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Mouse models of liver fibrosis mimic human liver fibrosis of different etiologies

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Abstract

The liver has the amazing capacity to repair itself after injury; however, the same processes that are involved in liver regeneration after acute injury can cause serious consequences during chronic liver injury. In an effort to repair damage, activated hepatic stellate cells trigger a cascade of events that lead to deposition and accumulation of extracellular matrix components causing the progressive replacement of the liver parenchyma by scar tissue, thus resulting in fibrosis. Although fibrosis occurs as a result of many chronic liver diseases, the molecular mechanisms involved depend on the underlying etiology. Since studying liver fibrosis in human subjects is complicated by many factors, mouse models of liver fibrosis that mimic the human conditions fill this void. This review summarizes the general mouse models of liver fibrosis and mouse models that mimic specific human disease conditions that result in liver fibrosis. Additionally, recent progress that has been made in understanding the molecular mechanisms involved in the fibrogenic processes of each of the human disease conditions is highlighted.

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Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

Conflict of Interest

Allyson K. Martínez, Luca Maroni, Marcio Marzioni, Syed T. Ahmed, Mena Milad, and Debolina Ray. declare they have no conflict of interest.

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Keywords

hepatic fibrosis; murine model; hepatic stellate cell; primary biliary cirrhosis; primary sclerosing cholangitis

Introduction

The repeated insult that occurs during the progression of many chronic liver diseases continuously activates the wound healing response; it is this chronic activation of the wound healing response that causes liver fibrosis [1, 2]. The activation of hepatic stellate cells (HSCs), which are the main collagen-producing cell in the liver, is a pivotal event during liver fibrogenesis. Provoked by chronic liver injury, activated HSCs display a myofibroblast phenotype and exhibit fibrogenic potential [1, 3, 2]. Activated HSCs set in motion a cascade of molecular, cellular, and tissue events that lead to the deposition and accumulation of extracellular matrix (ECM) components, especially collagen, to limit hepatic damage observed in chronic liver diseases [4–6, 3]. However, the accumulation of collagen and other ECM components that occurs in chronic hepatic injury results in the progressive replacement of the liver parenchyma by scar tissue, thus resulting in fibrosis [1].

While liver fibrosis is the outcome of many different chronic liver diseases including chronic hepatitis C virus (HCV) infection, alcoholic steatohepatitis (ASH), nonalcoholic steatohepatitis (NASH) and auto-immune liver diseases, the pathogenesis of liver fibrosis depends on the underlying etiology [1, 2]. Therefore, liver fibrogenesis must be studied in the context of each of the chronic liver diseases that result in fibrosis. Mouse models of liver fibrosis that mimic human liver fibrosis have contributed to this need and have greatly enhanced the study of liver fibrosis [7, 8].

Rodent models can address specific questions that are difficult to address in human studies. Due to the lack of an early diagnosis of liver fibrosis in human subjects and the invasive nature of liver biopsies, which is the standard for liver fibrosis assessment, multiple sampling at different stages of liver fibrogenesis in humans is challenging. However, mouse models provide researchers with the opportunity to conduct studies using multiple samples and at different stages of liver fibrogenesis [8]. The use of mice has the advantage that the whole organ and organism is intact, which is one of the limitations of *in vitro* studies with human tissues or cell lines [8]. Finally, genetic studies using knockout mice or the ability to knockdown specific genes can be used to determine the role of these genes in the progression of liver fibrosis [8].

Although the use of mouse models in the study of liver fibrosis is a powerful tool, these models are not without their disadvantages. Most notably, there is a lack of an appropriate mouse model for liver fibrosis caused by alcohol abuse and chronic HCV infection [8]. Also, there are species differences between humans and mice in the immune response, gene regulation, and metabolic, pharmacological, and tissue responses [8].

Despite these limitations, liver fibrosis research using both human subjects and mouse models has seen countless advancements in recent years. The purpose of this review article

is to discuss some of the most recent advances in the study of liver fibrosis and to specifically parallel the advancements in mouse models of liver fibrosis to their human liver fibrosis counterparts.

