Immunopathogenesis of Liver Cirrhosis

35

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Abbreviations

ACLF	Acute-on-chronic liver failure
ASK1	Apoptosis signal – regulating kinase 1
BAMBI	Bone morphogenetic protein (BMP) and activin membrane-bound inhibitor homolog
CCR2	C–C motif chemokine receptor 2
DAMP	Danger-associated molecular pattern
ER	Endoplasmic reticulum
EV	Extracellular vesicle
HMGB – 1	High – mobility group box 1
HSC	Hepatic stellate cell
IFN	Interferon
IL	Interleukin
MAIT	Mucosal-associated invariant T cells
Mdr2	Multidrug resistance gene 2
mtDNA	Mitochondrial DNA
NK	Natural killer
PAMP	Pathogen-associated molecular pattern
PDGFR	Platelet-derived growth factor receptor
STAT3	Signal transducer and transcription factor 3
TGF	Transforming growth factor
TIMP – 1	Tissue inhibitor of metalloproteinases-1
TLR	Toll-like receptor
TNFα	Tumor necrosis factor alpha
TRAIL	TNF-related apoptosis-inducing ligand
UPR	Unfolded protein response

Key Points

- Liver cirrhosis represents the end stage of chronic liver disease, characterized by excessive scar tissue (fibrosis), intense inflammatory cell infiltration, and liver loss-of-function, leading to multiple organ failure.
- Fibrogenic cells in the liver may be of different origins, but activated stellate cells represent the main source of extracellular matrix components.
- Fibrogenic cells sense changes in their microenvironment especially under inflammatory conditions and respond to a plethora of inflammatory stimuli including cytokines, danger-associated molecular patterns, and pathogen-associated molecular patterns.
- Both innate and adaptive immune cells are actively participating in the initiation, progression, and resolution of liver fibrosis. Current efforts are made to decipher the complex interplay between the immune system and liver disease.
- Immunomodulation represents a promising therapeutic approach in the control of liver fibrosis.

The classical development of chronic liver diseases, whatever the etiology, follows a well-established pattern. Chronic or severe acute liver injury leads to the initiation of various inflammatory processes that comprise the activation of local immune cells, as well as the recruitment and activation of circulating cells. The liver also possesses potent regenerative capacities, characterized by the ability for parenchymal or liver-resident progenitor cells to proliferate in response to hepatic function alteration. Additionally, fibrogenic cells of different origins may be activated and deposit scar tissue. When tissue scarring is excessive, this is termed "fibrosis" and serves as the soil for advanced liver disease or cirrhosis, ultimately leading to liver failure.

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A considerable amount of data supports the limitless relevance of studying the interactions between liver fibrosis and inflammation, in terms of virtually all immune cell types and phenotypes as well as immunological processes performed by professional or non-professional immune cells. Accordingly, new discoveries are made on a regular basis in the field of liver immunology and fibrosis and will further increase our knowledge of all the finely tuned cellular processes implicated and consequently lead to numerous advances for patients in the near future.

What Is Liver Fibrosis, and How Does It Evolve to Liver Cirrhosis?

Liver cirrhosis is defined as a final stage of chronic liver disease in which excessive parenchymal cell necrosis and scar tissue accumulation impedes blood flow, leading to liver loss-of-function and, consequently, the accumulation of toxics in the blood mainstream. Every year about 5–7% of previously asymptomatic cirrhotic patients exhibit multiple organ failure when decompensation has occurred [1, 2]. Complications include ascites, peritonitis, hepatic encephalopathy, hepatorenal syndrome, hepatopulmonary syndrome, and hypersplenism. Another important risk for cirrhotic patients is acute-on-chronic liver failure (ACLF), described as acute decompensation and a high short-term mortality occurring after an acute insult (e.g., drug-induced liver injury, viral hepatitis, or alcohol consumption) on a compensated cirrhotic liver [2, 3]. Importantly, systemic inflammation is constantly observed in patients with decompensated cirrhosis and ACLF patients, emphasizing the close interaction between immune cells and pathogenesis [2, 4]. Histologically, cirrhosis is characterized by regenerative nodules representing an attempt of the remaining liver cells to regenerate the organ and restore liver functions. These regenerative nodules are surrounded by fibrous septa made of extracellular matrix bridging the portal tracts. The fibrotic tissue is densely composed of fibrogenic cells, innate and adaptive immune cells, and pseudo-ductular structures, in a process termed as ductular reaction (see below) [5]. Hence, liver fibrosis and accompanying inflammation and ductular cell proliferation may be regarded as unbalanced regenerative processes [6]. At later stages, liver transplant may represent the only therapeutic option for cirrhotic patients, which represents a challenge due to organ donor shortage.

