

Bone marrow-derived fibrocytes contribute to liver fibrosis

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Abstract

Chronic liver injury often leads to hepatic fibrosis, a condition associated with increased levels of circulating TGF- β 1 and lipopolysaccharide, activation of myofibroblasts, and extensive deposition of extracellular matrix, mostly collagen Type I. Hepatic stellate cells are considered to be the major¹ but not the only source of myofibroblasts in the injured liver.² Hepatic myofibroblasts may also originate from portal fibroblasts, mesenchymal cells, and fibrocytes.³ Since the discovery of fibrocytes in 1994 by Dr. Bucala and colleagues, this bone marrow (BM)-derived collagen Type I-producing CD45⁺ cells remain the most fascinating cells of the hematopoietic system. Due to the ability to differentiate into collagen Type I producing cells/myofibroblasts, fibrocytes were implicated in the pathogenesis of liver, skin, lung, and kidney fibrosis. However, studies of different organs often contain controversial results on the number of fibrocytes recruited to the site of injury and their biological function. Furthermore, fibrocytes were implicated in the pathogenesis of sepsis and were shown to possess antimicrobial activity. Finally, in response to specific stimuli, fibrocytes can give rise to fully differentiated macrophages, suggesting that in concurrence with the high plasticity of hematopoietic cells, fibrocytes exhibit progenitor properties. Here, we summarize our current understanding of the role of CD45⁺Collagen Type I⁺ BM-derived cells in response to fibrogenic liver injury and septicemia and discuss the most recent evidence supporting the critical role of fibrocytes in the mediation of pro-fibrogenic and/or pro-inflammatory responses.

Keywords: Fibrocytes, myofibroblasts, myeloid cells, liver fibrosis, collagen type I, bone marrow

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Introduction

Pathogenesis of liver fibrosis

Hepatic fibrosis is an outcome of many chronic liver diseases, including hepatitis B virus (HBV), hepatitis C virus (HCV), alcoholic liver disease and non-alcoholic steatohepatitis (NASH).¹ Hepatic fibrosis results from dysregulation of wound healing with accumulation of extracellular matrix (ECM), mostly collagen type I, leading to scar formation. Several early events play an important role in the pathogenesis of liver fibrosis, including: 1) damage to hepatic epithelial and endothelial cells; 2) release of TGF- β 1, the major fibrogenic cytokine; 3) increase of intestinal permeability and endogenous bacterial products; 4) recruitment of inflammatory cells; 5) induction of reactive oxygen species (ROS); and 6) generation of ECM producing myofibroblasts, which are not present in the normal liver.^{1,4}

Chronic liver injury causes increasing of intestinal permeability and leakage of bacterial products, such as liposaccharide (LPS), into the circulation. LPS binds TLR4 on immune cells, and specifically liver resident

macrophages, Kupffer cells, facilitating their activation and production of pro-fibrogenic cytokine TGF- β 1, a key activator hepatic myofibroblasts.^{5,6}

Hence, myofibroblasts represent a primary target for antifibrotic therapy. The origin of fibrogenic cells (myofibroblasts) has been intensively discussed and studied, and several sources of myofibroblasts have been identified.^{7–10} In the fibrotic liver, hepatic stellate cells (HSCs) have been reported to contribute to >80% of the collagen producing cells.¹ Therefore, HSCs are currently considered to be the major, but not the only, source of myofibroblasts in the injured liver.² Hepatic myofibroblasts may also originate from portal fibroblasts, BM-derived mesenchymal cells and fibrocytes.³ Two mechanisms recently implicated in fibrogenesis are the epithelial-to-mesenchymal transition (EMT), when epithelial cells acquire features of mesenchymal cells and may give rise to fully differentiated myofibroblasts,^{11–14} and endothelial-to-mesenchymal transition (EndMT), when endothelial cells undergo a similar phenotypic change.^{15,16}

Liver fibrosis is characterized by intensive remodeling of liver tissue, formation of fibrous scar and appearance of

collagen Type I producing myofibroblasts.¹ Fibrogenic myofibroblasts are not present in the normal liver, but differentiate from other cellular populations in response to chronic liver injury. Although heterogeneous in their origin, hepatic myofibroblasts share similar cellular characteristics, such as expression of abundant pericellular matrix and fibrotic genes (vimentin, α -smooth muscle actin (α -SMA), non-muscle myosin, fibronectin).^{17–20} Studies of fibrogenesis conducted in different organs strongly suggest that resident myofibroblasts are the primary source of ECM.⁴ In response to fibrogenic stimuli, such as TGF- β 1, myofibroblasts may differentiate from other cell types, which under physiological conditions do not produce and secrete ECM.¹ Based on the extensive studies from our laboratory^{21–25} and others^{26–29} three sources of hepatic myofibroblasts critically contribute to liver fibrosis of distinct etiologies: hepatic stellate cells (HSCs), portal fibroblasts (PFs) and bone marrow-derived collagen producing fibrocytes.^{3,30} In response to various types of injury, the composition of myofibroblasts appears to be different.

