

Immunological Orchestration of Liver Fibrosis

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Abstract Fibrosis represents the main pathophysiological consequence of all chronic inflammatory liver diseases and ultimately leads to the life-threatening clinical consequences of cirrhosis. Regardless of the underlying etiology, fibrosis involves interplay between cells of the immune systems and the direct mediators of fibrosis, including hepatic stellate cells. However, the precise physiological mechanism of liver fibrosis remains largely on enigma. Primary biliary cirrhosis (PBC) is a unique chronic, fibrosing liver disease affecting predominantly women and characterized by an immune-mediated specific destruction of the small intrahepatic bile ducts. The presence of highly specific anti-mitochondrial autoantibodies (AMA) as well as T cell responses directed against the pyruvate dehydrogenase E2 complex (PDC-E2) provides a unique opportunity to assess the interaction between specific immune responses and fibrogenesis. In addition, mouse models of PBC have been developed allowing the dissection of mechanisms involved in breakdown of immune tolerance, bile duct injury, and

fibrosis. Recently, we reported that liver fibrosis could be induced in a xenobiotic-induced murine model of PBC by polyinosinic–polycytidylic acid (poly I:C) treatment as well as with the administration of α -galactosylceramide (α -GalCer). The aim of this article is to provide a current perspective of the immunological mechanisms that are significant in the fibrogenic processes, and potentially, for its regression by comparing PBC with other chronic inflammatory liver diseases.

Keywords Primary biliary cirrhosis · Fibrosis · Xenobiotics

Introduction

Fibrosis is the result of the wound-healing response. Physiologically, it is a scarring process that often correlates with repeated injuries, where extra cellular matrix (ECM) and myofibroblasts replace dead or damaged cells and impair organ function. Clinical manifestation of fibro-proliferative processes induces the pulmonary fibroses, systemic sclerosis, cardiovascular disease (myocardial infarction ischemic, dilated, and hypertrophic cardiomyopathies, and heart failure) [1], progressive chronic kidney diseases [2], macular degeneration, and liver cirrhosis.

Liver fibrosis is a response in virtually all chronic liver diseases. The result of progressive fibrosis is cirrhosis, which generally portends a poor outcome and high mortality rate. Liver cirrhosis is the twelfth leading cause of death in the USA, accounting for 29,963 deaths (1.2 % of total death) each year [3]. Genetic and environmental factors are both important in the etiology of liver fibrosis. The natural history of liver fibrosis is typically variable, slow, and develops over 20 to 40 years in patients with chronic liver injury. Currently, the only effective treatment for liver

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cirrhosis is liver transplantation. However, shortages of organs and the presence of concurrent diseases in transplant recipients greatly limit the practicality of this treatment and make clear the imperative to develop anti-fibrotic therapies [4–6].

The pathogenesis of liver fibrosis is a highly orchestrated, dynamic process that involves multiple players. Recent advances in liver immunology have begun to unveil the immunological mechanisms of this process. Indeed, the immune system plays significant roles in the modulation of injury response of the liver and it is not surprising that multiple immune players are involved in the regulation of liver fibrosis. The immunological activation of quiescent hepatic stellate cells (HSCs) by cytokines and their subsequent transformation into myofibroblast-like cells is believed to be the central process in the initiation and perpetuation of liver fibrosis [4, 6–9]. Interactions between HSCs, macrophages, and CD4 T cells (Th2) within a microenvironment of growth factors and cytokines that activate HSCs can lead to production of extracellular matrix (ECM), and subsequently liver fibrosis [4, 6–13]. More recently, the role of Th17 and Th22 CD4 T cells [14–17], natural killer (NK) cells, natural killer T (NKT) cells, and Toll-like receptor (TLR) signaling [18–23] have been identified in pathways to liver fibrosis.

In this article, we will review the immuno-physiology of liver fibrosis, with an emphasis on the interaction of the innate and adaptive arms of the immune response with the liver. Furthermore, we will discuss the current understanding of immunological mechanisms of liver fibrosis in primary biliary cirrhosis (PBC), and the dynamic nature of liver fibrosis by comparison of PBC with other chronic liver diseases.

