have been identified in a number of organs. Brittan et al. [67] demonstrated the presence of BM-derived pericytial myofibroblasts in the mouse intestine following irradiation and BM transplantation, and in the intestines of human patients suffering graft versus host disease following a BM transplant. Further work from the same group suggested that the BM can contribute to myofibroblast populations in multiple organs (stomach, lung, skin, kidney, adrenal gland, colon and small intestine), and that this contribution is exacerbated by injurious stimuli, such as skin wounding or paracetamol-induced lung injury [68]. Another study utilized adult mice durably engrafted with BM isolated from transgenic mice expressing EGFP [69]. Induction of pulmonary fibrosis by bleomycin caused large numbers of GFP-positive cells to appear in active fibrotic lesions, whereas only a few GFP-positive cells could be identified in control lungs. Flow cytometry of lung cells confirmed the bleomycin-induced increase in GFP-positive cells in chimaeric mice and showed a significant increase in GFP-positive cells that also expressed type I collagen, thus demonstrating that the collagen-producing lung fibroblasts in pulmonary fibrosis could also be derived from BM progenitor cells.

Recent work has also identified BM-derived myofibroblasts in human and mouse liver. Forbes et al. [70] analysed the origin of myofibroblasts within fibrotic liver in two scenarios: (i) seven male patients who received liver transplants from female donors and subsequently developed liver fibrosis; and (ii) a female patient who received a BM transplant from a male donor and subsequently developed hepatitis C-induced cirrhosis. Male cells of extrahepatic origin were tracked using the Y chromosome and immunophenotyped. Significant numbers of Y-chromosome-positive cells in fibrotic areas were positive for α-SMA, vimentin and fibrulin-2 and negative for CD45, thus demonstrating a myofibroblast phenotype. In the liver transplant cases, 6.8–22.2 % of α-SMA-positive myofibroblasts contained the Y chromosome. In the female recipient of a male BM transplant, 12.4 % of the myofibroblasts were Y-chromosome-positive, indicating a BM origin. Interestingly the distribution of these cells was pan-acinar with regard to areas of scarring. The authors [70] concluded that there was a significant contribution to liver cirrhosis in humans from extrahepatically derived myofibroblasts in liver disease of diverse aetiology.

In a study utilizing GFP transgenic mice, Baba et al. [71] examined the contribution of the BM to both the quiescent and activated HSC pools within the liver. BM cells from GFP transgenic mice were transplanted into age-matched C57BL/J mice. Ex vivo, GFP-expressing cells positive for HSC lineage markers comprised...
from Col 1α1rr mice, which have a mutated collagenase-
damaged liver. Moreover, when BM was transplanted
that the BM-derived cells transcribed collagen in the
collagen-I transcription. This technique clearly indicated
galactosidase reporter gene activated in the presence of
myofibroblasts, the authors [74] determined collagen-
in the liver of BM-transplanted mice, GFP-positive non-
parenchymal cells expressed GFAP. Following CCl4 liver
injury, these cells also co-expressed desmin and α-SMA.
This study therefore demonstrated a contribution of the
BM to the quiescent and activated HSC populations in
normal and injured mouse liver respectively.

A key question in this field relates to the functional
contribution of the BM to liver fibrosis, i.e. how important
(or not) are BM-derived cells in the pathogenesis of
liver fibrosis. For example, it has been suggested
that injection of fractionated BM can abrogate CCl4-
induced liver fibrosis in a murine model [72]. This type of
study has encouraged the investigation of BM therapy in
chronic organ injury and regeneration [73]. Therefore an
increased understanding of the specific BM-derived cells
mediating both the profibrotic and therapeutic response
in the injured liver is required to guide rational planning
of clinical trials of BM stem cell therapy.

In a recent study by Russo et al. [74], the temporal,
quantitative and functional role of BM-derived myo-
fibroblasts in mouse models of liver fibrosis were exam-
in. Female mice were lethally irradiated and received
male BM transplants. CCl4 and TAA were used to in-
duce cirrhosis. BM-derived cells were tracked through
in situ hybridization for the Y chromosome. In cirrhosis
secondary to chronic CCl4, the contribution of BM to
parenchymal regeneration was minor (0.6 %); however,
the BM contributed significantly to HSC (68 %) and
myofibroblast (70 %) populations. Interestingly, by
the time cirrhosis had developed, the majority of the
hepatic myofibroblasts were of BM origin, indicating
the importance of this axis in chronic hepatocellular liver
damage.

