Cellular and molecular mechanisms underlying bone marrow and liver fibrosis: a review

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ABSTRACT. Chronic fibroproliferative diseases are an important cause of morbidity and mortality in the world. Fibrotic diseases occur in a large variety of vital organs, and the process of fibrosis seems common to all tissues. In all of fibrotic reactions, the underlying cellular and molecular mechanisms involve leukocyte infiltration, the persistence of inflammation in the tissue, and the proliferation of cells with a myofibroblast phenotype. The different cell types participating to this process sustain production of growth factors, proteolytic enzymes, angiogenic factors, and fibrogenic cytokines, which together stimulate the deposition of connective tissue elements that progressively destroy and remodel normal tissue architecture. This review focuses on the comparison of two, major, chronic fibroproliferative diseases: the myelofibrosis which develops in bone marrow, a "fluid" tissue producing circulating haematopoietic cells, and liver fibrosis, which demonstrates all the features of solid tissue damage. We discuss the etiology and histological quantification of each type of fibrosis, the implication of cell partners, cytokines and growth factors, animal models developed to study fibrosis, and antifibrotic therapies for each of these two fibroproliferative disease models.

Keywords: fibroblasts, TGF-beta, extracellular matrix (ECM) components

Fibroproliferative diseases are an important cause of morbidity and mortality with nearly 45% of all deaths in the developed world being attributed to chronic forms of the disease. Indeed, fibrotic diseases occur not only in a large variety of vital organs including lung, liver, kidney, intestine, heart, skin and eye, but also in fluid tissues such as bone marrow (BM), with the loss of organ function leading to death. The diseases include pulmonary fibroses, systemic sclerosis, liver cirrhosis, cardiovascular disease, progressive kidney disease, macular degeneration and bone marrow fibrosis also called myelofibrosis (MF). Moreover, the fibrotic tissue remodelling that occurs in organs may be involved in the initiation/genesis of cancer, influence cancer metastasis and may accelerate chronic graft rejection in transplant recipients.

In all fibrotic reactions, the underlying cellular and molecular mechanisms involve: leukocyte infiltration, persistence of inflammation in the tissue, and proliferation of cells with a myofibroblast phenotype. These different cell types sustain the production of growth factors, proteolytic enzymes, angiogenic factors and fibrogenic cytokines, which together stimulate the deposition of connective tissue elements that progressively remodel and destroy normal tissue architecture. Thus, the understanding of mechanisms in which cells and a variety of factors are involved is critical for developing treatment strategies for these diseases.

The number of fibroproliferative diseases is large and varied. However, the goal of this review is to compare two types of fibrosis: liver fibrosis, which bears all hallmarks of tissue damage, and myelofibrosis which develops in the bone marrow, a "fluid" tissue generating circulating haematopoietic cells. After a brief definition of both types, this review will focus on the identification of the different or indeed common mechanisms involved in these two diseases. We shall analyse the etiology, histological quantification,, involvement of cell partners, cytokines and growth factors, animal models developed to study fibrosis, and antifibrotic therapies for each type.

DEFINITION AND AETIOLOGY

Fibrosis is attributed to excess deposition of extracellular matrix (ECM). This is a complex meshwork comprising various types of collagens, laminin, entactin, heparan sulfate and proteoglycans. It is often defined as a wound-healing response that has gone out of control and that does not lead to normal regeneration following tissue injury. Damage to tissues can result from a variety of acute or chronic stimuli, but pathogenic fibrosis typically results from chronic inflammatory reactions. Many fibrotic disorders have an infectious aetiology, with bacteria, viruses, fungi, and multicellular parasites driving chronic inflammation and the development of fibrosis. Moreover, fibrosis

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may also due to toxic agents such as smoke, chemicals, allergens, or alcohol. Finally, some fibroses arise from autoimmune disease.

Bone marrow fibrosis is a reactive process that can be associated with malignant as well as benign pathologies. It is a complication of several hematological neoplasms such as myeloproliferative disorders, myelodysplastic syndromes, lymphomas and lymphoid leukemias (hairy cell leukaemia, leukemic transformation of Hodgkin disease), systemic mastocytosis, lymphoid and myeloid acute leukemias, and is also seen in medullar cancer metastasis; in such cases, fibrosis is associated with significant morbidity and mortality. Myelofibrosis has also been described in association with nonmalignant pathologies such as chronic autoimmune diseases e.g. systemic lupus erythematosus, rachitis due to vitamin D deficiency, infectious syndromes or after exposure to toxins or radiation.

During the last few years, a number of studies performed in human and experimental models have improved our knowledge of the complexity of both normal and fibrotic bone marrow stroma, resulting in a better understanding of the development of bone marrow fibrosis. In this review, we will take the example of primary myelofibrosis (PMF), also called myelofibrosis with myeloid metaplasia, a Philadelphia chromosome-negative chronic myeloproliferative disorder (cMPD) that is characterized by the association of myeloproliferation and myelofibrosis [1, 2], to provide an overview of that which is currently known about the cellular and molecular mechanisms of bone marrow fibrosis. In PMF, the stromal reaction that accompanies clonal hematopoietic stem cell proliferation is characterized by a consistent myelofibrosis associated with osteosclerosis *(figure 1)* and neoangiogenesis. In contrast to the myelofibrosis associated with other MPDs such as chronic myeloid leukemia (CML) or polycythemia vera (PV), fibrosis is present in PMF at diagnosis, although it progresses slowly.

In contrast to bone marrow fibrosis, the causes of liver fibrosis/cirrhosis are better known. Indeed, liver fibrosis/cirrhosis represents the consequences of a sustained wound healing response to chronic liver injury from a variety of causes. The large majority of patients worldwide that have liver fibrosis or cirrhosis has chronic viral hepatitis (due to hepatitis C virus [HCV] or hepatitis B virus [HVB])., The World Health Organization (WHO) estimates that globally about 170 million people are chronically infected with HCV, with 3 to 4 million people being newly infected each year, while 350-400 million people are afflicted with chronic hepatitis B (see: http://www.who.int/en/). The steatohepatitis associated with either alcohol or obesity (non-alcohol steatosic hepatitis (NASH) represents another important cause of liver cirrhosis with an increasing incidence throughout the world. NASH is now estimated by WHO to affect over 50% of the world's morbidly obese population, involving some 60 million people. However, other etiologies, albeit with smaller numbers of patients, include parasitic diseases (*e.g.* schistosomiasis), autoimmune attack on