Immune Mechanisms and Players of Liver Fibrosis

Liver Component in Liver Fibrosis

Hepatic Stellate Cells (HSCs)

Although several cell types including myofibroblasts, bone marrow derived progenitor cells, and hepatocytes contribute to liver fibrosis, activated HSCs are now believed to be the major contributor to the fibrotic processes of the liver [4, 6–9]. Activated HSCs have been demonstrated to be the main source of hepatic collagens in fibrosis [24–26]. HSCs, also known as Ito cells, fat-storing cell, lipocytes, interstitial cells, or vitamin A-storing cells, are normally quiescent and store approximately 70 % of the body's supply of vitamin A. However, upon chronic liver injury and infection, quiescent HSCs becomes activated and transformed into myofibroblast-like cells, acquiring contractile, pro-inflammatory, and fibrogenic properties [27, 28]. The activation of HSCs leads to the accumulation of scar (fibrillar) ECM in the space of Disse [4],

a characteristic of advanced fibrosis. Furthermore, this activation induces the synthesis of fibrillar collagens, contractile activity, secretion of chemotactic and vasoactive factors, migratory activity, and the secretion of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) that construct and remodel extracellular matrix and lead to fibrosis [24, 27–35].

Immune Players in Liver Fibrosis

Kupffer Cells (Macrophages)

Kupffer cells (hereinafter referred to as macrophages) are tissue macrophages derived from circulating monocytes. They account for approximately 15 % of the total liver cell population and are predominantly found in the peri-portal areas [36]. Macrophages mediate phagocytosis, antigen presentation, and secretion of mediators during both innate and adaptive immune responses. During liver injury and infection, macrophages are activated and as part of this process produce transforming growth factor (TGF)- β , platelet-derived growth factor (PDGF), and interleukin (IL)-1 β [37–39]. These cytokines induce HSCs transformation from quiescent HSCs to activated HSCs (Fig. 1). During resolution of injury, phagocytosis by macrophages removes dead cells, microbes and waste, so that HSCs once again become inactivated. Macrophages also play crucial roles in restraining inflammation and fibrosis by providing a negative feedback signal through IL-10 production and hence stopping the production of TGF- β , PDGF, and IL-1 [4, 6–9, 40–43] (Fig. 1). Fibrosis resolves as activated HSCs die or return to a quiescent state and cease adding to the ECM and as macrophages degrade excess ECM.

CD4 T Cells

The orchestration of CD4 T cells in Th1/Th2, Th17, and Th22 immune responses and their regulatory roles in healing occupy a central role in hepatic fibrosis.

Th1/Th2 Generally, in the initiation and perpetuation of fibrosis, Th2 cell responses promote liver fibrosis more effectively than Th1 cell responses [43] (Table 1). Th2 cells coordinate the immune responses activating macrophages and inducing collagen production by HSCs with IL-4 and IL-12 [11–13] (Fig. 1). The combination of activating signals from the inflammatory environment, macrophages and Th2 cells stimulate HSCs to construct and remodel ECM and lead to fibrosis. On the contrary, in the resolution of fibrosis, Th1 cells secrete anti-fibrotic cytokines including IFN- γ that inhibits collagen synthesis by HSCs [44, 45] (Fig. 1), and the differentiation of naïve T lymphocytes into Th1 cells is promoted by IL-12. This mechanism was

