Animal models of chronic liver diseases

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Submitted 11 May 2012; accepted in final form 22 December 2012

Chronic liver disease (CLD) refers to a disease process that involves progressive destruction and regeneration of liver parenchyma leading to fibrosis and cirrhosis. Cirrhosis represents the final stage of liver fibrosis, characterized by disrupted liver architecture along with fibrotic bands, parenchymal nodules, and vascular distortion. Pathophysiologically, this often leads to portal hypertension and hepatic failure. Cirrhosis can be considered a premalignant condition and patients with liver cirrhosis are usually at high risk of developing hepatocellular carcinoma (HCC), which ranks as the fifth most common cancer, causing over 600 thousand deaths per annum worldwide. The major causes of CLDs include chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, alcoholic liver disease (ALD), nonalcoholic fatty liver disease (NAFLD), biliary fibrosis, and parasitic infection such as schistosomiasis in developing countries.

Improving our understanding of the pathogenesis of CLDs and development of novel diagnostic, prognostic, and therapeutic tools critically depends on robust and reproducible experimental animal models. However, to date no single animal model exhibits all attributes of human liver diseases. Currently used animal models for studying CLDs are aiming to imitate only particular characteristics of human diseases, and so it is especially important that the appropriate animal models are selected to answer specific research questions. Among a variety of experimental animals, rodents, particularly mice, are of special interest to the researchers because they are small, have a short life span and gestational period, and are therefore easy to maintain and breed in captivity. Furthermore, the remarkable genetic similarity of mice to humans, combined with the possibility and convenience of genetic manipulation, also account for mice often being the experimental animals of choice to model human diseases.

The present review will systematically summarize the most commonly used rodent models for studying CLDs. We roughly divided these animal models into two groups: 1) those generally used for studying mechanisms of liver fibrosis irrespective of disease etiologies and 2) those used to mimic specific CLDs including autoimmune and cholestatic liver diseases, chronic

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viral infection, NAFLD, and ALD. New models, technologies, and approaches as well as their potential impact in understanding etiopathological characteristics of different CLDs and development of therapeutic treatment will be particularly highlighted. In addition, the major strengths and shortcomings of specific models will be discussed.

**CLASSICAL ANIMAL MODELS OF EXPERIMENTAL LIVER FIBROSIS**

Liver fibrosis and its advanced form, cirrhosis, represent the common final pathway of most types of CLDs. The cellular and molecular mechanisms of hepatic fibrogenesis have been extensively investigated with the use of multiple complementary experimental animal model systems.

Fibrosis models were first developed in rats and then adapted to mice to take advantage of available genetic manipulation. Rats generally respond to fibrotic stimuli with development of a fibrotic reaction that is much more robust than that of inbred mice. However, the resistance to fibrosis in mice can be overcome by optimizing experimental procedures for some strains (12). Marked strain differences in fibrosis susceptibility often exist in inbred mice when using the same model (62), and, therefore, a second model is usually required to exclude model specific artifacts and to strengthen the data obtained.

**Chemically Induced Liver Fibrosis Using Hepatotoxins**

Repetition of a toxic insult to the liver is a classic way to induce liver fibrosis in experimental animals. **Carbon tetrachloride.** Carbon tetrachloride (CCl4) is one of the most widely used hepatic toxins for experimental induction of liver injury in laboratory animals. CCl4 directly impairs hepatocytes by altering the permeability of the plasma, lysosomal and mitochondrial membranes. Its metabolite, a toxic trichloromethyl (CCl3−), such as phenobarbitone, phospho-

**Bile duct ligation (BDL) is a classic experimental model for secondary biliary fibrosis (91). This technique requires a midventral laparotomy and isolation of the common bile duct above the duodenum, followed by double ligation of the bile duct and dissection between the ligatures (175). BDL stimulates the proliferation of biliary epithelial cells and oval cells, resulting in proliferating bile ductules, cholestasis, portal inflammation, and fibrosis (Fig. 1D), causing secondary biliary cirrhosis, and ultimately leads to liver failure (52, 91, 150). Rats are especially adapted to the BDL model because they lack the gall bladder; however, BDL is also widely used in mice. Relatively high mortality rates due to bile leakage and rupture of a biliary cyst (or gall bladder in mice) is a major drawback when performing BDL. Species and strain differences in the response to biliary obstruction are noteworthy. BDL may be difficult to apply to specific strains with altered wound healing or immune response (175). Spontaneous resolution of biliary fibrosis was observed in rats following bilo-
Fig. 1. A: bridging fibrosis in a 4-wk CCl4-treated C57/BL6 mouse. Arrows show bridging fibrosis between portal tracts [left, hematoxylin and eosin (H&E) staining]. Right panel highlights infiltrated inflammatory cells and damaged hepatocytes. B: histological changes after bile duct ligation (BDL) operation in C57/BL6 mice (H&E staining). Bile infarct demonstrates 18 h after BDL (arrow). Significant duct proliferation and fibrosis occur after 5 days of operation. C: typical biliary damage in 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC)-fed mice. Four weeks of DDC feeding induces protophorphyria deposits/plugs (I, H&E staining) and active ductular reaction [II, CK19 immunohistochemical (IHC) staining]. Three months of DDC feeding induces Mallory-Denk body in hepatocytes (III and IV, arrows, H&E staining). D: high-fat diet (HFD) induces inflammation and fibrosis in Sprague-Dawley rats (H&E staining). Macrosteatosis demonstrates in liver from a 12-wk HFD fed rat (left). Right; significant steatohepatitis and fibrosis after 24-wk HFD feeding. E: early liver damage in Tsukamoto-French model. Severe macrovesicular steatosis develops after 6-wk intragastric feeding of C57/BL6 mice with ethanol (p-Smad2 IHC staining).
jejunal anastomosis (73, 150), and therefore BDL has also been used to study fibrosis reversibility.

**Genetically Engineered Mice**

Transgenic animal technology allows germline insertion of exogenous genes or disruption of endogenous genes and has emerged as a powerful tool for in vivo analysis of gene function. Transgenic technology is usually adapted to mice to take advantage of mouse genetics. In the last decade, numerous genetically modified models or a combination of genetic modification with a profibrotic stimulus (e.g., CCl₄, BDL, chemical xenobiotics, dietary and environmental factors) have allowed investigators to evaluate the function of various candidate genes in progression of liver fibrosis. We summarized several genetically modified mice that spontaneously develop liver fibrosis in Table 1. A more detailed review about transgenic/knockout mouse models for studying liver fibrosis is provided by Hayashi and Sakai (58).

**ANIMAL MODELS MIMICKING SPECIFIC CHRONIC LIVER DISEASES**

Etiology deeply impacts on pathomechanisms and progression of liver fibrosis. Therefore, animal models mimicking specific attributes of CLDs are particularly useful resources for understanding disease pathogenesis and identifying diagnostic markers, as well as enabling development of therapeutic strategies.

**Schistosomiasis Model**

Schistosomiasis is a parasitic disease associated with liver injury and fibrosis. According to the World Health Organization, more than 200 million people are infected worldwide, resulting in around 200 thousand deaths annually. Despite the availability of chemotherapy with praziquantel, hepatic fibrosis remains among the most serious sequelae of chronic schistosoma infection and occurs in up to 20% of infected individuals.

Mice infected with *Schistosoma mansoni* develop granulomas in the liver from an immunological response against the eggs of the parasite that are deposited in the portal tract. Fibrosis follows the granulomatous inflammatory response and occurs mostly at the site of the resolving granulomatous reactions but also appears around portal veins (16). Clinical syndromes in murine hosts comprise hepatosplenomegaly, tissue fibrosis, portal hypertension, ascites formation, and bleeding that closely resembles those observed in human schistosomiasis (1). Murine schistosomiasis is thus widely used for studying the immunopathology of human schistosomiasis and the associated liver fibrosis (217).