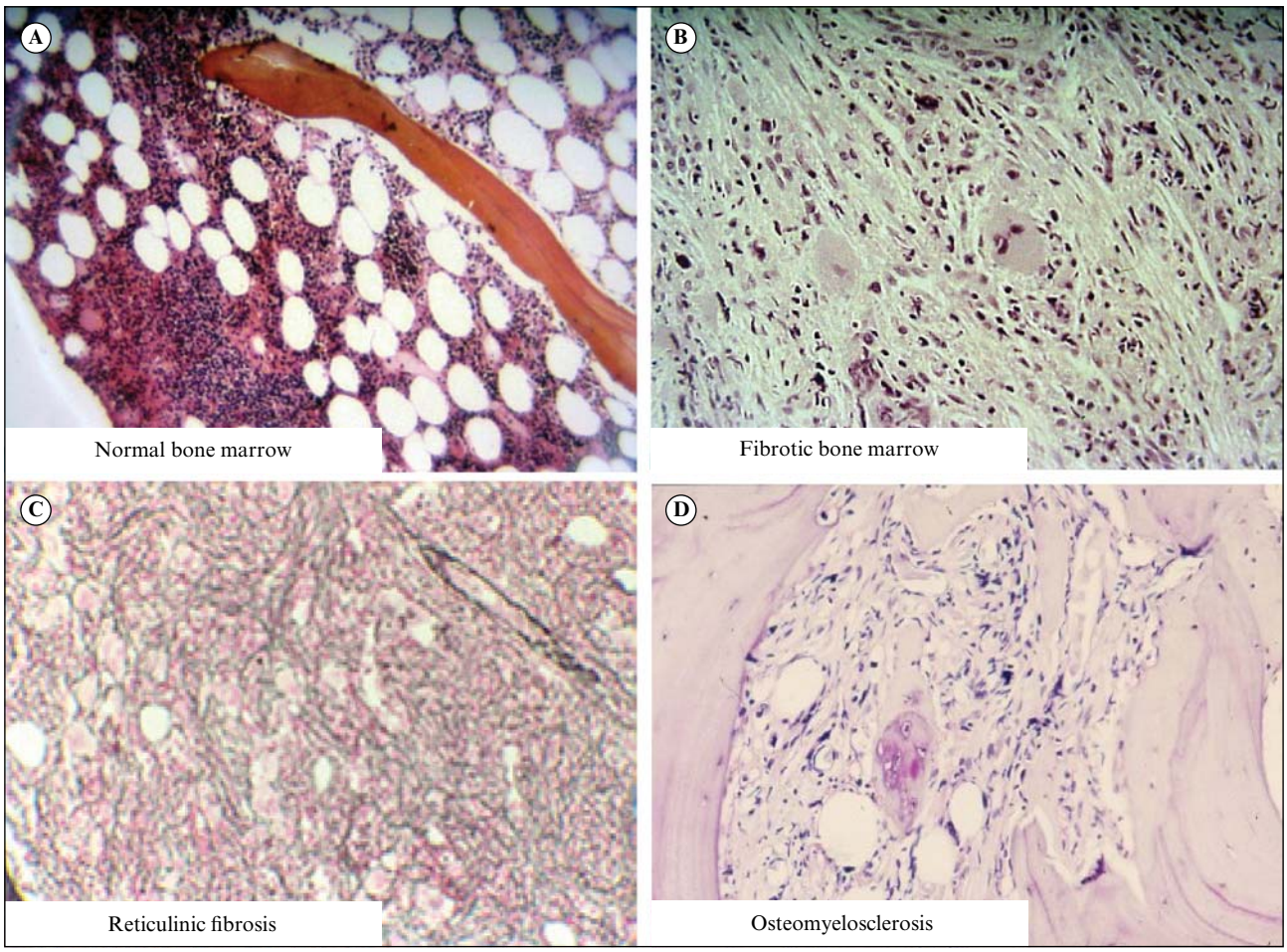

Figure 1

Histological sections of human bone marrow from healthy donors (**A**) or from patients with myelofibrosis (**B, C, D**).

hepatocytes or biliary epithelium, neonatal liver disease, metabolic disorders including Wilson's disease, hemochromatosis and a variety of other storage diseases, chronic inflammatory conditions (*e.g.* sarcoidosis), drug toxicity (*e.g.* methotrexate or hypervitaminosis A), and vascular disturbances, either congenital or acquired.

HISTOLOGICAL QUANTIFICATION

Myelofibrosis is defined as an increase in bone marrow fiber content without referring explicitly to the exact quantity or quality (reticulin *versus* collagen) [3]. In PMF, immunolocalisation studies show a marked neovascularisation [4] in the fibrotic bone marrow stroma together with an increased deposition of types I, III, IV, V and VI collagen, with types I and III being predominant [1, 2] and highly synthesized [5]. An increased deposition of interstitial and basement proteins, fibronectin, laminin, tenascin and vitronectin is also documented in the advanced stages of the disease [6], suggesting that myelofibrosis is a complex process that involves the accumulation and assembly of collagenous ECM components (reticulin) and noncollagenous ECM components (fibronectin). In the past, scoring methods were mainly based on subjective evaluations by individual pathologists using a variety of techniques. Recently, a consensus of European experts has solved this problem by proposing a semi-quantitative grading system regarding the quantity and quality of MF in chronic MPD (cMPD). Four main grades based on assessment of the fiber content and density within hematopoietic cellular areas, have been proposed [7]. These grades discriminate between a scattered, linear, reticulin network with no intersections corresponding to normal BM (MF-0), a loose network of reticulin with many intersections (MF-1), a diffuse and dense increase in reticulin with extensive intersections, occasionally (MF-2), or often (MF-3), associated with focal bundles of collagen.

For the evaluation of fibrosis in liver histology (stage and grade) *(figure 2)*, the most common scoring methods include Metavir [8] and the histological activity index (HAI) also called the Knodell [9]. Briefly, the Metavir scoring system was specially designed for patients with hepatitis C. The scoring consists of a grading and a staging system. The grade gives an indication of the activity or amount of inflammation and the stage represents the amount of fibrosis or scarring. The grade is assigned a number based on the degree of inflammation, which is usually scored from 0-4, with 0 being no activity and 3 or 4 considered as severe activity. The extent of inflammation is important because it is considered a precursor to fibrosis. The fibrosis score is also assigned a number from 0-4: 0 = no scarring, 1 = minimal scarring, 2 = scarring has occurred and extends outside the areas in the liver that contains blood vessels, 3 = bridging fibrosis spreading and connecting to other areas that contain fibrosis, 4 = cirrhosis or advanced

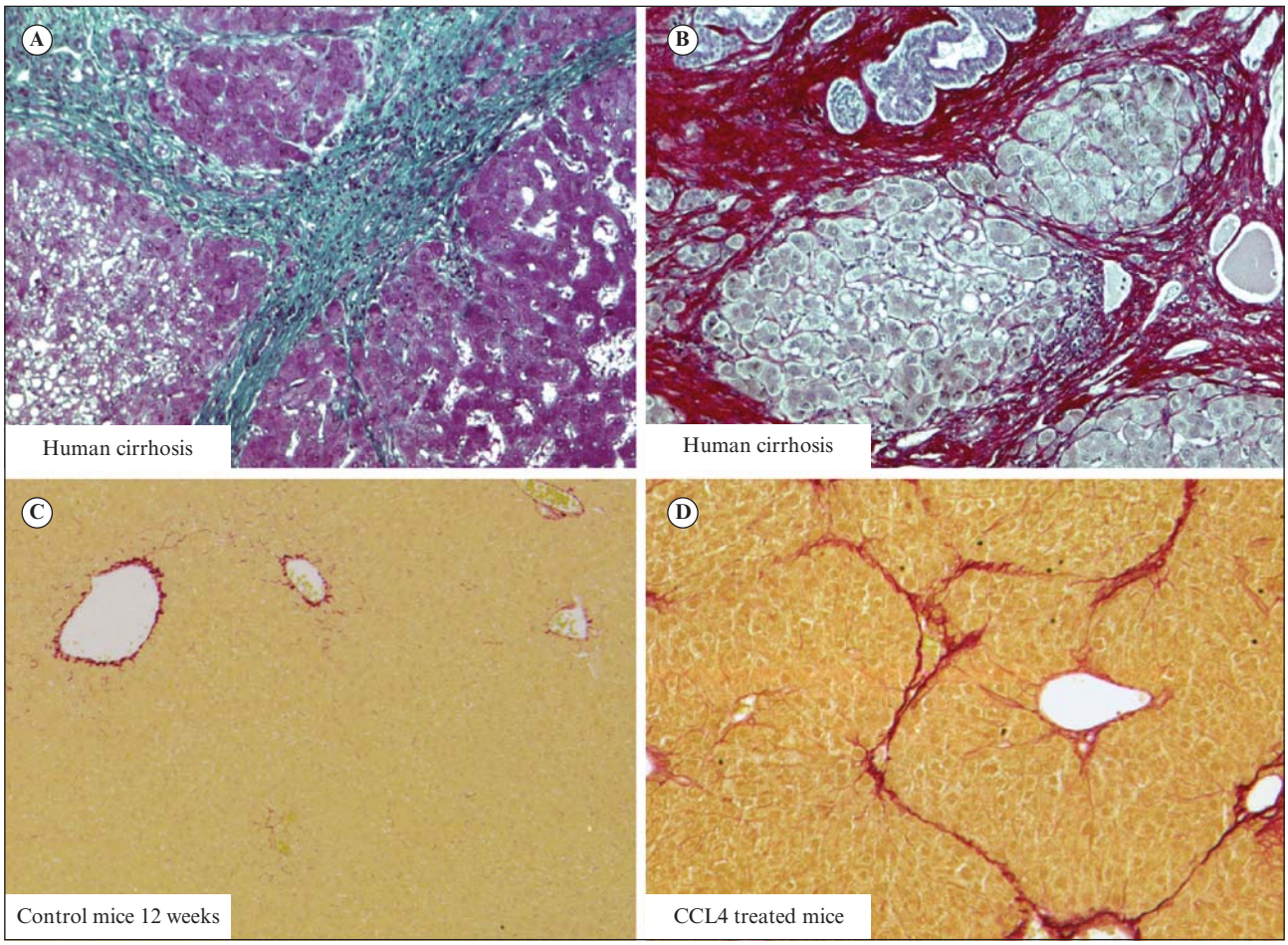

Figure 2
Histological sections of human cirrhotic liver stained with trichrome masson (**A**) or by red Sirius (**B**). Histological sections stained with red Sirius of liver from oil-treated mice (**C**) or from mice treated with an hepatotoxic agent, CCl_4, over 12 weeks (**D**).

scarring of the liver. The Knodell score or histological activity index (HAI) is also commonly used to stage liver disease. It is a rather more complex process, but some experts believe that it is a better tool for defining the extent of liver inflammation and damage. It is composed of four individually assigned numbers that make up a single score. The first component (peri-portal and/or bridging necrosis) is scored 0-10. The next two components (intralobular degeneration and portal inflammation) are each scored 0-4. The combination of these three markers indicates the amount of inflammation in the liver: 0 = no inflammation, 1-4 = minimal inflammation, 5-8 = mild inflammation, 9-12 = moderate inflammation, 13-18 = marked inflammation.

CELL PARTNERS

Two main types of cell partners have to be distinguished in the fibrotic process: cells synthesising fibrogenic growth factors that activate cells producing ECM components.

