Editorial Review

Pathogenesis of liver fibrosis

R. ALCOLADO, M. J. P. ARTHUR and J. P. IREDALE

University Medicine, University of Southampton, U.K.

1. Liver fibrosis is a common sequel to diverse liver injuries. It is characterized by an accumulation of interstitial collagens and other matrix components. The hepatic stellate cell is pivotal to the pathogenic process. Fibrotic liver injury results in activation of the hepatic stellate cell which undergoes a phenotypic change to a proliferative myofibroblast-like cell which synthesizes excess interstitial collagens and other matrix components.

2. The process of initiation of activation and its perpetuation result from complex, often interrelated series of signalling mechanisms which converge on this effector cell. Such mechanisms include alterations in matrix resulting in changed cell–matrix interactions and stimulation by cytokines released from damaged hepatocytes, infiltrating inflammatory cells, Kupffer cells and matrix. Foremost among the profibrotic cytokines is transforming growth factor-β1.

3. Once the hepatic stellate cell is activated the preceding matrix changes and recurrent injurious stimuli will perpetuate the activated state.

4. Despite the accumulation of excess collagens, the liver retains a capacity for matrix degradation. This capacity may be overwhelmed and any secreted matrix remodelling enzymes may be inhibited by the concurrently secreted tissue inhibitors of metalloproteinase-1 and α2-macroglobulin.

5. Our understanding of the molecular pathogenesis of liver fibrosis is increasing. It is anticipated that this knowledge will provide novel therapeutic avenues to treat this disease process.

INTRODUCTION

Liver fibrosis and cirrhosis represent the final common pathological pathway for a variety of liver diseases, irrespective of the underlying aetiology. In the fibrotic liver there is not only an increase in the total content of collagens and other matrix proteins compared with normal, but there are also qualitative changes in the nature of the matrix components and their distribution within the liver. Recent research has highlighted several key areas of the pathological process which has enhanced our understanding not only of liver fibrosis, but of the fibrotic process in other solid organs.

The key cellular and molecular events involved in the pathogenesis of liver fibrosis include activation of hepatic stellate cells (stellate cells, lipocytes, fat-storing cells or Ito cells) to a myofibroblast-like phenotype, their production of excess matrix proteins and their ability to regulate matrix degradation. The mechanisms controlling stellate cell activation and the factors regulating the synthesis and degradation of matrix proteins have thus become the principal focus of research activity in liver fibrosis. This work encompasses analysis of interactions between stellate cells and both parenchymal cells (hepatocytes) and non-parenchymal cells (Kupffer cells and endothelial cells) as well as examining the cell–matrix interactions that determine stellate cell and hepatocyte phenotype and behaviour, and the role of soluble cell messengers (cytokines, growth factors and other soluble messengers) released by inflammatory cells and resident liver cells.

The purpose of this article is to review the current state of knowledge of this complex pathological process and to describe the principal events that occur as the previously ordered liver microanatomy progresses to the distorted architecture that characterizes fibrosis.

MICROANATOMY, STRUCTURE AND CELLS OF THE HEPATIC SINUSOID

The functional unit of the liver is the acinus which consists of a central portal tract containing an efferent biliary radical and terminal branches of the portal and arterial blood supplies. The blood supplies feed into the hepatic sinusoid, which are bounded by plates of hepatocytes, and finally drain...
into the efferent central vein. Hepatocytes make up over 80% of the volume of the human liver and are the site of almost all liver synthetic and degradative functions. Normal hepatocytes are cuboidal in shape and are arranged in palisades along the sinusoid, in close contact with sinusoidal blood.

The vascular sinusoid is lined by a highly specialized fenestrated endothelium and contains within its lumen Kupffer cells and pin cells. Kupffer cells are the resident tissue macrophages found predominantly in the periportal areas of the sinusoid and are responsible for removing bacterial antigens and endotoxin from the portal blood. Endothelial cells in the liver are highly specialized, possessing many fenestrae which allow free passage of macromolecules from sinusoidal blood into the space of Disse, thus permitting contact with hepatocytes. Pin cells bear a strong resemblance to the natural killer cells in the spleen which play a major role in clearing opsonized particles and tumour cells from the blood.

Between the sinusoidal endothelium and the brush-bordered sinusoidal surface of the hepatocyte lies the space of Disse. This contains a specialized matrix with the same principal matrix components as a basement membrane (type IV collagen, laminin, proteoglycans and the stellate cells) (Fig. 1).