To define the in vivo functionality of BM-derived
myofibroblasts, the authors [74] determined collagen-
I gene expression by using donor mice that have a β-
galactosidase reporter gene activated in the presence of
collagen-I transcription. This technique clearly indicated
that the BM-derived cells transcribed collagen in the
damaged liver. Moreover, when BM was transplanted
from Col 1α1rr mice, which have a mutated collagenase-
resistant collagen-I, and then injured with CCl4, the mice
developed an extensive pericellular fibrosis characteris-
tic of the Col 1α1rr mouse [46]. In contrast, control mice
given wild-type BM had a more restricted linear collagen
deposition. Therefore manipulating the phenotype of
the BM-derived myofibroblasts influenced the injured
organ’s fibrotic response, suggesting that manipulation of
this axis may yield novel therapeutic options in hepatic
fibrosis.

Role of fibrocytes in hepatic fibrogenesis
A further population which has received attention
recently is a group of cells termed fibrocytes. The concept
that fibroblasts involved in wound healing can originate
from peripheral blood is not a new one and goes back
approx. 100 years [75]. In 1994, a distinct population of
circulating cells with fibroblast properties that specif-
cically enter sites of tissue injury was described, namely
fibrocytes. These circulating cells were characterized by a
distinctive phenotype (collagen+/vimentin+/CD34+), by
rapid entry from blood into subcutaneously implanted
wound chambers and by their presence in connective
tissue scars [76]. Fibrocytes comprise 0.1–0.5 % of non-
erythrocytic cells in peripheral blood and display an
adherent spindle-shaped morphology when cultured
in vitro [76]. These cells have a unique cell-surface pheno-
type expressing haemopoietic and myeloid markers
(CD11b+/CD13+/CD34+/CD45RO+/MHC class II+/CD86+)
marked with collagen [77, 78]. They also express
chemokine receptors, such as CXCR4 and CCR7, and
secrete collagen-I, collagen-III and fibronectin [79–
82]. Further work also demonstrated that blood-borne
ex vivo cultured precursor fibrocyte cells were able to
differentiate into α-SMA-positive TGF-β1-responsive
fibrocyte cells that exhibited characteristics similar to
those of wound-healing myofibroblasts, such as the
ability to contract collagen gels in vitro [77, 82].

However, until 2004, no study had examined whether
fibrocytes contributed to fibrosis in vivo. Phillips et al.
[83] demonstrated that a population of human CD45+/Col
1α1/CXCR4+ circulating fibrocytes migrated in response
to CXCL12 and could traffic to the lungs in a murine model of bleomycin-induced pulmonary fibrosis.
Maximal intrapulmonary recruitment of CD45+/Col1α1/
CXCR4+ fibrocytes correlated directly with increased
collagen deposition in the lungs. Furthermore, treatment
of bleomycin-exposed animals with specific neutralizing
anti-CXCL12 antibodies inhibited intrapulmonary recruit-
ment of CD45+/Col1α1/CXCR4+ circulating fibrocytes
and attenuated lung fibrosis, demonstrating that
 circulating fibrocytes contributed to the pathogenesis of
pulmonary fibrosis.

Only one study so far has implicated fibrocytes in liver
fibrogenesis [84]. This study tested the hypothesis that
HSCs and/or other collagen-producing cells arise from a
population of cells originating in the BM. They utilized
BM chimaeric mice expressing GFP under control of
the collagen α1(I) promoter to specifically examine the
contribution of BM to the population of HSCs and
non-HSC collagen-producing cells in response to BDL-
duced hepatic injury. Although there was a significant
increase in cells expressing GFP under the control of the
collagen α1 promoter in livers of chimaeric mice trans-
planted with BM from the collagen mice, these GFP-
positive cells did not express α-SMA or desmin. In
addition, GFP-positive cells were not isolated with the
HSC fraction. They concluded that the BM does not
contribute to the pool of HSC in response to BDL. This is
in contrast with the work by Baba et al. [71], cited earlier.
in this review, which proposed that BM cells differentiate into HSCs in response to CCL4-induced liver injury.

Furthermore, as the BM-derived collagen α1(I)–GFP-positive expressing cells did not possess features of HSCs or myofibroblasts, Kisseleva et al. [84] proposed that these cells represented a novel collagen-producing cell type in the liver constituting approx. 5% of the collagen α1(I)-producing population in the liver after BDL. Using immunocytochemistry, approx. 70% of BM-derived GFP-positive cells in the liver were CD45+α1(I)–GFP- whereas CD34/CD14 expression constituted approx. 1%, concordant with the previously described phenotype of fibrocytes.