Cells synthesising fibrogenic growth factors

The presence of a variety of immunological abnormalities in PMF first suggested that the lymphocytic lineage could be implicated in the induction and/or maintenance of the disease and favoured the hypothesis of an autoimmune origin. Immune-mediated bone marrow damage was suggested, particularly it was speculated that activation of platelets by circulating immune complexes would result in the release of fibrosis-promoting factors from the platelets and subsequent bone marrow fibrosis [10]. Whereas the pathogenic relevance of these findings has not been elucidated in PMF, the role of lymphocytes, and especially of B lymphocytes as a source of fibrogenic cytokines, has been strongly suggested in hairy cell leukemia [11, 12].

In contrast, a number of findings have led to the concept that the megakaryocytic lineage is pathogenically important in the promotion of myelofibrosis associated with PMF development [13]: *i)* dysplastic megakaryocyte (Mk) hyperplasia and its close association with the fibrotic tissue, *ii)* the increased number of circulating megakaryocytes and progenitors, *iii)* the possible transformation of PMF into acute megakaryoblastic leukaemia. However, the demonstration that megakaryocyte homogenates were able to stimulate the proliferation of bone marrow fibroblasts and the production of collagen has provided the first tangible evidence in support of the role of megakaryocytes and of an Mk-derived growth factor identified as platelet-derived growth factor (PDGF), in the genesis of marrow fibrosis [14]. Further studies have shown that the expression and production of transforming growth factor-beta (TGF-β) were increased in patients' peripheral blood mononuclear cells identified as megakaryocytic cells at different stages of differentiation/maturation [15]. The report of a steadily increasing percentage of myelofibrosis correlating with the proliferation of Mk in CML underlined the correlation of megakaryocytic cells with fibrosis, and stressed the link between TGF-β and megakaryocytic cells in the pathophysiology of myelofibrosis. Interestingly, megakaryocytes also produce and secrete vascular endothelial growth factor (VEGF), the expression of which can be up-regulated by PDGF, epithelial growth factor (EGF), basic fibroblast growth factor (bFGF) and TGF-β. In PMF, the clustering of Mk close to sinusoids and their intrasinusoidal localization support the concept of interactions between MK and endothelial cells that favour cytokine-mediated bone marrow reactions, including fibrosis and angiogenesis *(figure 3)*.

Apart from lymphocytes and megakaryocytes/platelets, monocytes could be alternative sources of fibrogenic growth factors. In PMF, monocytes are activated and overexpress IL-1 and TGF-β, suggesting that they may also contribute to the fibrotic process [16, 17]. Whereas contradictory results coming from experimental models of myelofibrosis induced in NOD/SCID mice that are deficient in functional monocytes [18, 19] illustrate that the issue is open, cooperation between MK and monocytes in the production of fibrogenic cytokines remains most likely. With regard to this assumption, it is interesting to note that NF-kB pathway activation is associated with TGF-β production in both cell types [16, 20].

Among the cell partners involved in the liver fibrosis process, the hepatic stellate cells (HSC), also known as Ito cells, which play a central role in fibrosis, synthesize fibrogenic growth factors. However, all neighboring cell types, such as sinusoidal endothelium, Kupffer cells, hepatocytes, platelets and leukocytes, produce cytokines and growth factors that directly or indirectly participate in the initiation and maintenance of the stellate cell phenotype. Activated HSC perpetuate their own activation through several autocrine loops, including the secretion of TGF-β1 and an upregulation of its receptors [21]. The HSC also express keratinocyte growth factor in chronic liver [22]. Endothelial cells are also likely to participate in activation, both by production of cellular fibronectin and *via* conversion of from the latent to active, profibrogenic form. Kupffer cell infiltration and activation also play a prominent role in liver fibrosis. Kupffer cells produce large amounts of cytokines, especially TGF-β1, and can also stimulate matrix synthesis, cell proliferation, and release of retinoids by stellate cells through the actions of both cytokines and reactive oxygen intermediates/lipid peroxides. Proliferation of HSC has been attributed to Kupffer cell-derived TGF-β. TGF-β derived from Kupffer cells markedly stimulates stellate cell ECM synthesis. Platelets are a potent source of growth factors, and are present in the injured liver [23]. Potentially important platelet mediators include PDGF [24] and TGF-β1 *(figure 3)*.

Cells synthesising ECM components

The key cellular mediator of the myelofibrotic process is the medullar fibroblast, while in liver fibrosis/cirrhosis the key cellular mediator is the HSC, both types of cells being mesenchymal cells.

Thus, in bone marrow fibrosis, fibroblasts usually found in close association with collagen fibers, are responsible for reticulin and collagen synthesis [25, 26]. Since no massive increase in fibroblast numbers is observed in the bone marrow of PMF patients, the increased production of reticulin and ECM proteins may rather be due to their activation into myofibroblasts [26] or to an acceleration of their differentiation in matrix-producing cells (collagen-

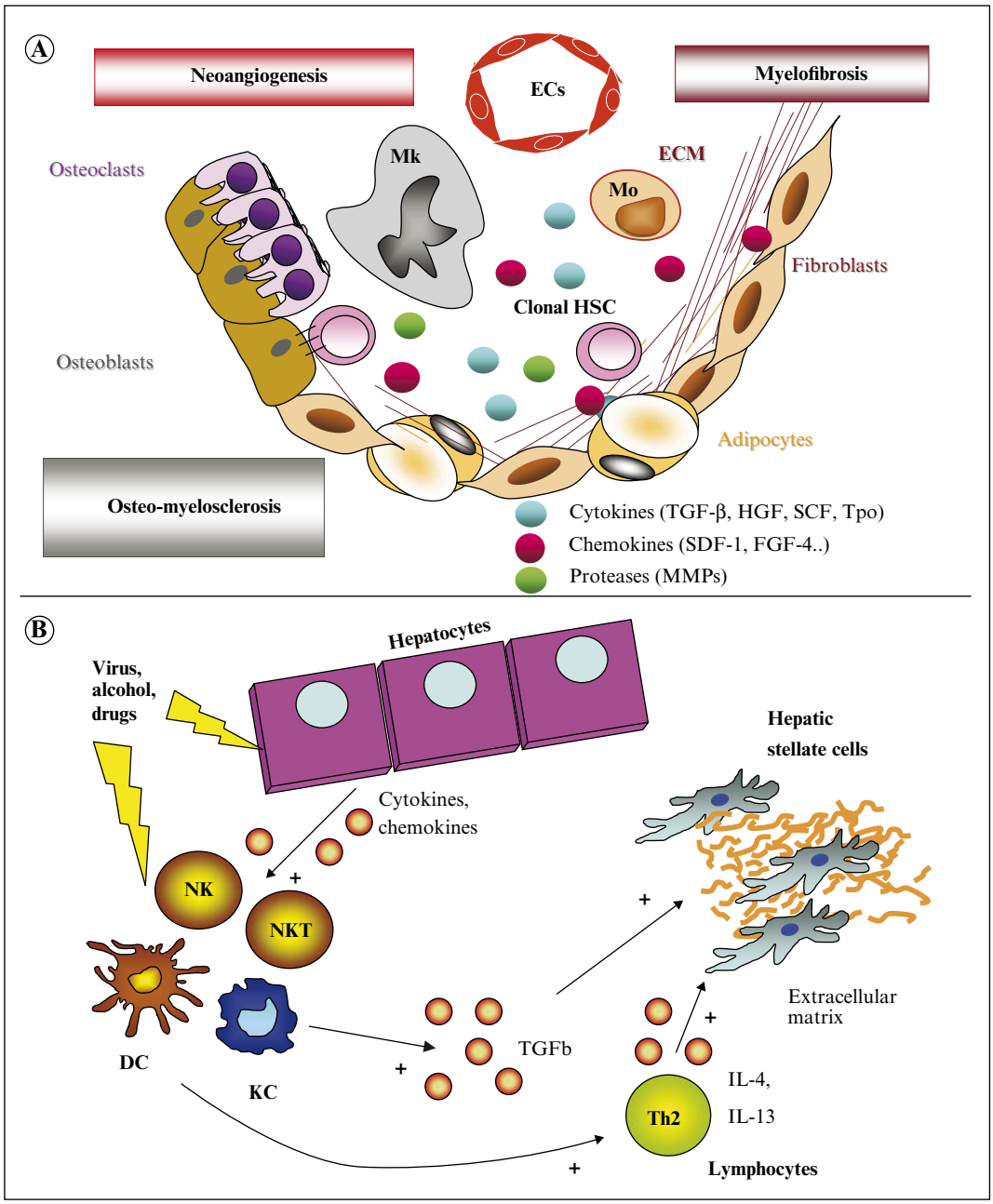

Figure 3

Schematic representation of cellular and molecular mechanisms underlying bone marrow (top) and liver fibrosis (bottom). Mk: megakaryocytes; Mo: monocytes; Ne: neutrophils; EMC: extracellular matrix component; HSC/PH: hematopoietic stem cells/ hematopoietic progenitors.

secreting α-SMA$^+$ fibroblasts) as reported in hairy cell leukaemia [27]. These myofibroblasts are mainly derived from resident mesenchymal stem cells; however, it has been shown that they can be also derived from epithelial cells in an epithelial-mesenchymal transition process [28, 29]. Recently, circulating fibroblast-like cells, termed fibrocytes, that define a new leukocyte subpopulation derived from bone marrow stem cells and that mediate tissue repair have been identified [30, 31], thus suggesting that cells with different origins, acting together, likely contribute to the collagen production. Interestingly, activated fibroblasts/fibrocytes also produce inflammatory cytokines, growth factors, and chemokines that participate in the persistence of inflammation linked to the fibrotic process.

