Gideon Hirschfield
David Adams
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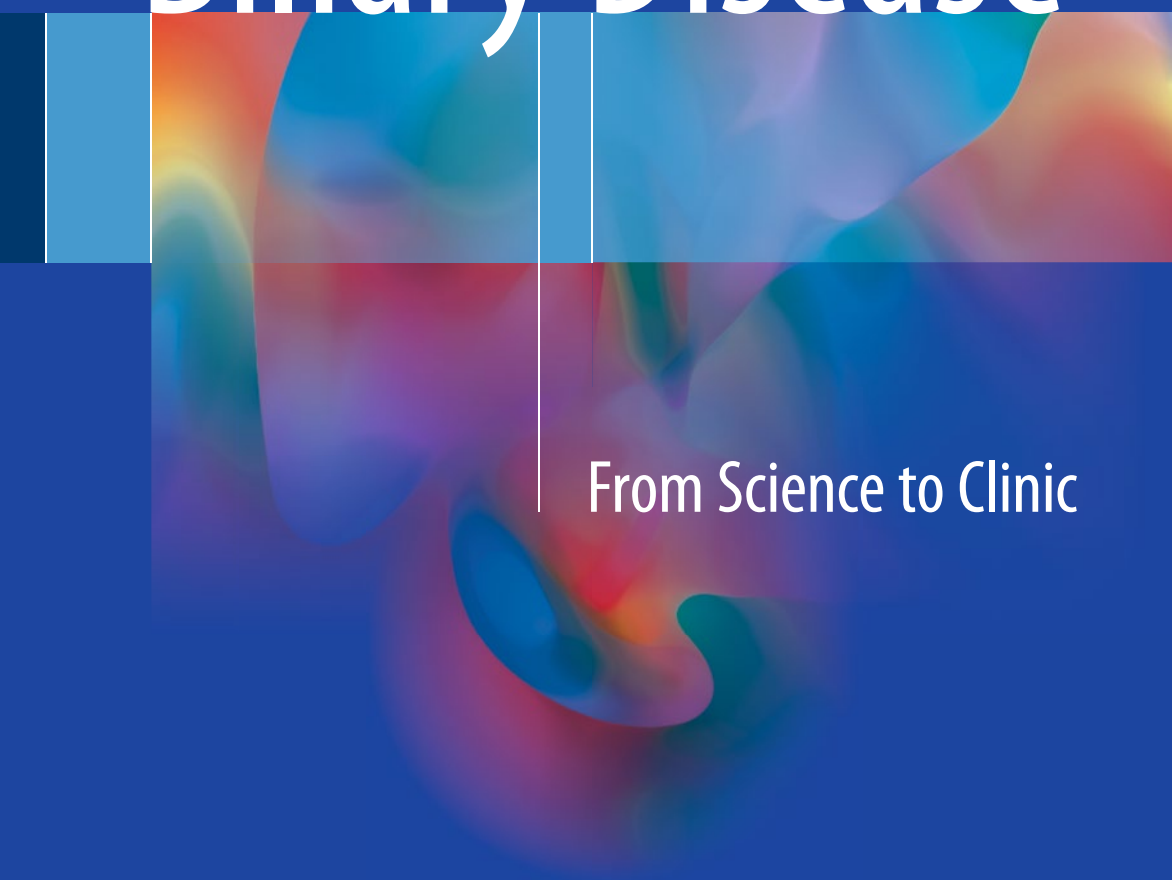
Editors

Biliary Disease



From Science to Clinic

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ISBN 978-3-319-50166-6 ISBN 978-3-319-50168-0 (eBook)
DOI 10.1007/978-3-319-50168-0

Library of Congress Control Number: 2017933709

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

Biliary diseases are important causes of acute and chronic liver illnesses that span all ages, both sexes, and individuals wherever they reside. Whilst our treatments remain presently limited, the biologic understanding of disease continues to improve. With insights into all facets of biliary disease from epidemiology, through genetics and environmental risk, to local tissue responses, a more detailed pathway can be mapped that helps patients and clinicians understand the challenges they encounter. In some cases diseases are restricted by age, or biliary location, in other facets disease features overlap (immune pathways, impact of the microbiome, resultant symptoms), and such similarities and distinctions have advanced therapeutic targets, such that treatment beyond the non-specific bile acid, ursodeoxycholic acid, is reaching the clinic environment. These continue to include efforts to redirect immune responses against the biliary tree, drugs positioned to enhance protective gut-liver axes (FGF19-FXR/PPAR signalling) as well as agents that complement inherent biliary epithelial cell tolerability to injury by improved bicarbonate production in the biliary tree. This short book provides the reader a thorough journey through biliary disease and will act as a current primer to what is a dynamic and exciting area of liver disease.