Stellate cells, the major focus of this review, are located in the space of Disse and are believed to be the liver equivalent of pericytes, with many cytoplasmic processes encircling the sinusoid. They contain many lipid-rich droplets which store over 75% of the body's store of retinoids, largely as retinyl esters. During liver injury stellate cells undergo activation to a myofibroblast-like phenotype, proliferate and express the matrix proteins which characterize fibrosis. The pathogenic role of stellate cell activation and its relationship to liver matrix and soluble cell messengers is considered in detail below.

**LIVER MATRIX IN NORMAL LIVER**

In normal liver, matrix proteins are distributed throughout the organ, but two principal types of matrix may be identified. Fibrillar or interstitial matrix proteins (collagens I, III and V) are located in the liver capsule, large and medium-sized blood vessels and the portal tracts. This contrasts with the basement membrane-like matrix located in the functionally important sinusoidal space of Disse. The latter matrix is not electron dense but is comprised of type IV collagen, laminin and proteoglycans as determined by immunochemistry and electron microscopy studies [1–5].

Of the many collagen subtypes described to date only five have been detected in liver; they are types I, III, IV, V and VI. The fibril-forming collagens make up the majority of collagen in normal liver [6] and are found predominantly in the perivascular spaces, around portal tracts, and subcapsular areas. Type I and III collagens form hybrid fibrils [7] condensing with type V fibres which forms the fibril core.

Type IV collagen is located in the space of Disse [8], together with type VI collagen [9], as well as in vascular and bile duct basement membranes. Type IV collagen together with laminin and proteoglycans forms a flexible lattice structure [10, 11] while type VI collagen monomers interact with each other to form chains of molecules within the space of Disse [12].

Periductal and perivascular areas are rich in fibronectin and vitronectin. The basement membrane-like matrix in the space of Disse contains fibronectin [2, 8, 13] and laminin in the same distribution as type IV collagen. Undulin is found in small amounts in association with the fibril-forming collagens [14].

Heparan sulphate, the commonest proteoglycan in normal liver [13], is found predominantly at the portal end of the sinusoid, with heparin proteoglycan at the venous end [15]. Dermatan and chondroitin sulphate are found in very small amounts in normal liver [15, 16].

**MATRIX CHANGES IN DISEASED LIVER**

The early response to liver injury is characterized by the activation of stellate cells. This event is common to a wide range of liver insults. A major consequence of stellate cell activation to a myofibroblast-like phenotype is that the synthetic activity of these cells alters and they secrete excess collagen I and III in addition to other matrix molecules. These matrix proteins are laid down in the space of Disse, initially causing an alteration in the appearance of the sinusoid. With further progression of fibrosis there are alterations in the phenotype of the endothelial cells which lose their fenestrae, so-called capillarization of the sinusoid, and perturbation of hepatocyte function (Fig. 1).

Further progression of the fibrotic process is
associated with a gross distortion of the sinusoidal architecture as the vascular structures at either end become linked by dense connective tissue. Connective tissue septae can also be visualized extending into the parenchyma. The final result of this process is the end-stage cirrhotic liver in which the whole anatomy is macroscopically distorted by thick bands of collagen, within which nodules of hepatocytes, often with regenerative foci, are surrounded.

Cirrhotic liver contains approximately six times the amount of matrix as normal liver [6]; there is an increase in the amounts of types I, III [8] and IV [1] collagen in the Space of Disse, but for type I collagen this increase is disproportionate so that the ratio of type I [17, 18] to types III and IV increases. There is an increase in laminin [6] within the space of Disse and alterations in both the total quantity and the type of fibronectin produced [6]. The spectrum of glycoproteins expressed changes with a relative decrease in heparin and heparan sulphate proteoglycans and an increase in dermatan and chondroitin sulphate proteoglycans. The latter contain the core proteins decorin and biglycan which have a potentially important role in cytokine binding within the extracellular matrix and are discussed in more detail below. Hyaluronan, which is only present in small quantities in normal liver, is increased more than 8-fold in diseased liver and is secreted by hepatic stellate cells [19].

