MINI REVIEW

Will we ever model PSC? — ‘‘It’s hard to be a PSC model!’’

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Summary Cholangiopathies such as primary sclerosing cholangitis (PSC) represent an important group of liver diseases of the intra- and extrahepatic bile ducts frequently causing end-stage liver disease with significant morbidity and mortality due to limited treatment options. The relatively low incidence of PSC and the difficult accessibility of the human bile duct system for longitudinal studies may represent some of the critical reasons for the lack of profound knowledge in regard to PSC pathophysiology. Therefore, there is an urgent need for reliable, well-defined and easily reproducible animal models to learn more about the pathophysiology of PSC and to test novel treatment modalities. In an ideal world, immunogenetically predisposed animals would develop fibrous-obliterative cholangitis of the intra- and extrahepatic bile ducts in association with inflammation of the gut (especially colitis) in a highly reproducible manner allowing to test new drugs. To date, however, no such animal model is available. We aimed to provide a systematic overview of current available rodent models for sclerosing cholangitis and biliary fibrosis and therefore critically analyzed the characteristics of models for

Abbreviations: Abcb4, ATP-binding cassette, subfamily B, member 4; AIH, Autoimmune hepatitis; ANCA, Anti-neutrophil cytoplasmic antibody; ANIT, Alpha-naphthylisothiocyanate; BDL, Bile duct ligation; BECs, Bile duct epithelial cells; Ccr5, Cysteine-cysteine chemokine receptor 5; CF, Cystic fibrosis; Cfr, Cystic fibrosis transmembrane conductance regulator; CMV, Cytomegalovirus; CR, Cryptosporidium parvum; CTGF, Connective tissue growth factor; DDC, 3,5-diethoxycarbonyl-1,4-dihydrocollidine; DSS, Dextrane sodium sulfate; EPP, Erythropoietic protoporphyrin; FECH, Ferrochelatase; FGF, Fibroblast growth factor; fMLT, N-formyl L-methionine L-leucin L-tyrosine; GCP5, Glypican; GVHD, Graft-versus-host disease; HLA, Human leukocyte antigen; IFN-γ, Interferon gamma; IBD, Inflammatory bowel disease; ICAM, Intercellular adhesion molecule; LCA, Lithocholic acid; MCP-1, Macrophage cationic peptide 1; Mdr2, Multidrug resistance protein-2; MHC, Major histocompatibility complex; MST-1, Macrophage-stimulating 1; NKT, Natural killer T-cell; PDG, Platelet-derived growth factor; PG-PS, Peptidoglycan-polysaccharide; PP, Protoporphyrin; PSC, Primary sclerosing cholangitis; PXR, Pregnane X receptor; ROS, Reactive oxygen species; SBBO, Small bowel bacterial overgrowth; SC, Sclerosing cholangitis; SCID, Severe combined immunodeficiency; SFBL, Self-filling blind loop; SKR, Steroid and xenobiologic receptor; TGFβ1, Transforming growth factor, beta 1; TGR5, G-protein coupled bile acid receptor-1; TLR, Toll-like receptor; TNBS, 2,4,5-trinitrobenzene sulfonic acid; TNF, Tumor necrosis factor; Tnfrsf1, Tumor necrosis factor receptor superfamily, member 5; Tnfrsf1, Tumor necrosis factor receptor superfamily, member 1; VCAM-1, Vascular cell adhesion molecule-1.

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Introduction

Cholangiopathies represent an important group of inborn or acquired liver diseases of the intra- and extrahepatic bile ducts frequently causing end-stage liver disease with significant morbidity and mortality [1]. Among others, this group of diseases includes PSC and a large group of secondary forms (i.e. secondary SC, SSC) [2–4]. PSC represents a chronic cholestatic liver disease of unidentified etiology and unknown pathogenesis characterized by inflammation, fibrosis and strictures of the intra- and extrahepatic bile ducts and may finally lead to biliary cirrhosis [2,5]. Since neither the etiology nor the pathogenesis of PSC have been clarified in detail, PSC still remains the "black box" in modern hepatology, which is also reflected by the lack of effective medical treatment for PSC and the frequent need for liver transplantation in PSC patients [2,5]. BECs, lining the bile ducts, may represent the primary pathogenetic victim in PSC leading to overexpression of adhesion molecules, cytokines and growth factors in those cells, infiltration of inflammatory cells into portal fields, and finally fibro-obliterative processes. However, the detailed sequence of these events is not well defined. Although the actual pathogenesis of PSC remains enigmatic, several different concepts exist covering some main areas including attempts to uncover the genetics of PSC, to examine the leaky gut and aberrant lymphocyte homing hypotheses (which are particularly attractive due to the close association of PSC with IBD), and to define a possible role for "toxic bile" in PSC. An overview of currently incriminated potential pathogenetic factors in PSC is summarized in Fig. 1.