**Animal Models of Autoimmune Hepatitis**

Autoimmune hepatitis (AIH) is a potentially severe and chronic inflammatory disease of the liver, characterized by a loss of self-tolerance leading to elevated serum transaminases, appearance of circulating autoantibodies, hypergammaglobulinemia, and chronic hepatitis (118). AIH is classified into three subtypes according to the seropositivity of autoantibodies, with the type I AIH being the most common type. AIH mostly affects women and is usually recognized during late disease stages, and so information about onset and course of the disease is limited. Therapeutic intervention that aims at dampening the overactive immune system often has potentially severe side effects. Therefore, convenient animal models of AIH are required to increase our knowledge of the disease and to explore new drugs for improved therapy. Such models representing AIH have recently been described in detail (57, 76), and, therefore, we just highlight here the most representative ones and some new approaches.

**Concanavalin A Hepatitis.** Concanavalin A (Con A) is a plant lectin that is purified from jack beans. Con A binds mannose residues of different glycoproteins and induces lymphocyte activation. Systemic application of Con A leads to nonspecific activation of T cells (CD4⁺ T cells, NKT cells, and Kupffer cells) and results in severe liver injury (188), which closely mimics the pathological changes in AIH patients. Major effectors cytokines involved in Con A-induced liver damage are TNF-α and IFN-γ (50, 96), whereas IL-10 plays a protective role in this model (34, 114). Blood levels of IL-2, IL-4, and IFN-γ increase dramatically upon Con A administration (214). Because of this, Con A hepatitis is considered a model for cytokine storm-induced acute liver injury, rather than a model of chronic autoimmune hepatitis. Major drawbacks of this model include lack of circulating autoantibodies and rapid hepatocyte damage after a single dose of Con A injection, which is not a typical feature of chronic AIH. Nevertheless, Con A hepatitis is suggested as a convenient animal model for developing and testing new drugs.

**TGF-β₁⁻/⁻ mice on Balb/c background.** TGF-β₁ exhibits a variety of anti-inflammatory activities and contributes to immune homeostasis and prevention of autoimmunity in the body.
ingly, mice lacking TGF-β1 develop severe inflammatory lesions involving several organs and die from cardiopulmonary failure at 3–5 wk of age (95, 169). However, TGF-β1−/− mice on the Balb/c background specifically develop aggressive necroinflammatory hepatitis that was not observed in TGF-β1−/− mice on a different genetic background (54). The phenotype of this model recapitulates many aspects of AIH, including spontaneous hepatitis development and involvement of the Th1 cytokine IFN-γ. Although TGF-β1−/− mice are not an ideal model for chronic hepatitis development because the mice usually die after a few weeks, this model demonstrates how genetic defects can lead to liver-specific autoimmunity on a defined genetic background.

**NTx-PD-1−/− mice.** Regulatory T cells (Tregs) are a specialized subpopulation of T cells that function as immune suppressors and maintain tolerance to autoantigens and immune system homeostasis. Performing neonatal thymectomy (NTx) on programmed death (PD)-1−/− mice induces characteristics of human AIH due to loss of PD-1 signaling and reduced Treg numbers. NTx-PD-1−/− mice produce antinuclear antibodies and develop fatal hepatitis with increased serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as well as hepatic T cell infiltrations with massive lobular necrosis (86). NTx-PD-1−/− mice represent the first model for spontaneous fatal AIH and they usually succumb around 3 wk after birth because of fulminant hepatic failure.

**Models of chronic AIH.** So far, very few models can achieve chronic autoimmune hepatitis that fulfills the AIH criteria in human patients. Zierden et al. (227) recently generated Alb-HA/Cl4-TCR double-transgenic mice that spontaneously develop chronic hepatitis. Alb-HA/Cl4-TCR mice express viral hemagglutinin (HA) under control of the liver-specific albumin promoter and a specific T cell receptor (TCR) for an major histocompatibility complex (MHC) class I-restricted epitope of HA on CD8+ T cells. These mice spontaneously develop necroinflammatory lesions, hepatic fibrosis and increased levels of aminotransferases, resembling those features of human AIH. A major weakness of this model is that only males develop hepatitis.

Cytochrome P-450 2D6 was previously identified as major autoantigen in type II AIH in humans. In line, injecting wild-type FVB/N mice with an adenovirus expressing human cytochrome P-450 2D6 (Ad-CYP2D6) leads to a chronic form of severe autoimmune liver damage with subcapsular fibrosis (63, 64). Interestingly, transgenic FVB/N mice expressing the identical human CYP2D6 in their liver failed to develop persistent hepatitis upon injection with Ad-CYP2D6, indicating the presence of a stronger immunological tolerance. The Ad-CYP2D6 model is the first mouse model that uses molecular mimicry (25) as a trigger for autoimmunity. Expression of human CYP2D6 breaks self-tolerance to the mouse CYP2D6 homologue and subsequently causes persistent autoimmune liver damage. This model is suggested as a platform for studying mechanisms of chronic human AIH and evaluating therapeutic intervention (24).

**Animal Models of Primary Biliary Cirrhosis**

Primary biliary cirrhosis (PBC) often develops in middle-aged women with unknown etiology (146). It is characterized by destruction of intrahepatic bile duct epithelial cells and a progressive portal lymphocytic inflammatory response. PBC is considered a prototype of autoimmune diseases of the liver with both humoral and cellular immunological features. Antimitochondrial antibodies (AMAs) that react with the pyruvate dehydrogenase complex (PDC), targeting the inner lipoyl domain of the E2 subunit (anti-PDC-E2), appear in the serum of almost all PBC patients. The primary lesion of PBC is caused by pathological destruction of interlobular bile ducts associated with lymphocyte and plasma cell infiltration. PBC typical granulomas are present in around 15% of patients. Fibrosis develops as the disease advances, appearing around the microsple duct and perportal areas. Ursodeoxycholic acid (UDCA) is currently the best treatment choice for PBC patients. Several studies suggest that UDCA is effective in improving liver biochemistry and helps to slow histological progression of the disease, although it is still controversial whether it improves disease prognosis (38).

Previously reported PBC models including PDC-immunized mice, neonatally thymectomized mice, antigen/PMBC (peripheral blood mononuclear cell) administration, MRL/lpr mice, and the graft-vs.-host disease model are now rarely used, because of their limitation in reflecting human PBC features and technical difficulties. Newly introduced PBC models can be roughly classified into spontaneous models and xenobiotic-immunized mice. Different aspects of these new models have been discussed in several recent reviews (26, 106, 146, 203), and we will discuss their major characteristics and some potential practical advantages to highlight their importance for current PBC model developments.

**Spontaneous mouse models.** NOD.c3c4 mice. NOD.c3c4 mice, the first spontaneous mouse model of PBC, were generated by introgression of B6- and B10-derived insulin-dependent diabetes regions (idd loci) into nonobese diabetic (NOD) mice, which thereafter are protected from diabetes (72). NOD.c3c4 mice spontaneously develop an autoimmune biliary ductular disease with lymphocytic portal tract infiltrates and autoantibodies to PDC-E2, which reflects several features of human PBC. Immunohistochemical staining demonstrated that the affected biliary epithelium is infiltrated with CD3+ and CD4+ cells, and CD8+ T cells. Depletion of T cells protects NOD.c3c4 mice from biliary disease, and, accordingly, adoptive transfer of splenocytes or CD4+ T cells to NOD-SCID mice causes autoimmune biliary disease, suggesting a central role of T cells in this model (72). NOD.c3c4 mice also develop biliary cysts, which are not known in PBC patients or in other PBC models (128, 132).