In liver fibrosis, although several hepatic cell types including the hepatocytes themselves can synthesize ECM proteins (mainly collagen types I and III in the liver parenchyma), HSC are the major source of increased ECM in chronic liver diseases [32-34]. Following liver injury, HSC undergo a phenotypic transformation, with the acquisition of myofibroblast-like features, including increased collagen production and cell contractility. These activated HSC migrate and proliferate at the sites of liver injury, perpetuating hepatic inflammation and playing a pivotal role in the formation of a fibrous scar. Thus, the HSC express virtually all of the key components required for pathological matrix degradation and therefore play a key role not only in matrix production, but also in matrix degradation.

MOLECULAR MECHANISMS OF FIBROSIS

Role of growth factors and cytokines

In PMF, whereas the clonality of the hematopoietic proliferation is well recognised [35], the proliferation of the medullary fibroblasts responsible for the development of the fibrosis is suggested to be polyclonal [36, 37]. Therefore, the stromal reaction appears to be secondary to the production of growth factors including TGF-β, PDGF, β-FGF, VEGF, EGF and platelet factor-4 (PF-4), by hematopoietic cells and especially by dystrophic megakaryocytes from the malignant clone. Interestingly, these cytokines that are powerful regulators of extracellular matrix biosynthesis are also mediators of fibroblast and endothelial cell proliferation, suggesting their involvement in the stromal reaction and reinforcing the hypothesis of a connection between fibrosis and angiogenesis as suggested in various fibrotic diseases including pulmonary and eye fibrosis as well as systemic sclerosis [28].

Historically, PDGF was the first growth factor suggested to participate in PMF bone marrow fibrogenesis through its role in the proliferation/activation of medullary fibroblasts [14, 38]. Several groups have evaluated PDGF activity in myeloproliferative disorders and have reported quantitative changes in platelet PDGF levels. An overall decrease of intraplatelet PDGF levels was observed in the different cMPD with the exception of PMF, where some groups have shown reduced platelet PDGF activity, while others have reported increased PDGF expression [14, 25, 38]. From these studies, it appeared that intraplatelet PDGF levels do not strictly correlate with the degree of fibrosis and that the release of PDGF solely could not account for the complexity of the myelofibrotic process. It seemed likely that additional growth factors were involved, among which TGF-β and bFGF have received particular attention. TGF-β, and especially TGF-β1, is one of the most powerful growth factors in the regulation of the activity of genes involved in the synthesis of the ECM components and of ECM-degrading enzymes [39, 40]. Similarly to its key role in the pathogenic process of tissue fibrosis [41], TGF-β has been also implicated in the development of fibrosis associated with hematological disorders. It has been reported to be highly expressed in hairy cell leukaemia [27] and in acute megakaryoblastic leukemia [11, 42], and to be involved in the pathogenesis of bone marrow reticulin fibrosis observed in these diseases. In PMF, the pathogenic relevance of TGF-β also lies in its capacity to increase the biosynthesis of type I, III and IV collagens, fibronectin, tenascin and proteoglycans and to block matrix degradation by reducing collagenase-like protease synthesis while enhancing protease inhibitor expression. Involvement of TGF-β in the promotion of fibrosis in PMF was first suggested by the demonstration of elevated intraplatelet levels in patients [15, 43]. The increase in TGF-β production by megakaryoblasts in acute megakaryoblastic leukemia [11, 42], and in TGF-β plasma concentrations in acute megakaryocytic leukemia [42], further strengthened the hypothesis of a possible contribution of this growth factor to the myelofibrotic process and of megakaryocytes in its production. The normal levels of TGF-β reported in essential thrombocythemia, an MPD in which the number of MK/platelets is abnormally elevated while myelofibrosis is uncommon, indirectly corroborate this assumption [44]. Finally, the demonstration of a prominent role for TGF-β released from megakaryocytes/platelets in the promotion of myelofibrosis came from studies performed in mouse models of myelofibrosis.

Involvement of additional growth factors, likely acting in conjunction with PDGF and TGF-β must also be considered. Among them, bFGF, is a powerful mitogen for human bone marrow stromal cells and which is a potent angiogenic factor, has been reported to be highly expressed in PMF patients and to participate in the medullar fibrotic reaction [45, 46]. By modulating the function of fibroblasts, IL-1, serum amyloid P substance and calmodulin may also take part in this process; interestingly Il-1β is increased in the serum of PMF patients (personal results) and the calmodulin calcium-binding protein is increased in the urine of MPD patients with fibrosis in contrast to non-fibrotic ones [47]. Finally, the level of VEGF that is particularly involved in angiogenesis, and which also participates in the promotion of fibrosis, has been reported to be higher in the plasma of PMF patients [48]. Actually, by increasing vascular permeability, VEGF enhances the extravasation of plasma proteins into the extravascular space. Such a process might lead to profound alterations in the EMC, which could result in fibrosis.

In liver fibrosis *(figure 3)*, PDGF is the most potent stellate cell mitogen identified [49]. Induction of PDGF receptors early in stellate cell activation increases responsiveness to this potent mitogen, and downstream pathways of PDGF signaling have been carefully characterized in stellate cells. TGF-β is the most potent stimulus for collagen I production by HSC [32-34]. The chemotactic cytokines are involved in the migration of HSC, an action that is characteristic of wound-infiltrating mesenchymal cells in other tissues too. Chemotaxis of stellate cells explains, in part, why HSC align within inflammatory septa *in vivo*. The several cytokines, produced by leukocytes, that are recruited to the liver during injury, joining with Kupffer cells, can modulate HSC behaviour and consequently liver fibrosis. The specific role of neutrophils as a stimulus for HSC was demonstrated in a co-culture experiment in which cells activated by fMLP were plated in direct contact with HSC. Activated neutrophils, which are an important source of reactive oxygen species (ROS), increase HSC collagen synthesis 3-fold over control levels via superoxide production [50]. Lymphocytes, including $CD4^+$ T-helper (Th) cells, residing in the liver, represent a further potential source of cytokines and therefore are able to modulate the liver fibrotic process [29]. Th lymphocytes help to orchestrate the host-response via cytokine production and can differentiate into Th1 and Th2 subsets. Thus, Th1 cells produce cytokines that promote cell-mediated immunity, including interferon (IFN)-gamma, tumor necrosis factor (TNF) and IL-2 and have been demonstrated as anti-fibrotic cytokines. In contrast, Th2 cells produce IL-4, IL-5, IL-6 and IL-13, promote humoral immunity, and have been demonstrated as pro-fibrotic cytokines. Th1 cytokines inhibit the development of Th2 cells and Th2 cytokines inhibit the development of Th1 cells; a phenomenon that increases the anti- or pro-fibrotic processes. Further evidence of the importance of Th2 cytokines in liver fibrogenesis has been demonstrated by studies using

mice that lack the Th2 cytokine IL-4 or its receptor IL-4Ra. All these animals show remarkably reduced liver fibrosis.

Mechanisms of fibrogenic growth factor production

As regards myelofibrosis, different studies have provided insight into possible mechanisms for the pathological release of fibrogenic growth factors in PMF. Abnormal expression and subcellular distribution of P-selectin, a specific platelet receptor for leucocytes and a component of the α-granule membrane, together with excessive and pathological emperipolesis (*i.e*. the passage of a cell into the cytoplasm of another cell) of polymorphonuclear leucocytes in Mk, have been observed in bone marrow from patients [51]. Such a phenomenon is reported to result in the progressive destruction of Mk, with degradation and lysis of α-granules and subsequent release of growth factors. Therefore, in PMF, abnormalities in Mk maturation and structure, with conspicuous proliferation and clustering, sublethal cell injury and/or cell lysis, probably account for the elevated fibrogenic growth factor levels detected in patients [46]. Once released, fibrogenic growth factors can be sequestrated in bone marrow stroma by binding to heparan sulfate proteoglycans in extracellular matrix and basement membranes [52, 53]. Their locally raised concentrations, together with their sustained release from abnormal Mk/platelets, would participate in the progressive alteration of bone marrow stroma and the recruitment of fibroblasts into wound sites through interactions with ECM component [54].

