Liver fibrosis: a bidirectional model of fibrogenesis and resolution

P. RAMACHANDRAN and J.P. IREDALE

From the University of Edinburgh/MRC Centre for Inflammation Research, The Queen’s Medical Research Institute, 47 Little France Crescent, Edinburgh EH16 4TJ, UK

Address correspondence to J.P. Iredale, University of Edinburgh/MRC Centre for Inflammation Research, The Queen’s Medical Research Institute, 47 Little France Crescent, Edinburgh EH16 4TJ, UK. email: john.iredale@ed.ac.uk

Summary

Liver fibrosis is the generic response to chronic injury of varying aetiologies. A number of common mechanisms link this response to the pathogenesis of fibrosis in other organs. While long thought to be relentlessly progressive, there is now excellent evidence in both human liver disease and animal models that hepatic fibrosis is potentially reversible. The liver therefore provides an excellent bidirectional model for the study of fibrogenesis and fibrosis resolution. In this article, we will review the evidence for the reversibility of liver fibrosis.

Liver fibrosis is the generic response to chronic injury of varying aetiologies, leading to cirrhosis, with clinical complications including liver failure, portal hypertension and hepatocellular carcinoma. Currently, no direct anti-fibrotic therapies exist with organ transplantation, the only curative option.

A number of common mechanisms link the pathogenesis of hepatic fibrosis and fibrosis seen in other tissues. Tissue fibrogenesis was long thought to be relentlessly progressive. However, emerging data indicates that even in advanced disease, fibrosis is potentially reversible. This evidence is best developed in human and experimental liver disease, establishing the liver as the paradigm for studying bidirectional fibrosis in a solid organ. Given that in clinical practice the majority of patients present with established tissue fibrosis, a greater understanding of the biology of fibrosis resolution is likely to inform novel treatment options. In this short review we will discuss the current knowledge of mechanisms mediating the reversal of hepatic fibrosis and highlight potential avenues for therapeutic translation.

Human liver fibrosis is potentially reversible

Anecdotal reports had suggested that human liver fibrosis was potentially reversible. However, it was only following the development of effective
treatments for chronic hepatitis B and hepatitis C infection that this was definitively shown in large cohorts of patients. Subsequent studies in chronic liver disease caused by alcohol, autoimmune disease, biliary obstruction, hereditary haemochromatosis and non-alcoholic fatty liver disease (NAFLD) confirmed these findings. Additionally, the wide range of aetiologies in which fibrosis resolution occurs suggests that generic rather than disease-specific mechanisms are at play.

Apoptosis of hepatic myofibroblasts accompanies the reversal of fibrosis

To study the mechanisms leading to the reversal of hepatic fibrosis, two tractable rodent models are widely utilized. In bile-duct ligation (BDL) followed by bilio-jejunal anastamosis and chronic carbon tetrachloride (CCl4) administration followed by cessation of dosing, a well-established hepatic fibrosis can resolve to near normal liver architecture within 4–6 weeks. Analysis of the histological changes during fibrosis resolution in both of these models identifies a rapid loss of activated hepatic myofibroblasts, the principal scar-producing cells in the fibrotic liver, by apoptosis.

A number of soluble signals, which may be released by neighbouring inflammatory cells or hepatocytes, have been shown to regulate hepatic myofibroblast apoptosis. Interestingly, the physical characteristics of the myofibroblast environment has important effects on cell survival. Specifically, contact between activated myofibroblasts and collagen 1 (the principal fibrillar collagen in hepatic scars) promotes cell survival and fibrogenic activity. Furthermore, transgenic mice expressing a non-degradable form of collagen 1, demonstrated a failure to spontaneously remodel hepatic scars and a persistence of activated myofibroblasts, the principal scar-producing cells in the fibrotic liver, by apoptosis.

TIMP-1 regulates matrix degradation during fibrogenesis and resolution

Clearly the loss of scar-producing myofibroblasts is not sufficient for adequate fibrosis resolution, and degradation of the extracellular matrix (ECM) is a prerequisite. Matrix metalloproteinases (MMPs), a group of endopeptidases, are capable of degrading a range of ECM components. Analysis of human and experimental animal fibrotic liver demonstrates an increase in a number of MMPs with a wide spectrum of activity. Therefore, even in fibrotic liver there remains the capacity for matrix degradation.

Tissue inhibitors of metalloproteinases (TIMPs), a family of protease inhibitors, are potent inhibitors of MMP activity in vivo. Hepatic myofibroblasts show a marked upregulation of TIMP-1 during activation, preceding collagen expression, and potently inhibiting MMP activity. In addition, elevated levels of TIMP-1 are seen during progressive fibrosis in humans and experimental models. Additionally, TIMP-1 has anti-apoptotic effects on hepatic myofibroblasts, indicating that loss of TIMP-1 during recovery may also contribute to a reduction in the scar-producing cells in the liver (Figure 1). Overall, these data indicate that TIMP production and consequent MMP inhibition is a key regulator in the progression and resolution of hepatic fibrosis.