**dnTGF-βRII mice.** dnTGF-βRII mice are currently suggested as the most useful animal model for PBC. Studies in TGF-β1 knockout mice demonstrated the critical role of TGF-β in regulation of survival, activation, proliferation, and effector function of several immune cell types (146). dnTGF-βRII mice overexpress a dominant-negative form of the TGF-β type II receptor under control of the CD4 promoter, resulting in specific abrogation of TGF-β signaling in CD4+ T cells (53). These mice exhibit several major serological and histological characteristics of human PBC, including interlobular bile duct destruction, lymphocytic cell infiltration, and periportal inflammation. Spontaneous production of AMA appears in all dnTGF-βRII mice, and increased serum levels of inflammatory cytokines including TNF-α, IFN-γ, IL-12p40, and IL-6, which are typical for human PBC, can also be detected (137). Practically, dnTGF-βRII mice show minimum interindividual pathophysiological variations, and the humoral and cellular immunological features are highly reproducible. dnTGF-βRII...
mice can also be used with other genetically modified mice to create a variety of specifically modulated PBC models. A major weakness of this model is the development of severe immunological abnormalities in multiple other organs because of the lack of TGF-β signaling in T cells, and therefore the mice have a relatively short lifespan (199).

**IL-2Rα−/− mice.** IL-2Rα−/− mice also show many characteristics of human PBC. IL-2Rα (CD25) is important for development, activity, and expansion of CD4+CD25+ Tregs known to suppress autoreactive T cells (133). Accordingly, lack of CD25 in IL-2Rα−/− mice leads to proliferation and activation of corresponding effector T cells, development of portal inflammation, and biliary ductular damage with profound lymphocytic infiltration of CD8+ T cells into the portal tract (210). AMA is present in serum of all IL-2Rα−/− mice. However, IL-2Rα−/− mice additionally develop ulcerative colitis that in humans is usually associated with primary sclerosing cholangitis (PSC), but not PBC. The reduced lifespan of IL-2Rα−/− mice may also account for its limited popularity as experimental PBC model.

**Ae2a,b−/− mice.** Ae2a,b−/− mice were generated based on the clinical observation that the anion exchanger 2 (Ae2) gene expression is reduced in liver specimens and blood mononuclear cells of PBC patients (160). Ae2 mediates Cl−/HCO3− exchange across the plasma membrane and is critical in regulating intracellular pH and transepithelial acid-base transport. Ae2a,b−/− mice show immunological and partial hepatobiliary morphological features resembling PBC in humans. The mice display increased numbers of CD8+ T cells but reduced numbers of natural regulatory T cells, suggesting Ae2 as potential pharmacological target to modulate T cell responses. Approximately 80% of Ae2a,b−/− mice develop AMA, but only one third develop extensive portal inflammation and portal tract fibrosis. Nevertheless, the observations in Ae2a,b−/− mice indicate a close relationship between biliary epithelial dysfunction and pathogenesis of PBC, although the underlying mechanisms leading to Ae2 deficiency in human PBC still remain to be explored.

**Chemical xenobiotics-immunized mice.** Accumulating evidence suggests that various environmental factors such as bacterial infection and exposure to xenobiotics are also important in the pathogenesis of PBC. It has been previously hypothesized that prolonged exposure to 2-octynoic acid (2-OA), a xenobiotic with a structure similar to that of the inner lipoyl domain of PDC-E2, could serve as a trigger for PBC development (154). Wakabayashi et al. (209, 211) demonstrated that immunization of C57BL/6 mice and the NOD congenic mouse strain 1101 with BSA-coupled 2-OA induces a human PBC-like lesion. High levels of AMA were detected in both mouse strains. Furthermore, significant bile duct lesions are found in the portal tract, accompanied by increased portal lymphocytic infiltrates enriched in CD8+ T cells. This model could be of special interest for future studies of PBC, since it is practically very simple (intraperitoneal injection) and the resulting PBC-like pathophysiology is highly reproducible. The model can also be used to generate derived models with genetically modified mice for specified questions. Peritonitis might be the major drawback upon introducing BSA-2-OA into the abdominal cavity of the mice, and therefore delivery methods may need to be further improved.

**Animal Models of PSC**

PSC is a progressive cholestatic liver disease that mainly affects male patients (male-to-female ratio 2:1) and characterized by chronic inflammation and obliteratorive fibrosis of both intra- and extrahepatic bile ducts (38). PSC is associated with autoimmune features, displaying perinuclear antineutrophil cytoplasmic antibodies (pANCA) in up to 90% of patients (207). Over 60% of affected subjects additionally suffer from an inflammatory bowel disease (IBD), mostly ulcerative colitis. Fibrosis presents as marked concentric periductular fibrosis (“onion-skinning”) that gradually progresses and eventually leads to obliteration and loss of interlobular bile ducts (146). In particular, cholangiocarcinoma may arise in ~15% of patients. The etiology of PSC remains unknown and pathomechanisms are still largely undefined. Therefore, development of causative treatment is challenging and no specific medical therapy is currently available (216). UDCA is the only medication to date showing some efficacy in PSC patients, primarily on liver biochemistry (117). Other drugs such as immunosuppressive, antifibrotic, or antibiotic agents were suggested but have not been assessed in large clinical trials (38, 216).

There is need for novel therapeutic strategies against PSC requiring reliable and well-defined animal models. Although there is no ideal animal model available that exhibits all of the characteristics of PSC, several can be used to mimic certain features of human PSC.

The previously described rat model of small bowel bacterial overgrowth provides compelling evidence that bacterial cell wall components of anaerobic bacteria participate in the pathogenesis of PSC and may help to explain the strong association between PSC and IBD (107). Other reported models of PSC include direct injury of biliary epithelial, peribiliary vascular endothelial or portal venous endothelial cells, biliary instillation of trinitrobenzene sulfonic acid (TNBS), oral administration of α-naphthylisothiocyanate (ANIT), and Helicobacter infection in mice. Because these rodent models have been reviewed in detail by Vierling (207), we will no longer describe them here.

Animal models based on the “toxic bile concept” (189) have been recently developed to mimic human PSC. Mdr2−/− mice and DDC diet feeding (see DDC diet below) have become the most frequently used animal models for studying PSC today.

**Mdr2−/− mice.** Mdr2−/− mice as a robust model for chronic cholangiopathies were generated and initially characterized by Smit and colleagues (121, 141, 170). The multidrug resistance gene (Mdr2 in rodents/MDR3 in humans) encodes a canalicular phospholipid flipase that mediates the transport of biliary phospholipids into the outer leaflet of the canalicular cell membrane, which facilitates their subsequent excretion into the bile. Accordingly, lack of this transporter in Mdr2−/− mice leads to the absence of phospholipids in the bile, thus increasing biliary concentration of non-micellar-bound free bile acids, which results in intoxication-based hepatocellular and bile duct injury. Mdr2−/− mice spontaneously develop liver injury morphologically resembling human PSC (41, 46, 149). Accumulation of toxic bile salts in the intrahepatic biliary system disrupts tight junction and basement membranes of bile ducts, causing bile leakage to the portal tract, which further induces inflammation, ductular proliferation, and finally the formation of periportal biliary fibrosis (149). In addition, Mdr2−/− mice...
spontaneously develop cholesterol hepatolithiasis and HCC (83, 98) and therefore also represent a valuable model for investigating the development of HCC in an inflammatory and fibrotic setting. Furthermore, the Mdr2−/− model has important implications for understanding the pathophysiology of human diseases resulting from MDR3 defects, since mutations of human MDR3 induce a wide spectrum of cholestatic liver diseases, ranging from neonatal cholestasis/cholangiopathy to adult biliary cirrhosis (189). Moreover, as a highly reproducible fibrosis model, Mdr2−/− mice provide a unique opportunity to be targeted by pharmacological strategies for evaluating potential antifibrotic drugs. UDCA treatment for PSC has been tested in Mdr2−/− mice, demonstrating a detrimental effect at high dosages (46). The same group further demonstrated the potential therapeutic mechanism of norUDCA, a side chain-modified UDCA derivative, with Mdr2−/− mice (45, 56). More recently, blocking β-adrenoceptors by propranolol treatment was found to improve liver architecture, to delay progression of sclerosing cholangitis and to reduce fibrosis in Mdr2−/− mice (176). These findings are now discussed as a potential new therapeutic option to treat human PSC.