Although fibrocytes may constitute only a small proportion (approx. 3.5%) of the collagen α1(I)-producing population in the liver after BDL, they could play a wider role in hepatic fibrosis. In addition to collagen production, fibrocytes are involved in other aspects of wound healing, such as angiogenesis [85], antigen presentation [79,86] and secretion of chemokines, growth factors and inflammatory cytokines [80,87]. However, further studies will be required to assess how much of a role fibrocytes play in the extracellular matrix deposition characteristic of liver fibrosis, perhaps examining fibrocytes in different models of liver injury and, where possible, specifically depleting the fibrocyte pool to determine whether this has a significant impact on the degree of liver fibrogenesis.

**EMT (epithelial–mesenchymal transition)**

EMT is a central mechanism for diversifying the cells found in complex tissues [88,89] and is an orchestrated series of events in which cell–cell and cell–extracellular matrix interactions are altered to release epithelial cells from the basement membrane and surrounding tissue, the cytoskeleton is re-organized to confer the ability to move through a three-dimensional extracellular matrix and a new transcriptional programme is induced to maintain the mesenchymal phenotype [90]. Although EMT has been studied extensively in the context of embryonic development where it is an essential process, it has recently received a great deal of attention with regard to its role in the formation of fibroblasts from epithelia during organ fibrosis in adult tissues. Secondary to morphogenic pressure generated by injured tissue, mature epithelia change their phenotype creating new fibroblasts and therefore contribute to organ fibrogenesis [89]. EMT can be induced by a mixture of cytokines and growth factors associated with proteolytic digestion of the basement membranes which support epithelia. Membrane assembly inhibitors [91] or metalloproteinases [92,93] kick-start the process by dismantling basement membrane, followed by local expression of TGF-β, EGF (epidermal growth factor), IGF-II (insulin-like growth factor-II) or FGF-2 (fibroblast growth factor-2), which facilitates EMT by binding epithelial receptors with ligand-inducible intrinsic kinase activity [89,94–97].

EMT has been extensively studied in the context of renal [98] and pulmonary [99–102] fibrosis. The most compelling evidence supporting a central role for EMT in organ fibrogenesis in vivo is derived from studies of renal fibrosis [88,103,104]. During renal fibrogenesis secondary to ureteric obstruction, approx. 36% of new fibroblasts derive from local EMT involving tubular epithelial cells under inflammatory stress, which then make the phenotypic transition to matrix-secreting myofibroblasts. The BM contributes 14–15% of fibroblasts in renal fibrosis and the rest come from local proliferation [88]. This suggests that, in the kidney at least, the epithelium is a major contributor to myofibroblast numbers and hence renal scarring, and highlights the remarkable plasticity of mature tubular epithelial cells in this organ.

Numerous examples of epithelial cell plasticity in the liver have been observed [105], but data examining the role of EMT in liver fibrosis are less abundant. HGF (hepatocyte growth factor), originally characterized as a potent mitogen for mature hepatocytes [106], has potent antifibrotic effects in various organs including the liver [107,108]. It has been shown previously that HGF specifically preserves renal tubular epithelial cell phenotype by inhibiting EMT both in vitro and in vivo [109]. Therefore Xia et al. [110] evaluated the therapeutic potential of HGF in blocking hepatic fibrosis induced by BDL and explored the possibility of biliary epithelial to myofibroblast transition in vivo and in vitro. A number of observations supported their claims of EMT occurring within the liver in response to BDL: BECs (biliary epithelial cells) co-expressed the epithelial marker cytokeratin-19 and the myofibroblast marker α-SMA following BDL, suggesting the presence of a transitional stage between two phenotypes. Single cells or loosely organized small cell clusters still positive for cytokeratin-19 were scattered in the periductal region. Vice versa, cell clusters with BEC morphology, but without cytokeratin-19, were also present. BECs at various transitional stages actively produced interstitial type I collagen in the fibrotic environment, and phenotypic conversion of the bile duct epithelium was recapitulated in cultured human BECs in vitro following incubation with TGF-β1. Blockade of biliary EMT by HGF prevented hepatic myofibroblast activation and biliary fibrosis. In addition, another recent study [111] demonstrated that hepatic epithelial progenitors are capable of co-expressing both epithelial and mesenchymal markers.