Birmingham, UK

Gideon Hirschfield
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The Clinical Burden of Biliary Disease: A Global Perspective

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Kirsten Muri Boberg

Abstract

Many biliary disorders are considered rare diseases (diseases affecting less than 50 per 100,000 inhabitants) according to the definition by the European Commission for Public Health. They still impose a burden on the patients affected and the health-care system. Although adequate population-based epidemiological studies on biliary diseases are scarce from several parts of the world, it is evident that there is a marked geographical variation in the incidence and prevalence rates of many of the conditions (Table 1.1). These observations are attributed to differences in the worldwide distribution of risk factors. Biliary diseases may be diagnosed in all age groups, even in the neonate. The disorders range from benign conditions with potential curative options, including liver transplantation, to devastating biliary tract cancers with very poor survival. Hopefully, ongoing efforts to elucidate the pathogenesis and define potential targets for therapy for biliary disorders will reduce the burden of these conditions in the future.

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G. Hirschfield et al. (eds.), *Biliary Disease*, DOI 10.1007/978-3-319-50168-0_1

Take-Home Points

- Gallstone disease is one of the most common digestive disorders, with a prevalence of gallstones in Europe and the USA in the range of 6–22% and even higher numbers in some ethnic subgroups like American Indians.
- Extrahepatic biliary atresia is the most frequent cause of morbidity of all childhood hepatobiliary diseases and the most common indication for liver transplantation in children.
- There is large variation in the incidence and prevalence rates of the chronic cholestatic disorders primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) worldwide, with the highest occurrence reported from Northern Europe and North America.
- Gallbladder cancer is the most common malignancy of the biliary tree and among the five to six most common cancers of the gastrointestinal tract. High-risk populations are found in Latin America and Asia, while incidence rates are lower in Northern Europe and the USA.
- The incidence of intrahepatic cholangiocarcinoma varies widely worldwide, most likely associated with the variable distribution of risk factors. Asians are affected almost twice as frequently as whites and blacks, with the highest incidence rates found in Northeast Thailand. Both cholangiocarcinoma and gallbladder cancers carry a poor prognosis with 5-year survival around 10%.

1.1 Pediatric Cholestatic Disorders

Cholestasis in children can be a manifestation of a number of disorders [1]. Some of the pediatric cholestatic conditions affect the neonate, and it is important to differentiate these from a benign, transient neonatal jaundice. Several of the cholestatic syndromes that occur in infancy can result in significant childhood disease. Early awareness facilitates relevant diagnostic workup and subsequent therapy for the conditions where this is a possibility. A detailed history, including the time of onset of jaundice, may be helpful in the differential diagnosis. The causes of infantile cholestatic syndromes can be classified as extrahepatic or intrahepatic disorders [1].

1.2 Extrahepatic Biliary Atresia

Extrahepatic biliary atresia, consisting of obliteration of the hepatic or common bile duct, is the most frequent cause of morbidity of all childhood hepatobiliary diseases [1]. Early diagnosis is essential so that surgical treatment by portoenterostomy (Kasai procedure) can be carried out in due time to achieve the best possible result. Despite this surgical intervention, 2/3 of patients experience progressive liver disease with development of cirrhosis. Extrahepatic biliary atresia is the most common indication for liver transplantation in childhood [1].

The incidence of biliary atresia varies between geographical regions and appears to be higher in Asian countries than in Europe. The annual incidence per 10,000 live births has been reported to be 1.03–1.08 in Japan, 1.06 in Hawaii, 0.65–0.85 in the USA, 0.71 in Sweden, 0.70 in Australia, 0.48–0.59 in the UK, 0.51 in metropolitan France, and 0.50 in the Netherlands [2] (Table 1.1). A very high incidence of 3.2 per 10,000 live births has been observed in French Polynesia, followed by 1.32–1.65 per 10,000 in Taiwan [2]. Girls are more frequently affected than boys.

The pathology and treatment of biliary atresia is discussed in Chapter 6.