**DDC diet.** 3,5-Diethoxycarbonyl-1,4-dihydrocollidine (DDC) feeding in mice is a well-established model to study Mallory-Denk body formation, which is specifically associated with alcoholic and nonalcoholic steatohepatitis, metabolic liver diseases, and chronic cholestatic liver diseases (44, 225). Recently, the DDC diet was also suggested to be used as a model for sclerosing cholangitis and biliary fibrosis. DDC feeding in mice causes cholangitis with a pronounced ductular reaction (Fig. 1C), onion-skin-type periductal fibrosis, and finally biliary fibrosis, therewith reflecting several specific pathological hallmarks of human PSC (42). This model is therefore especially useful to investigate the mechanisms of chronic cholangiopathies and their consequences, including biliary fibrosis, and to test novel therapeutic approaches for these diseases (42, 43).

**Animal Models for Studying HCV Infection-Associated Liver Diseases**

HCV is the only member of the Hepacivirus genus of the Flaviviridae family (127) and has infected ~170 million people worldwide. HCV is an enveloped virus with a positive-stranded RNA genome of ~9.6 kb, which serves as a template for both translation and replication. The virus encodes one long open reading frame that generates a single polyprotein precursor, subsequently processed into 10 individual gene products including structural (C, E1, E2, p7) and nonstructural (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) protein subunits (127). Replication of the HCV genome is mediated by the viral RNA-dependent RNA polymerase NS5B. NS5B lacks proof-reading functions, leading to a high mutation rate, and HCV thus exists as a genetically heterogeneous quasispecies in individual patients (7, 15).

There are six known HCV genotypes (1–6), with genotype 1 being the most common, accounting for about 60% of global infections. To date, a vaccine protecting against HCV is not yet available. The standard antiviral treatment with a combination of pegylated interferon-α (pIFN-α) and ribavirin is often ineffective and induces significant side effects (226), resulting in more than 70% of infected patients developing a chronic infection with a high risk of progression to liver cirrhosis, HCC, and end-stage liver disease. However, improved understanding of the HCV life cycle in recent years has supported the development of direct antiviral drugs that specifically target hepatitis C viral proteins. Inhibitors of the HCV NS3/4A protease are currently the most promising new agents in clinic. Two NS3/4A protease inhibitors, boceprevir (SPRINT-2) and telaprevir (ADVANCE), have progressed into phase III trials with a good safety profile and high antiviral efficacy. Several studies have thus been initiated to combine antiviral agents with different novel therapies, which may lead to the eventual possibility of interferon-free treatment (94, 172, 206).

Investigation of HCV pathogenesis has been complicated since the only widely recognized animal model of HCV infection is the chimpanzee. However, human HCV-infected chimpanzees rarely develop chronic liver disease, making this model less than ideal for studying the pathogenesis of HCV (9).

Over the years, a long list of transgenic mice constitutively overexpressing one or more HCV proteins have been generated to study effects of specific viral proteins on liver pathology. Despite some contradictory results, three features of HCV pathogenesis could be demonstrated with these HCV transgenic mouse models: 1) the link between HCV core protein expression and hepatic steatosis, 2) the implication of HCV core protein in HCC development, and 3) the modulation of interferon signaling pathways by HCV polyproteins. For detailed descriptions of these studies, we refer the reader to some recently published review articles (8, 92, 105).

**Inducible-HCV transgenic mice.** One of the shortcomings of using transgenic mice with constitutive expression of HCV proteins to study pathogenesis of HCV is the fact that transgenic mice are immunotolerant to these proteins and thus lack an immune response against the virus. To overcome this limitation, Kohara’s laboratory (213) generated an HCV transgenic mouse that allows conditional expression of core, E1, E2, and NS2 proteins by using the Cre/loxP switching system upon a timed infection with a Cre-recombinase expressing adenovirus. A humoral response to core protein and an HCV-specific T cell response were detected after transgene activation, indicating that the transgenic mice were immunocompetent for HCV proteins. In a follow-up study, they further demonstrated that these inducible HCV transgenic mice are resistant to Fas-mediated apoptotic cell death in the liver. This finding suggests that HCV can evade the innate antiviral mechanism of apoptosis to maintain a persistent infection (116). More recently, using this model, the same group provided evidence that NK cells participate in the eliminating core protein expressing hepatocytes during innate immune response against acute HCV infection (163). Furthermore, they generated another inducible transgenic mouse that expresses the full HCV genome in B cells (RzCD19Cre mice) and therewith established a new model for studying mechanisms related to the development of HCV-associated B cell lymphoma (81, 198). With these inducible HCV transgenic mice, the host immune response against HCV proteins can be investigated in further detail. However, since adenoviral vectors themselves induce strong inflammatory responses in murine livers due to elimination of infected cells (79, 212), the expression of HCV proteins in such transgenic mice only persist for a short time (6, 79, 212). Chiyo and colleagues (23), therefore, further modified their model by constructing an adenovirus vector expressing Cre recombinase under the control of the EF1α promoter.
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instead of the previous CAG promoter. Adenovirus carrying the EF1α promoter does not induce a severe inflammatory response in liver and thus enables persistent expression of HCV proteins without elimination of infected hepatocytes by the host immune system, providing a potentially useful model to study HCV pathogenesis.

**Xenograft models.** To develop a rodent model in which HCV is infectious and efficiently replicates is highly complex because of its extremely narrow species tropism. The species barriers that prevent HCV infection in nonhuman hosts are still not completely defined, thus nearly all HCV-permissive rodent models require xenografting of human hepatocytes and a permanent lack of immune rejection toward these engrafted cells.

**Immunotolerized rat model.** This model is based on the fact that the rat immune system does not develop until days 15–17 of gestation. If human hepatocytes are introduced before or during this period, rats can be immunotolerant to these foreign cells (142). Wu et al. (219) immunotolerized rat embryos to allow the transplantation of a human hepatoma cell line (Huh7) after birth and the following inoculation with HCV-positive human serum. Nearly 30% of the transplanted human hepatocytes were positive for HCV core protein expression. Elevated ALT levels and inflammatory infiltrates on liver histology were observed, indicating a response of the rat immune system culminating in liver damage. However, a genuine adaptive immune response toward HCV cannot be expected in this model because of a mismatch between the rat immune system and the human MHC on the transplanted Huh7 cells. Thus, this model is restricted for studying some details of HCV infection, such as the involvement of receptors and intracellular host factors (219).

**Human liver chimeric Alb-uPA/SCID mice.** The urokinase plasminogen activator (uPA) transgenic mouse model was first described by Heckel et al. around 20 years ago (60). Overexpression of uPA in hepatocytes is cytotoxic, leading to a continuous liver regeneration process. Under these conditions, uPA-negative cells possess a strong survival advantage over the resident cells. In this setting, the diseased liver could be efficiently replaced by healthy transplanted hepatocytes from different donors, once uPA transgenic mice were backcrossed on an immunodeficient background, such as SCID mice or Rag2 mice (15, 153, 162). On this basis, Mercer et al. (124) transplanted normal human hepatocytes into SCID mice carrying the uPA transgene under the hepatocyte-specific albumin promoter (Alb-uPA). Morphological and biochemical analyses indicated that repopulation with human hepatocytes may reach 70% and the chimeric mice reveal a satisfying hepatic architecture (126). Such Alb-uPA/SCID mice developed prolonged HCV infections with high viral titers after inoculating human serum from HCV patients (124). The Alb-uPA/SCID model is physiologically able to mimic a natural human viral infection with HBV, HCV, and other hepatotropic viruses once human hepatocytes are stably engrafted (29, 124, 126, 129, 191), and thus it can be widely used to evaluate various novel anti-HBV/HCV therapies (125). However, Alb-uPA/SCID mice lack an immune system. Since the major mechanism of HCV-related liver damage occurs as a result of the host immune response to the infection, Alb-uPA/SCID mice fail to develop chronic liver injury. Therefore, this model is not suitable for studying immunopathology of HCV infection and the associated CLDs. In addition, breeding efficiency of the mice is low, the time window for transplantation is narrow, and efficacy of human hepatocyte engraftment can be highly variable (185, 200). Renal disease in repopulated mice has also been reported (185). All these disadvantages account for limiting its widespread use and application, and thus this model system still needs improvement.