**CELLULAR SOURCES OF MATRIX**

Early studies of matrix secretion by isolated cells indicated that hepatocytes were a significant source of matrix components. More recently, access to rigorously characterized cell populations has cast doubt on this finding, suggesting that it may have resulted from overgrowth of contaminating stellate cells, and has given a clearer picture of the role of individual cell types in matrix synthesis. These findings have been confirmed by studies using in situ hybridization or analysis of cell fractions extracted from normal and injured liver to confirm the origin of matrix components.

Hepatocytes contain the mRNAs for collagens I [20] and IV although not all workers [21] have been able to reproduce these results. Cultured hepatocytes produce plasma fibronectin [22] and the proteoglycan heparan sulphate [4].

Current studies indicate that stellate cells are the major source of matrix proteins in the diseased liver [21–23]. They produce a broad range of matrix components – mRNA for collagen type I, III and IV [24–26] has been isolated along with mRNAs for laminin [5, 25], fibronectin [22, 24], heparan sulphate [4] and dermatan and chondroitin sulphate [4, 27] proteoglycan and the collagen synthetic enzyme, prolyl hydroxylase.

mRNA transcripts for type IV collagen [21, 28] and fibronectin [29] have been identified in endothelial cells, while Kupffer cells and Pit cells do not appear to elaborate any matrix components on the basis of present data. Doubt has been cast on earlier data suggesting that hepatocytes are a major source of matrix after it was demonstrated that hepatocyte cultures are often contaminated with stellate cells [30].

**BIOLOGICAL EFFECT OF MATRIX CHANGES IN FIBROSIS AND THEIR RELATIONSHIP TO STELLATE CELL ACTIVATION**

In addition to the described architectural changes, the shift in matrix constitution to one rich in collagen I and III is associated with specific changes in the phenotype of parenchymal and non-parenchymal cells.

The evidence for a biological activity of matrix lies in a series of elegant experiments which have demonstrated that hepatocytes retain a differentiated phenotype in which they continue to express albumin and cytochrome P-450, when cultured on a model basement membrane. This biological effect of the whole-model matrix is greater than each of its constituent parts, when these are used as individual culture substrata. In contrast, culture of hepatocytes on type I collagen or tissue culture plastic results in a flattening and spreading of the cells, in association with which, tissue-specific gene expression is lost [31, 32]. Interestingly, more simple matrices which result in cultured hepatocytes retaining their three-dimensional shape, including a gelatin sandwich, have been demonstrated to maintain gene-specific function, suggesting that the maintenance of normal shape is important for the persistence of a differentiated phenotype [33].

The implications of these studies are that hepatocyte function may be disrupted comparatively early in the progression of liver fibrosis and before gross changes occur, by virtue of altered cell–matrix interactions. In addition, the findings are consistent with the hypothesis that hepatocellular dysfunction may remain a feature of liver injury even after withdrawal of the injurious agent in the absence of adequate matrix remodelling.

Evidence also exists for important cell–matrix interactions involving endothelial cells [16] and, pertinent to this article, stellate cells. Of importance for both the initiation and progression of fibrosis, stellate cells respond to a change in substratum by undergoing a phenotypic change [34]. Stellate cells in normal liver lie in the space of Disse. They are rich in retinoids and fat droplets and can be readily activated to a myofibroblast-like phenotype [35]. They shed their retinoids after hydrolysing the esters and start to express α-smooth muscle actin [36–39]. This phenotypic change is central to the pathogenesis of fibrosis because it is in this activated phenotype that stellate cells express and secrete excess
matrix proteins during fibrosis and downregulate their subsequent degradation.

The activation of stellate cells is a general response to injury and can be demonstrated to be temporally related to an insult (after CCl₄ intoxication, stellate cells can be demonstrated to pass through stages of activation, so-called transitional cells). However, activation is also related to the site of an injury; thus after CCl₄ intoxication, stellate cell activation will occur in association with hepatocyte degeneration pericentrally, while bile duct ligation is associated with perportal stellate cell activation. In the presence of recurrent or progressive injury, stellate cell activation becomes more generalized [21, 37, 38, 40–42].