The relatively low incidence of PSC, the difficult accessibility of the human bile duct system for longitudinal studies, and the large variation in the clinical course of PSC patients may represent some of the critical reasons for the lack of profound knowledge in regard to PSC pathophysiology. Therefore, there is an urgent need for reliable, well-defined and easily reproducible animal models for PSC. In an ideal world, such models should allow standardized testing of novel treatment strategies for PSC. The major attributes of an "ideal PSC model" have been summarized in a thoughtful review by John Vierling [6]. Accordingly in brief, immunogenetically predisposed animals would develop fibrous-obliterative cholangitis of the intra- and extrahepatic bile ducts in association with inflammation of the gut (especially colitis). In addition, special immunological phenotypes of inflammatory cells infiltrating portal tracts (in parallel to the human situation) as well as atrophy of BECs should be observed. To date, however, no animal model has been established exhibiting all of the claimed attributes. The aim of this review is to provide an overview of current available rodent models for SC and biliary fibrosis.

Methods

We performed a systematic Medline search for suitable animal models of SC with biliary fibrosis, with focus on rodent models due to limitations of space. Articles discussed in this review were selected according to the following criteria: full-length report in peer-reviewed medical journals; published in English language; report of sufficient information about methodology used; and development of at least two major pathological features of SC, namely cholangitis and peribiliary fibrosis. Using the combined search terms "cholangitis" and "animal model" identified 192 articles. According to the selection criteria 28 [7–34] were chosen for further review. For systematic and didactic reasons models were divided arbitrarily into six different groups: chemically-induced cholangitis; knock-out mouse models; cholangitis induced by infectious agents; models of experimental biliary obstruction; models involving enteric bacterial cell-wall components or colitis; and models of primary biliary epithelial and endothelial cell injury. However, we have to knowledge that there may be substantial overlap between those groups. Subtypes of models, respective characteristics and respective references are summarized in Table 1.

Chemically-induced cholangitis

Retrograde biliary injection of 2,4,5-trinitrobenzene sulfonic acid

Retrograde biliary injection of TNBS in Sprague-Dawley rats led to histological and cholangiographic features resembling SC [7]. Subtotal ligation of the common bile duct prior to TNBS-injection produced comparable findings and the appearance of anti-neutrophil cytoplasmic antibodies (ANCA) in Lewis rats [8]. TNBS haptenization was considered to be the principal mechanism for autoantibody production [8]. A one-year follow-up study after single TNBS-injection together with subtotal ligation of the common bile duct did not show morphological signs of SC, indicating that multiple insults might be inevitable to trigger chronic SC [9]. This model has limitations due to a high mortality rate caused by the combined surgical/chemical trauma.

Oral administration of alpha-naphthylisothiocyanate

Oral administration of ANIT in Sprague-Dawley rats induced histological, immunohistochemical, and cholangiographic characteristics of human SC together with a progressive increase in CD4 Th1 cytokine expression [10,11]. However,
Table 1  Subtypes of models, respective characteristics and respective references summarized.