Virtually any chronic liver disease can lead to cirrhosis, whether caused by alcohol abuse, viral hepatitis, chemical toxicity, autoimmune liver diseases, non-alcoholic fatty liver disease, or genetic predispositions, among others. Whatever the etiology, the common soil for liver cirrhosis is fibrosis [3]. Thus, a lot of efforts are being put toward limiting or even reversing liver fibrosis. Among the different strategies, fibrogenic cell inhibition and immune system modulation represent the most promising approaches.

Liver fibrosis is defined as an excessive accumulation of extracellular matrix, mainly consisting of type I and III collagens, laminin, and hyaluronic acid. This scar tissue progressively occupies larger areas and replaces functional liver parenchyma. Myofibroblasts derived from activated hepatic stellate cells (HSCs) are considered to be the main source of extracellular matrix in the liver [7, 8]. HSCs are located in the space of Disse in the healthy liver, between the hepatic sinusoids lined by liver sinusoidal endothelial cells, and the basolateral surface of the hepatocytes. One of their main functions in the healthy liver is the storage of retinoids (vitamin A) in perinuclear droplets. Upon activation by various stimuli (detailed below), HSCs progressively lose their vitamin A droplets and adopt a myofibroblast phenotype notably defined by intense extracellular matrix deposition, alpha-smooth muscle actin expression, as well as migratory properties [8]. Myofibroblasts are characterized by their contractility, participating in the increase of portal resistance observed upon liver fibrosis. This contractility is induced by enthothelin-1 and angiotensin-II [9, 10]. The signals that activate or inhibit HSCs have been extensively reviewed previously [11].

Collagen deposition typically occurs around the remains of hepatic parenchyma and fibrotic septa expand from the periportal area in advanced stages of liver fibrosis, a feature referred to as bridging fibrosis. It is thus remarkable that HSCs, considered to be the main producers of extracellular matrix, seem to migrate to the perilobular areas before laying down collagen fibers. These facts raised doubts on the origin of the putative fibrogenic cells in the liver. Thus alternative cellular sources of fibrogenic cells have been identified, namely, portal fibroblasts and bone-marrow-derived circulating fibrogenic cells, or fibrocytes, among other potential candidates [12–16]; even epithelial cells (i.e., hepatocytes and biliary epithelial cells) undergoing epithelial-to-mesenchymal transition have been studied [15, 17, 18]. Although there is still some debate regarding the relative contribution of each cell type, HSC-derived myofibroblasts are still considered as the main collagen-producing cells in chronic liver diseases.

A process called ductular reaction is a hallmark in most chronic liver diseases [5]. It is defined as immune cell accumulation, fibrosis, and ductular cell proliferation in the periportal area. Numerous studies have reported close interactions between these three events. In brief, liver injury leads to the recruitment of immune cells including monocyte-derived macrophages and T lymphocytes, which in turn favor fibrogenic cell activation, as well as ductular cell (liver progenitor cells, or biliary cells) proliferation [19–28]. Fibrogenesis has been suggested to favor ductular cell proliferation, and reciprocally, ductular cells are known to release fibrogenic factors