By culture on a model basement-like matrix, stellate cells can be maintained in a normal quiescent, retinoid-rich phenotype. When type I collagen or uncoated tissue culture plastic is substituted the stellate cells rapidly undergo a phenotypic transformation to activated cells in a manner that is very similar to the process in vivo [25, 34, 43]. These findings have led to the hypothesis that alterations in the matrix substratum, specifically the degradation of the normal basement membrane-like matrix and its replacement with a matrix rich in interstitial collagens, may be important in both the activation and perpetuation of activation of stellate cells. Furthermore, the demonstration that activation can be mimicked in tissue culture, by plating primary cultures of stellate cells onto plastic, has greatly facilitated the study of stellate cell expression and synthesis of matrix proteins and matrix remodelling enzymes.

Another important matrix change which occurs early in the course of experimental liver injury and is important for stellate cell activation is the production, by endothelial cells, of fibronectin spliced to contain the EIII-A variable region [29]. This isofrom is virtually absent from normal liver. The peak in EIII-A fibronectin production occurs within 12 h of experimental bile duct ligation and remains elevated for 7 days. Stellate cells cultured with endothelial cells isolated from bile duct-ligated livers become activated more rapidly than equivalent controls. This action of the endothelial cell can be blocked by antibody directed against the EIII-A region of fibronectin [29], suggesting that the endothelial cell response to injury may directly activate stellate cells via changes in matrix environment.

The mechanisms of cell–matrix interactions involving stellate cells, hepatocytes and endothelial cells are likely to be mediated via integrin and non-integrin receptors. Integrins are transmembrane heterodimeric proteins which mediate cell adhesion and cell signalling and may be important in the secretion of ordered matrix by synthetic cells [44, 45]. Normal basement membrane constituents contain well-characterized integrin recognition sequences. The repeated Arg–Gly–Asp motif which occurs in the triple helical region of the type I [46] and type IV [47] collagen molecule is recognized by the α₁β₁ integrin receptor which occurs on hepatocytes [47]. Fibronectin [48] bears a number of integrin recognition sequences for the α₁β₁ [49] (Arg–Gly–Asp) and α₁β₁ [50] (Leu–Asp–Val, or Arg–Glu–Asp–Val) receptors. It also contains a number of recognition sequences for other matrix components as well as for the transmembrane proteoglycan syndecan. Laminin [49] has eight cell adhesion or recognition sequences, many of which recognize other matrix components, especially fibrillar collagens, and are thought to be responsible for the arrangement of collagen into fibrils. Laminin also contains epidermal growth factor-like peptide repeats which, when hydrolysed, are capable of stimulating cellular epidermal growth factor receptors [51]. Proteoglycans interact with other matrix components via non-integrin receptors but their major interaction with cells may be mediated via their ability to bind cytokines. Heparan sulphate proteoglycan, found in normal liver, is capable of binding β-fibroblast growth factor [52], which prevents its rapid degradation by membrane-bound plasmin and enhances binding to specific β-fibroblast growth factor receptors. Decorin and biglycan, the protein core of chondroitin and dermatan sulphates, are capable of binding TGF-β1 reversibly [53].

**OTHER FACTORS INFLUENCING STELLATE CELL ACTIVATION**

In addition to alterations in the matrix substratum, there is evidence that stellate cell activation is influenced by a wide variety of cytokines and cell messengers. Kupffer cells are probably central to the response of the liver to many injuries. Indeed, experimental liver injury can be ameliorated by prior depletion of Kupffer cells. Foremost among the bioactive substances released by Kupffer cells is the cytokine TGF-β1 (Fig. 2). Extensive studies of this cytokine elsewhere in the body indicate that its expression is fundamental to the scarring response. Transforming growth factor β, so-called because of its ability to transform a non-proliferating fibroblast cell line in soft agar culture [54], inhibits the proliferation of rat stellate cells [55] but promotes the platelet-derived growth factor (PDGF)-mediated proliferation of human stellate cells [56]. TGF-β₁ is one of a family of related peptides, TGF-β₁–TGF-β₃, of which only TGF-β₁, β₂ and β₃ have been detected in liver [57].

In disease models including CCl₄ intoxication, bile duct ligation, iron and alcohol intoxication, streptococcal cell wall administration and murine schistosomiasis [58–62], an increase in the level of mRNA for TGF-β₁ has been documented. Fibrotic liver from patients with a variety of chronic liver diseases has been shown to contain increased levels of TGF-β₁ mRNA [63]. In chronic liver injury TGF-β₁
is produced by a number of non-parenchymal cells [64]. TGF-β1 may be derived from activated stellate cells [65], Kupffer cells [55] and endothelial cells [60]. In addition, TGF-β1 may be produced by migrating mononuclear cells [58].