Fah+/−/Rag2−/−/Il2rg−/− mice (FRG mice). The recently reported Fah+/−/Rag2−/−/Il2rg−/− mice as well represent a broadly useful hepatic xenorepopulation system (5, 13). An immunodeficient strain lacking the liver tyrosine catalytic enzyme fumarylacetoacetate hydratase (FAH) develops liver disease in the absence of the protective drug 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC). The Fah mutation bearing mice were crossed into Rag2−/−/Il2rg−/− background mice to generate Fah/Rag2/Il2rg (FRG) triple mutants (5, 13). FRG mice can be readily and reproducibly engrafted with human hepatocytes. In the absence of NTBC, FAH-deficient mouse hepatocytes die because of accumulation of toxic tyrosine metabolites, whereas transplanted human hepatocytes remain healthy. Furthermore, these mice can be highly engrafted (up to 90%) with human hepatocytes from multiple sources, including liver biopsies (5). FRG mice exhibit a higher level of human liver chimerism, when compared with uPA/SCID mice, and they also propagate both HBV and HCV, which can be sustained for more than 6 mo (14). In contrast to uPA/SCID mice, mutant breeders of FRG mice are fully viable, hepatocyte repopulation can be done at any age, and no renal disease was observed (13). Therewith, Fah+/−/Rag2−/−/Il2rg−/− mice are promising for HBV and HCV infection studies and antiviral therapy testing, although further studies still need to determine whether it is superior to the uPA/SCID mouse model.

**Immune competent HCV-permissive mouse models.** Reconstruction of a functional immune system in HCV-permissive mice is a big challenge for studying HCV immunopathology and development of vaccination strategies. In a recent study, Washburn and colleagues (215) generated mice expressing a fusion protein of the FK506 binding protein and caspase 8 under control of the albumin promoter (AFC8) on Balb/C Rag2−/−/γC-null background mice. These mice were then cotransplanted with CD34+ human hematopoietic stem cells (HSC) and hepatocyte progenitor cells (Hep) from a human fetal liver, resulting in efficient engraftment of both human leukocytes and liver cells (AFC8-hu HSC/Hep mice). The authors further showed that AFC8-hu HSC/Hep mice are sensitive for HCV infection in the liver, generate a humanized T cell immune response against HCV, and develop hepatitis and fibrosis (215). Activated hepatic stellate cells were detected in HCV-infected AFC8-hu HSC/Hep mice, accompanied by elevated collagen production and TIMP-1 (tissue inhibitor of matrix metalloproteinases 1) expression. This promising study makes the first step toward the development of a HCV mouse model containing a human immune system, although it has several shortcomings that need to be improved, such as the low level of viral RNA detection, the relatively poor hepatocyte repopulation, and the lack of an efficient antibody response due to incomplete B cell function. Nevertheless, AFC8-hu HSC/Hep mice represent a useful model to study mechanisms of HCV-related liver fibrosis.

More recently, a genetically humanized HCV model was generated by Dorner et al. (37) based on the in vitro observa-
tion that CD81 and occludin (OCLN) comprise the minimal human factors required for permissiveness of rodent cells to HCV entry (147). The same group further constructed a bicistronic HCV genome expressing Cre recombinase (HCV-Cre), which activates a loxP reporter gene in Rosa26-Fluc mice (158). They next generated Rosa26-Fluc mice expressing human CD81 and OCLN, or expressing all four human HCV entry factors previously identified as required for the virus to enter hepatocytes, CD81, scavenger receptor type B class I (SCARB1), claudin 1 (CLDN1), and OCLN (37), and inoculated these mice with cell culture-derived HCV-Cre. All mice expressing at least human CD81 and OCLN became successfully infected by HCV. Furthermore, the authors were able to show that HCV entry into hepatocytes of these mice can be blocked by specific antibodies and that a recombinant vaccinia virus vector induces humoral immunity in these mice, indicating utility of this model for developing passive immunization strategies and therapeutic vaccines. This genetically humanized mouse is the first reported HCV-permissive model that recapitulates the viral life cycle in a fully immunocompetent mouse and provides a promising platform for studying HCV immunopathogenesis as well as for developing effective antiviral treatment. Further evaluation and improvement of this innovative model is eagerly awaited.

Animal Models for Studying HBV Infection-Associated Liver Diseases

Human HBV is a member of the Hepadnaviridae family, which comprises a variety of avian and mammalian viruses that share similar genomic organization and a unique asymmetric strategy of genome replication (166). There are ~360 million people chronically infected with HBV worldwide, with a high risk for cirrhosis and HCC (168). Although a safe and effective vaccine is available, persistent infection develops in ~5% of adults and 95% of neonates infected with HBV (97).

HBV is an enveloped virus. The HBV genome comprises of a partially double-stranded DNA of ~3.2 kb, organized in four open reading frames. The covalently closed circular DNA (cccDNA) serves as template for both viral transcription and replication. The HBV genome is transcribed into four pre-RNAs: C, S, P, and X. C encodes hepatitis B core protein, soluble and secreted HBeAg; S encodes the three viral envelope proteins known as HBsAgs; P pre-RNA encodes the viral DNA polymerase (Pol); and the X encodes the hepatitis B X protein (HBx). The viral reverse transcriptases (P pre-RNA) of HBV lack a proofreading function and as a result, similar to HCV, HBV populations exist in the host as quasispecies (204).

Like HCV, human HBV infects only human and chimpanzees. HBV infected chimpanzees do not establish chronic liver diseases but develop a cellular immune response similar to that observed in humans with acute HBV infection (10), thus being useful for vaccine development studies.

Several viruses of the Hepadnaviridae family are closely related to HBV and therefore, most advances in HBV research have been made with studies of two such HBV-like viruses, the woodchuck HBV (WHBV), which infects Eastern American woodchucks, and the duck HBV (DHBV), which infects Peking ducks. These models provided knowledge about HBV infection, persistence, hepatocarcinogenesis, and viral life cycles (31). However, for testing anti-HBV drugs, WHBV and DHBV models are of limited value because of species-specific features of different Hepadnaviridae family members and their corresponding hosts.

Transgenic mice expressing complete or partial HBV genomes also provide a useful tool to address specific questions concerning mechanisms of HBV replication, control of HBV replication by the host immune system, function of certain viral products such as HBeAg and the X protein, and their potential oncogenic effect during chronic infection. For detailed description of HBV transgenic mice, we refer the reader to two recent reviews by Dandri et al. (31, 32).

HBV-transfected mice. As transgenic mice are not permissive for HBV infection, they cannot be used for studying viral entry and spread. Thus investigators have generated HBV-transfected mice to break the species barriers of HBV infection. Two strategies have been successfully used for the in vivo delivery of HBV DNA into mice, i.e., via adenoviral vectors (173) and via hydrodynamic HBV DNA injection (222). In contrast to transgenic mice, HBV DNA is not integrated into the host genome in transfected mice and, therefore, immune system-mediated viral clearance or antiviral treatment can be investigated. Furthermore, replication of different HBV mutants can be analyzed in these systems. A common drawback of transfected mouse models is the relatively short duration of viral replication, which is limited by the host immune response against viral vectors (173), or because of quick clearance of HBV replications by appearance of antiviral antibodies and CD8+ T cells (222). Therefore, the HBV-transfected mouse model is more suitable for studying acute HBV infection.