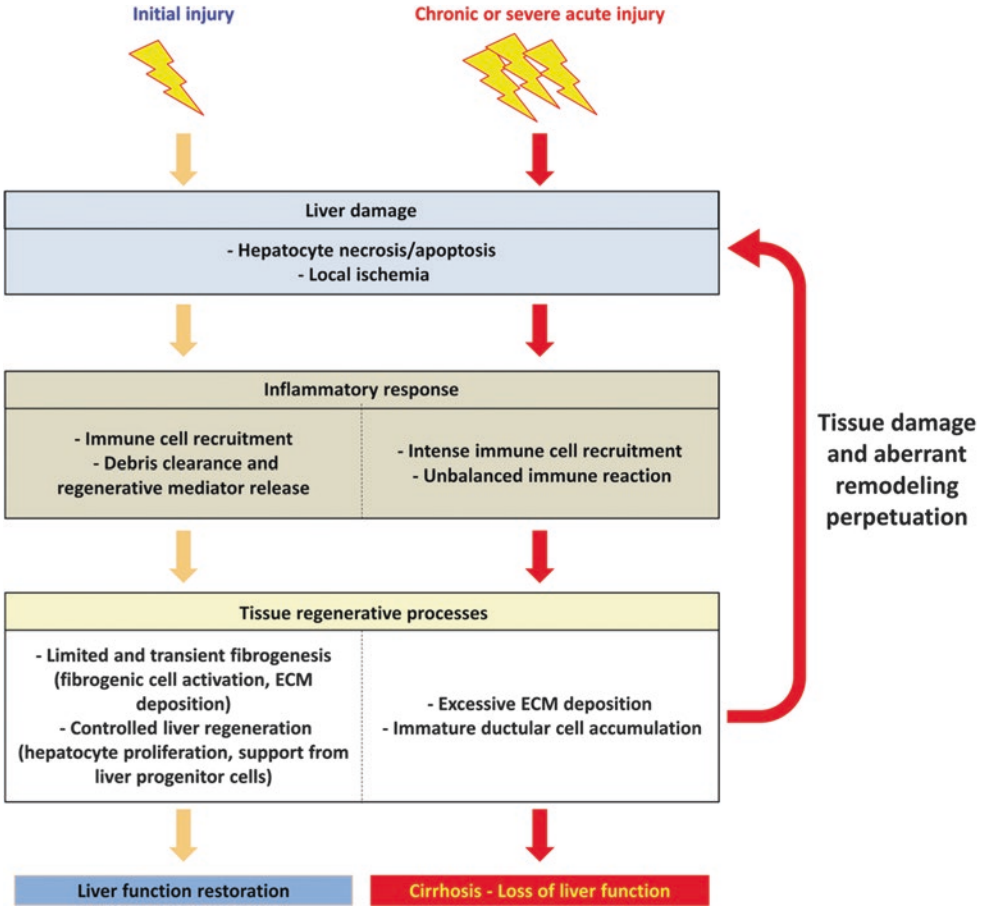

Fig. 35.1 Classical liver disease history. Inflammation plays a critical role not only in inducing liver fibrogenesis during chronic liver injury but also in promoting liver fibrosis resolution and liver regeneration. *ECM* extracellular matrix

[5, 29–31]. Moreover, fibrogenic cells and ductular cells release pro-inflammatory mediators, thus participating in the maintenance of an inflammatory microenvironment and tissue injury [32–35]. This vicious circle may hold the key to the control of chronic liver disease progression, and numerous efforts are put toward identifying key therapeutic targets that may impede these processes (Fig. 35.1).

HSCs Sense Changes in Their Microenvironment Under Inflammatory Conditions

HSCs are well located and equipped to sense changes in their microenvironment. Indeed, their cytoplasmic protrusions expand toward the liver sinusoidal cells and hepatocytes [8, 36, 37]. HSCs, and by extension myofibroblasts, possess a complete arsenal of sensing receptors that detect changes in their microenvironment especially inflammatory conditions (please see reference [38]). Toll-like receptors (TLRs) are among these key receptors, and TLR types 1–9 have been proposed to be expressed by HSCs [33, 39, 40]. Most notably, direct TLR 2, 3, 4, and 9 activation on HSCs have been described as some of the mechanisms leading to inflammation and fibrosis progression (as detailed below) [41]. The TLRs and injury-related changes in the liver microenvironment have been described as crucial mediators of numerous inflammatory and fibrogenic processes through immune or parenchymal cell stimulation, but that will not be discussed in this section (reviewed elsewhere [42, 43]).

Danger-Associated Molecular Patterns (DAMPs) (Such as High-Mobility Group Protein 1 [HMG-1], Mitochondrial DNA [mtDNA])

DAMPs are molecules that are released upon cell death or exposed atypically at the cell membrane under stressing conditions. DAMPs are often described as the mediators of sterile inflammation, a process that initiates immune responses and tissue regeneration/fibrosis, independently of pathogens [44]. A variety of DAMPs have been implicated in liver disease and fibrosis, from nucleus or mitochondrial DNA to acute-phase proteins and protein chaperones [42]. For instance, TLR9 stimulation on HSCs, by apoptotic hepatocyte-derived nucleus DNA, led to the immobilization of migrating HSCs at the site of injury and to their activation into a collagen-producing phenotype [45]. Another example

has been the proposed mechanism that TLR3 activation would induce the release of exosomes from HSCs, which would then stimulate interleukin (IL)-17A production by γδ T cells – a potent pro-inflammatory and pro-fibrogenic cytokine [46]. High mobility group box 1 (HMGB1) is similarly regarded as a crucial enhancer of liver fibrosis. Indeed, it has been shown that HMGB1 released by damaged hepatocytes activates HSCs toward a pro-fibrogenic phenotype, through TLR4 activation and endoplasmic stress induction [47]. However, liver (i.e., hepatocyte and biliary cell) HMGB1-deficient mice had similar liver inflammation and fibrosis in a hepatocarcinogenesis model [48].

Pathogen-Associated Molecular Patterns (PAMPs) (Such as Bacterial Products)

It has been shown that LPS-mediated TLR4 stimulation on HSCs favors their activation toward collagen-producing cells through reduced TGFβ pseudo-receptor bone morphogenetic protein (BMP), bone morphogenetic protein (BMP), and activin membrane-bound inhibitor homolog (BAMBI) expression, thus rendering them more responsive to transforming growth factor (TGF)β1 stimulation [33]. This study also demonstrated the importance of systemic inflammation and more specifically of intestinal bacterial product leakage in initiating and perpetuating liver fibrosis. Indeed, antibiotic treatment reduced bile duct-ligation-induced liver damage and tissue fibrosis [33]. These effects have been specifically attributed to TLR4 expression on HSCs and not Kupffer cells. Moreover, TLR9 stimulation by bacterial- or mitochondrial-derived DNA is known to promote liver fibrosis [49]. Lastly, it has been demonstrated that lipopolysaccharides (LPS) treatment (i.e., TLR4 activation) on HSCs downregulates miR-29 expression, favoring HSC activation and increased collagen expression [50].