TGF-β1 has profound effects on matrix production in a number of well-characterized cell lines [66, 67] including fibroblasts, osteoblasts and, to a lesser extent, myofibroblastic stellate cells [24, 68]. TGF-β1 not only causes an increase in the production of type I collagen but also in types III and IV [24]. In addition, fibronectin [69, 70] and proteoglycan [55, 71] synthesis is enhanced. In primary stellate cell culture, addition of TGF-β1 24 h after plating results in an accelerated transformation to the activated phenotype [69].

TGF-β1 also alters the balance of matrix turnover against matrix degradation and in favour of matrix accumulation, by inhibiting production of interstitial collagenase [72, 73], stromelysin and plasminogen activators, and promoting the production of the potent collagenase inhibitor, tissue inhibitor of metalloproteinase-1 (TIMP-1) [72] and plasminogen activator inhibitor-1 [74]. Fibroblasts will also respond to TGF-β1 by upregulating gelatinase A [73]. However, this has not been demonstrated in stellate cells [75]. A further significant effect of TGF-β1 on stellate cells is the stimulation of a positive autocrine loop [24, 65]. TGF-β1 increases the facility for stellate cell-matrix interactions by increased transcription of mRNA for the α and β subunits of integrin receptors and increased assembly of these subunits to form the active receptors [76].

Relative levels of mRNA cannot be directly correlated with active TGF-β1 because the protein is released in a latent form. The mechanism of activation of TGF-β1 in liver is probably via the insulin-like growth factor II/mannose-6-phosphate (IGF-II/M6P) receptor. Moreover, it has been demonstrated that stellate cells extracted from CCl4-treated rats but not normal liver bear the IGF-II/M6P receptor on the cell surface [77].

The balance of activated versus inactivated cytokine must be of central importance as TGF-β1 is detectable in normal liver [77] and almost all normal liver cells exhibit receptors for active TGF-β1, while mRNA levels for receptor types 1, 2 and 3 are all increased in activated stellate cells [78]. Latent TGF-β1 may also exist in a pool within the liver matrix. The type 3 TGF-β1 receptor has long been known to be a non-signalling receptor: it has recently been identified as membrane proteoglycan β-glycan [79, 80]. In addition, the matrix component decorin binds and inactivates TGF-β1. The binding of TGF-β1 to these matrix constituents may both prevent activation and protect the latent form from breakdown. The characteristics of this binding in vivo are not understood, particularly the conditions required for the release of latent TGF-β1 from these receptors. Nonetheless, decorin has been demonstrated to have an anti-fibrotic effect in renal fibrosis in vivo [81]. Moreover, the position of TGF-β1 as a central cytokine to the fibrotic/scarring process has now been demonstrated in a series of mechanistic studies in which fibrosis was reduced by the addition of decorin and neutralizing antibodies to cell culture and in vivo models [81–84]. Perhaps predictably, a transgenic line of TGF-β1 overexpressor animals developed evidence of hepatic and renal fibrosis [85].

Another very important cytokine is PDGF [86] (Fig. 2). This consists of A and B peptide chains. Three forms exist, the homodimeric AA and BB forms or the heterodimer. PDGF is a potent stellate cell mitogen [87] which is produced by endothelial cells [88] and by activated stellate cells [89] in culture. Quiescent stellate cells express the α-PDGF receptor subunit, but do not express the β-subunit until activated. This is observed in both inflammatory liver injury and after bile duct ligation [90]. The β-receptor subunit only interacts with the B chain of PDGF, and the PDGF dimers containing at least one B-chain are significantly more potent to activated stellate cells than the PDGF-AA dimers. Expression of the PDGF β-receptor subunit is enhanced in response to TGF-β1. Furthermore, there is evidence that Kupffer cells may direct stellate cell PDGF receptor expression by means of an as yet uncharacterized soluble factor [91].

PDGF, like TGF-β1, is able to induce transcription of its own mRNA and PDGF BB has been shown to induce transcription of both A and B chains in culture-activated stellate cells [89].