1.3 Progressive Familial Intrahepatic Cholestatic Syndromes

Progressive familial intrahepatic cholestasis (PFIC) comprises a heterogenous group of autosomal recessive disorders that are characterized by intrahepatic cholestasis. The conditions display characteristic clinical, biochemical, and histologic features. Genetic studies have revealed mutations in genes encoding hepatocanalicular transport proteins that are required for normal canalicular bile flow [3]. Mutations in the genes *ATP8B1*, *ABCB11*, *and ABCB4* are associated with the clinical entities PFIC syndromes type 1, 2, and 3, respectively [4]. Patients often present with cholestasis in the neonatal period or the first year of life, but PFIC3 may become apparent later in childhood or even during young adulthood [5]. Most PFIC patients will become liver transplant candidates.

Mutations in the *ATP8B1* gene can also cause benign recurrent intrahepatic cholestasis (BRIC1), characterized by episodic cholestasis at any age. A proportion of patients suffering from intrahepatic cholestasis of pregnancy have mutations in the *ABCB4* gene [3].

PFIC is a rare disorder, with an estimated incidence between 1 per 50,000 and 1 per 100,000 births, although exact numbers are not known [5]. PFIC appears to affect girls and boys equally frequent. All PFIC types have a worldwide distribution.

1.4 Drug-Induced Cholestasis

Drugs and other substances, including herbs and dietary supplements, may cause a wide range of liver damage. It is therefore important to consider drug-induced liver injury (DILI) as a differential diagnosis in all cases of hepatobiliary disease with uncertain etiology. DILI is usually classified as being of a hepatocellular, cholestatic, or mixed type, and the various drugs are usually associated with a predominating type of reaction. Genetic variants of biliary transporters (e.g., MDR3 and BSEP) have been associated with a predisposition to drug-induced cholestasis [3]. In most cases, the cholestatic drug-induced liver injury is mild and reversible, but persistent injury with biliary fibrosis and cirrhosis develops in some cases [6, 7].

Table 1.1 Examples of reported incidence and prevalence rates of biliary diseases

Disorder	Gender distribution	Incidence rates	Prevalence rates
Extrahepatic biliary atresia	Girls > boys	Per 10,000 live births: French Polynesia 3.2 Taiwan 1.32–1.65 Japan 1.03–1.08 USA 0.65–0.85 Sweden 0.71 Australia 0.70 UK 0.48–0.59 France 0.51 The Netherlands 0.50	n.a.
Progressive familial intrahepatic cholestasis	Girls ~ boys	1 per 50,000–1 per 100,000 live births	n.a.
Drug-induced cholestasis		Per 100,000 inhabitants/year: France: 8.1 (47% cholestatic plus mixed type) Spain: 3.4 (20% cholestatic, 22% mixed type) Iceland: 19.1 (32% cholestatic, 26% mixed type)	n.a.
Primary biliary cholangitis	Females:males ~ 10:1	Per 100,000 inhabitants/year: UK (Newcastle upon Tyne) 5.8 USA (Olmsted County) 2.7 Iceland 3.4 Finland 1.7 Norway 1.6 Sweden 1.3–2.4 The Netherlands 1.1	Per 100,000 inhabitants: UK (Newcastle upon Tyne) 39.2 USA (Olmsted County) 40.2 Iceland 38.3 Finland 18.0 Norway 14.6 Sweden 9.6–15.1 The Netherlands 13.2 Japan 2.7–5.4 Israel 5.5 Australia 1.91

Primary sclerosing cholangitis	Females/males ~ 1:2	Per 100,000 inhabitants/year: UK 0.41–0.91 USA (Minnesota) 0.90 Canada 0.92 Norway 1.31 Sweden 1.22 The Netherlands 0.5 Southern Europe and Asia <0.1	Per 100,000 inhabitants: Northern Europe and the USA 8–16 The Netherlands 6.0 Spain 0.22
Autoimmune pancreatitis (no specific data are available on IgG4-associated cholangitis)	Females<males	Per 100,000 inhabitants/year: Japan 0.9	Per 100,000 inhabitants: Japan 2.2
Intrahepatic cholangiocarcinoma	Males 1.3–3.3 × females	Per 100,000 inhabitants/year USA (1995–1999) 0.85 USA (2004–2007) 0.89 England and Wales (1995–2008) 1.62	n.a.
Extrahepatic cholangiocarcinoma		Per 100,000 inhabitants/year USA (1998) 0.82 USA (2004–2007) 0.99 England and Wales (1995–2008) 0.47	n.a.
Gallbladder cancer	Females 2–6 × males	Per 100,000 inhabitants/year India (Dehli, females) 21.5 Pakistan (South Karachi) 13.8 Ecuador (Quito) 12.9 Northern Europe, USA, Canada <3 (females)	n.a.
Gallstone disease	Females>males	n.a.	Per 100 inhabitants: USA (gallstones or previous cholecystectomy) 14.3 North American Indians 60–70 Europe 6–22 South America 9–29 Black Africans <5 Asia 3–6