Hepatic Stellate cells

HSCs are believed to be a major source of myofibroblasts induced during chronic toxic liver injury in patients³¹ and in experimental models of toxic liver injury in mice, such as administration of hepatotoxin carbon tetrachloride (CCl₄) or intragastric alcohol feeding (Tsukamoto-French model).^{4,32} Quiescent HSCs (qHSCs) are perisinusoidal cells that normally reside in the space of Disse and contain numerous retinoid and lipid droplets.^{33,34} Under physiological conditions, HSCs reside in the space of Disse, exhibit a quiescent phenotype and express neural markers, such as glial fibrillar acidic protein (GFAP), synamin, synaptophysin,¹ nerve growth factor receptor p75,^{35,36} secrete HGF, and store vitamin A.³⁷ HSCs are also implicated in phagocytosis and antigen presentation.^{38,39} In response to injury, qHSCs lose Vitamin A containing oil droplets, acquire contractility and activate into collagen type I- and α -SMA-expressing myofibroblasts.^{1,22,31,40–46} Upon upregulation of typical myofibroblast-like markers, HSC-derived myofibroblasts can still be identified by residual expression of GFAP and vitamin A, and expression of Desmin, PDGFR β and p75 (which are absent in myofibroblasts of other origins and therefore serve as HSC-specific markers).^{21,41,47} **Portal fibroblasts.** Portal fibroblasts are spindle-shaped “periductular fibroblasts” (or portal/periportal mesenchymal cells that are not related to sinusoids”) and participate in the turnover of ECM under physiological conditions.^{2,27,48–50} Very few portal fibroblasts are present in the normal liver. In response to cholestatic liver injury caused by primary and secondary biliary cirrhosis in patients, or experimental model of bile duct ligation (BDL) in mice,^{27,51–54} Portal fibroblasts proliferate and differentiate into α -SMA-expressing myofibroblasts (aPFs). Studies, including those from our laboratory,²¹ have demonstrated that aPFs significantly contribute to collagen Type I deposition, and secrete factors that promote activation of HSCs into myofibroblasts.²¹ aPFs can be identified by expression of gremlin, Thy1, fibulin 2, IL-6, elastin, the ecto-ATPase

nucleoside triphosphate diphosphohydrolase-2 (NTPD2), and cofilin-1. Our recent data suggest that aPFs also express mesothelin, asporin, and uroplakin 1 β ,²¹ although the importance of these proteins for aPF functions remains to be characterized. In addition, aPFs lack expression of desmin, cytoglobin, α 2-macroglobulin, neural proteins (GFAP, p75, synaptophysin), and lipid droplets distinguishing them from HSCs.^{1,55–59}

EMT

Although the contribution of EMT to fibrogenic myofibroblast has been recently questioned, this topic remains controversial. Several evidence indicated that, under prolonged *in vitro* culturing, hepatocyte and cholangiocytes upregulate myofibroblast marker α SMA and suppress epithelial cellular marker.^{3,11,12} Hence, fate mapping-based studies have clearly demonstrated that hepatocytes and cholangiocytes, or their precursors do not undergo EMT in response to experimental models of liver fibrosis and do not give rise to myofibroblasts.^{60–62} These studies have shown that genetic labeling of hepatocytes (using Albumin-Cre mice), cholangiocytes (using cytokeratin 19 (K-19)-Cre mice) and their precursors did not yield generation of myofibroblasts *in vivo*. Furthermore, expression of S100A4 (Fsp1), a marker which is associated with EMT progression, and widely used to detect cells undergoing EMT, has been found to be expressed not only by subsets of fibroblasts but also by myeloid cells, suggesting that the role of this protein in pathogenesis of liver fibrosis has to be reevaluated.