Hepatocytes, representing the vast majority of the liver cell mass, would be a potentially very large reservoir for EMT-derived myofibroblasts. In vitro studies have demonstrated EMT of cultured neonatal rat hepatocytes under various conditions [112–114]. Although the main focus of previous work examining hepatocyte EMT has been related to liver carcinogenesis [115–117], the...
findings from these studies are of clear relevance to hepatic fibrogenesis. Pertinent to both pathological processes, hepatocyte EMT involves loss of typical epithelial differentiation markers, re-organization of the cytoskeleton, acquisition of a migrating morphology and altered expression of genes involved in matrix regulation. However, definitive in vivo studies specifically examining the role of hepatocyte EMT in liver fibrosis have not been published.

Full evaluation of the extent of EMT in liver fibrosis secondary to biliary disease and, additionally, hepatic scarring secondary to other forms of liver injury could be interesting. If EMT turns out to be a ‘major player’ and significant contributor to the hepatic myofibroblast pool, then it will quickly ascend the list of research priorities in the search to develop effective anti-fibrotic therapies.

These studies advance our understanding of myofibroblast heterogeneity within the liver, but also raise some interesting questions. Are all liver myofibroblasts identical? The answer is almost certainly no, and any differences may alter a therapeutic approach based on targeting the myofibroblast cells. The cell biology of these subpopulations may well vary in respect of responsiveness to specific growth factors, chemoattractants and death signals. As noted above, however, the key similarities or differences relating to myofibroblasts of different lineages are those functional attributes that impact on fibrogenesis. Differences in the origin of the myofibroblast population may, for example, be irrelevant for a therapy based on regulating collagen expression or breakdown. In this context, the plasticity of resident myofibroblasts with respect to collagen-expressing phenotype may be more important than the lineage origin of the cell. Further research in many of these areas should aid our efforts to unravel further the apparent complexity of the fibrogenic cell populations within the liver.

**RESOLUTION OF LIVER FIBROSIS**

Resolution of hepatic fibrosis has attracted more and more interest since it has been demonstrated in human and animal models that liver fibrosis can be considered a potentially bidirectional process and can be reversible, although it is important to stress that current evidence indicates that cirrhosis is not completely reversible.

A key study [118] described a group of patients who underwent surgical decompression of an obstructed biliary system. In some patients, liver fibrosis significantly regressed after decompression, implying that fibrosis caused by biliary obstruction is reversible in some instances. An important feature of this study was that 19 of the 22 liver biopsies were large wedge specimens taken from the third segment of the left hepatic lobe under direct vision at the time of surgery, thereby negating concerns regarding sampling variation. Fibrosis regression has also been documented in patients with hepatitis B [119], hepatitis C [120], delta hepatitis [121] and immunosuppressive therapy for autoimmune hepatitis [122]. Chronic HCV infection is the most extensively studied condition, and therapy (IFN-α plus ribavirin) with viral clearance results in fibrosis improvement. Importantly, nearly half of patients with cirrhosis exhibit reversal of fibrosis to a significant degree. Therefore studies that used pathological specimens and paired biopsies from trials of antiviral treatments in chronic hepatitis have shown that matrix degradation occurs in advanced human cirrhosis. Nevertheless, incontrovertible evidence for complete spontaneous reversal of advanced cirrhosis does not exist. Moreover, experimental models in rodents indicate that aspects of fibrosis in advanced cirrhosis are truly irreversible (see below).

As well as supporting the findings of fibrosis reversibility in humans, rodent models in which spontaneous recovery from liver fibrosis and cirrhosis occur have allowed the in-depth analysis that is needed to identify the critical features of this process [34,123,124]. The mechanisms regulating HSC survival and apoptosis and the consequences of stellate cell apoptosis in the resolution of liver fibrosis are summarized diagrammatically in Figure 3. HSCs, other liver myofibroblasts and inflammatory cells involved in the fibrotic process, including macrophages and Kupffer cells, secrete a repertoire of matrix-degrading MMPs [125]. These enzymes degrade collagen and other matrix molecules, and their presence in the fibrotic liver highlights the potential dynamic nature of scarring within the liver. Molecular studies of the expression of mRNA for these enzymes (including those with collagenolytic activity) have demonstrated that they are expressed in the liver even in cirrhosis, but their activity is limited by powerful inhibitors, TIMP-1 and TIMP-2 [126]. In recovery, expression of TIMP-1 and TIMP-2 decreases rapidly while matrix-degrading MMPs continue to be expressed, resulting in increased collagenase activity and consequent matrix degradation within the liver.