Other Inflammatory Mediators (Such as Apoptotic Bodies, Extracellular Vesicles)

HSCs can be activated when phagocytosing damaged hepatocyte-derived apoptotic bodies [51]. Of note, macrophages that phagocyte apoptotic bodies also adopt an anti-inflammatory phenotype, notably characterized by enhanced TGFβ1 release [52]. This macrophage polarization has been questioned by another study reporting that cell debris phagocyting monocyte-derived macrophages adopt a phenotype favoring fibrosis resolution, through increased matrix-metalloproteinase expression [53]. Extracellular vesicles (EVs) allow for intracellular component sharing among cells, and EVs are increasingly studied in the field of liver diseases and fibrosis [54–56]. EVs are implicated in cell-to-cell communication and can also be used to deliver therapeutic agents to targeted cell populations. Indeed, studies using mesenchymal stromal/stem cell-derived EVs have reported promising results in ameliorating liver fibrosis and inflammation in rodent models [57, 58]. More recently, it has been shown that liver stem cell-derived EVs reduced ductular reaction and liver fibrosis in the multidrug resistance 2 knockout (*Mdr2*−/−) mice, via Lethal-7 microRNA and notably by reducing NF-κB and IL-13 signaling pathways in liver tissue [59]. EV cargos may also prove to be detrimental, since another group demonstrated that HSC-derived platelet-derived growth factor receptor (PDGFR) α-enriched EVs directly promote liver fibrosis in vivo [60]. As stated above, it has been demonstrated that apoptotic body engulfment by HSCs leads to fibrosis progression, thus shedding the light on the need for state-of-the-art EV isolation protocols to appropriately discriminate between EVs and apoptotic bodies [61, 62].

Cytokines Regulate Liver Fibrosis Initiation, Progression, or Resolution

Liver fibrosis is the consequence of a multitude of events, to include chronic tissue injury, inflammation, and fibrogenic cell activation. Here, we mainly discuss several cytokines that play an important role in regulating HSC activation in the liver. Some factors are produced by multiple cell types and have been shown to have redundant functions. Table 35.1 summarizes the main cytokine implications in liver fibrosis.

Major Cytokines That Promote Liver Fibrosis

Transforming growth factor beta 1 (TGFβ1) and platelet-derived growth factor (PDGF) are the two major cytokines that promote HSC activation and proliferation, respectively. TGFβ1 is considered the most prominent fibrogenic factor that favors HSC activation and fibrogenesis through the activation of Smads 2 and 3. Many types of cells can release TGFβ1, including HSCs, hepatocytes, T cells, and macrophages [63, 64]. In addition, TGFβ1 possesses potent anti-inflammatory properties that may direct immune cells toward a pro-fibrogenic response.

PDGF is long recognized as a potent mitogen for HSCs by targeting PDFGRα on these cells [65, 66]. Different sources of PDGF have been identified, such as Kupffer cells and proliferating cholangiocytes [67]. PDFGRα expression is highly upregulated after HSC activation and is induced by TGFβ1 [68]. Moreover, it has been shown that targeting PDFGRα in hepatocytes may result in lowering PDGFRα expression on HSCs, thus reducing their activation and liver fibrosis [69]. Therapeutic approaches such as the use of dom-

Table 35.1 Main cytokines regulating HSC activation and liver fibrosis

Cytokine	Main source(s)	Direct effects on HSCs/MFBs	Other effects on the liver
IL-1β	Macrophages	Induces fibrogenic gene expression	Pro-inflammatory
IL-4	Granulocytes, NK cells, Th2 lymphocytes	Stimulates collagen production	Protection against infection
IL-6	Macrophages	HSC survival and proliferation	Hepatoprotective and pro-inflammatory
IL-10	Macrophages, dendritic cells, T cells	HSC senescence	Anti-inflammatory
IL-13	Granulocytes, Th2 lymphocytes	Stimulates collagen production	Protection against infection
IL-17A	Th17 lymphocytes, γδT cells, neutrophils, MAIT cells	Induces collagen production and release of pro-inflammatory mediators, sensitizes HSC to TGFβ1	Pro-inflammatory
IL-22	Th17, Th22 lymphocytes	Induces HSC senescence	Hepatoprotective
IL-33	LSECs, activated HSCs	Induces HSC activation and collagen production	Biliary cell proliferation
TNFα	Macrophages	Increases HSC survival and increases TGFβ1 signaling	Pro-inflammatory
TGFβ1	Macrophages, myofibroblasts	Induces HSC activation and collagen production	Anti-inflammatory
PDGF	Kupffer cells, proliferating cholangiocytes	Mitogenic on HSCs	Angiogenic
IFNγ	CD8+ T cells, NK cells, Th1 lymphocytes	Decreases fibrogenic gene expression	Increases liver damage and inflammation, thus fibrosis

Abbreviations: *HSC* hepatic stellate cell, *IL* interleukin, *IFN* interferon, *LSECs* liver sinusoidal endothelial cells, *NK* natural killer, *PDGFR* platelet-derived growth factor receptor, *TGF* transforming growth factor, *TNFα* tumor necrosis factor alpha

inant negative soluble PDGFβ receptor or PDGF receptor signaling inhibitor (imatinib) have generated promising results in counteracting liver fibrosis [70, 71].