Fibrocytes

Development of hepatic fibrosis is closely associated with increased intestinal permeability and diffusion of bacterial products into the blood.⁶ TGF- β 1 and endogenous lipopolysaccharide (LPS) induce rapid recruitment of bone marrow (BM) derived cells to the injured liver.³ Fibrocytes are one population of BM-derived cells that are recruited to an injured organ (Figure 1). Fibrocytes were first described as “CD45 and collagen type I (Col⁺) expressing leukocytes that mediate tissue repair and are capable of antigen presentation to naive T cells.”⁶³ Although fibrocytes were initially identified in fibrotic tissues, they have also been isolated from peripheral blood. Due to their ability to differentiate into α -smooth muscle actin (α -SMA)⁺ myofibroblasts *in vitro* and *in vivo*, fibrocytes are implicated in the fibrogenesis of skin, lungs, kidneys, and liver fibrosis.^{3,64,65} It has been proposed that human serum amyloid protein (hSAP) inhibits fibrocyte differentiation into myofibroblasts,⁶⁶ and may provide a potential therapy to prevent fibrocyte recruitment into injured tissues.⁶⁷ In addition to collagen, fibronectin and vimentin, fibrocytes express CD45, CD34, MHCII, MHCI, CD11b, Gr1, Ly6c, CD54, CD80, CD86, CCR2, CCR1, CCR7, CCR5 and secrete growth factors (TGF- β 1, MCP-1) that promote deposition of ECM,^{68,69} indicating that fibrocytes indeed exhibit dual characteristics of fibroblasts and hematopoietic cells. In response to liver injury, fibrocytes also migrate to the lymphoid organs, such as spleen,⁷⁰ suggesting that

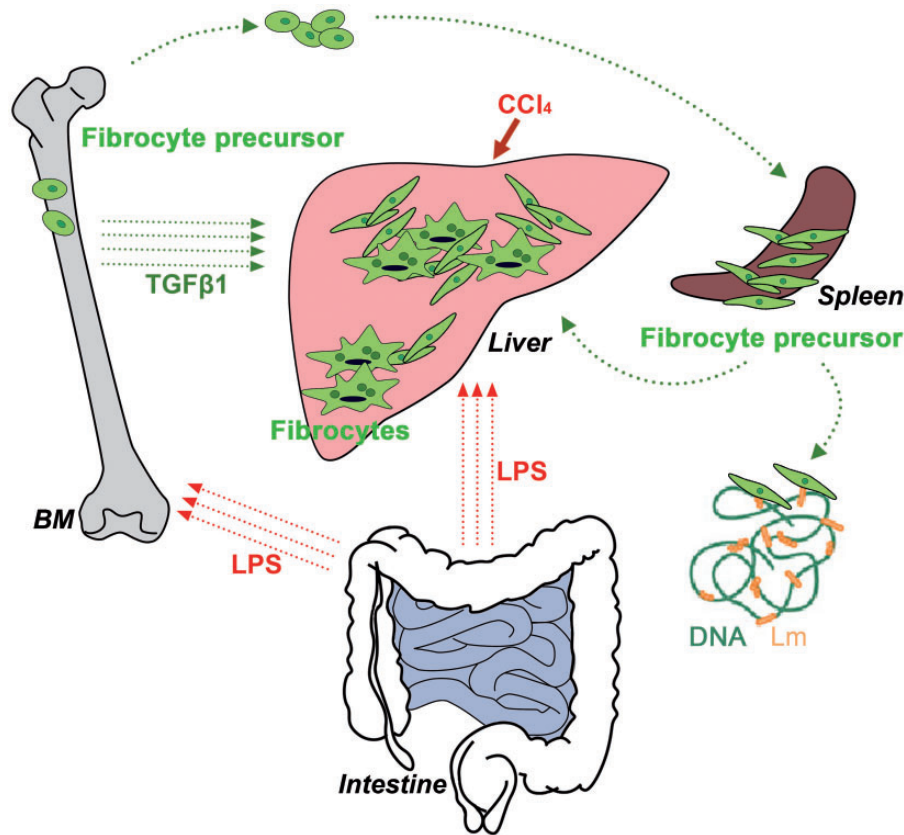


Figure 1 Contribution of fibrocytes to liver fibrosis. Toxic liver injury causes death of hepatocytes, which form formation of apoptotic bodies and release factors, including TGF- β 1, that trigger activation of systemic inflammatory response, increase of intestinal permeability and release of bacterial LPS into the blood stream. Pro-inflammatory cytokines, TGF- β 1, and LPS promote proliferation of CD45⁺ Collagen Type I⁺ fibrocytes in the bone marrow (BM). BM fibrocytes migrate to the spleen, where they undergo maturation/activation. Spleen is considered to serve as a storage of fibrocytes. Splenic fibrocytes possess antimicrobial properties and are capable of entrapping and killing bacteria. In response to chronic liver injury, fibrocytes migrate to fibrotic liver, where they give rise to number of myofibroblasts.