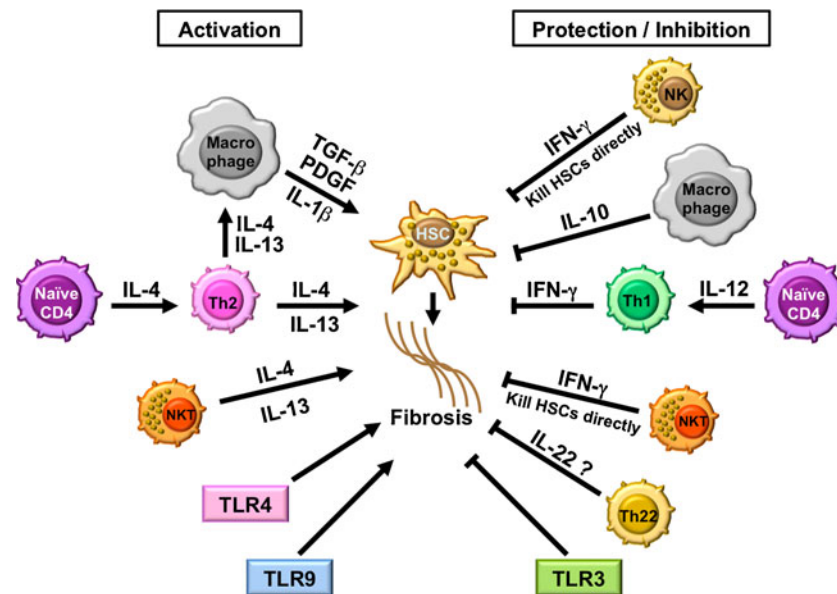


Fig. 1 General liver fibrosis. In the initiation and perpetuation of fibrosis, macrophages are activated by injury and infection. During the process, transforming growth factor (TGF)- β , platelet-derived growth factor (PDGF), and interleukin (IL)-1 β are produced. These cytokines induce liver fibrosis. In the resolution of fibrosis, macrophages also reverse HSCs activation with IL-10 by stopping production of TGF- β , PDGF, and IL-1 β , and reduce fibrosis. Generally, in the initiation and perpetuation of fibrosis, Th2 cell responses promote liver fibrosis more effectively than Th1 cell responses. Th2 cells coordinate the immune response activating macrophages and inducing collagen production by hepatic stellate cells (HSCs) with IL-4 and IL-13. On the contrary, Th1 cells secrete well-known anti-fibrotic cytokines such as IFN- γ . Th22 cells, a

new type of inflammatory cell that mainly secretes interleukin (IL)-22; member of the IL-10 family, may provide hepato-protection in the development of liver inflammation and fibrosis. NK cells inhibit liver fibrosis by killing early-activated and senescent-activated HSCs and producing IFN- γ . NKT cells can destroy activated HSCs directly and by producing IFN- γ . On the contrary, NKT cells also produce pro-fibrotic cytokines such as IL-4 and IL-13. TLR4, which acts as receptor for lipopolysaccharide (LPS); a cell-wall component of Gram-negative bacteria, induces liver fibrosis. TLR9, whose main ligand is bacterial unmethylated CpG containing DNA, induces liver fibrosis. TLR3 signaling which is strongly activated by synthetic polyinosinic-polycytidylic acid activates NK cells to inhibit liver fibrosis

illustrated by Mentink-Kane et al. in *Schistosoma mansoni*-infected IL-10^{-/-} mice. In this setting, liver fibrosis occurred only when mice were also deficient in IL-12p40 [46]. Thus, IL-10 and the interferon (IFN)- γ -inducing cytokine IL-12 interact synergistically to inhibit the development of liver fibrosis [47].

Th17 cells Th17 cells have been identified as a distinct subset of CD4⁺ T cells characterized by production of IL-17A (also called IL-17), IL-17F, and IL-22 [48]. IL-23, IL-6, and TGF- β promote the differentiation of naïve T lymphocytes into Th17 cells, which promote auto-immunity and inflammation. In fact, Th17 cells have been found to be involved in PBC [49] and alcoholic liver disease (ALD) [14]. Furthermore, the association of Th17 with liver fibrosis has been documented in patients with autoimmune hepatitis (AIH), chronic hepatitis B (CHB), ALD, and hepatitis C virus (HCV)-induced liver allograft fibrosis [14–17]. Th17 cells are increased in both peripheral blood and livers of AIH, ALD, CHB and liver allografts in HCV (Table 1), and may exacerbate liver injury and Th17-secreting cell infiltrates have been correlated to the severity of fibrosis in ALD. However, recombinant IL-17 does not directly induce

HSC SMA or α 1 pro-collagen I gene expression in vitro suggesting an indirect mechanism of action [14].