<table>
<thead>
<tr>
<th>Agens / Knockout</th>
<th>Application / Observation period</th>
<th>Species / Background</th>
<th>Face validity</th>
<th>&quot;Clinical data&quot;</th>
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<td>Surgical injury</td>
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<tr>
<td>Single intracholedochal injection (1 mL 10% EtOH + 10 mg TNBS) / 30 d</td>
<td>Sprague-Dawley rats</td>
<td>+</td>
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<tr>
<td>Single injection into incompletely ligated common BD (50 mg/kg TNBS in 0.9% EtOH) / 12 w</td>
<td>Lewis rats</td>
<td>+</td>
<td>+</td>
<td>n.d.</td>
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<tr>
<td>Single injection into incompletely ligated common BD (50 mg/kg TNBS in 0.9% EtOH) / up to 12 m</td>
<td>Lewis rats</td>
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<td>ANIT 0.001% suppl. diet / 14 d</td>
<td>Sprague-Dawley rats</td>
<td>+</td>
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<td>DDC 0.1% suppl. diet / 8 w</td>
<td>Swiss albino</td>
<td>+</td>
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<td>Agents / Knockout</td>
<td>Application / Observation period</td>
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<td>Knockout mouse models</td>
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<td>Mdr2&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>8 w</td>
<td>FVB/N</td>
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<td>Cftr&lt;sup&gt;−/−&lt;/sup&gt; DSS-treated Cftr&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>24 m 100 mg for 9 d + 85 mg for 5 d / 14 d</td>
<td>C57BL/6J  Cftr&lt;sup&gt;−/−&lt;/sup&gt; exon10</td>
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<td>fch/fch</td>
<td>Life time</td>
<td>BALB/c</td>
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<td>Infectious agents</td>
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<tr>
<td>Crypto-sporidium parvum</td>
<td>Gastric gavage of 10&lt;sup&gt;7&lt;/sup&gt; oocysts on two successive weeks / 16 w</td>
<td>BALB/c nu/nu</td>
<td>+</td>
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| Infectious agents |
| | Single intragastrical inoculation with 10<sup>6</sup> oocysts of adult and 5 × 10<sup>4</sup> of neonatal mice / 12 w | Neonatal and adult BALB/c SCID and NIH-III nu/nu | + | + | + | + | n.d. | n.d. | n.d. | [22] |
Table 1 (Continued)

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<thead>
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<th>Agens / Knockout Application / Observation period</th>
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<td>C57BL/6-SCID</td>
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<td>C57BL/6-CD40−/−</td>
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<td>C57BL/6-iIFNγ−/−</td>
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<td>C57BL/6-Tnfrsf5−/−</td>
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<td>C57BL/6-Tnfrsf1b−/−</td>
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<td>C57BL/6-Tnfrsf1a/1b−/−</td>
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<td>Helicobacter hepaticus</td>
<td>Single intraperitoneal injection / 32 w (A/J mice), 12 w (SCID mice)</td>
<td>A/JCr, C3H/HeNCr and C57BL/6NCr</td>
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<td>Oral inoculation of 100 μL of 10^7 CFU/mL / 17 m</td>
<td>A/J</td>
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<td>BDL</td>
<td>Common BDL / up to 6 w</td>
<td>C57BL/6J +/+/+/+</td>
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<td><strong>Models involving enteric bacterial cell-wall components or colitis</strong></td>
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<td>SBBO</td>
<td>Surgical creation of SFBL / 4 w (Lewis rats), 12 w (Wistar rats)</td>
<td>Lewis and Wistar rats +/+/+</td>
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<td>+</td>
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<tr>
<td>fMLT</td>
<td>Single intrarectal administration of 15% acetic acid followed by daily intrarectal infusion of 500 μl / 4 w</td>
<td>Wistar rats +/+</td>
<td>+</td>
<td>+</td>
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<td>n.d.</td>
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<td>Cholangiography</td>
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<td>DSS</td>
<td>Administration of 2.5% DSS via the drinking water / 28 w</td>
<td>CD-1 mice + + + - - n.d. n.d. n.d. +</td>
<td>[31]</td>
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<td>TNBS + ANIT</td>
<td>Single intracolonic administration of TNBS / after 7 w: single oral administration of ANIT for 24 h</td>
<td>Sprague-Dawley rats + + + - - n.d. n.d. ↑ALT, ↑Bili +</td>
<td>[32]</td>
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<td><strong>Models of biliary epithelial and endothelial cell injury</strong></td>
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<td>Experimental GVHD</td>
<td>Single injection of spleen and BM-cells of B10.D2 mice into sublethally irradiated BALB/c mice / up to 14 m</td>
<td>BALB/c + + + + + n.d. n.d. n.d. n.d.</td>
<td>[33]</td>
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<tr>
<td>TNBS</td>
<td>Single infusion of TNBS of 5, 50, 100, 150 mg/kg into the portal vein / 5 w</td>
<td>Lewis rats + + + + + n.d. n.d. Initially ↑AST and ↑Bili, ALP peak at day 15</td>
<td>[34]</td>
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ALP: alkaline phosphatase; ALT: alanine transaminase; ANIT: alpha-naphthylisothiocyanate; BD: bile duct; BDL: bile duct ligation; Bili, bilirubin; Cftr: cystic fibrosis transmembrane conductance regulator; d, days; DDC: 3,5-diethoxycarbonyl-1,4-dihydrocollidine; DSS: dextrane sodium sulfate; EtOH: ethanol; fch: ferrochelatase; fMLT: N-formyl L-methionine L-leucin L-tyrosine; GVHD: graft-versus-host disease; h: hours; LCA: lithocholic acid; m: months; Mdr2: multidrug resistance protein-2; n.d.: not determined; PG-PS: peptidoglycan-polysaccharide; SBBO: small bowel bacterial overgrowth; SFBL: self-filling blind loop; w: weeks.
Animal models for sclerosing cholangitis