fibrocyte function may not be limited to ECM deposition. While the roles of fibrocytes in wound healing and fibrosis has been studied, fibrocyte functions related to their hematopoietic origin have not been explored. Here we refer the “fibrocyte precursors” as bone marrow (BM)-resident, circulating, and splenic CD45+Col+ cells, while we define “fibrocytes” or “mature fibrocytes” as tissue (liver resident) fibrocytes as they become Collagen Type I producing cells in fibrotic liver.

This review will discuss the current understanding of fibrocyte biology and outline future prospects of using fibrocytes as targets for anti-fibrotic therapy.

Contribution of fibrocytes to chronic liver injury

BM-derived fibrocytes migrate to fibrotic livers

Development of hepatic fibrosis is closely associated with increased intestinal permeability and diffusion of bacterial products into the blood.⁶ TGF- β 1 and endogenous lipopolysaccharide (LPS) induce rapid recruitment of BM-derived cells to the injured liver.³ Fibrocytes are hematopoietic cells that are recruited to the injured liver.⁶³ Although fibrocytes were initially identified in fibrotic tissues, they have also

been isolated from peripheral blood. Originating in the BM, fibrocytes comprise a small subset (0.1%) of mononuclear cells, and are defined by simultaneous expression of CD45 and collagen type I. Upon injury or stress, fibrocytes proliferate and migrate to the injured organ.^{63,68}

Fibrocytes are implicated in fibrogenesis of parenchymal organs

Based on original observations, human fibrocytes isolated from peripheral blood have a spindle-like shape but obtain a myofibroblastic phenotype upon differentiation on plastic or in response to TGF- β 1.^{64,68,71,72} Since then, several studies have implicated fibrocytes in the pathogenesis of fibrogenesis of parenchymal organs.^{64,71,72} Fibrocytes have mostly been studied in pulmonary fibrosis in patients with IPF (idiopathic pulmonary fibrosis). Elevated numbers of circulating fibrocytes in the blood of patients with IPF correlates with the severity of the disease and may serve as a prognostic marker.⁷³ In a bleomycin-induced model of pulmonary fibrosis in mice, the number of BM-derived fibrocytes recruited to the injured lung constituted up to 25% of the collagen producing cells,³ suggesting that fibrocytes play an important role in the development of pulmonary fibrosis.⁷⁴ BM-derived collagen producing cells were also shown to

migrate to fibrotic kidney, where they contribute up to 15% of the total myofibroblast population.³ In addition, activation and trafficking of fibrocytes may be important in the pathogenesis of nephrogenic systemic fibrosis.⁷⁵ On the other hand, a collaborative study with our lab demonstrated that interstitial fibrosis induced by ureteral obstruction recruit only a small number of fibrocytes to fibrotic kidneys, and fibrocyte recruitment does not significantly contribute to collagen deposition.⁷⁶ Our laboratory has shown that fibrocytes migrate to the liver in response to toxic (carbon tetrachloride, CCl₄) and cholestatic (BDL) injury of liver fibrosis.^{24,70} Hepatic fibrocytes comprise only $\approx 4\text{--}6\%$ of the collagen type I-producing cells⁷ in these models of liver fibrosis, and *in situ* differentiate into $\alpha\text{-SMA}^+$ myofibroblasts⁷⁷ (Figure 1). Based on the results from two models of liver injury, we conclude that fibrocyte recruitment to the liver is a universal mechanism in the pathogenesis of liver fibrosis. However, contribution of fibrocytes to hepatic myofibroblasts activated in response to BDL or CCl₄ might be minimal (compared to the numbers of activated Hepatic Stellate cells and/or Portal fibroblasts), and therefore, fibrocytes might not serve as a significant source of ECM and Collagen Type I deposition. However, that might not be true for liver fibrosis of different etiologies. In fact, an independent research group assessed fibrocyte recruitment in *Abcb4* knockout mice, a genetic model of spontaneous liver fibrosis, and observed a significant flux of fibrocytes to the liver in these mice, such that fibrocytes contributed $\approx 50\%$ to the liver myofibroblast population.⁷⁸ Although the mechanism of fibrocyte recruitment caused by this genetic deficiency is not completely understood, it demonstrates that fibrocytes have a strong potential to differentiate into myofibroblasts under specific circumstances. For example, adoptively transferred fibrocytes that differentiate into collagen producing cells when cultured on plastic, exacerbate pulmonary fibrosis in mice.⁷¹ This phenomenon emphasizes fibrocyte plasticity, fibrogenic potential and organ-specific differentiation. The number of circulating fibrocytes correlates with the severity of fibrosis induced by hepatitis C virus (HCV)⁷⁹ and lung fibrosis,⁷⁴ suggesting that fibrocytes may serve as a prognostic marker, and become a novel target for anti-fibrotic therapy in fibrosis.