General mouse models of liver fibrosis

Repetitive toxic insults

Carbon tetrachloride—Carbon tetrachloride (CCl_4) is a hepatic toxin that is commonly used to induce toxic liver injury in mice. CCl_4 is converted to a free radical by reductive dehalogenation catalyzed by cytochrome p450 2E1 (CYP2E1) in hepatocytes, which induces lipid peroxidation and membrane damage that causes centralobular necrosis [9–11]. CCl_4 is a fast acting toxin with morphological changes appearing at 15 minutes [12]. Acute administration (single dose) of CCl_4 results in centralobular necrosis and reversible injury that triggers a wound healing response [13, 14]. In addition to hepatocyte necrosis, acute administration of CCl_4 triggers apoptosis of large cholangiocytes, which is followed by the activation of proliferation and compensatory *de novo* expression of secretin receptor in small cholangiocytes [15, 16]. Liver fibrosis develops progressively during repetitive administration of CCl_4 [17–19]. Fibrosis appears initially in pericentral areas, which then progresses to bridging fibrosis, cirrhosis and eventually hepatocellular carcinoma [17–20]. CCl_4 has been administered to mice via different routes including intraperitoneal [18], subcutaneous[19] and oral gavage [19]. Each route has distinct advantages and disadvantages that have been reviewed elsewhere [8, 21, 19]. In addition to the progression of fibrosis and cirrhosis, the CCl_4 model has been used to study the mechanisms regulating the reversibility/resolution of fibrosis [22, 23].

Thioacetamide—Thioacetamide (TAA) is an organosulfur compound that has metabolic intermediates that are toxic to the liver. One intermediate, thioacetamide-S-oxidase, is a reactive oxygen species (ROS) that covalently bind to hepatic macromolecules resulting in necrosis of hepatocytes [21]. CPY2E1 has been shown to mediate TAA-induce hepatoxicity in mice [24, 25]. Chronic treatment of mice with TAA induces liver damage, fibrosis and eventually cirrhosis, which is associated with elevated oxidative stress and activation of hepatic stellate cells [26–28]. TAA can be administered by intraperitoneal injections or in the drinking water. The disadvantage of TAA is that it takes a relatively long time to induce liver fibrosis and there is the potential for the development of hepatocellular carcinoma [28–30].

Dimethyl or Diethylnitrosamine—Dimethyl or diethylnitrosamine (DMN and DEN) are highly toxic to the liver and are hydroxylated by CYP2E1 to form bioactive diazonium ions that react with nucleic acids to form alkylation products [31, 32]. DMN and DEN models are characterized by centrilobular and periportal liver damage with the subsequent development of liver fibrosis and cirrhosis [33–35]. These models provide a unique opportunity to study the pathogenesis of liver fibrosis to hepatocellular carcinoma [33–35].

Bile duct ligation (BDL)

Model of Secondary Biliary Fibrosis—Ligation of the common bile duct (BDL) stimulates the proliferation of biliary epithelial cells (i.e., cholangiocytes) that line the bile ducts along with cholestasis, portal inflammation and subsequently portal fibrosis [36]. Although the model is characterized by extensive ductular proliferation, portal myofibroblasts have been proposed to be an important contributor to the progression of biliary fibrosis [37, 38]. Rats are more suitable for the model due to a lack of a gall bladder. However, despite higher surgical complications and mortality the model is commonly used in mice [8].

Abcb4$^{-/-}$

The ATP-binding cassette subfamily B member 4 (ABCB4) is a gene that encodes the multidrug resistance 3 (MDR3) protein (MDR2 in mice), which is a canalicular translocator for phosphatidylcholine [39]. A mutation in the ABCB4 gene can cause progressive familial intrahepatic cholestasis (PFIC3) and primary biliary cirrhosis (PBC). Due to the lack of protection against bile acids [40–42], these individuals experience increased damage of the biliary epithelium, ductular proliferation and potential progressive portal fibrosis [43]. ABCB4 knockout mice (Abcb4$^{-/-}$) have been used to study the pathophysiology of PFIC3 and PBC, and their potential therapies. Abcb$^{-/-}$ mouse models have also been used to study cholestasis of pregnancy and drug-induced cholestasis [44].

Recent studies using Abcb$^{-/-}$ mice have provided insight into the pathophysiology behind chronic cholestatic liver disease and have explored new therapeutic options for the treatment of these diseases. Alterations in lipid metabolism and in the expression of canalicular transporters that regulate bile composition contribute to the progression of cholestatic liver disease in Abcb$^{-/-}$ mice [45, 46]. Recently, a derivative of ursodeoxycholic acid (UDCA), norUDCA, has been found to decrease hepatobiliary injury in BDL mice, raising the possibility that norUDCA could be used as a therapeutic in the treatment of cholestatic liver disease [47].