IL-17A (IL-17)-producing cells are frequently observed in the liver of patients suffering from alcoholic steatohepatitis and a variety of other chronic liver diseases associated with liver fibrosis [19, 72]. IL-17 levels are strongly increased upon liver fibrosis, and IL-17 has also been shown to correlate with disease progression and a poor prognosis in a variety of liver diseases [19, 73, 74]. More specifically, IL-17A-deficient animals exhibited reduced fibrosis and inflammation in the bile duct ligation model [73, 74]. Recombinant IL-17 strongly increased production of pro-inflammatory mediators such as IL-6 and TNFα in macrophages [74]. IL-17-receptor is ubiquitously expressed in the organism and has been shown to directly induce fibrogenic cell activation by favoring HSC to myofibroblast activation and enhancing collagen expression [73]. Furthermore, IL-17 directly induced collagen type I production in myofibroblasts through signal transducer and transcription factor 3 (STAT3) activation. Another study reported that IL-17A sensitizes HSCs to TGFβ1-mediated activation [75]. Interestingly, Th17 cells are also a potent source of IL-22, which exerts anti-fibrotic effects (see below) [73, 76].

Defined as Th2-profile cytokines, IL-4 and IL-13 are often associated and display similar functions [77]. Although both cytokines are crucial in host defense against infection, they also exert potent fibrogenic functions that have been long described. Indeed, IL-4 has been shown to increase TGFβ1 production in fibroblasts, and IL-13 is a potent pro-fibrogenic cytokine that directly acts on myofibroblasts [78–80]. Both IL-4 and IL-13 have also been shown to directly stimulate collagen expression and production in cultured fibroblasts [81, 82]. Lastly, IL-4 levels have been correlated with advanced fibrosis development in HCV patients [83].

Major Cytokines That Attenuate Liver Fibrosis

Interferon-gamma (IFN-γ) is considered a major negative regulator of liver fibrosis. IFN-γ directly reduces myofibroblast activation and collagen production in culture [84–87]. Moreover, IFN-γ is known to induce a cytotoxic NK cell phenotype in the liver, directed against activated HSCs [88]. Additionally, IFN-γ is known to increase liver injury in acute models such as Concanavalin A or lipopolysaccharides [89, 90]. Accordingly, in a model of methionine- and choline-deficient high-fat, or in a model of primary sclerosing cholangitis (Mdr2−/− mice), it has been reported that IFN-γ-deficient mice had reduced liver inflammation and fibrosis as compared to IFN-γ-expressing mice, possibly due to reduced tissue injury [91, 92]. These results highlight the complex roles of IFN-γ in favoring both tissue injury and repair mechanisms and orientating the immune system toward an anti-fibrotic response and inhibiting fibrogenic gene expression on HSCs.

IL-22, mainly produced by Th17 and Th22 cells, opposes the anti-fibrogenic effects of IL-17 by inducing HSC senescence and protecting against hepatocellular damage [76]. Mechanistically, IL-22-induced HSC senescence was pre-

vented when STAT3 signaling was blunted. Accordingly, IL-22 deletion exacerbated liver fibrosis and IL-22 administration prevented bile-duct ligation liver fibrosis [73]. In addition, IL-22 has potent hepatoprotective roles, by favoring hepatocyte survival through STAT3 activation, thereby inhibiting liver fibrosis [93, 94].

Other Cytokines That May Have Dual Roles in the Control of Liver Fibrosis

TNFα, one of the most potent inflammatory cytokines, is upregulated during tissue injury responses and is participating in tissue injury by favoring hepatocyte apoptosis. TNFα administration enhanced liver fibrosis through increasing HSC survival through induction of tissue inhibitor of metalloproteinase 1 (TIMP-1) expression, an effect that has been reported to be mediated by Kupffer cell activation [95]. TNFα and LPS stimulated HSCs harbored reduced BAMBI expression, resulting in increased TGFβ1 signaling [96]. Additionally, HSCs isolated from TNR-receptor 1- and/or TNR-receptor 2-deficient mice showed reduced proliferation in response to PDGF and a reduced expression of collagen [97]. Contrastingly, direct treatment of isolated HSCs by TNFα led to reduced collagen expression but increased cell proliferation in other studies [98–100]. Therefore, TNFα seems to have contradictory effects on HSCs, which may be linked to their integration into a more complex microenvironment that exposes fibrogenic cells to a multitude of activating and inhibitory signals. TNFα stimulation induced activation of apoptosis signal-regulating kinase 1 (ASK1) and subsequently activated the p38/JNK signaling pathway, promoting liver fibrosis [101, 102]. Although an early phase II trial reported that selonsertib, an ASK1 inhibitor, showed promising results in reducing liver fibrosis in nonalcoholic steatohepatitis patients [103], selonsertib failed to reduce liver fibrosis in a phase III clinical trial (STELLAR-4, NCT03053063).