Figure 1 shows cumulative data on the currently incriminated potential pathogenetic factors in PSC. PSC occurs in genetically susceptible individuals as a result of simultaneous occurrence of multiple events that promote sustained immunopathogenetic mechanisms, finally cumulating in fibrous-obliterative cholangitis and biliary cirrhosis. Extrahepatic bile ducts remained normal [11]. Pathogenesis of ANIT-induced cholangitis still remains elusive. ANIT may lead to BEC injury and consequently processing and presentation of antigens with a predominant CD4 Th1 adaptive immune response. However, direct evidence for this hypothesis is lacking.

Feeding of 3,5-diethoxycarbonyl-1,4-dihydrocollidine

Feeding of DDC in Swiss albino mice induced characteristic microscopic features of SC in a highly reproducible manner [12]. The cholestatic phenotype was related to increased porphyrin secretion and the induction of a reactive phenotype of BECs with induced expression of VCAM, TNF-α, and osteopontin, leading to periductal fibrosis and biliary type of liver fibrosis. However, bile duct plasminization lacked beading and pruning of large ducts typical for PSC. Although the detailed underlying mechanisms of DDC-induced cholangitis still have to be elucidated, this model is attractive for in vivo studies on the cellular crosstalk between hepatocytes, BECs and mesenchymal cells in cholangiopathies and biliary fibrosis [35–37].

Feeding of lithocholic acid (LCA)

Feeding of LCA induced bile infarcts, destructive cholangitis, periductal edema and fibrosis in Swiss albino mice, closely resembling characteristics of early-stage SC [13]. The destructive cholangitis is most likely caused by the physiochemical properties of LCA (i.e. high hydrophobicity and lithogenicity) together with obstruction of bile ducts through LCA precipitates [13]. LCA feeding leads to periductal fibrosis via an efflux of ‘‘toxic bile’’ into the portal field and subsequent activation of BECs and periductal myofibroblasts. Large duct morphology has to be determined in this model. Since LCA-feeding causes morphological alterations within days, this model can be used as a valid short-term model to study acute pathogenetic mechanisms involved in cholangiopathies but information on a tolerable long-term protocol for LCA feeding is missing.

Knock-out mouse models

Mice with targeted disruption of the Mdr2 (Abcb4) gene encoding a canalicular phospholipid flippase (Mdr2−/− mice) spontaneously develop cholangitis and onionskin type periductal fibrosis mirroring key features of human SC [14–16]. These features are most likely linked to the lack of biliary phospholipid secretion and consequently increased concentration of free non-micellar-bound bile acids, which subsequently cause BECs damage [38]. The current working model for the pathogenesis of SC in Mdr2−/− mice represents a multistep process with regurgitation of bile from leaky ducts into portal tracts, causing the induction of periductal inflammation and fibrosis [15]. This model is suitable to
develop and test novel treatment strategies for SC and liver fibrosis of the biliary type [39–41].

Data on CF transmembrane conductance regulator knock out mice (Cftr−/− mice) harboring a mutation of exon 10 of the Cftr gene are conflicting. Durie and colleagues describe the development of progressive liver disease with steatosis, focal cholangitis, inspissated bile and bile duct proliferation, finally resulting in biliary cirrhosis in CF-mice within one year of age [17]. However, this specific model is in striking contrast to other murine CF-models with predominantly intestinal phenotype but mild or no pathological changes in other organs [42–45]. Disparate results may originate in different genetic backgrounds used and different ages of mice studied [17,42–45]. Exon 10 Cftr−/− mice are at high risk to develop intestinal obstruction, making it difficult to generate mice with advanced liver disease. As proof of concept that reduced Cftr function in the setting of colitis may predispose to bile duct injury, Cftr+/− and Cftr−/− were challenged by oral administration of dextran sodium sulfate (DSS), which led to a significant increase in bile duct injury in this genotypes [18]. However, fibrotic changes were very mild.