While Kupffer cell-derived factors may be important to the PDGF- and TGF-β1-mediated effects on stellate cells, recent work has suggested that substances released directly from damaged hepatocytes are mitogenic for cultured stellate cells...
Moreover, IGF and IGF-binding protein released from injured hepatocytes may act in a paracrine manner to activate stellate cells locally. Studies of stellate cell IGF-I and II receptors indicate that there is a diminution of quantity (IGF-Ir) and binding affinity (IGF-IIr) with transformation to activated phenotype. This suggests that any IGF-mediated effect is only likely to be important early on in the process of stellate cell activation [93].

Tumour necrosis factor-α is a known mitogen [68, 69] for stellate cells and also has an effect on transcription of mRNA for chondroitin sulphate [24], interstitial collagenase [94, 95] and TIMP-1 [95].

Cytokines and cell messengers released during injury and inflammation may also moderate stellate cell activation, proliferation and synthetic function. The addition of interferon γ to a culture medium of myofibroblastic stellate cells results in reversion to a more quiescent phenotype in which the cell regains its lipid droplets. It also affects collagen production [96] with falls in procollagen I and type IV collagen and significant reduction in the mRNA level for total fibronectin [96].

Addition of retinoids to stellate cells in culture can maintain a quiescent phenotype, and when added to activated stellate cells causes a reversion of phenotypic changes together with a decrease in the rate of cell proliferation [97, 98]. Retinoids also modulate collagen production by culture-activated lipocytes – total collagen production is reduced in a dose-dependent fashion [98]. Further important effects of retinoids on culture-activated stellate cells include the inhibition of both TGF-β1, mRNA production [98] and PDGF-mediated proliferation [99]. However, a paradox exists: although retinoids display this potent cytoprotective role in models of fibrosis, the ingestion of large amounts of retinoids is known to induce liver fibrosis [100].

It is apparent that there are many factors which play a role in the activation of stellate cells to the proliferating fibrogenic phenotype seen in chronic liver disease. There is a complex interaction between each individual cell type, the local matrix milieu and the production and activation of many cytokines, all of which converge on the stellate cell. This is summarized in Fig. 2. Key to the development and progression of fibrosis is the activation and persistence of activation of the stellate cell. The presence and persistence of particular matrix components is probably fundamental to the perpetuation of stellate cell activation and the progression of fibrosis. The role of matrix remodelling is therefore considered below.

**MATRIX REGULATION**

The control of matrix turnover is central to any understanding of the pathogenesis of liver fibrosis, since in profibrotic liver injury either the regulatory mechanisms are overwhelmed by collagen production or the normal response to increasing collagen secretion is altered such that the excessive matrix is not degraded. Accumulating evidence indicates that progressive liver fibrosis is characterized by changes in the pattern of matrix degradation in addition to synthesis.

The turnover and remodelling of matrix by mesenchymal cells is mediated by a family of zinc-dependent neutral proteinases, the matrix metalloproteinases (MMPs) [101]. In addition, during inflammatory liver injury, matrix degradation may be mediated by matrix-degrading cathepsins.

In the analysis of matrix degradation during liver fibrosis two major groups of MMPs are believed to be important. The first, comprising the gelatinases and stromelysins, have activity against type IV collagen and other components of the normal basement membrane-like matrix [101]. These enzymes may therefore significantly alter stellate cell activation through degradation of the normal matrix. There is evidence that members of these groups are expressed by activated Kupffer cells (gelatinase B) [102], stellate cells (gelatinase A and stromelysin) [103–105] and endothelial cells (stromelysin) [106]. Moreover, expression of both stromelysin and gelatinase A has been described in acute liver injury in vivo in addition to studies of non-parenchymal cells in culture [106, 107].

The second group comprises the collagenases [101]. These are derived from two sources: interstitial collagenase, derived from fibroblastic cells including activated myofibroblastic stellate cells and neutrophil collagenase, released from neutrophil phagosomes in areas of inflammation [101]. Because none of the other MMPs can initiate breakdown of interstitial collagen it is axiomatic that interstitial or neutrophil collagenase must be expressed for matrix remodelling to occur in established fibrosis [101]. Studies of cultured stellate cells indicate that these cells express interstitial collagenase in response to specific cytokines and early in models of activation [94, 95, 108]. One group suggested recently that gelatinase A has degradative activity against type I collagen [109]. Moreover, studies of mRNA expression in fibrotic human liver and in animal models of liver fibrosis indicate that transcripts for interstitial collagenase are present and indeed may be upregulated in certain pathologies [95, 108, 110]. These studies have also indicated that fibrosis is accompanied by a significant upregulation in mRNA transcripts of gelatinase A [110].