IL-6 not only plays an important role in protecting against hepatocellular damage and promoting liver regeneration but also acts as a pro-inflammatory cytokine. However, IL-6 can also promote HSC activation and survival via the activation of STAT3, thereby enhancing liver fibrosis [104–106]. Therefore, the effect of IL-6 on liver disease progression depends on the balance between its beneficial and detrimental functions.

Several studies reported that IL-1-receptor or IL-1β deficient mice were resistant to liver fibrosis [107, 108]. It has been questioned, however, whether these effects were due to direct effects of IL-1β or IL-1α on HSCs or indirect by favoring a pro-inflammatory environment. In addition, macrophage-derived IL-1β was demonstrated to induce fibrogenic gene expression in myofibroblasts from the liver [109].

IL-10 is a potent anti-fibrotic cytokine, and accordingly, IL-10-deficient mice develop a stronger immune response and a more severe fibrosis than wild-type mice following repeated CCl4 injections [110, 111]. Direct effects of IL-10 on HSCs were related to senescence induction and a decrease in HSC viability [112].

IL-33 is generally associated with tissue regeneration. For instance in the liver, it has been shown to promote biliary cell proliferation [113]. Moreover, IL-33 expression is higher in human and mouse fibrotic livers as compared to normal liver samples [114]. Accordingly, IL-33 has been characterized as a pro-fibrogenic factor being produced by activated HSCs and increasing their collagen production, as well as a pro-fibrogenic immune environment [114–118].

Immune Cells Regulate Liver Fibrogenesis

Liver fibrosis is seemingly always associated with liver inflammation (with the apparent exception of hemochromatosis) [119, 120]. Virtually all myeloid and lymphoid immune cells are implicated in liver fibrosis initiation, progression, and/or resolution (Fig. 35.2) [55, 121]. While immune cells respond to tissue injury by clearing DAMPs and PAMPs, an unbalanced response or chronic inflammation can enhance tissue damage and lead to liver fibrosis. Indeed, inflammatory processes are tightly regulated, and a slight imbalance results in either aggravated pathology or recovery. While specific factors produced by immune or parenchymal cells have been discussed (see above), we herein briefly describe the putative and sometimes contradictory roles of immune cell populations in the liver over the course of liver fibrosis.

Liver-Resident Macrophages

Kupffer cells are the liver-resident macrophages and are renowned to exert sentinel functions in healthy conditions. Kupffer cells are thus considered to be among the first immune cells to sense changes associated with liver injury [122]. During liver disease initiation, they are notably a potent source of chemokines for other immune cell types. Due to the difficulties in distinguishing Kupffer cells and monocyte-derived macrophages upon chronic liver injury, some reports may inadvertently confound these two cell types, and macrophage-depleting approaches may impact both compartments [123]. Accordingly, macrophage depletion by using clodronate-loaded liposomes at early stages of liver fibrosis prevents excessive scarring, while macrophage depletion at later stages dampens fibrosis resolution in the CCl4 model [124].