Mice homozygous for a point mutation in the ferrochelatase gene (fch/fch)

Mice homozygous for a point mutation in the ferrochelatase gene (fch/fch) develop cholangitis and severe biliary fibrosis, reflected by ductular proliferation and portal bridging and progression to cirrhosis within a few months [19,20] and mirrors the DDC model in numerous aspects which may be explained by the porphyrinogenic properties of DDC. In humans, deficiency of the enzyme ferrochelatase, responsible for insertion of iron into PP, results in EPP via PP accumulation in various organs; severe liver disease occurs rarely but shows rapid progression to cirrhosis and liver failure [46–49]. In fch/fch mice, variable amounts of PP deposition were present small bile duct lumina, resulting in incomplete obstruction [19,20]. Liver disease is associated with the formation of bile showing high concentrations of hydrophobic bile salts and PP with reduced cholesterol, phospholipid and glutathione content, which may be causative for BEC injury in this model [19]. A detailed time-course study including visualization of the bile duct system to better understand longitudinal changes and underlying pathogenetic mechanisms of this interesting model system is pending.

Cholangitis induced by infectious agents

Cryptosporidium parvum

Infections of the biliary tract with CP may lead to SC and biliary fibrosis in patients suffering from acquired immunodeficiency syndrome (AIDS) [50–52]. Establishment of chronic cryptosporidiosis in mouse models requires immunodeficiency [21]. Earlier studies with oral CP-infection using mice with SCID on C57Bl/6 and BALB/c backgrounds (lacking T and B lymphocytes) as well as BALB/c- and NIH-III-nu/n mice (with dysfunctional T cells) [21–23] induced unequal hepatic responses [21–23]. Whereas SCID mice developed mild portal lymphocytic inflammation with spontaneous recovery [21,23], nude mice (particularly those on the NIH-III background) showed severe cholangitis, pericholangitis and biliary fibrosis with porto-portal bridges [22,23]. These findings indicate that mice with dysfunctional T cells tend to have more severe SC than SCID animals, suggesting a crucial role for cell-mediated immunity in this model [21]. More recently, single gavage with CP in mice with disrupted genes for IFNγ−/−, TNF receptor CD40 (CD40−/−) and its ligand CD154 (CD154−/−) revealed severe SC [21]. The majority of IFNγ knockouts died of fatal enteritis and survivors developed chronic SC with ductular proliferation [21]. In half of CD154−/− mice, chronic portal inflammation, periductal sclerosis of intrahepatic bile ducts, ductular proliferation and lobular hepatitis was observed and all CD40−/− mice developed chronic portal inflammation [21]. The high susceptibility of CD40−/− and CD154−/− mice might be explained by reduced apoptosis of CP-infected cells [21]. To determine the role of TNF in this model, mice harboring genetic ablation of the TNF superfamily member 5 (Tnfsf5−/−) and TNF receptor 1 knockout mice, (Tnfrsf1a−/−, Tnfrsf1b−/−) as well as double and triple knockout mice were challenged [24]. SC was present in Tnfsf5/Tnfrsf1a and Tnfsf5/Tnfrsf1b double knockouts, whereas triple knockout mice were spared from cholangitis despite present intestinal and biliary CP infection, suggesting that prevention of SC in this model requires the disruption of both TNF receptors [24]. The CP model is therefore useful to determine the role of several cytokines and their receptors in SSC. Systematic cholangiographic data are missing for this model.