In liver fibrosis, TGF-β is produced by Kupffer cells and hepatic stellate cells [55], and its inhibition prevents progression of fibrosis [56]. Convincing proof of the role of Kupffer cells in fibrogenesis was demonstrated when Kupffer cells were suppressed using a mutated form of MCP-1 (CCL2), which functions as a dominant negative mutant. Thus, the blockade of macrophage infiltration inhibits activation of HSC and leads to suppression of liver fibrogenesis [57]. Therefore, while TGF-β is required for the attraction of HSC, production of MEC and survival of activated HSC, little is known concerning inductors able to increase the secretion of TGF-β by Kupffer cells. In rat, it was shown that TGF-β mRNA could not be detected in parenchymal and nonparenchymal cells from normal liver. Nevertheless, human Kupffer cells are an important source of TGF-β1 production even without any stimulation [58, 59]. Thus, large amounts of TGF-β1 were produced by unstimulated Kupffer cells. Among the regulators of TGF-β expression, eicosanoids, a fatty acid, could modulate TGF-β production [60]. Octreotide, a somatostatin analog that significantly decreases TGF-β1, has been implicated in the reduction of liver fibrosis [61]. Controversially, works have been reported on the role of LPS in liver fibrosis and in the TGF-β expression by Kupffer cells. *In vitro*, similar to rat KC (CXCL1) in culture, LPS-stimulated human Kupffer cell release TGF-β [60]. However, Xidakis *et al*. [61] have shown that LPS stimulation seems to significantly decrease the TGF-β production in primary culture of human Kupffer cells. Recently, the role of LPS and TLR4 has been further elucidated *in vivo* using TLR4 chimeric mice. In quiescent HSC, TLR4 activation by LPS challenge induces chemotaxis of Kupffer cells, and downregulates the Bambi TGF-β pseudoreceptor to sensitize HSC to TGF-β-induced signals and allow for unrestricted activation by Kupffer cells. Here, the modulation of TGF-β signaling by a TLR4-MyD88-NF-kappaB axis is a novel link between proinflammatory and profibrogenic signals [62]. An autocrine or short-loop paracrine effect of TGF-β or PDGF has been proposed as a mechanism contributing to the expression of these growth factors by HSC.

Alteration of the proteolytic environment

In most cells, TGF-β is synthesized as a latent, biologically inactive form that has to be activated to be recognized by its cell-surface receptors in order to trigger biological responses. Mechanisms leading to TGF-β activation are complex and not well understood. Although proteolytic processing by plasmin and matrix metalloproteases (MMP), as well as exposure to ROS may participate in its activation, the conformation change of the latent complex induced by molecular interactions appears to be a prominent mechanism *in vivo*. Integrins such as αvβ6 and αvβ8 and thrombospondin have been demonstrated to be major activators of TGF-β *in vivo* [63-65]. In PMF, TGF-β activation is restricted to the hematopoietic (medullar and spleen) environment and the underlying mechanisms are still unclear. It cannot be ruled out that aberrations in the complex enzymatic machinery that regulates latent TGF-β activation may lead to the prolonged release of TGF-β, thus making the myelofibrotic process even more complex. Therefore, it is most likely that activation of proteolytic enzymes paves the way for the increasing collagen meshwork. Studies investigating plasma from patients with PMF and related Philadelphia chromosome-negative cMPD for aberrant MMP and tissue inhibitors of metalloprotease (TIMP) protein levels, have suggested that elevated TIMP together with decreased MMP levels might be essential for fibrosis formation [66, 67]. Interestingly, the increased levels of MMP-14, a key pericellular collagenolysin in pathological vessel remodeling and angiogenesis, observed in progressive myelofibrosis shed new light on a potentially important candidate in the PMF stromal reaction, including fibrosis and angiogenesis [6]. It is most likely that the role of other molecules, including members of the urokinase-type plasminogen activator system, elastases and stromelysins, concomitantly interacting in the process of ECM remodeling, complicates the network of aberrant proteolytic mechanisms in PMF [5]. The JAK/STAT pathway has been reported to be involved in the activation of genes responsible for metalloprotease synthesis. Interestingly, a single point mutation in the cytoplasmic tyrosine kinase JAK2 (JAK2V617F) has been discovered in half of PMF patients [68-71]. However, whereas this JAK2 mutation is suggested to be responsible for the altered haematopoiesis in the mutated patients, it does not modify the matrix-modeling gene expression that is predominantly related to the stage of disease [5].

In liver, quantitative and qualitative changes in matrix protease activity also play an important role in the ECM remodeling accompanying fibrotic injury. HSC express almost all of the key components required for pathological matrix degradation, and therefore play a key role not only in matrix production, but also in matrix degradation. A

growing family of matrix-metalloproteinases has been identified that are calcium-dependent enzymes, which specifically degrade collagens and noncollagenous substrates [72, 73]. These enzymes include: interstitial collagenases (MMP-1, MMP-8 and MMP-13); gelatinases (MMP-2 and MMP-9; stromelysins (MMP-3, MMP-7, MMP-10 and MMP-11); membrane-type (MMP-14, MMP-15, MMP-16, MMP-17, MMP-24 and MMP-25); and a metalloelastase (MMP-12). Inactive metalloproteinases can be activated through proteolytic cleavage by either membrane-type matrix metallproteinase 1 (MT1-MMP) or plasmin, and inhibited by binding to specific inhibitors known as tissue inhibitors of metalloproteinases (TIMP). Thus, net collagenase activity reflects the relative amounts of activated metalloproteinases and their inhibitors, especially TIMP. HSC are an important source of MMP-2, as well as increases in the specific MMP inhibitor molecules, TIMP-1 and TIMP-2, leading to a net decrease in protease activity, and therefore, more matrix accumulation.

EXPERIMENTAL MODELS

To date, there are several experimental models of myelofibrosis in mouse. One of the first models described, induced by the myeloproliferative sarcoma virus (MPSV), shares many clinical and biological characteristics with PMF, and especially a massive fibrosis observed not only in the bone marrow, but also in the spleen and the liver of infected mice [74, 75]. Very interestingly, in this model, the role of growth factors and cytokines produced by stromal cells in the genesis of fibrosis closely mimics that observed in the human disease. More recently, two other murine models have been reported: one is induced by administration of suprapharmacological doses of thrombopoietin (TPO), a physiological regulator of megakaryocytopoiesis (TPO^{high} mice) [76, 77]; the other is observed in mice harbouring a decreased expression of the GATA1 megakaryocytic transcription factor ($GATA\text{-}1^{low}$ mice) [78]. In these models, the development of fibrosis is associated with increased numbers of Mk cells and high levels of TGF-β [79]. However, the most significant demonstration of the central role of TGF-β in the myelofibrotic process comes from studies in which mice were grafted with either bone marrow stem cells from homozygous TGF-β-null ($TGF\text{-}\beta^{-/-}$) or wild-type (WT) littermates infected with a retrovirus encoding the murine TPO protein. In this model, myelofibrosis occurred only in mice repopulated with WT cells while no reticulin deposition was seen in animal reconstituted with $TGF\text{-}\beta^{-/-}$ cells [80]. Finally, most recently, mouse models of MPD associated with myelofibrosis, in which the JAK2-V617F mutation was overexpressed by retroviral transduction [81, 82] or by transgenesis [83, 84], have been described.

Several animal models have been developed to study liver fibrosis *(figure 2)*. Challenges of mice with carbon tetrachloride (CCl_4), a hepatotoxic molecule that generates a trichloromethyl-free radical, leading to an oxidative stress-mediated lipid peroxidation and membrane damage, results in the development of liver fibrosis. In the same way, injection of parasites, such as *Schistosoma mansoni* causes granulomas around schistosome eggs and, liver fibrosis. Bile duct ligation (BDL) is also used in mouse to induce liver fibrosis which mimicks human chronic cholestatic liver disease. Finally, the use of transgenic (conventional or "knockout") mice has allowed the study of liver fibrosis against different genetic background. Thus, the impact of TGF-β1 on liver fibrosis has been well documented in a TGF-β1-knockout mouse model [85], in the remarkable attenuation of the development of liver fibrosis by using soluble type II TGF-β receptor [86], and in adenoviral delivery of dominant-negative TGF-β receptor [87].