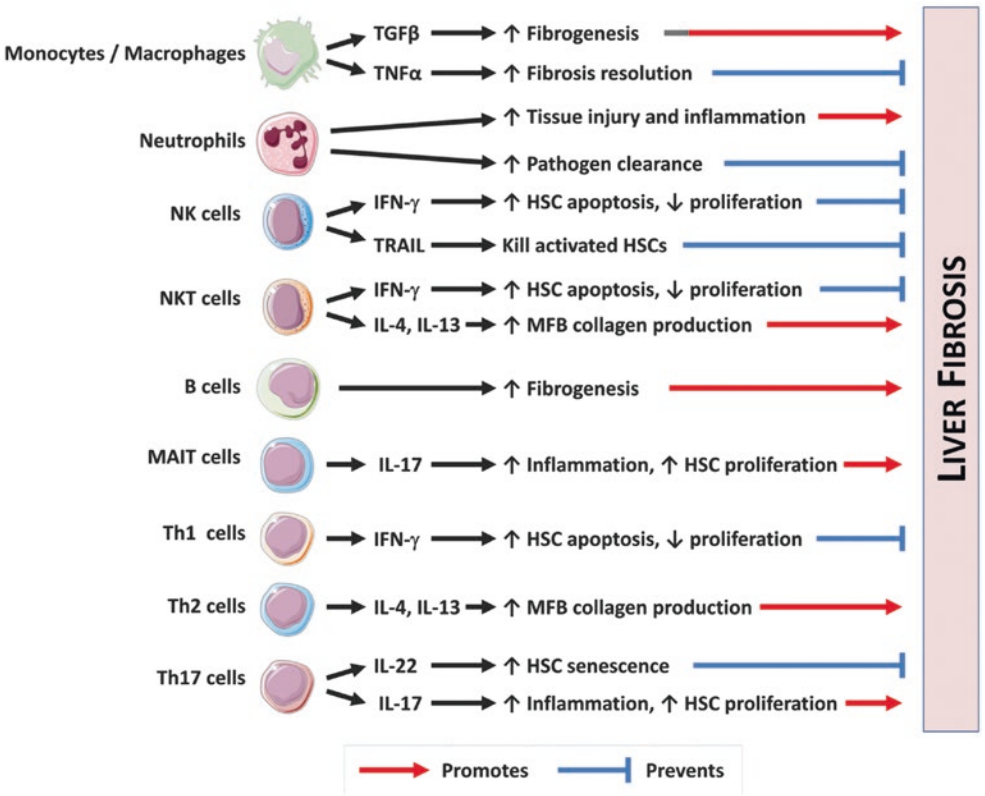

Fig. 35.2 Immune cell implication in liver fibrosis prevention or promotion. This figure represents the main immune cell types and subpopulations implicated in liver fibrosis progression or resolution. *HSC* hepatic stellate cell, *IL* interleukin, *IFN* interferon, *MAIT* mucosal-associated invariant T cells, *MFB* myofibroblast, *NK* natural killer, *TGF* transforming growth factor, *Th* Helper T cell, *TNFα* tumor necrosis factor alpha

Monocyte-Derived Macrophages

Mononuclear cell infiltration (more specifically monocyte accumulation) is a classical feature of liver fibrosis. Monocytes can activate toward a plethora of phenotypes, including pro- or anti-inflammatory and pro- or anti-fibrogenic phenotypes [123, 125]. They are thus regarded as crucial orchestrators of liver disease due to their potent cytokine secretion. C–C motif chemokine receptor 2 (CCR2) is crucial for monocyte recruitment, since CCR2-deficient mice had reduced numbers of liver macrophages after bile duct ligation [126]. Accordingly, the use of a CCR2/CCR5 antagonist (cenicriviroc) has shown promising effects for the treatment of nonalcoholic steatohepatitis with fibrosis [127]. Nonetheless, monocytes are also crucial in liver regeneration, and monocyte/macrophage-depleting methods have been proven to sometimes delay or prevent tissue repair mechanisms [128–132].

Neutrophils

Neutrophils are among the first responders to liver injury and are mainly characterized by their potent roles in aggravating tissue injury through reactive oxygen species release [133, 134]. Consequently, neutrophil recruitment and activation are generally regarded to promote chronic pro-inflammatory and pro-fibrogenic environment. On the other hand, neutrophils are crucial in pathogen clearance that is necessary for inflammation resolution [135].

Natural Killer (NK) and NKT Cells

A clear role of NK cells in liver fibrosis is to control fibrosis progression through killing activated HSCs and producing IFN-γ that induces HSC apoptosis and cell cycle arrest [88, 136, 137]. In contrast, CD1d-dependent invariant NKT cells play dual roles in regulating liver fibrogenesis; for example, NKT cells not only can promote fibrosis progression, through IL-4 and IL-13 production [138], but may also attenuate liver fibrosis by killing HSCs and producing IFN-γ [139, 140].

T Lymphocytes

$CD4^+$ T lymphocytes, also termed T-helper cells, are regarded as immune response orchestrators due to their very distinct and intense immune system-mediating cytokine release. Th1, Th2, and Th17 are the most studied and well-characterized activation phenotypes in liver disease. While Th1 cells are classically regarded as anti-fibrogenic through the promotion of anti-fibrogenic responses and IFN-γ-mediated fibrogenic cell death, Th2 cells are considered to be pro-fibrogenic

through IL-4 and IL-13 production [141–143]. Th17 cells, on the other hand, have been described as having contradictory roles in liver disease. Th17 cells are mainly characterized by IL-17A and IL-22 production, which exert opposing functions in liver disease, IL-17A being pro-inflammatory and pro-fibrogenic and IL-22 favoring tissue regeneration and inducing HSC senescence (discussed above) [74, 76]. Cytotoxic CD8+ T lymphocytes play detrimental roles in alcoholic liver disease, notably by directly killing parenchymal cells [144]. Furthermore, autoreactive CD8+ T cells are considered to be the main drivers of biliary cell damage in primary biliary cholangitis, by targeting biliary epithelial cells [145].