Helicobacter species

Helicobacter species cause chronic infections of the gastrointestinal tract in humans and animal species including the bile duct system [53,54]. The pathogenesis of hepatic *H. hepaticus* infection is not fully understood. Based on findings with other Helicobacter species, the bacteria probably colonize the gastrointestinal tract, primarily the large bowel [53,55]. The mode of transfer to the liver and bile canaliculi is unknown. Species differences in the susceptibility to infectious liver diseases exist between different mouse strains with SCID/Ncr, A/JCr and C3H/HeNcr background) showed severe cholangitis, pericholangitis and biliary fibrosis with porto-portal bridges [22,23]. These findings indicate that mice with dysfunctional T cells tend to have more severe SC than SCID animals, suggesting a crucial role for cell-mediated immunity in this model [21]. More recently, single gavage with CP in mice with disrupted genes for IFNγ−/−, TNF receptor CD40 (CD40−/−) and its ligand CD154 (CD154−/−) revealed severe SC [21]. The majority of IFNγ knockouts died of fatal enteritis and survivors developed chronic SC with ductular proliferation [21]. In half of CD154−/− mice, chronic portal inflammation, periductal sclerosis of intrahepatic bile ducts, ductular proliferation and lobular hepatitis was observed and all CD40−/− mice developed chronic portal inflammation [21]. The high susceptibility of CD40−/− and CD154−/− mice might be explained by reduced apoptosis of CP-infected cells [21]. To determine the role of TNF in this model, mice harboring genetic ablation of the TNF superfamily member 5 (Tnfsf5−/−) and TNF receptor 1 knockout mice, (Tnfrsf1a−/−, Tnfrsf1b−/−) as well as double and triple knockout mice were challenged [24]. SC was present in Tnfsf5/Tnfrsf1a and Tnfsf5/Tnfrsf1b double knockouts, whereas triple knockout mice were spared from cholangitis despite present intestinal and biliary CP infection, suggesting that prevention of SC in this model requires the disruption of both TNF receptors [24]. The CP model is therefore useful to determine the role of several cytokines and their receptors in SSC. Systematic cholangiographic data are missing for this model.

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Models of experimental biliary obstruction

Since its first description in 1889 [56,57], BDL has been widely used to induce obstructive cholestasis and bil-
Biliary fibrosis in rodents [11,16,27,57,58]. Biliary pressure is immediately increased after BDL [59,60] and accompanied by characteristic morphological features [58,61]. Early changes include the development of a specific biliary type of hepatocyte necrosis (i.e. bile infarcts), portal inflammation (mainly neutrophils) and periductal edema of bile ducts [16,27], followed by a proliferative response of BECs (as part of the ductular reaction) and hepatocytes [27]. Proliferating cholangiocytes secrete proinflammatory cytokines and chemokines. Neutrophils themselves contribute to cholestatic liver injury after BDL for 3 days [62], whereas Kupffer cells are believed to abrogate acute cholestatic liver injury through the release of interleukin 6 [27,63]. It is well established that BDL induces fibrosis in the liver, showing increasing levels of type I collagen, TIMP-1, and TGF-β1 [27,64]. BDL in rats induces progressive fibrosis, leading to liver cirrhosis within two weeks after BDL [65]. However in mice, combined BDL and cholecystektomie (up to 8 weeks depending on mouse strains used) are necessary to induce advanced liver fibrosis [64]. To some degree, effects of biliary obstruction are present in any mouse model addressing biliary tract injury or cholangitis [6]. To account for this issue and for reasons of specificity, future studies addressing mouse models for SC should include a “control group” of BDL rodents for comparison.

Models involving enteric bacterial cell-wall components or colitis

Small bowel bacterial overgrowth

Bacterial translocation via an increased intestinal permeability (leaky gut) from the inflamed gut to the portal circulation resulting in activation of hepatic immune cascades and subsequent bile duct injury is believed to represent a causative pathogenic link between PSC and IBD [66]. In a rat model of SBBO, genetically susceptible animals (e.g. Lewis, Wistar and Sprague-Dawley strains) developed hepatobiliary injury after SFBL creation of the jejunum [28] with portal inflammation, ductular proliferation, bile duct destruction with peribiliary fibrosis. Cholangiography revealed involvement of intra- and extrahepatic bile ducts [28]. The underlying mechanisms of the model still remain partly elusive. In genetically susceptible rats, bacterial cell wall components of anaerobic bacteria (e.g. PG-PS) might induce innate immune responses by activation of Kupffer cells [67]. Since concomitant metronidazole or tetracycline treatment prevented liver injury in this model, Bacteroides species appeared to be obligatory [68]. The elevation of IgA and IgM antibodies against PG-PS in the sera of experimental animals further support this hypothesis [67]. This model suggests that an abnormal amount of enteric anaerobic bacteria may trigger pathologic immune responses in the liver in susceptible hosts when the colonic epithelium is compromised as seen in IBD, which might help to determine the pathogenetic role for the liver-gut axis in PSC/IBD.