D-galactosamine (d-GalN)

D-galactosamine (d-GalN) is a hepatotoxin, which causes acute hepatic injury and has been a good model for monitoring the progression of chronic biliary diseases. d-GalN causes UDP glucose and UDP galactose deficiency, loss of intracellular calcium homeostasis, inhibition of energy metabolism of hepatocytes, and injuries of the mitochondrial enzymes affecting lipoprotein interactions[48–50]. To understand the inflammation induced pathway in hepatocytes, d-GalN/Lipopolysaccharide treatment was performed to show that hepatic injury is facilitated by TNF-α. For therapeutic purposes, S-adenosyl-L-methionine (SAMe) was found to have protective effects *in vivo* and *in vitro* on liver cell damage caused by d-GalN [51], including enhanced bile secretion, improved liver function tests and amelioration of symptoms of d-GalN induced hepatotoxic mice [52, 53]. Glucuronidation was found to be an important step in the pathogenesis of ethinylestradiol (EE)-induced cholestasis. When administered with d-GalN, there was an improvement in cholestasis because d-GalN decreases the UDP-GA availability required by EE 17 β-glucuronide, thus showing that these molecules are involved in cholestasis [54]. Hepatoxins, such as d-GalN, can cause

inhibition of mature hepatocytes. When this occurs, a regenerative process occurs where hepatic stem/progenitor cells become activated eventually forming hepatocytes or biliary epithelial cells (BEC) [55–60]. Using the D-galactosamine injury model, the Thy 1+ cells would differentiate into hepatocytes and cholangiocytes on day 2 and 3 of hepatic injury to aid in the recovery process [61].

Methione and choline deficient (MCD) and choline-deficient L-amino acid defined diet (CDAA)

The methionine and choline deficient diet (MCD) is a common dietary mouse model that is used for studying the pathophysiology of NASH [21]. The diet contains about 40% of high sucrose and 10% of high fat. This diet lacks methionine and choline, which are required in mitochondrial β-oxidation and synthesis of low-density lipoprotein (LDL) [62]. It can cause more reactive oxygen species, mitochondrial DNA damage and apoptosis compared to other NASH models [63]. Wistar rats are more susceptible to this diet, but Long-Evans and Sprague-Dawley rats are also used in presenting steatosis [64]. The disadvantage regarding this model is that the metabolic profile has differences compared to human NASH [65–67].

Recent studies using the MCD diet model have elucidated factors involved in the progression or reduction of NASH. Mesenchymal epithelial transition factor (c-met) receptor signaling has been shown to activate anti-apoptotic pathways in hepatocytes (74). Caspases, most recently Caspase 3, have been shown to be involved in the proapoptotic and proinflammatory processes in NASH (75). Finally, a therapeutic study was done regarding Sitaglipin in MCD fed mice. Sitagliptin showed attenuation of hepatic steatosis, inflammation and fibrosis insinuating future therapy application [68]. However, the next step would be to check long-term side effect profiles of Sitagliptin in NASH mouse models.

Choline-deficient L-amino-acid-defined (CDAA) is a well-known dietary model that induces pathogenesis related to NASH. During continuous consumption of a CDAA diet, animal models have shown induction of steatosis, lobular inflammation and fibrosis [69, 70]. In one study, CDAA fed Wistar rats continued to have fibrosis even after reverting back to a choline sufficient diet, but the steatosis and lobular inflammation improved. The persistent fibrosis was likely due to the hypoxic damage and oxidative stress the CDAA diet caused (79). Choline is an essential nutrient that is involved in VLDL production via phosphatidylcholine. Without choline, hepatic lipidosis can occur causing a decrease in lipid and cholesterol excretion [71–73].

Choline deficiency can also cause oxidative stress, mitochondrial dysfunction and endoplasmic reticulum stress. Consequently, the animal model is more susceptible to hypoxic damage resulting in significant hepatocellular death [74, 71, 75]. Lack of choline can also cause induction of a proinflammatory cascade that eventually activates HSC causing fibrosis [76, 77].

Mouse models that mimic specific human diseases

Auto-immune fibrosis

Primary biliary cirrhosis (PBC)—Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease that predominantly affects middle age women [78]. Intrahepatic small bile ducts are progressively destroyed by an immune-mediated attack and the disease may slowly progress until liver cirrhosis. PBC is considered an autoimmune disease. Antimitochondrial antibodies (AMAs) directed against the E2 component of the pyruvate dehydrogenase complex (PDC-E2) are present in the sera of about 95% of the patients, and are detectable years before the appearance of clinical symptoms [79, 80, 78].