Migration of fibrocytes is mediated by chemokine receptors

Fibrocytes migrate to sites of tissue injury where they give rise to fibroblast-like cells.⁶⁸ Egress of fibrocytes from the BM and homing to peripheral organs is regulated on several levels, including profibrogenic growth factors (e.g. TGF- β 1) and chemokines (CCL2, CCL3, and CCL12).⁸⁰ Fibrotic changes in the injured lung are closely associated with elevated levels of TGF- β 1, one of the most potent fibrogenic factors for all parenchymal organs. Consistent with this, fibrocytes express TGF- β -RI and II, and stimulation with TGF- β 1 triggers fibrocyte mobilization and differentiation into $\alpha\text{-SMA}^+$ myofibroblasts, suggesting that TGF- β 1 is a chemoattractant and activator of fibrocytes.⁸⁰ In addition, other chemokines (CCL2, CCL3, CCL12, and CCL21) bind

and activate their cognate receptors to regulate fibrocyte migration into fibrotic tissues.^{69,74} Chemokines in the CC family promiscuously bind multiple receptors.⁶⁵ Thus, CCL2, CCL7 and CCL12 signal through the CCR2 chemokine receptor; CCL19 and CCL21 signal through CCR7; while CCL3 binds CCR1 and CCR5.^{65,68,71} Recruitment of fibrocytes is attenuated in CCL3 and CCR5 deficient mice, but not in CCR1^{-/-} mice, suggesting that signaling through CCL3-CCR5 axis facilitates fibrocyte recruitment to fibrotic lungs.⁸¹ Moreover, CCR2 deficient mice are protected from fluorescein isothiocyanate (FITC)-induced lung fibrosis. Protection from fibrosis correlates with impaired recruitment of fibrocytes, which is mostly mediated by the CCL12-CCR2 axis.^{82,83} Fibrocytes are detected in the human fibrosing diseases, nephrogenic fibrosing dermopathy and renal fibrosis.⁸⁴ Upon migration to fibrotic kidneys, fibrocytes express chemokine receptors such as CCR7, CXCR4, and CCR2.^{84,85} Experimental renal fibrosis studies demonstrate that CCR7 is involved in fibrocyte recruitment to the fibrotic kidney,⁸⁴ supporting the notion that CCL21-CCR7 axis may have an important role in the recruitment of fibrocytes to fibrotic kidney.

Despite extensive studies, the mechanism of fibrocyte recruitment to fibrotic tissues is not completely understood. In particular, the mechanism of fibrocyte homing into fibrotic liver has not been elucidated. However, migration of fibrocytes is highly restricted to the damaged organs. Thus, bile duct ligation-induced liver injury triggers fibrocyte migration to the liver, but not kidney or lungs.⁷⁰ Therefore, differential expression of chemokines or their receptors may regulate fibrocyte homing specifically to the injured organ. In concordance, our study of chimeric mice, in which BM-derived fibrocytes express Luciferase (and could be monitored in real time in live mice upon luciferin administration) had demonstrated that homing of fibrocytes to fibrotic liver depends on CCR2 (and in part CCR1) expression.²³ Based on our results and other reports,^{86,87} CCR2 may be a general regulator of fibrocyte migration to fibrotic organs. Interestingly, using the same BM-chimeric Luciferase reporter mice, we have demonstrated that fibrocytes are recruited to fibrotic liver in two phases. In response to toxic liver injury, the peak of fibrocytes migration to the damaged liver has been observed 2 weeks after the onset of injury. The second peak of fibrocyte flux was observed after cessation of toxic liver injury, similar to that demonstrated for CD11b⁺ monocytes,⁸⁸ suggesting that fibrocytes might also have a role in regression of liver fibrosis. This observation is consistent with myeloid origin of fibrocytes. Only recently have fibrocytes been linked to circulating monocytes.⁶⁹ However, the hematopoietic origin and hematopoiesis-related functions of fibrocytes have not been well investigated.