More recently, the role of Th1 or Th2 cytokines has been assessed in liver fibrosis induced by repeated CCl_4 administration or schistosoma infection [29]. Thus, the use of severe, combined, immunodeficiency (SCID) mice for T-cell depletion, the C57/BL6 mice for Th1 predominance and BALB/c mice for Th2 predominance shows that the degree of fibrosis is modified significantly in immunocompetent mice from both strains. Immunocompetent C57/BL6 mice, whose lymphocyte cytokine profile includes IFN-gamma, actually exhibit less fibrosis than SCID mice from the same background. When C57/BL6 mice, with targeted disruption of IFN-gamma, are treated with CCl_4, fibrosis returns to the level seen in C57/BL6 SCID mice. However, immunocompetent BALB/c mice, whose lymphocyte cytokine profile includes the fibrogenic compounds IL-4 and TGF-β, exhibit more fibrosis than BALB/c SCID mice. Among all of the studies examining immunoregulation of fibrosis, those demonstrating modulation by T lymphocytes appear the most convincing. Furthermore, the relevance of the Th2 cytokines, IL-4 and IL-13 in liver fibrosis, has been investigated in mice infected with *Schistosoma mansoni*. Thus, treatment with neutralizing mAb against IL-4 has allowed evaluation of the role of this cytokine in the generation of parasite egg-induced, cell-mediated responses and hepatic pathology. Animals treated with anti-IL-4 before egg deposition showed decreased IL-4, IL-5, and IL-10 production, and anti-IL-4-treated, infected mice showed consistent and marked reductions in hepatic collagen deposition [88]. In the same way, use of IL-13 inhibitor allows the development of hepatic fibrosis to be blocked [89, 90]. IL-4-deficient, IL-13-deficient, and IL-4/13-deficient mice, when infected by *Schistosoma mansoni*, presented a reduction in hepatic fibrosis, compared to wild type mice [91, 92].

CLINICAL IMPACT AND THERAPEUTIC CHALLENGE

Despite the substantial progress made over the past few years, there are currently no approved treatments that directly and effectively target the complex mechanism(s) of fibrosis. This raises the issue of the existence of common mechanisms that regulate fibrosis in the various fibroproliferative diseases. The extreme complexity of the cellular network involved in ECM secretion and in fibrogenic growth factor production can explain why targeting only one cell type is not sufficient for the effective treatment of fibrosis.

Whereas the long and chronic evolution of fibrosis is difficult to reproduce in mice, the use of the experimental models of myelofibrosis has been very helpful for testing the efficacy of new therapeutic strategies such as adenoviral-mediated TGF-β1 inhibition [93] or proteasome inhibitor bortezomib [94] targeting either the cytokine (TGF-β) distribution or production related to NF-kB

activation, respectively. However, whereas these strategies are promising in mouse models, preliminary data from therapeutic trials have not demonstrated the efficiency of such inhibitors in reducing fibrosis, suggesting that removing the etiological agent is not sufficient to treat a multifactorial disease such as this. Therefore, other therapeutic strategies, including molecules targeting cytokine-mediated signalling, are currently being explored [95], their rational lying in the recent discovery of the JAK2 mutation in MPD that constitutively activates the JAK2 pathway [2]. Whereas preliminary data show significant clinical activity of such inhibitors in MPD patients including those with PMF, their effect on fibrosis has not yet been reported. Thus, until now, allogenic haematopoietic cell transplantation offers the only therapy available for patients with PMF that has the potential to reverse bone marrow fibrosis between six and 12 months after transplantation [96]. Interestingly, the efficiency of bone marrow cell transplantation in reducing fibrosis is not restricted to bone marrow fibrosis, as it has been recently proposed as a potential treatment for liver fibrosis in mice [97, 98].

As TGF-β plays a major role in liver fibrosis, several therapeutic strategies have been developed to target this molecule *in vivo* [99, 100]. Anti-TGF-β approaches were established and successfully utilized for the treatment of experimental fibrogenesis in animals. Dominant negative TGF-β receptors (TbetaR), generated by fusing the Fc domain of human IgG and the N-terminal (extracellular) fragment of TbetaRII (Fc:TbetaRII) were applied to suppress fibrosis. Further, the over-expression of Smad7, a transcriptional factor involved in intracellular TGF-β signalling, achieved efficient inhibition of liver fibrosis. Likewise, soluble TGF-β receptor II expression reduced experimental fibrogenesis *in vitro* and *in vivo*, partially by decreasing intracellular ROS and inhibiting NADH oxidase. Similar approaches, aimed at reducing endogenous IL-13 or suppressing IL-13 receptor signalling, have been developed. Thus, IL-13 immunoneutralization reduces liver fibrosis in mice [89, 90].

In conclusion, several approaches that specifically target pro-fibrogenic TGF-β signalling are effective at reducing liver fibrosis in mice and therefore show promise for the treatment of liver disease in humans. However, to ensure patient safety approaches with strong specificity need to be established.

CONCLUSION

Whereas bone marrow and liver fibrosis occur in different organs, analysis of their pathophysiological mechanisms reveals a number of similarities including: a predominant implication of TGF-β, and a key role of fibroblasts as both a cellular target of TGF-β and a central cell for the production of ECM components. Furthermore, in both types of fibrosis, the chronic inflammatory response seems to be required for induction of the fibrotic process. In contrast, the etiology and the nature of the cells producing the fibrogenic factors are different. However, the complexity and the specificity of the cellular and humoral network participating in these fibrotic processes, as well as their long and chronic evolution, account for the current lack of effective antifibrotic therapies, challenging both researchers and the pharmaceutical industry to intensify and gather their efforts.

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REFERENCES

1. Reilly JT. Idiopathic myelofibrosis: pathogenesis, natural history and management. *Blood Rev* 1997; 11: 233.
2. Tefferi A. Pathogenesis of myelofibrosis with myeloid metaplasia. *J Clin Oncol* 2005; 23: 8520.
3. Thiele J, Kvasnicka HM. Myelofibrosis in chronic myeloproliferative disorders--dynamics and clinical impact. *Histol Histopathol* 2006; 21: 1367.
4. Mesa RA, Hanson CA, Rajkumar SV, Schroeder G, Tefferi A. Evaluation and clinical correlations of bone marrow angiogenesis in myelofibrosis with myeloid metaplasia. *Blood* 2000; 96: 3374.
5. Bock O, Neuse J, Hussein K, Brakensiek K, Buesche G, Buhr T, Wiese B, Kreipe H. Aberrant collagenase expression in chronic idiopathic myelofibrosis is related to the stage of disease but not to the JAK2 mutation status. *Am J Pathol* 2006; 169: 471.
6. Hasselbalch HC. Idiopathic myelofibrosis. Clinical aspects and studies on extracellular matrix metabolism. *Dan Med Bull* 1993; 40: 39.
7. Thiele J, Kvasnicka HM, Facchetti F, Franco V, van der Walt J, Orazi A. European consensus on grading bone marrow fibrosis and assessment of cellularity. *Haematologica* 2005; 90: 1128.
8. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology* 1996; 24: 289.
9. Knodell RG, Ishak KG, Black WC, *et al.* Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology* 1981; 1: 431.
10. Cappio FC, Vigliani R, Novarino A, Camussi G, Campana D, Gavosto F. Idiopathic myelofibrosis: a possible role for immune-complexes in the pathogenesis of bone marrow fibrosis. *Br J Haematol* 1981; 49: 17.
11. Terui T, Niitsu Y, Mahara K, *et al.* The production of transforming growth factor-beta in acute megakaryoblastic leukemia and its possible implications in myelofibrosis. *Blood* 1990; 75: 1540.
12. Gruber G, Schwarzmeier JD, Shehata M, Hilgarth M, Berger R. Basic fibroblast growth factor is expressed by CD19/CD11c-positive cells in hairy cell leukemia. *Blood* 1999; 94: 1077.
13. Martyre MC. TGF-beta and megakaryocytes in the pathogenesis of myelofibrosis in myeloproliferative disorders. *Leuk Lymphoma* 1995; 20: 39.
14. Castro-Malaspina H, Jhanwar SC. Properties of myelofibrosis-derived fibroblasts. *Prog Clin Biol Res* 1984; 154: 307.
15. Martyre MC, Romquin N, Le Bousse-Kerdiles MC, *et al.* Transforming growth factor-beta and megakaryocytes in the pathogenesis of idiopathic myelofibrosis. *Br J Haematol* 1994; 88: 9.
16. Rameshwar P, Denny TN, Stein D, Gascon P. Monocyte adhesion in patients with bone marrow fibrosis is required for the production of fibrogenic cytokines. Potential role for interleukin-1 and TGF-beta. *J Immunol* 1994; 153: 2819.
17. Rameshwar P, Narayanan R, Qian J, Denny TN, Colon C, Gascon P. NF-kappa B as a central mediator in the induction of TGF-beta in monocytes from patients with idiopathic myelofibrosis: an inflammatory response beyond the realm of homeostasis. *J Immunol* 2000; 165: 2271.

18. Frey BM, Rafii S, Teterson M, Eaton D, Crystal RG, Moore MA. Adenovector-mediated expression of human thrombopoietin cDNA in immune-compromised mice: insights into the pathophysiology of osteomyelofibrosis. *J Immunol* 1998; 160: 691.

19. Wagner-Ballon O, Chagraoui H, Prina E, *et al.* Monocyte/macrophage dysfunctions do not impair the promotion of myelofibrosis by high levels of thrombopoietin. *J Immunol* 2006; 176: 6425.

20. Komura E, Tonetti C, Penard-Lacronique V, *et al.* Role for the nuclear factor kappaB pathway in transforming growth factor-beta1 production in idiopathic myelofibrosis: possible relationship with FK506 binding protein 51 overexpression. *Cancer Res* 2005; 65: 3281.