There is a relatively large literature suggesting that MMP activity in liver decreases as fibrosis progresses [111–113]. This may in part result from the expression of the powerful MMP inhibitors the TIMPs and α2-macroglobulin by stellate cells and hepatocytes [95, 108, 110, 114–118]. Detailed studies of culture-activated stellate cells have demonstrated that these cells express TIMP-1 and 2, and that these inhibitors exert a profound inhibition of
co-expressed gelatinase activity [114]. Expression of mRNA for TIMPs in diseased human liver and animal models of fibrosis indicates that transcripts for both TIMP-1 and 2 are significantly upregulated [108] and, in the case of TIMP-1, are probably stellate cell derived [108].

TIMPs have been immunolocalized in diseased liver and co-localize with cells expressing interstitial collagenase [110]. In addition there are several studies which indicate that serum TIMP levels are elevated in fibrotic liver disease [119], and that TIMP-1 may be expressed by hepatocytes as a feature of the acute phase response [115]. α2-Macroglobulin has also been demonstrated to be expressed and secreted by stellate cells and hepatocytes [120].

Stellate cells may also influence both the degradation of matrix and the activation of pro-MMPs by manipulating the plasmin/plasminogen cascade. Stellate cells express urokinase plasminogen activator (uPA) during activation and through expression of this protein can generate active plasmin from plasminogen [121]. The plasmin in turn cleaves the propiece from pro-stromelysin, thereby also initiating the activation of interstitial collagenase. By simultaneously expressing uPA receptors, stellate cells are able to activate plasmin in a focused pericellular manner [121]. As activation of stellate cells becomes more advanced, expression of uPA decreases and is replaced by the plasminogen activator inhibitor with a resulting decrease in uPA activity [121].

Taken together, these data provide evidence that the potential for matrix degradation exists, even in comparatively advanced fibrosis. However, the process of matrix degradation is either overwhelmed by synthesis, and/or that matrix degradation is inhibited as a result of TIMP and plasminogen activator inhibitor expression.

CONCLUSION

There has been significant progress in our understanding of the cellular and molecular events which characterize the response of the liver to injury and the consequent development of liver fibrosis. Central to the process is the stellate cell which undergoes a phenotypic change to a proliferative myofibroblast-like cell which then synthesizes excess interstitial collagens and other matrix components. The process of initiation of activation and its perpetuation result from a complex, often interrelated, series of signalling mechanisms which converge on this important effector cell. These mechanisms include alterations in matrix resulting in changed cell–matrix interactions and stimulation by cytokines released from damaged hepatocytes, infiltrating inflammatory cells, Kupffer cells and matrix. Once the stellate cell is activated the preceding matrix changes and any recurrent injurious stimuli will perpetuate the activated state. The activated stellate cell is highly synthetic and secretes interstitial collagens which accumulate initially in the space of Disse, but ultimately result in gross liver fibrosis. Despite these effects, the liver retains a capacity/potential for matrix degradation/remodelling. However, this capacity may be overwhelmed and any secreted matrix remodelling enzymes may be inhibited by the concurrently secreted TIMPs and α2-macroglobulin.

Future work in each of the areas highlighted in this review will ultimately provide a detailed dissection of the molecular mechanism of stellate cell activation and the process of matrix secretion and assimilation. Specific targets are likely to include mechanisms to reverse stellate cell activation or inhibit matrix synthetic activity and intervention to promote matrix degradation. This may be accomplished directly, i.e. by enhancing expression of collagenase and other MMPs, or indirectly by reducing the level of MMP inhibitory factors such as TIMPs. Such an approach, based on control of matrix degradation, is attractive because the majority of patients with chronic liver disease will present clinically when fibrosis is already established and may be relatively advanced. Tools to intervene directly in the process of experimental fibrosis (e.g. transgenic and gene knockout mice) are now becoming available, and it is anticipated that these data will highlight suitable avenues for therapeutic intervention.

REFERENCES

3. Abrahamsson DR, Caulfield JP. Distribution of laminin within rat and mouse renal, splenic, intestinal, and hepatic basement membranes identified after the intravenous injection of heterologous antilaminin IgG. Lab Invest 1985; 52: 169.


Pathogenesis of liver fibrosis