Abbreviations: *USA* United States of America, *UK* United Kingdom, *n.a.* not applicable

There is only limited information on the incidence of DILI. In general, the frequency of drug-induced adverse events appears to be underestimated due to incomplete ascertainment of cases. In large DILI series, a cholestatic type of drug injury has been noted in 20–40% of patients [6]. In a population-based study from France from 1997 to 2000, the global crude annual incidence rate for hepatic adverse drug reactions was 13.9 ± 2.4 per 100,000 inhabitants, with a corresponding standardized annual global rate of 8.1 ± 1.5 [8]. For comparison, the annual detection rate of hepatitis C in the same region was approximately 30 per 100,000. Among the 34 cases of DILI recorded, cholestatic and mixed injury patterns accounted for 16 (47%). In a prospective study in Southern Spain during 1994–2004, 461 cases of toxic liver injury were collected. The estimated annual incidence rate in the period 1998–2003 (only reported from the coordinating center) was 3.4 ± 1.1 cases per 100,000 inhabitants, about half of these being classified as serious adverse hepatic reactions [9]. Among the 461 cases, a hepatocellular damage pattern was most common (58%), whereas the mixed and cholestatic types were equally frequent (22% and 20%, respectively). Patients with a cholestatic pattern were significantly older than patients with other patterns of liver injury, as has also been observed by others [7]. A prospective, population-based survey from Iceland during 2010–2011 reported a crude annual incidence rate of DILI as high as 19.1 (95% CI 15.4–23.3) cases per 100,000 inhabitants (excluding cases with acetaminophen toxicity) [10]. Hepatocellular injury was most frequent (42%), followed by the cholestatic (32%) and mixed types (26%). In a recent study from the DILIN Prospective Study in the USA, including 899 cases considered as DILI enrolled between 2004 and 2013, the pattern of damage was hepatocellular in 54% and cholestatic or mixed in 23% each [7]. Among the 899 patients, 10% died or underwent liver transplantation, underscoring the potential serious outcome of DILI. As many as 31% of patients with cholestatic injury had signs of chronic or unresolved injury 6 months after onset, compared with an overall frequency of 18%.

The above studies were carried out in Western countries; even less information on the occurrence of DILI is available from other parts of the world. The real impact of cholestatic DILI on the clinical burden of cholestatic liver disease is thus difficult to assess.

Chapter 7 focuses on the mechanisms and importance of drug-induced cholestasis.

1.5 Primary Biliary Cholangitis

Primary biliary cholangitis (PBC), previously known as primary biliary cirrhosis, is a chronic, non-suppurative, destructive cholangitis involving the small- to medium-sized intralobular bile ducts [11]. The disease leads to fibrosis and may progress to liver cirrhosis and end-stage liver disease. The diagnosis is conventionally based on a history of elevated cholestatic liver tests for at least 6 months, the presence of antimitochondrial antibodies, and a compatible liver biopsy [12]. According to current guidelines, a liver biopsy is not necessary to diagnose PBC [12]. This disorder predominantly affects females, with a female to male ratio of approximately 10:1. The median age of diagnosis is around 50 years; PBC does not affect children.

The pathogenesis of PBC appears to comprise a complex interaction between genetic and environmental factors, also involving autoimmunity. Ursodeoxycholic acid (UDCA) improves liver tests and delays the disease progression with extended liver transplant-free survival [13]. Around 40% of patients do not, however, display a biochemical response to UDCA. Hepatocellular carcinoma is a rare complication of PBC but occurred more frequently among nonresponders than responders to UDCA in a recent survey that included a large international cohort of 4565 PBC patients [14]. The overall incidence rate of hepatocellular carcinoma was 3.4 cases per 1000 patient-years. PBC patients who develop end-stage liver disease are good candidates for liver transplantation.