21. Bataller R, Brenner DA. Liver fibrosis. *J Clin Invest* 2005; 115: 209-18.

22. Steiling H, Muhlbauer M, Bataille F, Scholmerich J, Werner S, Hellerbrand C. Activated hepatic stellate cells express keratinocyte growth factor in chronic liver disease. *Am J Pathol* 2004; 165: 1233.

23. Burt AD, Oakley CL. Cellular and molecular aspects of hepatic fibrosis. *J Pathol* 1993; 170: 105.

24. Shiraishi T, Morimoto S, Koh E, Fukuo K, Ogihara T. Increased release of platelet-derived growth factor from platelets in chronic liver disease. *Eur J Clin Chem Clin Biochem* 1994; 32: 5.

25. Bock O, Loch G, Busche G, von Wasielewski R, Schlue J, Kreipe H. Aberrant expression of platelet-derived growth factor (PDGF) and PDGF receptor-alpha is associated with advanced bone marrow fibrosis in idiopathic myelofibrosis. *Haematologica* 2005; 90: 133.

26. Brouty-Boye D. Developmental biology of fibroblasts and neoplastic disease. *Prog Mol Subcell Biol* 2005; 40: 55.

27. Shehata M, Schwarzmeier JD, Hilgarth M, Hubmann R, Duechler M, Gisslinger H. TGF-beta1 induces bone marrow reticulin fibrosis in hairy cell leukemia. *J Clin Invest* 2004; 113: 676.

28. Wynn TA. Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. *J Clin Invest* 2007; 117: 524.

29. Wynn TA. Fibrotic disease and the T(H)1/T(H)2 paradigm. *Nat Rev Immunol* 2004; 4: 583.

30. Bucala R, Spiegel LA, Chesney J, Hogan M, Cerami A. Circulating fibrocytes define a new leukocyte subpopulation that mediates tissue repair. *Mol Med* 1994; 1: 71-81.

31. Quan TE, Cowper SE, Bucala R. The role of circulating fibrocytes in fibrosis. *Curr Rheumatol Rep* 2006; 8: 145.

32. Safadi R, Friedman SL. Hepatic fibrosis--role of hepatic stellate cell activation. *Med Gen Med* 2002; 4: 27.

33. Friedman SL. Stellate cells: a moving target in hepatic fibrogenesis. *Hepatology* 2004; 40: 1041.

34. Friedman SL. Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. *Physiol Rev* 2008; 88: 125.

35. Buschle M, Janssen JW, Drexler H, Lyons J, Anger B, Bartram CR. Evidence for pluripotent stem cell origin of idiopathic myelofibrosis: clonal analysis of a case characterized by a N-ras gene mutation. *Leukemia* 1988; 2: 658.

36. Bilhou-Nabera C, Brigaudeau C, Clay D, *et al.* Does cytogenetic mosaicism in CD34+CD38low cells reflect the persistence of normal primitive hematopoietic progenitors in myeloid metaplasia with myelofibrosis? *Blood* 2003; 102: 1551.

37. Wang JC, Lang HD, Lichter S, Weinstein M, Benn P. Cytogenetic studies of bone marrow fibroblasts cultured from patients with myelofibrosis and myeloid metaplasia. *Br J Haematol* 1992; 80: 184.

38. Castro-Malaspina H. Pathogenesis of myelofibrosis: role of ineffective megakaryopoiesis and megakaryocyte components. *Prog Clin Biol Res* 1984; 154: 427.

39. Massague J. The transforming growth factor-beta family. *Annu Rev Cell Biol* 1990; 6: 597.

40. Verrecchia F, Mauviel A. Transforming growth factor-beta signaling through the Smad pathway: role in extracellular matrix gene expression and regulation. *J Invest Dermatol* 2002; 118: 211.

41. Border WA, Noble NA. Transforming growth factor beta in tissue fibrosis. *N Engl J Med* 1994; 331: 1286.

42. Reilly JT, Barnett D, Dolan G, Forrest P, Eastham J, Smith A. Characterization of an acute micromegakaryocytic leukaemia: evidence for the pathogenesis of myelofibrosis. *Br J Haematol* 1993; 83: 58.

43. Martyre MC, Magdelenat H, Bryckaert MC, Laine-Bidron C, Calvo F. Increased intraplatelet levels of platelet-derived growth factor and transforming growth factor-beta in patients with myelofibrosis with myeloid metaplasia. *Br J Haematol* 1991; 77: 80.

44. Zauli G, Visani G, Catani L, Vianelli N, Gugliotta L, Capitani S. Reduced responsiveness of bone marrow megakaryocyte progenitors to platelet-derived transforming growth factor beta 1, produced in normal amount, in patients with essential thrombocythaemia. *Br J Haematol* 1993; 83: 14.

45. Le Bousse-Kerdiles MC, Chevillard S, Charpentier A, *et al.* Differential expression of transforming growth factor-beta, basic fibroblast growth factor, and their receptors in CD34+ hematopoietic progenitor cells from patients with myelofibrosis and myeloid metaplasia. *Blood* 1996; 88: 4534.

46. Martyre MC, Le Bousse-Kerdiles MC, Romquin N, *et al.* Elevated levels of basic fibroblast growth factor in megakaryocytes and platelets from patients with idiopathic myelofibrosis. *Br J Haematol* 1997; 97: 441.

47. Dalley A, Smith JM, Reilly JT, Neil SM. Investigation of calmodulin and basic fibroblast growth factor (bFGF) in idiopathic myelofibrosis: evidence for a role of extracellular calmodulin in fibroblast proliferation. *Br J Haematol* 1996; 93: 856.

48. DiRaimondo F, Azzaro MP, Palumbo GA, *et al.* Elevated vascular endothelial growth factor (VEGF) serum levels in idiopathic myelofibrosis. *Leukemia* 2001; 15: 976-80.

49. Davis BH, Rapp UR, Davidson NO. Retinoic acid and transforming growth factor beta differentially inhibit platelet-derived-growth-factor-induced Ito-cell activation. *Biochem J* 1991; 278(Pt 1): 43.

50. Svegliati-Baroni G, Saccomanno S, van Goor H, Jansen P, Benedetti A, Moshage H. Involvement of reactive oxygen species and nitric oxide radicals in activation and proliferation of rat hepatic stellate cells. *Liver* 2001; 21: 1.

51. Schmitt A, Jouault H, Guichard J, Wendling F, Drouin A, Cramer EM. Pathologic interaction between megakaryocytes and polymorphonuclear leukocytes in myelofibrosis. *Blood* 2000; 96: 1342.

52. Smadja-Joffe F, Legras S, Girard N, *et al.* CD44 and hyaluronan binding by human myeloid cells. *Leuk Lymphoma* 1996; 21: 407; (color plates following 528).

53. Le Bousse-Kerdiles MC, Martyre MC. Myelofibrosis: pathogenesis of myelofibrosis with myeloid metaplasia. French INSERM Research Network on Myelofibrosis with Myeloid Metaplasia. *Springer Semin Immunopathol* 1999; 21: 491.

54. Zhu H, Mitsuhashi N, Klein A, *et al.* The role of the hyaluronan receptor CD44 in mesenchymal stem cell migration in the extracellular matrix. *Stem Cells* 2006; 24: 928.

55. De Bleser PJ, Niki T, Rogiers V, Geerts A. Transforming growth factor-beta gene expression in normal and fibrotic rat liver. *J Hepatol* 1997; 26: 886.

56. Nakamura T, Sakata R, Ueno T, Sata M, Ueno H. Inhibition of transforming growth factor beta prevents progression of liver fibrosis and enhances hepatocyte regeneration in dimethylnitrosamine-treated rats. *Hepatology* 2000; 32: 247.

57. Imamura M, Ogawa T, Sasaguri Y, Chayama K, Ueno H. Suppression of macrophage infiltration inhibits activation of hepatic stellate cells and liver fibrogenesis in rats. *Gastroenterology* 2005; 128: 138.

58. Hata J, Ikeda E, Uno H, Asano S. Expression of hepatocyte growth factor mRNA in rat liver cirrhosis induced by N-nitrosodimethylamine as evidenced by in situ RT-PCR. *J Histochem Cytochem* 2002; 50: 1461.

59. Luckey SW, Petersen DR. Activation of Kupffer cells during the course of carbon tetrachloride-induced liver injury and fibrosis in rats. *Exp Mol Pathol* 2001; 71: 226.

60. Roland CR, Goss JA, Mangino MJ, Hafenrichter D, Flye MW. Autoregulation by eicosanoids of human Kupffer cell secretory products. A study of interleukin-1, interleukin-6, tumor necrosis factor-alpha, transforming growth factor-beta, and nitric oxide. *Ann Surg* 1994; 219: 389.

61. Xidakis C, Ljumovic D, Manousou P, Notas G, Valatas V, Kolios G, Kouroumalis E. Production of pro- and anti-fibrotic agents by rat Kupffer cells; the effect of octreotide. *Dig Dis Sci* 2005; 50: 935.