**Human liver chimeric mouse models for HBV.** Development of human liver chimeric mice also provides a platform to study HBV infection.

**Trimera mouse model.** The Trimera mouse model is the first chimeric mouse model that supports HBV replication (31, 70), and it has also been validated for HCV infection (69). To generate a Trimera mouse, a normal mouse is preconditioned by total body irradiation to break down the immune system, with subsequent reconstitution using SCID mouse-derived bone marrow. Then, transplantation with ex vivo HBV infected liver fragments under the kidney capsule or into the ear pinna is performed. The implanted human hepatocytes can be maintained functional for several weeks and low level HBV DNA is detectable in mouse sera for ~20 days. Thus this model can be used for short-term antiviral studies of HBV infection (70).

**Alb-uPA mice for studying HBV.** Since Alb-uPA mice were introduced for successful transmission of both HCV and HBV (124, 191), several investigators have reported to use this model for studying HBV infectivity (179), viral clearance, pathways of spread (31, 191), and evaluation of antiviral treatment (30, 208). Moreover, the biological properties of two newly identified HBV strains have been determined in Alb-uPA mice (183, 184).

Animal Models for Studying Nonalcoholic Fatty Liver Disease

NAFLD is defined as a presence of hepatic steatosis in the absence of alcohol abuse. NAFLD encompasses a wide spectrum of liver diseases ranging from simple steatosis to nonalcoholic steatohepatitis (NASH). NASH is a progressed form of NAFLD, characterized by steatosis, lobular inflammation, hepato cellular
ballooning, and fibrosis (59). Fibrosis typically starts perisinusoidally in zone 3 as chicken-wire pattern, spreading to the portal area, and subsequently leading to portal-portal and portal-central bridging fibrosis (18). Long-standing NASH may progress to liver cirrhosis, end-stage liver disease, and HCC (59).

NAFLD/NASH is regarded as one of the most common causes of liver dysfunction in developed countries today. Major risk factors are overnutrition and thereof resultant metabolic abnormalities concerning visceral obesity, insulin resistance, glucose intolerance, dyslipidemia, and an altered adipokine profile (40, 119). Until now, knowledge about etiopathological mechanisms of NASH remains incomplete; thus therapies to prevent or delay disease progression are still very limited.

Currently available animal models for NASH are of value for investigating some specific issues of disease progression, but none of them alone reflects the natural etiology and pathology of human fatty liver disease. Those animal models have been extensively reviewed by others (3, 39, 59, 99, 113, 131, 164, 181), and thus only the most commonly used and some newly described models are mentioned here. NASH models can be roughly classified into three broad categories: 1) genetic models, 2) dietary models, and 3) models combining genetic factors with nutritional factors.

**Genetic models.** *Ob/ob mice, db/db mice (or fa/fa rats).* Leptin is a satiety hormone synthesized by the white adipose tissue that regulates energy intake and expenditure. Mice or rats that have mutations in the gene encoding leptin (ob/ob mice, leptin deficient) or the leptin receptor Ob-Rb (db/db mice or fa/fa rat, leptin resistant) become grossly obese, hyperphagic, inactive, and insulin resistant and spontaneously develop hepatic steatosis and Type 2 diabetes (3, 20, 112). Although metabolic abnormalities reflect NAFLD in both ob/ob and db/db mice, spontaneous development of hepatocellular damage and hepatic inflammation does not occur. An interesting finding with the ob/ob mice is that they are resistant to liver fibrosis, even when exposed to CCl₄ or TAA (65, 102), suggesting that leptin is an essential mediator of hepatic fibrogenesis.

**KK-Ay/a mice.** KK-Ay/a mice are a cross strain of diabetic KK and lethal yellow (Ay) mice and carry a heterozygous mutation of the agouti gene (67). The phenotype of KK-Ay/a mice comprises altered adipokine expression, obesity, dyslipidemia, and insulin resistance, resembling the metabolic abnormalities in NAFLD patients. KK-Ay/a mice spontaneously develop mild hepatic steatosis. However, progression into steatohepatitis and severe hepatic fibrosis in KK-Ay/a mice still requires a second insult (68, 139).

**PPARα⁻/⁻ mice.** Many genes encoding enzymes involved in the mitochondrial and peroxisomal fatty acid β-oxidation pathways in the liver are regulated by peroxisome proliferator-activated receptor α (PPARα). Under conditions of normal feeding, fat does not accumulate in the liver of PPARα-deficient mice. However, PPARα⁻/⁻ mice exhibit severe hepatic steatosis when subjected to fasting, indicating that a defect in PPARα-inducible fatty acid oxidation accounts for severe fatty acid overload and steatosis (84, 152).

**PTEN⁻⁻/⁻ mice.** PTEN is a multifunctional phosphatase and tumor suppressor that serves as a negative regulator of several signaling pathways, including PI3K/Akt. Liver-specific PTEN⁻⁻/⁻ mice (PTEN flox/flox) develop steatohepatitis with a histological phenotype similar to human NASH. However, those mice exhibit low serum insulin levels and even have improved insulin sensitivity, which does not adequately reflect the pathophysiological situation of the majority of NAFLD patients. Liver-specific PTEN deletion is associated with hepatocarcinogenesis, and thus this model may be more suitable for studying aspects of disease progression from NASH to HCC (66).

**Dietary models.** Dietary manipulation is commonly used to acquire a NAFLD/NASH phenotype. Basically, metabolic defects in rodents can be induced by either nutrition deficiency or overnutrition. A major limitation of most dietary models is the severity of induced histopathological changes that may be strongly species and strain dependent. Thus, although some models appear suited to simulate pathology of human NASH, a remaining challenge is reproducibility between different laboratories, which currently limits their utility and popularity.

**Methionine- and choline-deficient diet.** Methionine- and choline-deficient (MCD) diet is the classic dietary model for studying NASH. It contains high sucrose (40%) and fat (10%) but lacks two components, methionine and choline, that are essential factors for hepatic mitochondrial β-oxidation and for synthesis of very low-density lipoprotein (3). MCD diet rapidly induces hepatic steatohepatitis with subsequent necroinflammation, as well as pericellular and pericentral fibrosis in rodents. The development of chicken-wire fibrosis resembles that seen in human NASH. MCD diet induces more ROS production, mitochondrial DNA damage, and apoptosis than most other NASH models (51) and therefore is considered one of the best-established models for studying NASH-associated inflammation, oxidative stress, and fibrosis. The severity of NASH induced by MCD diet may vary depending on sex, species, and strain of enrolled rodents. Male rodents fed a MCD diet usually develop more steatosis than females. In rat species, Wistar rats with MCD feeding showed greater propensity to develop steatosis than Long-Evans and Sprague-Dawley rats. Compared with Wistar rats, male C57/BL6 mice develop more inflammation, necrosis, lipid peroxidation, and mitochondrial injury, while showing less steatosis (87). The main disadvantage of the MCD diet model is that the metabolic profile is generally a conversion of human NASH. Rats or mice fed the MCD diet show significant loss of body weight (up to 10% in 10 wk) and reductions in plasma triglyceride and cholesterol levels. Serum levels of insulin, leptin, and glucose are also decreased, serum adiponectin remains unchanged or even increases, and the animals are peripherally insulin sensitive, although they exhibit hepatic insulin resistance (101, 103, 155).