Th22 cells Recently, the Th-22 cell, a new type of inflammatory Th cell that mainly secretes IL-22 has been identified [50, 51]. IL-22, produced by Th17 and NK cells, plays an important role in controlling bacterial infection and tissue repair [52, 53]. IL-22 belongs to member of the IL-10 family of cytokines and binds to a heterodimeric receptor (IL-10Rb and IL-22R). IL-22R is highly expressed on hepatocytes (and epithelial cells), but not on leukocytes, suggesting a potential physiological effect on function in the liver [52]. However, knowledge on the role of IL-22 in liver injury and fibrosis is still rudimentary.

It has been reported that exogenous IL-22 provides protection in acute hepatitis induced by Con-A [54], carbon tetrachloride (CCl₄), or FasL [55] in vivo. Conversely, severe hepatitis is observed in Con-A-induced hepatitis in IL-22^{-/-} mice [56]. Ki et al. demonstrated that treatment with IL-22 recombinant protein activated hepatic signal transducer and activator of transcription 3 (STAT3), and ameliorated alcoholic fatty liver, liver injury, and hepatic oxidative stress in a mouse model of alcohol induced liver injury [57]

Table 1 Immune cell activation in liver fibrosis

	Th1	Th2	Th17	Treg	Th22	NK cell	NKT cell	TLR3
PBC	↑ IFN-γ ↑ Th1/Th2 cytokines imbalance Th1 dominant (98, 122)	↓ IL-4 ↓ Th1/Th2 cytokines imbalance (98, 122)	↑ IL-17 ↑ Th17/Treg imbalance (105, 132)	↓ Th17/Treg imbalance (131) (105, 132)	?	↑ Activity ↑ IFN-γ ↓ IL-6 ↓ (104)	↑ Contribute to Th1/Th2 cytokines imbalance? (99, 100, 134)	↑ IFN-α, -β ↑ (112, 135)
ALD	↓ IFN-γ ↓	↑ IL-4, IL-13 ↑	↑ IL-17 ↑ (14)		? Protect liver injury (57)	? Function ↓ IFN-γ ↓ (59, 69–73)	↑ Accelerate liver injury (80) (137)	
CHB	↓ IFN-γ ↓	↑ IL-4, IL-13 ↑	↑ IL-17 ↑ (16)	↑ (131)	↓ IL-22 ↓ (58)	?	↑ IL-4, IL-13 ↑ (81)	
CHC	↓ IFN-γ ↓	↑ IL-4, IL-13 ↑	↑ IL-17 ↑	↑ (17, 138)	?	↓ Activity ↓ Cytokine production ↓ (74–79)	↓ (77, 139–141)	↓ (135)

PBC primary biliary cirrhosis, ALD alcoholic liver disease, CHB chronic hepatitis B, CHC chronic hepatitis C, TLR Toll-like receptor

(Table 1). Xiang et al. reported that IL-22 expression was inversely correlated with the histological activity; in particular, there is significant negative correlation between the fibrosis stage and IL-22 in patients with chronic hepatitis B (CHB) patients [58] (Table 1). Altogether, these studies suggested that IL-22 might provide hepato-protection in the development of liver inflammatory injury and perhaps fibrosis (Fig. 1).

NK Cells and NKT Cells

Hepatic lymphocytes are abundant in NK cells (20–30 %) and NKT cells (10–25 %) that play important roles in antiviral and anti-tumor defenses and in the pathogenesis of chronic liver disease [59]. In general, NK and NKT cells accelerate liver injury by producing pro-inflammatory cytokines and killing hepatocytes. However, the role of NK cells on the proliferation of liver progenitor cells and the role of NKT cells in liver regeneration remains controversial [60–64].