Animal models of PBC aim to specifically recapitulate the complex pathophysiological characteristics of the human disease. Despite remarkable advances in the last decade, to date none of the proposed models can perfectly resemble the complexity of the human disease.

Murine models of PBC can be divided into spontaneous models, if biliary alterations appear in genetically modified animals without additional interventions, and induced models, in which biliary damage appears after breakdown of tolerance to PDC. Koarada et al. described the NOD.c3c4 congenic mouse as the first spontaneous model for autoimmune biliary diseases [81, 82]. NOD.c3c4 mice develop lymphocytic peribiliary infiltrates, autoantibodies, and progressive cholestasis [81]. The full-blown disease is present in 50% of the females and in 25% of male within one year of age [83]. Moreover, 55% of the NOD.c3c4 mice develop antibodies against the PDC-E2 complex before the biliary lesions are completely formed. The microscopic alterations in the liver include the infiltration of CD3+, CD4+, and CD8+ T cells with sporadic formation of granulomas [83]. Despite the fact that NOD.c3c4 mice recapitulate several features of the human disease, the appearance of alterations that are not found in PBC patients is reported too. Biliary cysts in the intrahepatic bile ducts develop in the majority of the animals, and seem related to a B cells response [84]. Moreover, unlike PBC, common bile duct dilatation is also present in NOD.c3c4 mice [83].

An alternative model for PBC is the dominant-negative TGF-β receptor II (dnTGF-βRII) mouse [85]. $CD4^+$ and $CD8^+$ T cells of dnTGF-βRII mice over-express a mutated form of the TGF-βRII that is unable of signal transduction. As a result, the immune homeostasis is altered and clusters of immune cells infiltrate the liver parenchyma [86]. Gershwin et al. showed that dnTGF-βRII mice develop several features of the human PBC, including spontaneous production of antimitocondrial antibodies to PDC-E2, $CD4^+$ and $CD8^+$ T cells infiltration within the portal tract of 6- to 7-month-old mice, and increased levels of IFN-γ, TNF-α, IL-6, and IL-12p40 [85, 87]. However, the general dysregulation of self-tolerance in dnTGF-βRII mice should not be overlooked. In fact, starting from 3–4 months of age, dnTGF-βRII mice develop a wasting syndrome associated with diarrhea due to marked to severe inflammatory bowel disease, with infiltration of lymphocytes, macrophages, and plasma cells in the gut [86]. Mild inflammatory infiltrates appear also in the lungs, stomach, duodenum, pancreas, and kidney [86]. The development of cholangitis in dnTGF-βRII mice seems does not seem to be related to abnormalities of the biliary tree. Indeed, bile duct inflammation occurs also if splenocytes of dnTGF-βRII mice are transferred into

recombinase-deficient ($Rag1^{-/-}$) mice, which lack a diversified B and T cell receptor repertoire [88]. In addition, $CD8^+$ T cells seem to be the primary effectors of the inflammation [88].

The importance of immune regulation in the pathogenesis of PBC has been further emphasized in a subsequent murine model, the IL-2R$\alpha^{-/-}$ mouse. Wakabayashi et al reported the development of autoimmune cholangitis and AMAs in IL-2R$\alpha^{-/-}$ mice [89]. However, severe anaemia, lymphoproliferative disorders and inflammatory bowel disease are prevalent in these mice, especially after 2 months of age [90]. Similarly to dnTGF-βRII mice, $CD8^+$ T cells have been involved in the pathogenesis of the biliary injury, while $CD4^+$ T cells are responsible for the colitis [91].

The $AE2^{-/-}$ mouse is considered an additional mouse model of PBC [92]. The Anion Exchanger (AE) 2 is a Cl^-/HCO_3^- exchanger expressed in different cells, where it regulates the intracellular pH [93]. In cholangiocytes, AE2 is located at the apical membrane and is the main responsible for bicarbonate secretion in bile [94]. At 15 months of age, most of $AE2^{-/-}$ mice develop AMA against the PDC-E2 inner lipoyl domain, increased alkaline phosphatase levels in plasma and different degrees of portal inflammation. However, the liver damage is not progressive, a slight fibrosis is reported only in mice with florid portal infiltrates, and there are no gender differences [92]. Together with the liver phenotype, $AE2^{-/-}$ mice develop alterations in the immune system. Enlarged spleen, reduced $CD4^+/CD8^+$ ratio and altered cytokine production have been described in $AE2^{-/-}$ mice, possibly as a consequence of defective pH regulation in immune cells [95, 92]. Interestingly, natural regulatory T cells (Tregs) are also reduced in this mouse model. This finding is in accordance with what described in PBC patients [96] and in dnTGF-βRII and IL-2R$\alpha^{-/-}$ models [85, 89], underlying an important pathogenic role of loss of tolerance in PBC.