**High-fat diet.** Diet with high-fat content is widely used to induce hepatic steatosis in different experimental animals. The effect of a high-fat diet (HFD) is more pronounced in rats than mice (59). In a rat HFD model (71% of calories from fat, 11% from carbohydrates, and 18% from proteins), pathological changes include obesity, increased insulin levels, and insulin resistance, which closely resemble the situation of NAFLD patients (110). Also increased ALT and AST serum levels, hepatic steatosis, inflammation, and fibrosis have been observed in different studies using HFD feeding (20, 74, 178, 229), whereas other studies with HFD models failed to develop histological evidence of hepatocellular injury, steatohepatitis, and disease progression to fibrosis (99, 157). Similar as in the MCD model, rodent species and strain differences may strongly affect the degree of steatosis, inflammation, and fibrosis obtained when fed a HFD. Sprague-Dawley rats appear...
more susceptible to steatohepatitis development with a HFD (99, 221) (Fig. 1D), whereas even long term high-fat feeding did not induce NASH in Wistar rats (157). Male BALB/c mice accumulate more hepatic lipids than C57/BL6 mice when fed a HFD (135). Intragastric overfeeding of C57/BL6 mice with a HFD replicated the histopathological and pathophysiological features of human NASH (33). However, in general, application of fats via Chow feeding leads to a milder form of liver injury and thus it can be widely used to evaluate whether a genetic or a pharmacological manipulation is able to aggravate liver disease in this setting.

**Atherogenic diet (cholesterol and cholate).** Elevated blood triglyceride and cholesterol levels are critical factors for an increased risk to develop cardiovascular disease, which is usually associated with NASH. Genetic susceptibility to atherosclerosis or feeding animals with an atherogenic diet, typically containing cholate and cholesterol as well as an increased fraction of fat, results in steatohepatitis and liver damage (36, 78, 136). Pathological features comprise steatosis, inflammation, perivenular and pericellular fibrosis, and ballooned hepatocytes, very similar to those found in human NASH (120). Interestingly, cholesterol and cholate components of the atherogenic diet have been shown to induce distinct stages of hepatic inflammatory gene expression (205).

Comparison of an atherogenic diet with 30% fat content and a diet containing 30% fat but no increased cholesterol or cholate supplementation allows the stimulation of simple steatosis vs. NASH that mimics pathology of NAFLD patients (36). Still, even with an extremely high-fat content (60%) atherogenic diets do not cause elevated serum triglyceride levels as frequently found in NAFLD patients, and even upon very long feeding periods systemic insulin sensitivity is only modestly affected (120).

**Fructose.** Increased levels of fructose consumption, especially in form of corn syrup in soft drinks, are associated with NAFLD (143). Hepatic metabolism of fructose can promote de novo lipogenesis and inhibition of long-chain fatty acid (LCFA) β-oxidation in mitochondria, and, consequently, may cause hepatic steatosis, insulin resistance, and hyperglycemia. Owing to the molecular instability of the furanose ring, fructose can also promote protein fructosylation and formation of ROS (111). Addition of 30% fructose to water resulted in increased levels of hepatic triglycerides and steatosis in mice (174). Fructose also promoted intestinal bacterial overgrowth, leading to increased endotoxin levels in portal blood, subsequent Kupffer cell activation, and inflammation in the liver (174). Furthermore, combination of high-long-chain trans-fat solid diet and fructose-enriched water caused obesity, insulin resistance, and severe hepatic steatosis with associated necro-inflammatory changes in the liver (186). However, these mice were protected from fibrosis. A further improved model, the combination of a high-calorie diet with predominantly medium-chain saturated fatty acids diet and high-fructose enriched drinking water, resulted in an almost complete human NASH like phenotype with steatosis, severe hepatic inflammation, and significant fibrosis (2, 88).

**Fast food diet.** Recently, a diet based on the composition of “fast food” (FF), i.e., highly saturated fats, cholesterol, and fructose, was reported to recapitulate features of the metabolic syndrome and NASH in C57BL/6 mice. Obesity, insulin resistance, steatohepatitis with pronounced hepatocellular ballooning, and progressive fibrosis were observed in mice with prolonged FF diet feeding. Hepatocellular ballooning is the most significant feature of this model, since the majority of reported diet models fail to develop hepatocellular injury. Moreover, FF diet-induced NASH progresses similar to human pathophysiological condition, namely chronic overnutrition with a high caloric intake, thus providing a novel platform for studying mechanisms of NAFLD and NASH as well as for testing of potential therapies (22).

**oxLDL + HFD.** Yimin et al. (223) established a novel model for NASH by combining HFD with supplementing oxidized low-density lipoprotein (oxLDL) in C57BL/6J mice. oxLDL is regarded as an extracellular source of ROS and induces intracellular oxidative stress in cultured cells (122). Combination of HFD feeding and oxLDL administration between weeks 21 and 23 not only aggravated lipid metabolism, hepatic steatosis, and fibrosis but also resulted in severe inflammation, including hepatic injury and inflammatory cell infiltration, accompanied by increased TNF-α and IL-6. Furthermore, the livers of regular diet-fed mice treated with oxLDL were characterized by foamy macrophages and inflammatory cell infiltration along with an elevated IL-6 mRNA level. These findings suggest that an increased oxidative state, including HFD-induced intracellular lipid peroxidation and its extracellular source from ox-LDL, is the actual trigger for hepatic inflammation in which liver injury is mediated by TNF-α and inflammatory cell infiltration is dependent on IL-6 (223).

**GTG + HF diet.** Ogasawara et al. (138) recently reported a new model combining HFD and gold thioglucose (GTG) administration. GTG is known to induce lesions in the ventromedial hypothalamus, leading to hyperphagia and obesity. Combination of GTG treatment with a 12-wk HF consumption in C57BL6 mice resulted in exacerbated obesity with increased abdominal adiposity, insulin resistance, and enhanced oxidative stress. This leads to a more severe histological steatohepatitis with hepatocyte ballooning, Mallory-Denk body formation, and pericellular fibrosis. Although the progression to advanced fibrosis was not shown with GTG + HFD administration, this model may facilitate studies of the transition from simple steatosis to steatohepatitis and the initiation of fibrogenesis while realistically resembling many fundamental pathophysiological processes occurring in human NAFLD/NASH.

**Combination models (genetic modification plus dietary manipulation).** Since most animal models do not fully reflect the whole pathogenetic spectrum of hepatic and extrahepatic pathologies observed in human NAFLD, combinations of genetic modification and dietary manipulation are set up to bridge the phenotypic gap that exists between available animal models and the human situation. The literature reports numerous of such combinations (Table 2). Generally these combination models are claimed to mimic most of the major characteristics of human NASH and induce more severe steatohepatitis, insulin resistance, and various degrees of fibrosis. Notably, in a very recent publication, Trevaskis et al. (190) reported that obese mice fed with a high-trans-fat (HTF), high-fructose, and high-cholesterol diet, are able to develop severe pathophysiological features of fibrotic NASH, indicating a leptin-independent mechanism involved in NASH associated liver fibrogenesis.
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HF, high-fat; HFD, high-fat diet; KO, knockout; MCD, methionine- and choline-deficient diet; NASH, nonalcoholic steatohepatitis.
Animal Models for Studying Alcohol-Induced Chronic Liver Diseases

ALD, caused by heavy and prolonged consumption of alcohol, contributes to the bulk of liver disease burden worldwide. Progression of ALD is a multifactorial and multistep process involving both genetic and environmental risk factors (167). The spectrum of ALD ranges from simple steatosis to hepatic inflammation, necrosis, liver fibrosis, and cirrhosis. Alcohol abstinence is clearly the most effective prevention and therapy for ALD. However, for those who cannot achieve abstinence from alcohol, e.g., because of addiction, and for patients with severe forms of ALD, there is still no universally accepted therapy available.

The development of new drugs for ALD is, in part, limited by the drawbacks of existing laboratory animal models. This is, to some degree, related to the aversion of rodents for alcohol, the low blood alcohol concentration due to rapid metabolism and the imbalance of nutritional consumption and alcohol intake (175). From the numerous models available to date, none could precisely reproduce clinical manifestations of human ALD. However, animal models have provided valuable information to understand mechanisms how alcohol damages the liver, including consequences of alcohol metabolism, alcohol-related oxidative stress, or immune variations.