NK Cells NK cells can inhibit liver fibrosis through multiple mechanisms (Fig. 1) including killing early-activated and senescent-activated HSCs and producing IFN-γ [18, 19, 65–67]. Animal models of liver fibrosis primarily using carbon tetrachloride have demonstrated that NK cells have a critical role in inhibiting liver fibrosis. Melhem et al compared quantitative fibrosis in wild type, T and B cell deficient SCID, and T, B, and NK cell deficient Beige-SCID mice after CCL4 treatment and found that mice lacking NK cells had significantly more fibrosis [19]. In addition, after fibrosis, hepatic NK cells increased while there was a relative decrease in activating killer cell immunoglobulin-like receptor (KIR) and MHC class I on HSC which were more susceptible to apoptosis. NK induced death of activated

HSC may also be induced by binding of retinoic acid early inducible (REA)-1 on activated HSC by the NK-activating receptor NKG2D [18]. This effect as well as TRAIL-mediated cytotoxicity of NK cells on HSC is augmented by polyinosinic-polycytidylic treatment. Further, NK cells can suppress activated HSCs through the production of IFN-γ which induces HSC cell cycle arrest and apoptosis in a STAT1-dependent mechanism [66, 67]. Interestingly, NK cells selectively kill early-activated or senescent-activated HSCs because of high expression of NK cell-activating ligands (RAE-1 in mice; MICA in human), but not quiescent or fully activated HSCs [18, 68].

There is no evidence supporting a role for NK cells in alcoholic liver injury [59], but NK cell functions are inhibited in alcoholic cirrhosis [69] and in ethanol fed mice [70, 71] (Table 1). Alcohol's blunting effect on the inhibitory mechanisms of NK/IFN-γ in liver fibrosis may provide an explanation for the observed acceleration of liver fibrosis in patients with chronic hepatitis C virus (CHC) infection who also consume excessive alcohol [72, 73]. CHC infection changes the expression of NK cell receptors on NK cells and thereafter decreases NK cytolytic activity [74] and the ability of producing cytokines [75] (Table 1). Finally, NK cells are depleted significantly in patients with CHC infection which has been reported in a multitude of studies [76–79]. These inverse correlations suggest that NK cells may negatively regulate liver fibrosis in CHC (Table 1). In hepatitis B virus (HBV) patients, elevation and activation of NK and NKT cells have been observed only in the early stages, so the role of NK cells in chronic stages and fibrosis remains unclear and should be addressed in future studies.

NKT Cells Similar to NK cells, NKT cells can destroy activated HSCs directly and indirectly through the production of

IFN- γ [21] (Fig. 1). However, NKT cells may act as a double-edged sword by promoting liver injury under certain conditions. In the mouse model of CCl₄-induced acute liver injury, natural activation of hepatic NKT cells inhibited acute liver injury and inflammation likely by suppression of activated HSC. In contrast, strong activation of NKT cells by α -galactosylceramide (α -GalCer) accelerated CCl₄-induced acute liver injury, inflammation, and fibrosis. Notably, these effects were present only during acute injury, but not in the chronic phase when NKT cells are depleted [21].

In alcohol-induced liver injury, NKT cells appear important in the severity of injury and inflammation. Following alcohol consumption, NKT cells increase in the liver and activation with α -GalCer exacerbates the liver injury. In contrast, NKT cell deficiency delays the liver injury [80] (Table 1). Data on the role of NKT cells on fibrosis in this model is lacking. However, in humans with chronic viral hepatitis, NKT cells may promote fibrosis through the production of the profibrotic cytokines IL-4 and IL-13 (Fig. 1). Lalla et al. demonstrated that liver derived NKT cells from virally infected patients secreted greater amounts of IL-4 and IL-13 compared to NKT cells from non-inflamed livers [81] (Table 1). In addition, CD1d-dependent activation of NKT cells from healthy donor elicited IL-4 and IL-13 expression as well.

TLRs

Toll-like receptors (TLR) are structurally conserved proteins that activate innate immune responses. They are a type of pattern recognition receptors that recognize a broad range of microbial molecules known as pathogen-associated molecular patterns (PAMP). The liver's unique vasculature in which blood from intestines flows to the liver via the portal vein and is exposed to hepatocytes through large sinusoidal fenestrae is postulated to deliver microflora and microbial products into the liver causing acute and chronic liver inflammation through TLR and other PAMP signaling. To date, 13 different TLR have been identified and several of them are important in regulating the development and progression of liver fibrosis.