Fibrocytes are antigen-presenting cells

Expression of CD45, HLA-DR, and co-stimulatory molecules by circulating human fibrocytes has prompted a study of their antigen presenting properties.⁶⁸ In addition to MHC II, human fibrocytes express CD11a, CD11b, CD18, CD54, CD71, CD80, and CD86, suggesting that they express

the full variety of surface proteins required for antigen presentation.^{68,89} Although several tissue-derived cells (e.g. fibroblasts, endothelial cells, melanocytes) present antigen to memory T cells,⁸⁹ human fibrocytes possess unique features and are capable of sensitization of naïve CD4 T cells,⁸⁹ a function that had been attributed to dendritic cells (DC), professional antigen presenting cells.⁹⁰ However, fibrocytes differ from dendritic cells due to expression of collagen type I⁺, CD13⁺, CD34⁺, and lack of CD25,⁹¹ CD10, and CD38 surface antigens. In addition, fibrocytes are an actively proliferating cellular population, whereas dendritic cells are usually terminally differentiated. Similar to human fibrocytes, murine fibrocytes are potent stimulators of antigen-specific T cells *in vitro* and *in vivo*, capable of migrating to lymph nodes and *in situ* sensitizing naïve CD4 T cells.⁸⁹ Moreover, fibrocytes can stimulate anti-virus cytotoxic CD8 T cells and induce their proliferation.⁹² The fact that fibrocytes have a specialized and potent antigen presentation activity suggests that they may play a critical role in the initiation of immunity during tissue injury and repair.⁸⁹

Development of liver injury is accompanied by recruitment of fibrocytes to the spleen

Development of liver fibrosis is strongly associated with elevated levels of TGF- β 1, increased intestinal permeability and release of endogenous LPS (Figure 1). In addition to the injured organ, recruitment of CD45⁺Col⁺ fibrocytes to the spleen has been documented in liver^{93,94} and kidney fibrosis.⁸⁴ Hepatotoxic injury (CCl₄), cholestatic injury (BDL), administration of TGF- β 1, and administration of LPS trigger migration of fibrocytes from the BM to the spleen and liver.⁹⁵ Moreover, spleens were suggested to function as a major reservoir of immature fibrocytes.⁹⁶ Splenic CD45⁺Col⁺ fibrocytes express myeloid markers and resemble CD115⁺CD11b⁺ monocytes.⁹⁵ Although the biological significance of splenic fibrocytes is not understood, our recent study suggests that CD45⁺Col⁺ fibrocytes are capable of differentiating according to the changing micro-environment, giving rise to different subtypes of fibrocyte-like cells with distinct roles during tissue repair and fibrosis.⁹⁷ Further insights into the function of splenic fibrocytes were provided by gene expression microarray which compared splenic fibrocytes to quiescent and activated macrophages.²⁴ Unexpectedly, splenic fibrocytes expressed genes that are preferentially found in quiescent and activated macrophages and are functionally linked to wounding, cytokine metabolism, chemotaxis, and antigen presentation. However, even though fibrocytes expressed lineage-specific markers (CD11b, Gr-1), they lacked markers of maturation (CD68, CD14), uniquely expressed fibroblast-like genes (collagen 1 α 1, collagen 7 α 1, collagen 11 α 2 and collagen 14 α 1, TGF β -RI and RII and TGF- β 1). Further analysis of splenic fibrocytes generated the concept that they are poised for transmigration, and contribute to antimicrobial responses and antigen presentation. First, expression of mRNAs encoding S100A9/A8, CD11b, ICAM-1, ICAM-2, VCAM-1, β -integrins, proteoglycan 2, RAGE, CD36 and mannose receptor genes makes splenic fibrocytes highly suitable for transmigration through the blood

stream.^{98–100} Second, fibrocytes express many genes that encode antimicrobial enzymes and peptides (myeloperoxidase, lactotransferrin, α -defensins, chitinase like 3 and 4, lipocalin, lysozymes, complement 3, cathelicidin), typically produced by myeloid or other immune cells to confine bacterial spread and kill microbial pathogens.^{101–104} Third, expression of mRNAs that encode cytotoxic granule proteins (perforins and granzymes), lysosome-associated peptides (serglycin, CD63, CD107, CD81, CD9), and leukocyte peptidase inhibitors (Slpi, Serpina 3F), to prevent autolysis, link fibrocytes to cytotoxic effector cells.^{105,106} Fourth, consistent with earlier findings, expression of H2-K1, H2-Q8, H-13, CD83, CD86 and CD11b genes, associates with antigen presentation, and links fibrocytes to adaptive immunity functions.^{107,108}