The induced mouse models of PBC rely on the breakdown of tolerance to PDC, the mitochondrial autoantigen against which AMA are directed. Jones et al. reported that immunization of SJL/L mice with intraperitoneal injections of bovine PDC-E2 emulsified in complete Freund's adjuvant (CFA) containing 10 mg/ml of *Mycobacterium Tuberculosis* is able to induce AMA formation and non-suppurative destructive cholangitis [97, 98]. Some authors have however questioned the specificity of the immune response in SJL/L mice [99, 100]. Recently, an additional model for PBC has been successfully induced by the immunization of mice with 2-octynoic acid (2OA) coupled to bovine serum albumin [101]. Immunized mice manifest typical autoantibody formation and cholangitis but fail to develop fibrosis. 2OA is a chemically synthetized compound which is widely present in cosmetic products. This model offers therefore an intriguing conceptual support to an environmental origin of PBC [101]. To this extent, previous work showed that xenobiotic modification of PDC-E2 is able to generate new antigens that react with the autoantibodies present in PBC sera [102].

In conclusion, considerable advances in the understanding of PBC pathophysiology have been made in recent years through the study of animal models [103]. Since UDCA still represent the only recommended medical treatment for PBC, murine models offer also the possibility to evaluate potential new drugs. Different compounds have indeed shown

promising effects in attenuating the biliary damage in a number of PBC models, suggesting new therapeutic approaches that deserve further studies [104, 105].

Primary sclerosing cholangitis (PSC)—Primary Sclerosing Cholangitis (PSC), first described in the mid-1850's is characterized grossly by chronic cholestasis accompanied by inflammation of the biliary epithelium resulting in multifocal biliary strictures. PSC is asymptomatic in 50% of the diagnosed patients [106].

Diagnosis of PSC is made through liver enzyme assessment where the most commonly dysregulated candidate is alkaline phosphatase. Total bilirubin remains normal in most of the cases whereas the level of amino transferases is elevated in a very small number of patients [107]. One of the major risk factors contributing to the development of PSC is Inflammatory Bowel Disease (IBD) [108]. A multitude of factors have been deemed responsible for the development and progression of this disease. Considering the heterogeneity of PSC and all the variable factors contributing to this disease, a single animal model mimicking human PSC is hard to develop. Hence, there are a few different models that are used to study the mechanisms involved PSC pathogenesis.

MDR2$^{-/-}$: Liver cirrhosis resulting from chronic cholangiopathies such as PSC is marked by a massive increase in liver fibrosis. The MDR2$^{-/-}$ mice develop severe biliary fibrosis, and thus have been used has a model of liver fibrogenesis in PSC [109].

Decreased phosphatidylcholine in the bile of MDR2$^{-/-}$ mice might potentiate the toxicity of other bile acids. It is a multistep process where there is leakage of bile (from disrupted tight junctions and basement membranes of bile ducts) into portal tracts causing inflammation and fibrosis [110]. Fibrosis in MDR2$^{-/-}$ mice is caused by a time dependent alteration in expression of pro and anti-fibrotic genes [110, 109]. The inflammatory response in MDR2$^{-/-}$ mice varies according to the age of the animal, although there is over expression of at-least some factors such as TNF-α, IL-1β, Il-6, TGF- β1 and interferon-γ when compared to MDR2$^{+/+}$ control mice. The MDR2$^{-/-}$ animals can thus serve as a good model to intervene for developing treatment strategies to tackle the fibrotic response in PSC. norUDCA or UDCA treatment has already proved to ameliorate fibrosis in PSC models, though controversies exist in regard to their applicability in human subjects [111].