TLR4 TLR4 is a receptor for lipopolysaccharide (LPS), a cell-wall component of Gram-negative bacteria. Macrophages in the liver are the major target of LPS because they are the first point of contact for bacterial products, including endotoxin, that are derived from the gastrointestinal tract [82, 83]. A number of studies have implicated TLR4 signaling in the development of liver fibrosis. For example, the level of LPS in peripheral blood and the portal vein have been reported to be elevated in patients with liver cirrhosis [84]. In addition, in animal studies where fibrosis was induced by bile duct ligation (BDL) and chronic CCl₄ or

thioacetamide, deficiency of functional TLR4 showed a marked reduction in liver inflammation and fibrosis [22]. Mice deficient in CD14 and LPS-binding protein, TLR4-associated cell surface molecules, also showed reduced liver fibrosis induced by BDL [85].

TLR4-mediated signals are transduced through two major pathways, which are either MyD88 dependent or MyD88 independent, the former signaling through NF- κ B, MAPK, and PI3K/Akt [86]. Most resident liver cells, including HSCs, express TLR4. Trans-located into the portal vein, microflora-derived LPS stimulates TLR4 on HSCs. Activated human HSCs respond to LPS by secreting cytokines and activating I κ B kinase/NF- κ B and JNK [87]. Moreover, high levels of the TGF- β pseudo-receptor BAMBI limit TGF- β signaling in quiescent HSCs. However, LPS down regulates BAMBI, thereby promoting TGF- β signaling and enhancing hepatic fibrosis [22]. The production of chemokines (MCP-1, MIP-1 β , and RANTES) in HSCs is induced by TLR4 signaling, engaging macrophages via chemokine receptors CCR1 and CCR2. TGF- β is then produced by these engaged macrophages. Contemporaneously, downregulation of BAMBI and miR-29 is induced by TLR4 signaling, bringing on full-activation of HSCs [22]. Thus, TLR4 could play an important role in regulating fibrogenic signaling in HSCs and induces liver fibrosis (Fig. 1).

TLR9 A major natural ligand for TLR9 is unmethylated bacterial DNA containing CpG and is expressed on HSCs. TLR9 signaling induces MCP-1 and collagen production and inhibits PDGF-mediated chemotaxis in HSCs. TLR9 can also be activated by apoptotic hepatocyte DNA, which leads to cellular activation and collagen production [88] (Fig. 1). Accordingly, TLR9-deficient mice displayed decreased liver fibrosis in experimental liver injury [89]. Furthermore, a recent study showed that TLR9 on dendritic cells (DC) is crucial for liver fibrosis. DC-depleted animals exhibited a significant reduction of liver fibrosis. DC in the fibrotic liver, but not in the normal liver, produced TNF- α , IL-6, and chemokines in response to CpG-DNA [90]. In addition, CpG-DNA treatment stimulated DC of fibrotic livers to produce TNF- α , which in turn activated HSCs and enhanced NK cells cytotoxicity [90].

TLR3 A natural ligand for TLR3 is double-stranded RNA (ds RNA) which is generated during the replication of virus [91]. TLR3 signaling induces type I interferons (IFN- α , - β). Radaeva et al. reported that in mice the synthetic TLR3 ligand polyinosinic-polycytidylic acid (poly I:C) induced NK cells to kill activated HSCs and ameliorated liver fibrosis in 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) diet-induced liver fibrosis. In addition, NK cells depletion diminished the anti-fibrotic effect of poly I:C. These experiments suggest that NK cells play a role in TLR3 signaling

and the inhibition of liver fibrosis [18]. Furthermore, Gao et al. reported that poly I:C treatment stimulated NK cells to produce IFN- γ [86]. However, Jeong et al. compared liver fibrosis induced by a 2-week or a 10-week CCl₄ challenge. They observed NK activation HSC killing with Poly I:C treatment only in the early stage of fibrosis [92]. Previously, they reported that poly I:C-mediated inhibition of liver fibrosis was diminished in ethanol fed mice with CCl₄ treatment [73], suggesting that TLR3-mediated NK activation is ineffective in advanced stage of liver fibrosis and alcoholic liver fibrosis.