Anti-microbial defense provided by neutrophils, macrophages and fibrocytes

Microbial products have a significant impact on liver fibrosis,⁶ Translocation of bacterial products from the intestine into the portal circulation synergistically facilitate other fibrogenic factors such as TGF- β 1, oxidative stress, and mechanical injury.⁴ Effector cells such as neutrophils and macrophages have well-documented antimicrobial functions.^{109,110} Neutrophils are terminally differentiated cells that are programmed to die a few hours after they enter into circulation.¹¹¹ In response to inflammatory stimuli, circulating neutrophils migrate to infected tissues, where they efficiently bind, engulf, and inactivate bacteria.¹¹¹ Neutrophils, as well as macrophages, primarily kill bacteria by phagocytosis. Neutrophils continuously synthesize multiple cytotoxic granules, containing cathelicidin, neutrophil elastase, cathepsin G, myeloperoxidase, lactoferrin and gelatinase. Activated neutrophils degranulate, release inflammatory and pro-fibrogenic cytokines, and apoptose. A variety of antimicrobial peptides have also been detected in macrophages.^{101–104} For example, lysozymes, complement 3 and myeloperoxidase, kill bacteria directly upon contact,¹¹² or make conductive pores in bacterial membranes (such as cathelicidin and α -defensins).¹⁰¹ An additional mechanism of antibacterial activity has recently been discovered in phagocytic cells, the formation of bacteriocidal extracellular traps, and has been recognized as an important mechanism of the host innate immune response against infections.¹¹⁰ Originally identified in neutrophils,¹¹¹ phagocytosis-independent formation of DNA traps has been described in mast cells and eosinophils.^{113,114} In response to bacterial infection, neutrophils release extracellular nuclear DNA-based structures into the environment to entrap and kill bacteria. They secrete cathelicidin, myeloperoxidase, lactoferrin and histones into the DNA-based framework to aid in bacterial killing. Formation of DNA traps is accompanied by death ("ETosis") of neutrophils and mast cells.¹⁰⁹ Antimicrobial activity mediated by the formation of DNA-traps has been discovered only recently,¹¹¹ but has already been linked to several pathological conditions. Thus, several studies have provided *in vivo* evidence of DNA-trap formation during infections,

including appendicitis, pneumococcal pneumonia, and sepsis (reviewed in¹¹⁰).

Until recently, fibrocytes have not been implicated in any direct antimicrobial activity. Despite a similarity in the expression of surface markers (CD11b, Gr-1, F4/80) with cells of myeloid lineage, fibrocytes lack phagocytic activity and markers of maturation CD14, CD68, and CD11c.⁶⁴ Consistent with this observation, infection with *Listeria monocytogenes* (*Lm*), a Gram⁺ bacterial pathogen that infects liver and spleen and induces the recruitment of inflammatory monocytes,¹¹⁵ also causes migration of fibrocytes specifically to spleen of *Lm*-infected mice, supporting the observation that fibrocytes may mediate innate immunity. Remarkably, significant number of fibrocytes was recruited to spleens and livers (but not kidneys) just 24 h post infection, and correlated with bacterial load, indicating that infection triggered a proportional fibrocyte migration to the target organs. Furthermore, isolated splenic fibrocytes lacked phagocytic activity, but when co-cultured with *L. monocytogenes*, released extracellular traps (Figure 1), formed by DNA fibers and containing the antimicrobial peptide cathelicidin (mCRAMP, a critical effector of mammalian innate immunity against invasive bacterial pathogens including *L. monocytogenes*),¹⁰¹ and effectively entrapped and killed bacteria. In addition to secreted and DNA-bound mCRAMP, the cathelicidin peptide was expressed in the cytoplasm of splenic fibrocytes. Similar to neutrophils, formation of these traps was accompanied by death of fibrocytes ("ETosis"),¹¹⁶ determined by release of nuclear (vs. mitochondrial) DNA. Formation of extracellular traps by splenic fibrocytes was associated with reduction of bacterial viability, directly implicating splenic fibrocytes in mediation of immediate innate antimicrobial responses at the site of infection.