B Lymphocytes

B lymphocytes represent a major lymphocyte population in the liver [146]. Despite identical initial injury, B-cell-deficient (*JH−/−*) mice showed reduced fibrosis 16 weeks after CCl4-induced liver fibrosis [146]. Furthermore, B cells accumulate in the liver from Mdr2−/− mice, and B-cell ablation by intravenous injections of anti-mouse CD20 monoclonal antibody promoted HSC senescence-mediated fibrosis resolution and was also associated with reduced TNFα and NF-κB activation [147].

Mucosal-Associated Invariant T (MAIT) Cells

MAIT cells have recently gained a lot of interest due to their antibacterial activity and are especially enriched in the human liver [148]. They are innate-like T cells mostly characterized as CD161+CD8+ T-cells and by the invariant TCR-chain, Vα7.2-Jα33 [149, 150]. MAIT cells have notably been shown to accumulate at the portal tracts around biliary ducts in human cholangiopathies and have thus been suggested to play a role in bile duct diseases [151]. Moreover, IL-7 is produced by hepatocytes under inflammatory conditions, and IL-7-stimulated MAIT cells dramatically upregulated their IL-17A production [148]. Similarly, repetitive IL-12 stimulation upregulated IL-17A production by MAIT cells [152]. In this same study, the authors demonstrated that although MAIT cells are less frequent in fibrotic than in the healthy liver, the remaining MAIT cells have adopted a pro-fibrogenic phenotype that further accentuates liver fibrosis, notably through IL-17A [152].

Roles of Immune Cells in Fibrosis Resolution

Despite the crucial roles of inflammation in initiating liver fibrosis, there is considerable amount of data enlightening the role of immune cells in fibrosis resolution. Accordingly, it has been demonstrated that macrophage depletion at early stages prevents CCl4-induced liver fibrosis, while macrophage depletion during liver fibrosis resolution stage, on the other hand, leads to fibrosis perpetuation [124]. The role of macrophage-mediated fibrosis resolution could be explained by the fact that bone-marrow-derived macrophages are required for natural killer (NK) cell recruitment. NK cells will then release TNF-related apoptosis-inducing ligand (TRAIL) and IFN-γ and subsequently induce fibrogenic cell apoptosis [88, 153]. Another major function of NK cells is the production of IFN-γ, which is known to oppose TGFβ1 fibrogenic signaling and inhibit HSC fibrogenicity through STAT1 activation [154].

Potential Anti-fibrotic Therapeutic Approaches by Targeting Immune Components

Withdrawal of the causative agents of liver injury has been shown to effectively prevent disease worsening and even allow for fibrosis or cirrhosis regression in hepatitis B and C infected patients, autoimmune diseases, non-alcoholic steatohepatitis, and more disputably in alcoholic patients [155–158]. As discussed above, many immune components have been implicated in the pathogenesis of liver fibrogenesis. Some of these components could be used as therapeutic targets for the treatment of liver fibrosis. For example, one potential approach to modulate HSC activation is to alter the TGFβ1 and unfolded protein response (UPR) [159]. More specifically, upon extracellular matrix protein assembly, fibrogenic cells experience increased ER stress, leading to the UPR and allowing for a proper protein folding and trafficking out of the cell while favoring cell survival. TGFβ1 is known to increase ECM protein production and to induce ER stress as well as UPR [159]. Procollagen I export blockade through transport and Golgi organization protein 1 (TANGO1) impairment led to HSC death in basal conditions due to enhanced ER stress, which was further increased upon concomitant TGFβ1 stimulation [159]. In addition, targeting the UPR through pharmacological inhibition of C/EBPβ-p300 may result in limiting fibrogenic cell activation and liver fibrosis [160].

IFN-γ is one of the most potent anti-fibrotic cytokines and was examined in clinical trials for the treatment of liver fibrosis with some beneficial effects, but long-term benefits were not observed [161, 162]. These disappointing results were likely due to low efficacy and adverse effects from IFN-γ therapy because IFN-γ strongly inhibits liver regeneration by targeting hepatocytes and induces inflammation by targeting immune cells. Researchers have been trying to develop fibroblast-targeted IFN-γ via the fusion of PDGF-β

receptor recognizing peptide and IFN-γ, which had increased anti-fibrotic potency and improved safety profile in experimental models of liver fibrosis in vivo [163].

IL-22 has many beneficial functions in the liver, including hepatoprotective, proliferative, anti-oxidative, and anti-fibrotic functions [76]. More importantly, IL-22 therapy may generate limited side effects because IL-22 mainly targets epithelial cells as well as HSCs without affecting immune cells. Thus, IL-22 is a promising drug for the treatment of liver failure and may also have therapeutic potential for the treatment of liver fibrosis [164]. Indeed, a clinical trial shows promising results regarding IL-22Fc treatment for severe alcoholic hepatitis [165].

In summary, many immunological factors play important roles in controlling liver fibrogenesis. Further understanding of their functions may help identify novel therapeutic targets and effective strategies for the treatment of liver fibrosis in the future.

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