Fibrosis in PBC

Liver fibrosis and cirrhosis are histological characteristics in patients with advanced stage PBC [93–96], but rarely reported in murine PBC models. We have vigorously investigated the immunological mechanisms of fibrosis in human PBC [49, 97–109] and in various murine models of PBC [110–115], in particular in mice expressing the dominant negative form of TGF- β receptor type II (dnTGF β RII) in T cells [116–121]. Recently, we reported that the successful induction of liver fibrosis with poly I:C [112] and also with activation of iNKT cells by the administration of α -galactosylceramide (α -GalCer) in a 2-oxalic acid (OA)-BSA xenobiotic model of PBC [113]. Current data showed that Th1 profile is predominant in human PBC livers [98, 122], and NKT and TLR3 activation (poly I:C) contribute to the liver fibrosis in murine models of PBC [112, 113, 123]. On the other hand, Th17 elevation, Treg diminution, and NK cell elevation are characteristic and essential for pathogenesis in PBC [104], but their significance in liver fibrosis is still unclear.

Th1 Dominance and Th17/Treg Imbalance

Generally, in the initiation and perpetuation of liver fibrosis, Th2 cell responses are more dominant than Th1 cell responses [43]. However, Th1 cells are dominant over Th2 cells in PBC patients [98] even in advanced stage PBC patients [122]. Thus Th1 cytokines may be important in the initiation and/or perpetuation of liver fibrosis in PBC. However the mechanisms are still unknown. Previous studies by our group demonstrated that IFN- γ expressing cells, when compared to IL-4 expressing cells, were predominant around injured bile ducts in PBC, suggesting that PBC is Th1-dominant disease [98] (Table 1).

Taking advantage of an autoimmune murine model of dnTGF β RII that exhibited characteristic autoimmune biliary ductular disease of PBC, we have begun to dissect the immunological mechanism of autoimmune mediated biliary damage. The dnTGF β RII mice are engineered for directed expression of a dominant negative form of the TGF- β

receptor type II (dnTGF β RII), under the control of the CD4 promoter lacking the CD8 silencer, and spontaneously develop immunological, histological and serological characteristics that closely resemble human PBC [116].

Importantly, IL-12p40 deficiency eliminated biliary disease in dnTGF β RII mice [119]. The IL-12 family, composed of IL-12, IL-23, IL-27, and IL-35, is an important group of cytokines in the cytokine network of the innate and adaptive immune system [124, 125]. Specially, p40 chain (IL-12R β 1) is shared by IL-12 and IL-23. The lack of the p40 subunit of IL-12 results in the absence of both IL-12 and IL-23 [126–128]. In our dnTGF β RII mice, TGF- β signaling has already been blocked and is incapable of Th17 induction which is mediated by IL-23, demonstrating that IL-12 plays a dominant role in portal inflammation. Furthermore IL-12 promotes the differentiation of naïve T lymphocytes into Th1 cells consistent with PBC being a Th1-dominant disease. On the contrary, the presence of clinical features of PBC including AMA and bile duct destruction in a child with IL-12 deficiency [129] and the association of SNPs in the intronic regions and regions downstream of p35chain (IL-12R β 2) with PBC suggest a protective role for IL-12 in PBC pathogenesis [130].

Compared with other liver diseases (AIH, ALD, CHB, and liver allograft in HCV), PBC typically demonstrates a distorted balance from a Treg response towards Th17 in liver infiltrating lymphocytes as well as circulating T-helper cell subtype [131] (Table 1). Specifically, there is a decreased frequency of Tregs in peripheral blood samples of patients with PBC, compared with other diseases (AH, CHC) [105] (Table 1). In addition, it has also been reported that biliary epithelial cells can directly respond to IL-17, express IL-17 receptors and upon engagement, produces acute inflammatory cytokines such as IL-1 β , IL-6, and IL-23 which are theoretically prone to Th17 differentiations and averse to Treg differentiation [132, 133]. Thus, a Th17/Treg imbalance with Th17 high, Treg low is also supported by the immune-histological detection of IL-17⁺ cells infiltration in the vicinity of injured bile ducts in PBC [49, 132].