62. Seki E, De Minicis S, Osterreicher CH, Kluwe J, Osawa Y, Brenner DA, Schwabe RF. TLR4 enhances TGF-beta signaling and hepatic fibrosis. *Nat Med* 2007; 13: 1324.

63. Crawford SE, Stellmach V, Murphy-Ullrich JE, Ribeiro SM, Lawler J, Hynes RO, Boivin GP, Bouck N. Thrombospondin-1 is a major activator of TGF-beta1 *in vivo*. *Cell* 1998; 93: 1159.

64. Yang Z, Mu Z, Dabovic B, Jurukovski V, Yu D, Sung J, Xiong X, Munger JS. Absence of integrin-mediated TGFbeta1 activation *in vivo* recapitulates the phenotype of TGFbeta1-null mice. *J Cell Biol* 2007; 176: 787.

65. Munger JS, Huang X, Kawakatsu H, *et al.* The integrin alpha v beta 6 binds and activates latent TGF beta 1: a mechanism for regulating pulmonary inflammation and fibrosis. *Cell* 1999; 96: 319.

66. Wang JC, Novetsky A, Chen C, Novetsky AD. Plasma matrix metalloproteinase and tissue inhibitor of metalloproteinase in patients with agnogenic myeloid metaplasia or idiopathic primary myelofibrosis. *Br J Haematol* 2002; 119: 709.

67. Wang JC. Importance of plasma matrix metalloproteinases (MMP) and tissue inhibitors of metalloproteinase (TIMP) in development of fibrosis in agnogenic myeloid metaplasia. *Leuk Lymphoma* 2005; 46: 1261.

68. James C, Ugo V, Le Couedic JP, *et al.* A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. *Nature* 2005; 434: 1144.

69. Baxter EJ, Scott LM, Campbell PJ, *et al.* Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *Lancet* 2005; 365: 1054.

70. Kralovics R, Passamonti F, Buser AS, *et al.* A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med* 2005; 352: 1779.

71. Levine RL, Wadleigh M, Cools J, *et al.* Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell* 2005; 7: 387.

72. Iredale JP. Tissue inhibitors of metalloproteinases in liver fibrosis. *Int J Biochem Cell Biol* 1997; 29: 43.

73. Benyon RC, Arthur MJ. Extracellular matrix degradation and the role of hepatic stellate cells. *Semin Liver Dis* 2001; 21: 373.

74. Le Bousse-Kerdiles MC, Smadja-Joffe F, Klein B, Caillou B, Jasmin C. Study of a virus-induced myeloproliferative syndrome associated with tumor formation in mice. *Eur J Cancer* 1980; 16: 43.

75. Le Bousse-Kerdiles MC, Souyri M, Smadja-Joffe F, Praloran V, Jasmin C, Ziltener HJ. Enhanced hematopoietic growth factor production in an experimental myeloproliferative syndrome. *Blood* 1992; 79: 3179.

76. Villeval JL, Cohen-Solal K, Tulliez M, *et al.* High thrombopoietin production by hematopoietic cells induces a fatal myeloproliferative syndrome in mice. *Blood* 1997; 90: 4369.

77. Yan XQ, Lacey D, Hill D, Chen Y, Fletcher F, Hawley RG, McNiece IK. A model of myelofibrosis and osteosclerosis in mice induced by overexpressing thrombopoietin (mpl ligand): reversal of disease by bone marrow transplantation. *Blood* 1996; 88: 402.

78. Vannucchi AM, Bianchi L, Cellai C, *et al.* Development of myelofibrosis in mice genetically impaired for GATA-1 expression (GATA-1(low) mice). *Blood* 2002; 100: 1123.

79. Vannucchi AM, Bianchi L, Paoletti F, *et al.* A pathobiologic pathway linking thrombopoietin, GATA-1, and TGF-beta1 in the development of myelofibrosis. *Blood* 2005; 105: 3493.

80. Chagraoui H, Komura E, Tulliez M, Giraudier S, Vainchenker W, Wendling F. Prominent role of TGF-beta 1 in thrombopoietin-induced myelofibrosis in mice. *Blood* 2002; 100: 3495.

81. Wernig G, Mercher T, Okabe R, Levine RL, Lee BH, Gilliland DG. Expression of Jak2V617F causes a polycythemia vera-like disease with associated myelofibrosis in a murine bone marrow transplant model. *Blood* 2006; 107: 4274.

82. Lacout C, Pisani DF, Tulliez M, Gachelin FM, Vainchenker W, Villeval JL. JAK2V617F expression in murine hematopoietic cells leads to MPD mimicking human PV with secondary myelofibrosis. *Blood* 2006; 108: 1652.

83. Tiedt R, Hao-Shen H, Sobas MA, Looser R, Dirnhofer S, Schwaller J, Skoda RC. Ratio of mutant JAK2-V617F to wild-type Jak2 determines the MPD phenotypes in transgenic mice. *Blood* 2008; 111: 3931.

84. Hellerbrand C, Stefanovic B, Giordano F, Burchardt ER, Brenner DA. The role of TGFbeta1 in initiating hepatic stellate cell activation *in vivo*. *J Hepatol* 1999; 30: 77.

85. Xing S, Wanting TH, Zhao W, *et al.* Transgenic expression of JAK2V617F causes myeloproliferative disorders in mice. *Blood* 2008; 111: 5109-17.

86. George J, Roulot D, Koteliansky VE, Bissell DM. *In vivo* inhibition of rat stellate cell activation by soluble transforming growth factor beta type II receptor: a potential new therapy for hepatic fibrosis. *Proc Natl Acad Sci USA* 1999; 96: 12719.

87. Qi Z, Atsuchi N, Ooshima A, Takeshita A, Ueno H. Blockade of type beta transforming growth factor signaling prevents liver fibrosis and dysfunction in the rat. *Proc Natl Acad Sci USA* 1999; 96: 2345.

88. Cheever AW, Williams ME, Wynn TA, *et al.* Anti-IL-4 treatment of Schistosoma mansoni-infected mice inhibits development of T cells and non-B, non-T cells expressing Th2 cytokines while decreasing egg-induced hepatic fibrosis. *J Immunol* 1994; 153: 753.

89. Chiaramonte MG, Cheever AW, Malley JD, Donaldson DD, Wynn TA. Studies of murine schistosomiasis reveal interleukin-13 blockade as a treatment for established and progressive liver fibrosis. *Hepatology* 2001; 34: 273.

90. Chiaramonte MG, Donaldson DD, Cheever AW, Wynn TA. An IL-13 inhibitor blocks the development of hepatic fibrosis during a T-helper type 2-dominated inflammatory response. *J Clin Invest* 1999; 104: 777.

91. Fallon PG, Richardson EJ, McKenzie GJ, McKenzie AN. Schistosome infection of transgenic mice defines distinct and contrasting pathogenic roles for IL-4 and IL-13: IL-13 is a profibrotic agent. *J Immunol* 2000; 164: 2585.

92. Kaviratne M, Hesse M, Leusink M, *et al.* IL-13 activates a mechanism of tissue fibrosis that is completely TGF-beta independent. *J Immunol* 2004; 173: 4020.

93. Gastinne T, Vigant F, Lavenu-Bombled C, *et al.* Adenoviral-mediated TGF-beta1 inhibition in a mouse model of myelofibrosis inhibit bone marrow fibrosis development. *Exp Hematol* 2007; 35: 64.

94. Wagner-Ballon O, Pisani DF, Gastinne T, *et al.* Proteasome inhibitor bortezomib impairs both myelofibrosis and osteosclerosis induced by high thrombopoietin levels in mice. *Blood* 2007; 110: 345.

95. Pardanani A. JAK2 inhibitor therapy in myeloproliferative disorders: rationale, preclinical studies and ongoing clinical trials. *Leukemia* 2008; 22: 23.

96. Deeg HJ, Gooley TA, Flowers ME, *et al.* Allogeneic hematopoietic stem cell transplantation for myelofibrosis. *Blood* 2003; 102: 3912.

97. Higashiyama R, Inagaki Y, Hong YY, *et al.* Bone marrow-derived cells express matrix metalloproteinases and contribute to regression of liver fibrosis in mice. *Hepatology* 2007; 45: 213.

98. Inagaki Y, Higashiyama R, Okazaki I. Treatment strategy for liver fibrosis through recruitment and differentiation of bone marrow stem/progenitor cells. *Hepatol Res* 2007; 37: 991.

99. Lotersztajn S, Julien B, Teixeira-Clerc F, Grenard P, Mallat A. Hepatic fibrosis: molecular mechanisms and drug targets. *Annu Rev Pharmacol Toxicol* 2005; 45: 605.

100. Breitkopf K, Haas S, Wiercinska E, Singer MV, Dooley S. Anti-TGF-beta strategies for the treatment of chronic liver disease. *Alcohol Clin Exp Res* 2005; 29: 121S.