CFTR mutation: Mouse models with mutations in the exon 10 of the cystic fibrosis (CF) transmembrane conductance regulator (CFTR) gene have been shown to develop focal cholangitis and biliary cirrhosis [112]. Specifically, loss of function of the CFTR gene in biliary epithelial cells results in decreased bile flow and alkalinization in subjects with cystic fibrosis. In a study to evaluate the role of CFTR gene in development of cholangitis, mice with disrupted CFTR gene (*cftr*$^{-/-}$) were fed Dextran sodium sulfate (DSS) to induce colitis. DSS caused biliary damage and portal inflammation as displayed by enhanced ductular reaction and high reactivity of cholangiocytes (isolated from the *cftr*$^{-/-}$ mice) towards LPS treatment [113]. After DSS treatment, intestinal permeability to microbial products as well as endotoxins is increased which reach the liver via portal circulation resulting in inflammation and fibrosis[114, 115]. Taken together, these results suggest that the CFTR mutation is not the only cause for biliary cirrhosis and portal hypertension. This is

statistically supported by patient data where it is found that among patients with CF disorder, about 40% display abnormal hepatic imaging and biochemistry and among which only 5–10% develop focal biliary cirrhosis and portal hypertension[116]. This is an indication that CFTR dysfunction predisposes to liver diseases.

3,5-diethoxycarbonyl-1,4-dihydrocholine (DDC): To study the pathological alterations occurring in the earlier phases of PBC and PSC, a slowly progressing model is essential. Fickert and colleagues have demonstrated that continuous feeding of 3,5-diethoxycarbonyl-1,4-dihydrocholidine (DDC) induced chronic cholangiopathy that progressed slowly over a period of time. Mice fed with this xenobiotic agent for a week showed ductal proliferation, which progressed slowly over time, and by four weeks post treatment the bile ducts contained pigment plugs. The intraductal plugs showed autofluorescence generated from biliary protoporphyrin secretion [117]. Infiltration of neutrophils around both large and small bile ducts, an increase in serum transaminases and an induction of reactive phenotype of the biliary epithelial cells was also observed in the DDC fed mice. Recently, morphological studies confirmed hepatocellular necrosis and phagocytosis of these necrotic cells by Kupffer cells and showed compensatory hepatocyte proliferation in response to DDC-induced injury. This study also revealed that bile canalicular abnormalities occur prior to ductular reactions and periductal fibrosis in this novel xenobiotic induced model of primary sclerosing cholangitis [118]. This was the first study showing these characteristics associated with progression of PSC.

Autoimmune hepatitis (AIH)—Autoimmune hepatitis (AIH) is a form of chronic hepatitis. It is characterized by histological findings (most commonly interface hepatitis), elevated serum aminotransferases, hypergammaglobulinemia, and seropositivity for ANA, anti-LKM-1, and SMA, after the exclusion of other causes of chronic hepatitis [119]. It is important to exclude other causes of chronic hepatitis by checking viral serologies, obtaining a good history for substance abuse including alcohol, and ruling out biliary sources of chronic hepatitis. Disease severity can range from asymptomatic hepatitis [120] to severe, fulminant hepatic failure [121]. Although the cause of AIH remains unknown, the working model of pathogenesis is recognition of self-antigen or autoantibody/antigen complexes by $CD4^+$ T cells [122] resulting in loss of tolerance and progressive necroinflammation and fibrosis in a host with genetic pre-disposition [123]. HLA genes have increasingly been implicated in the genetic link of AIH [123].

Cytokines, specifically TGF-β, have been shown in murine models to play a large role in immune tolerance. TGF-β is secreted by phagocytes that are exposed to apoptotic T cells. This contributes to immune tolerance by inducing CD4+Foxp3+ regulatory T cells via CD3-specific antibody [124]. Additionally, TGF-β is found to have increased expression in hepatic inflammation. This overexpression is thought to play a role in the suppression of an auto-immune response. Impairment of this signaling pathway has been shown to increase susceptibility of AIH based on histological findings in murine models [125]. This pathway has therefore been used to produce animal models of AIH. TGF-$\beta1^{-/-}$ mice spontaneously develop necroinflammatory hepatitis recapitulating human aspects of AIH [126, 127].

Conclusion

Liver fibrosis is the consequence of many chronic liver diseases and regardless of the etiology is the result of a highly coordinated process. Murine models of liver fibrosis recapitulating fibrogenesis in human liver disease conditions are valuable tools for studying the fibrogenic process of specific diseases. Although recent advances have been made in understanding the molecular mechanisms involved in fibrogenesis and in discovering novel tools that can aid in the diagnosis and treatment of liver fibrosis, additional studies are still needed. Specifically, the efficacy of these diagnostics and therapeutics in human patients still needs to be explored.

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