Macrophages are critical for fibrogenesis and resolution

Cells of the monocyte/macrophage lineage are central proponents of the innate immune response following tissue damage. A number of experimental studies implicate macrophages in promoting hepatic inflammation and fibrosis. Notably, macrophages are located in close proximity to activated hepatic myofibroblasts during fibrogenesis and may produce factors such as TGF-β, IL-1β, PDGF and CCL2 which can enhance the pro-fibrogenic nature of the myofibroblasts by promoting activation, proliferation, chemotaxis and survival. Functional studies using CCL2 (the principal chemoattractant for the recruitment of inflammatory monocytes) inhibitors or CCL2 knockout animals demonstrated reduced hepatic macrophage infiltration, reduced inflammation and reduced fibrogenesis following chronic injury. In seminal work, selective depletion of macrophages during fibrogenesis, using a transgenic CD11B-DTR system, resulted in reduced fibrosis, confirming the importance of macrophages in scar formation. Further elegant work identified the specific Gr-1 subset of hepatic macrophages, derived from recruitment of inflammatory

814 P. Ramachandran and J.P. Iredale
monocytes via CCL2/CCR2 interactions, as responsible for the pro-fibrogenic effects (Figure 1). Emerging evidence also implicates macrophages as central mediators of fibrosis resolution. Intriguingly, macrophage depletion during the resolution phase following chronic CCl₄ administration caused a failure to degrade the hepatic scar, the opposite effect to that seen with depletion during fibrogenesis. Similarly, CCR2 knockout mice subjected to chronic injury with CCl₄ had diminished macrophage recruitment and fibrogenesis, but also a delayed ability to resolve fibrosis. The mechanisms underpinning macrophage-mediated fibrosis resolution are likely to be multifactorial. Macrophages can produce a range of MMPs and macrophage-derived MMP-13, the major rodent collagenase, has been shown to be important for degrading the hepatic scar. Macrophages are also capable of producing molecules such as TRAIL and MMP-9 which can promote myofibroblast apoptosis, although in vivo studies demonstrating this as a relevant mechanism are still lacking (Figure 1).

How can one cell type have such divergent functional effects? While this question remains incompletely answered, it is probable that heterogeneity in macrophage populations will be critical. It is well recognized that macrophages can adopt distinct functional characteristics depending on the stimuli to which they are exposed. It is likely that a specific macrophage phenotype will predominate during fibrosis resolution, and this will be distinct from the phenotype which promotes fibrogenesis. Studies aimed at characterizing the macrophage population responsible for fibrosis reversal will yield novel mechanistic insights. Specifically, determining if the same macrophage population switches from a pro-fibrotic to pro-resolution phenotype
in situ and identifying the factors mediating this switch may enable the development of novel therapies designed to promote this change in vivo and thus induce fibrosis resolution.

**What renders a fibrosis irreversible?**

Having discussed the factors mediating the resolution of hepatic fibrosis, it is also important to consider what might render fibrosis irreversible. In a rat CCl₄ model, an increasing duration of injury induced hepatic fibrosis which was resistant to full resolution, even with protracted recovery times of up to 1 year. Careful analysis of the non-degraded scar tissue indicated that matrix cross-linking was a key feature, making the fibrotic bands more resistant to proteolytic degradation. Additionally, the presence of elastin fibres in the more mature scars suggests a change in the biochemical composition of the fibrotic bands may be relevant. Another striking feature of the persistent scars was the relative hypocellularity. This raises the intriguing possibility that a degree of ongoing inflammation and thus the influx of macrophages around the fibrotic bands is essential for adequate scar remodelling.

**Potential for therapy**

Clearly, using the liver as a model organ, our knowledge regarding the mechanisms orchestrating scar resolution has improved greatly. But are we any closer to the ‘holy grail’, a therapy which can reverse established fibrosis and improve organ function? A number of strategies have been employed in experimental fibrosis, variously targeting myofibroblast activation, myofibroblast apoptosis and the critical MMP–TIMP balance. While no large-scale clinical studies have yet been conducted, promising results are seen in the experimental models. Interestingly, drugs such as sulphasalazine, angiotensin receptor antagonists and PPAR-γ agonists, already widely used in clinical practice, show some early promise as anti-fibrotic agents and may speed up the route to translation.

An alternative strategy is the use of cell therapy to treat hepatic fibrosis, which has shown some beneficial effects. Specifically, the use of macrophages as a therapy has the potential to harness the pro-resolution capabilities of this cell type and can be anti-fibrotic in vivo. However, given the plasticity of macrophages, caution should be used with this approach, as macrophages delivered at an inappropriate time in the disease course have the potential to exacerbate inflammation and fibrosis. Thus, treatments aimed at inducing the pro-resolution macrophage phenotype in vivo may represent a useful methodology in the future.

**Conclusions**

In using the liver as a paradigm for studying fibrosis resolution, we have made significant advances in our understanding of the key cellular and molecular mechanisms at play. However, further work is needed to determine the specific phenotype of the cells involved, which of these mechanisms are relevant in other fibrotic organs and to develop methods to enhance this process with a therapeutic benefit.

**Funding**

The Wellcome Trust (to P.R.); the Medical Research Council (to J.P.I.).

**Conflict of interest:** None declared.

**References**