NK Cells and NKT Cells

NK cells have been associated with many autoimmune diseases, but the role of NK cells in the pathogenesis of autoimmune liver disease is unknown. We reported NK cell frequencies and numbers increased significantly in the peripheral blood and liver of PBC patients [104]. Furthermore, the cytotoxic activity and perforin expression of isolated NK cells from PBC patients were significantly increased and were associated with increased levels of plasma IL-8 and CD128a expression (IL-8 receptor) on NK cells [104]. By contrast, the production of IFN- γ , IL-6, and IL-8 in stimulated

NK cells from PBC patients were significantly reduced when compared to other liver diseases [104] (Table 1). Thus, NK cells may have a distinct mechanistic and perhaps even an accelerative role in the pathogenesis of PBC.

Our previous works in human PBC and in the dnTGF β R2 mouse model of PBC suggested that activation of NKT cells is a critical factor in accelerating disease development [99, 100, 117]. Increased intrahepatic NKT cells could be detected in liver tissue particularly around injured interlobular bile ducts in PBC, compared with normal livers and other diseased livers [99, 100, 134] (Table 1). Furthermore, CD1d expression is increased in small bile duct epithelium and epithelioid granulomas in the liver of PBC patients [99]. Recently, Harada et al. reported that the numbers of CD57⁺CD3⁺ NKT cells were increased in portal tracts, especially around injured interlobular bile duct in PBC. On the other hand, NKT cell deficiency attenuated the development of PBC in the dnTGF β R2 mouse [117] and *Novosphingobium aromaticivorans* infection mouse model of PBC [123].

Recently, we have reported the importance of NKT cells in the liver pathology in the 2OA-BSA-induced mouse model of PBC. Particularly, the activation of NKT cells by α -GalCer aggravated the cholangitis and increased anti-mitochondrial autoantibodies (AMA) production, portal inflammation and bile duct damage [113]. Especially, significant fibrosis was induced by multiple treatments of α -GalCer.

TLR Function

Recent reports suggested that several TLR are implicated in the pathogenesis of human PBC [101, 103, 106]. For example, memory B cells from patients with PBC were hyper-responsive to bacterial CpG-B (TLR9) stimulation, which, in part, might be responsible for elevated IgM and AMA levels in the sera of patients with PBC [101, 106]. Moreover, we reported that the innate immune system sensitivity is increased in patients with PBC, particularly in monocyte responses to TLR ligation [103].

A number of studies have suggested that the TLR3 pathway is critical in the pathogenesis of PBC. Specifically, the livers from PBC patients have been shown to express markedly higher levels of TLR3 than other liver diseases, i.e., AH and CHC (Table 1), and TLR3 and type I IFN signaling pathways are active in both the portal tract and liver parenchyma of early-stage PBC [135]. Contrary to general liver fibrosis, activation of TLR3 with poly I:C produced eosinophil infiltration and bridging fibrosis in the liver of our 2OA-BSA-induced murine model [112]. The role of TLR3 in PBC is still unknown, but our results suggest that TLR3 activation could be a critical factor in modulating the natural history of PBC.

Conclusion

Liver fibrosis is a physiological manifestation of extensive liver damage in chronic liver diseases including PBC. The complexity in the natural history and etiology of liver fibrosis has made it difficult to suggest any standard treatment modalities. To date many anti-fibrotic therapeutic targets have been proposed for, or used in, clinical trials [7, 136], but their effect is limited to controlling the pathological development and have thus far fallen short of a cure. We believe that continuous growth in the knowledge of the immunological mechanisms of liver fibrosis with both human and animal studies will shed light on to possible common pathways that are critical in the initiation and perpetuation of this fibrosis and may help to identify novel therapeutic targets.

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