Administration of N-formyl L-methionine L-leucin L-tyrosine

Daily administration of fMLT, a chemotactic peptide produced by Escherichia coli, into the colon of Wistar rats with acetate-induced colitis induced a profound hepatic mononuclear cell infiltrate in the portal area, preferentially around small intrahepatic bile ducts [29]. While only neutrophils and macrophages are strongly activated by fMLT [29], T-lymphocytes were found attached to BECs by day four and appeared to be responsible for BEC injury [29]. Activated macrophages themselves seem to be necessary for this subsequent T-cell migration to the bile ducts, since pharmacologic inhibition of macrophage function resulted in a decrease in lymphocyte infiltration [30]. Since it is known that fMLT is taken up from the portal vein, secreted into bile by hepatocytes and undergoes enterohepatic circulation [29,30], the preferential involvement of small bile ducts in this study might originate in differences in the blood supply between small and large bile ducts. fMLT could also be absorbed by BECs, thereafter transferred to the extra-cellular space and consequently attract leukocytes to BECs [29]. Neither periductal fibrosis nor obliterating cholangitis were observed in this study probably due to the short observation period. Of note, acute life-threatening colitis due to intrarectal infusion of acetate and fMLT and resulting very high mortality rates complicate long-term studies in this model.

DSS treatment of CD-1 mice

DSS treatment of CD-1 mice induced chronic experimental colitis accompanied by hepatobiliary lesions in one third of animals studied with portal inflammation and focal hepatocellular necrosis [31]. No SC developed within an observation period of one month. Flow cytometry data indicated a stimulation of natural killer T-cells (NKT) and activation of Th1 cells in the liver and a main Th2 immune response in the colon. Further mechanistic studies are needed to elucidate the link of colitis and hepatobiliary disorders in this model.

Models of biliary epithelial and endothelial cell injury

Graft-versus-host disease

Bile ducts are major targets in acute and chronic GVHD and the principal hepatic lesion is non-suppurative destructive cholangitis, mediated by T-cells [6]. Experimental GVHD across minor histocompatibility antigens was developed by injecting spleen and bone marrow cells of congenic B10.D2 mice into sublethally irradiated BALB/c mice [33]. Both the intra- and extrahepatic bile ducts showed severe cholangitis with mainly lymphocytes infiltrating bile ducts in the early phase. Later on distinct periductal lamellar fibrosis with a decreasing amount of inflammatory cells invading the bile duct wall was observed [33]. The degree of fibrosis remained relatively stable over time. The close morphological similarities between this GVHD-model and human PSC suggests
that PSC and GVHD share at least to some extent common immunological mechanisms.

**Single TNBS-infusion into the portal vein**

Single TNBS-infusion into the portal vein of rats induced acute necrotizing liver injury accompanied by the production of ANCA mainly directed against catalase [34], frequently observed in PSC patients. TNBS, a chemical hapten reagent, has been used to induce T-cell and B-cell immunoresponses and for induction of experimental IBD [69]. It is believed that the mechanisms of immune response to hapten determinants depend on haptenization of autologous proteins and subsequent presentation to T-cells by antigen-presenting cells [70]. Liver histology revealed mild dose-dependent portal inflammation, ductular proliferation and chronic cholangitis. The autoantibody induction showed no correlation with the TNBS-dose or with the extent of liver damage, suggesting the hapten function of TNBS as the primary mechanism of autoantibody induction [34]. Since the specificity for ANCA testing in PSC is low and the liver phenotype in TNBS-challenged rats is mild, the general validity of this model may be limited.

Taken together, currently no animal model fulfills all criteria for "the ideal PSC model" [6,71]. The major goal of future studies could be to strike a new path by for instance challenging mice with targeted disruption of potential susceptibility genes (e.g. TGR5−/−) and screening of already available knockout mice (e.g. Foxo3a−/−) for their susceptibility to bile duct injury. Since PSC represents a long-standing disease with complex genetic background, it seems most unlikely that one single model will perfectly mirror PSC, but we will rather need different models to study particular pathogenetic steps of PSC. Future studies should also aim at careful cross-validation of findings in models and the human situation.

**Disclosure of interest**

The authors declare that they have no conflicts of interest concerning this article.

**References**