Fibrocytes are not macrophages

As mentioned above, fibrocytes express myeloid cell markers, but are not terminally differentiated, and are not capable of killing bacteria by phagocytosis. Interestingly, when cultured in the presence of M-CSF, splenic fibrocytes downregulated expression of collagen- $\alpha 1(I)$ and differentiated into Col⁻CD11b⁺CD14⁺F4/80⁺ macrophages, capable of phagocytosing *E. coli* as efficiently as BM-derived macrophages.²⁴ Furthermore, adoptive transfer of purified CD45.2⁺ splenic fibrocytes into CD45.1⁺ mice produced a mixed population of CD11b⁺F4/80⁺, CD11b⁺CD11c⁺, and CD11b⁺Gr-1⁺ cells, indicating that fibrocytes might possess progenitor properties and their differentiation/maturation is associated with the downregulation of collagen expression.²⁴ Collectively, these observations propose that fibrocytes may serve as a significant source of mature myeloid cells.

Collagen $\alpha 1(I)$, Cytoskeletal proteins and Adhesion Molecules Facilitate Fibrocyte Migration

Up-to-date it remains unknown why fibrocytes express Collagen Type I, and how expression of Collagen Type I affects fibrocyte functions. On one hand, release of collagen fibers into DNA-based extracellular traps was observed in

fibrocytes exposed to *L. monocytogenes*.²⁴ We can speculate that collagen fibers may serve as a scaffold to support these DNA-traps to maximize bacterial killing. On the other hand, egress of fibrocytes from the BM is associated with rearrangement of cytoskeletal proteins required for transmigration. We can speculate that expression of collagen (and other fibroblast-specific proteins) may facilitate the ability of fibrocytes to rapidly respond to emerging changes in their environment.

Hematopoietic cells have specific machinery to migrate through the bloodstream,¹¹⁷ including 1) specific cytoskeletal proteins (such as cytoplasmic β -actin, α -filamin and tubulin- $\beta 5$) that coordinate cellular conformation according to changes in the environment, 2) different types of intracellular or secreted matrix proteins (collagens and fibronectins) that together with 3) adhesion molecules (CD11b, ICAM-1, ICAM-2, VCAM-1, β -integrins, proteoglycan 2, RAGE, CD36) facilitate transmigration of circulating cells. Typically, cells of myelo-monocytic lineages upregulate such genes. For example, S100A9/A8 proteins (Calprotectin) are involved in chemotaxis and transendothelial migration of myeloid cells to the site of inflammation. On the other hand, β -integrins and mannose receptors, which represent distinct families of collagen-receptors, contribute to cellular binding and migration through the collagen-rich stroma.^{99,118} In addition, macrophages were suggested to upregulate and secrete collagen type VIII and VI and other collagens,¹¹⁹ but their contribution to collagen production in fibrotic livers seems to be rather insignificant.⁸⁸ Inability to migrate and impaired wound healing was reported in fibroblast strains obtained from patients with Ehlers-Danlos syndrome (EDS) bearing mutations in COL5A1 allele.¹²⁰ Therefore, besides cytoprotection,¹²¹ expression of collagen may facilitate cell migration. In support of this notion, expression of matrix proteins have been reported in progenitor cells¹²² and circulating monocytes and macrophages, and appear to modulate cell-cell and cell-matrix interactions.¹¹⁹

Conclusions

Recent studies have provided convincing evidence that fibrocytes play an important role in mediation of pro-fibrogenic and pro-inflammatory signals in fibrotic liver. Both TGF- $\beta 1$ and LPS play a critical role in liver fibrogenesis, and these factors also appear to trigger fibrocyte recruitment to the injured liver, and promote their differentiation into collagen Type I producing myofibroblasts. Meanwhile, fibrocytes recruited to the spleen in response to acute liver injury, septicemia or bacterial infection are involved in regulation and mediation of innate immune responses rather than promoting *in situ* ECM deposition. Future studies are required to gain a better insight in fibrocyte functions, plasticity and differentiation dependent on surrounding microenvironment.

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Abbreviations

Col	collagen α 1(I)
α -SMA	α -smooth muscle actin
BDL	bile duct ligation
CCl ₄	carbon tetrachloride
qHSCs	quiescent Hepatic Stellate Cells
aHSCs	activated Hepatic Stellate Cells

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