Liquid diet model (Lieber-DeCarli model). Of the various methods of alcohol exposure to animals, oral alcohol administration via drinking water is obviously the most relevant to clinical ALD. Simplicity and low technical demands are the biggest advantages of ad libitum alcohol feeding, whereas a major concern is the strong aversion of rodents to the taste and smell of ethanol. Because of this, animals do not consume enough alcohol to produce significant liver pathology, even when using alcohol-prefering rodent strains (156, 171). To overcome the aversion for alcohol, Lieber and DeCarli developed a “forced choice” model of alcohol exposure, in which ethanol was incorporated into a liquid diet and offered to the animals as the only source of calories (108, 109). In this model, ethanol constitutes 36% of the total calories and, although rats and mice generally dislike alcohol, when provided with this liquid diet as the only source of food and water, their natural aversion could be partially overcome. Compared with the model that ethanol is only included in the drinking water, the Lieber-DeCarli model produced higher blood ethanol levels and the daily ethanol intake was sufficient to induce early stages of liver injury with slight elevation of serum ALT and steatosis, but more serious forms of liver damage such as severe necroinflammation and fibrosis have not been observed without supplementation of a second insult (104). However, the Lieber-DeCarli liquid diet model has provided an excellent tool for studying the early hepatic lesions of ALD, including steatosis, activation of Kupffer cells, ROS generation, and hepatocyte cell death.

A number of modified Lieber-DeCarli models have further advanced our knowledge about the contribution of gut-derived endotoxins (i.e., lipopolysaccharide) and the immune response in ALDs. Recently, a 10-day ethanol feeding model was adapted to C57BL/6 mice to increase liver damage (47, 55, 115). In this model, mice are allowed to access a modified Lieber-DeCarli diet low in carbohydrates with ~6.3% (vol/vol) ethanol (28% ethanol-derived calories) for 10 days. The mice initially lost weight but gradually regained their body weight after 7 days of diet. Mice show increased early liver damage compared with the classic Lieber-DeCarli diet. Serum ALT levels are significantly elevated in ethanol-fed mice and hepatomegaly was associated with the development of moderately pronounced macro- and microvesicular steatosis. However, no significant evidence of inflammatory cell infiltration or necrosis was observed by histology (47, 55). Further modification of this protocol by feeding mice with an ethanol (5%) diet for 10 days plus a single gavage of ethanol (a chronic-binge model) could induce a more severe form of liver injury and fatty liver with markedly elevated serum ALT and AST levels as well as significant microsteatosis. Liver inflammation and necrosis were also been observed in chronic-binge ethanol-fed mice (85).

Enteral ethanol infusion model (Tsukamoto-French model). Because oral alcohol feeding-mediated liver injury cannot go beyond the stage of fatty liver, Tsukamoto and colleagues developed an enteral ethanol feeding model, which is based on the hypothesis that rats have a higher metabolic rate than humans and may require sustained high blood levels of ethanol to induce liver damage (192, 196, 197). To build this model, a catheter is implanted into the stomach, and the alcohol liquid diet as set up in the Lieber-DeCarli protocol is infused directly into the stomach at a constant rate with the aid of an infusion pump (202). Rats and mice fed an enteral ethanol diet have a significantly higher blood ethanol level compared with the Lieber-DeCarli model. The resulting liver damage, including fatty liver, local necrosis, inflammation, and mild portal fibrosis (Fig. 1E), mimics early and intermediate stages of ALD in humans (193). More serious forms of fibrosis and cirrhosis can be achieved by adding carbonyl iron into the diet or by prolonging the time of enteral feeding (48, 180, 194). A major limitation of this model is the high technological demand required to perform the catheter implantation surgery and the necessity of constant monitoring. However, absolute control of nutritional intake is obviously a big advantage of this model.

In particular, the Tsukamoto-French model is a useful tool for studying the molecular mechanisms involved in ALD. Kono et al. (89) were able to translate the Tsukamoto-French model from rat to mice, thus enabling extraordinary possibilities for transgene and knockout studies. In chronic ALD, endotoxin activated Kupffer cells have been suggested to play a key role by releasing proinflammatory and profibrogenic cytokines like TNF-α and TGF-β. The application of knockout mouse studies has further confirmed the major role of Kupffer cell activation in the development of ALD, releasing NADPH oxidase-derived free radicals and secreting TNF-α (90, 224).

Table 3 summarizes the specific chronic liver diseases related to animal models described above.

FROM ANIMAL MODELS TO THE CLINIC

Distinct from in vitro cell culture systems, experimental animal models enable researchers to study cell biology in a complex system, including whole organs and living organisms. Thus animal models offer researchers irreplaceable opportunities to study biological, pathological, and histological characteristics of human CLDs and provide a useful platform for developing novel therapeutic approaches and testing new drugs.
Table 3. Animal models mimicking specific chronic liver diseases

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AIH, autoimmune hepatitis; ALD, alcoholic liver disease; Con A, concanavalin A; DDC, 3,5-diethoxycarbonyl-1,4-dihydrocollidine; FF diet, fast food diet; GTG, gold thio-glucose; HBV, hepatitis B; HCC, hepatocellular carcinoma; HCV, hepatitis C; NAFLD, nonalcoholic fatty liver disease; 2-OA, 2-octynoic acid; PBC, primary biliary cirrhosis; PSC, primary sclerosing cirrhosis; Tregs, T regulatory cells.
However, animals are not small humans. The species differences between human and animals may rule out the possibility of directly translating the results from animal experiments to the clinic trials. CLDs develop slowly in humans, usually taking decades to evolve and progress, but rodents have a very short lifespan, and disease progression may take only several weeks or months in the experimental system. The pathogenesis of disease in animal models, therefore, could be quite different from those occurring in human CLDs, although many typical histological features closely resemble those seen in humans. Furthermore, rodents have a distinct immune system from humans, making it difficult to properly evaluate whether the immune response to the environmental stimuli (e.g., viral infection) can be considered identical to the human immunological reaction. Moreover, the metabolic rates of rodents are much faster, which may also cause problems when we use these animals for studying the progression of metabolism-related CLDs such as NAFLD and ALDs. It could be imagined that toxic stimuli will either lead to an extreme “overshooting” response due to dramatically enhanced levels in the blood or result in no obvious effect because of a fast metabolic resolution. Furthermore, therapeutic intervention may be severely affected by the metabolic differences between human and rodents. The potential effect and toxicity of therapeutic drugs to patients are hard to evaluate by using only animal models. Finally, human CLD development is a very complicated process involving a variety of factors and may even be a result of disorders from different tissues, organs, or systems. This feature is also hard to mimic by using a single animal model. All these differences may account for the recurrent failures of therapeutic interventions that appear promising in animal models to be translated into the clinic.

In summary, no animal model to date entirely recapitulates the human disease; however, animal models are valuable for us to answer specific questions with regard to disease etiology and pathology, as well as to test those therapeutic approaches that cannot safely be applied to patients. Animal models are highly specific to a particular study; therefore it is especially important for the researchers to choose the appropriate models for designing their experiments.

ACKNOWLEDGMENTS

Our research on chronic liver disease is supported by Marie Curie Initial Training Network (ITN) IT-Liver grant, the Netherlands Organization for Scientific Research (NWO), Netherlands Institute for Regenerative Medicine (NIRM) and Centre for Biomedical Genetics, National Natural Science Foundation of China, 81100278 (C. Xu), Zhejiang Provincial Natural Science Foundation of China, Y2110026 (C. Xu), German Research Foundation programs “SFB TRR7 Liver Cancer” and “Do373/8-1,” and Federal Ministry of Education and Research grants “The Virtual Liver” and “Cell Therapy in Liver Regeneration.”

The present review is not exhaustive and the animal models and references are chosen as illustrations. We apologize to the authors whose work is not cited here.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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