A further key contemporaneous process in the resolution of hepatic fibrosis is apoptosis of HSCs [34,123]. During iterative liver injury, when HSCs are activated in the normal wound-healing response, HSC apoptosis is forestalled, probably through signals from soluble factors and changes in the surrounding matrix. When the injurious stimulus is withdrawn and remodelling of matrix is required, the loss of these survival factors causes the activated HSCs to undergo apoptosis, which facilitates the remodelling process by removing a major cellular source of collagen and TIMP. Manipulating matrix degradation or enhancing HSC apoptosis might be expected to reduce fibrosis and promote a return to normal liver architecture and function. In addition to the pivotal role of HSC apoptosis in fibrosis resolution, various studies have examined the importance of the composition
Summary of the processes mediating recovery from liver fibrosis and the mechanisms regulating HSC survival and apoptosis

For example, inability to degrade collagen-I results in failure of recovery from CCl₄-induced liver fibrosis, with impairment of HSC apoptosis and diminished hepatocyte regeneration [46]. Furthermore, the quality, as well as the quantity, of the extracellular matrix within the liver has a large bearing on the efficiency of scar repair. When micronodular cirrhosis is induced in rats following 12 weeks of CCl₄ intoxication and the rats are then allowed to recover for 1 year, their livers remodel to a macronodular cirrhosis, demonstrating that only a degree of recovery from comparatively advanced cirrhosis is possible. Resolution was characterized by apoptosis of HSCs, predominantly at the margins of fibrotic septa. Residual septa, which had not remodelled after 1 year, were characterized by tissue-transglutaminase-mediated cross-linking and relative hypocellularity. Therefore incomplete resolution may be a result of qualitative changes in hepatic matrix, such as tissue-transglutaminase-mediated matrix cross-linking, as well as failure of HSC apoptosis [124].

A potential therapeutic avenue in the promotion of fibrosis resolution would be to selectively deplete HSCs. To this end, a number of studies have investigated agents which induce HSC apoptosis. For example, addition of gliotoxin to activated (α-SMA-positive) rat and human HSCs results in morphological alterations typical of apoptosis and caspase 3 activation [127]. Apoptosis was widespread as judged by FACS analysis and TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling) staining in both rat and human HSCs at concentrations that had no effect on the viability of rat hepatocytes. In vivo, gliotoxin treatment significantly reduced the number of activated HSCs and mean thickness of bridging fibrotic septae in livers from rats treated with CCl₄. Furthermore, drugs that selectively target the inhibitor of κB kinase may have potential as antifibrotics. The inhibitor of κB kinase suppressor, sulphasalazine, induced apoptosis of activated rat and human HSCs. In vivo, a single administration of sulphasalazine promoted accelerated recovery from fibrosis, as assessed by improved fibrosis score, selective clearance of α-SMA-positive myofibroblasts, reduced hepatic procollagen-I and TIMP-1 mRNA expression, and increased MMP-2 activity [128].

Although loss of the activated HSCs/myofibroblasts from the fibrotic areas of the liver through apoptosis has been defined, there is little evidence for a role for the inflammatory cells described above in matrix remodelling. The sole exception for this is the macrophage. Identified as the major source of the rodent collagenase MMP-13 in murine models of fibrosis, macrophage depletion at the onset of spontaneous recovery is clearly associated with a failure of fibrosis resolution [4].

CONCLUSIONS

The component parts of hepatic inflammation are exquisitely complex; however, significant progress has been made in unravelling inflammation both in terms of how individual inflammatory cell populations contribute to the liver wounding response and how different inflammatory cell types cross-talk in the evolution and
resolution of scarring. Adding further complexity to the process of hepatic fibrogenesis is the discovery that myo-fibroblasts may arise from an increasingly heterogeneous population within the liver or indeed be recruited from the BM.

Liver fibrosis and cirrhosis represents a huge health care burden worldwide. Liver transplantation is the only effective therapy for end-stage liver disease, but it is available to only a very small minority of the people suffering from liver failure globally. Increasing our understanding of how inflammation interdigitates with the key cell types involved in matrix deposition within the liver will provide important data upon which the rational design of novel antifibrotic therapies can be based.

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