There is a large variation in the incidence and prevalence rates of PBC worldwide, ranging from 0.33–5.8 per 100,000 inhabitants/year and 1.91–40.2 per 100,000 inhabitants, respectively [15]. The highest incidence and prevalence rates have been reported from Northern Europe and North America, in particular including the findings in Newcastle upon Tyne, UK (incidence 5.8 per 100,000; prevalence 39.2 per 100,000) [16], and Olmsted County, USA (incidence 2.7 per 100,000; prevalence 40.2 per 100,000) [17]. Incidence rates per 100,000 for PBC from other northern European countries include 1.7 in Finland, 1.6 in Norway, 1.3–2.4 in Sweden, and 3.4 in Iceland [15, 18]. Corresponding prevalence figures per 100,000 inhabitants are 18.0 in Finland, 14.6 in Norway, 9.6–15.1 in Sweden, and 38.3 in Iceland. The largest population-based study on the epidemiology of PBC published until now was performed in the Netherlands during 2000–2008, comprising a population of around 5.8 million [18]. The mean annual incidence was 1.1 per 100,000, and the point prevalence at study end was 13.2 per 100,000. Incidence and prevalence rates increased significantly over time. Apparent increases in incidence and prevalence rates for PBC have also been noted by others, although several contributing factors may be implicated [19].

Good epidemiological studies on PBC from other parts of the world are scarce, but reports indicate generally lower incidence and prevalence numbers. Examples of published prevalence rates per 100,000 include studies from Japan (2.7–5.4), Israel (5.5), Australia (1.91), and Brunei Darussalam (Southeast Asia) (2.6) [15, 19].

Chapter 8 elaborates on the science and practice of PBC.

1.6 Primary Sclerosing Cholangitis

Primary sclerosing cholangitis (PSC) is characterized by inflammatory and fibrotic processes that primarily affect the medium-sized and large bile ducts, resulting in bile duct irregularities with strictures and dilatations [20]. PSC is more common in males than in females (2:1). The disease affects a rather young population, with median age at diagnosis around 40 years. PSC may also present in childhood and then often with associated features of autoimmune hepatitis [21]. PSC is in most cases a progressive disorder that leads to liver cirrhosis, with estimated survival from diagnosis until PSC-related death or liver transplantation in the range 13–21 years [22]. There is no medical therapy that effectively halts the disease progression, but PSC patients are considered good candidates for liver transplantation. In the Nordic countries, PSC has until recently been the most important indication

for liver transplantation [20]. PSC is strongly associated with inflammatory bowel disease (IBD). The highest frequencies of concomitant IBD (70–80%) have been reported from Northern Europe and the lowest (20–50%) from Asia [23].

PSC patients carry an increased risk of cancer. The high risk of cholangiocarcinoma (CCA) development (6–13% in population-based series) in particular adds to the disease burden in PSC patients. In a Swedish cohort of PSC patients, the risk of hepatobiliary malignancy was 161 times higher compared to the general Swedish population and that of cancer of the colon-rectum was ten times increased [24]. The risk of any gastrointestinal cancer was 29 times higher than in the general population. It is recognized that the risk of colorectal malignancies is higher in PSC-IBD than in IBD without hepatobiliary disease.

Epidemiological studies of PSC are hampered by several factors. First, PSC has an insidious onset and can have a prolonged preclinical course before being diagnosed. Approximately 50% of patients are asymptomatic at the time of diagnosis. Second, the diagnosis relies on typical findings by cholangiography and requires that such investigations have been carried out. Incidence and prevalence estimates therefore must be considered minimum numbers. Based on six population-based studies from North America and Europe, the combined incidence rate of PSC was 1.0 (0.82–1.17) per 100,000 [25]. The prevalence of PSC in these regions is around 10 per 100,000 [20]. More specifically, studies have shown an incidence rate per 100,000 of 1.31 in Norway, 1.22 in Sweden, 0.92 in Canada, from 0.41 to 0.91 in the UK, and 0.90 in Minnesota, USA [26]. A lower incidence rate of 0.5 per 100,000 was however noted in a recent study from the Netherlands, based on the identification of 590 PSC patients in an area of almost eight million inhabitants [22]. Markedly lower incidence rates have been observed in Southern Europe (0.07 per 100,000 in Spain) and Asia [20]. An increasing trend in the incidence of PSC has been noted [15]. The prevalence ranges from 8 to 16 per 100,000 inhabitants in Northern Europe and the USA (6.0 in the above study from the Netherlands), while reported numbers are lower in Southern Europe (0.22 per 100,000 in Spain) and Asia.

Small-duct PSC is characterized by the same presenting features as the classical (large-duct) PSC, but displays a normal cholangiogram. The proportion of small-duct PSC relative to classical PSC has been reported to be in the range 5.8–16.3% [26]. Small-duct PSC appears to have a more benign course than classical PSC.

Increasing efforts are directed toward the understanding of disease pathogenesis in PSC, with the ultimate aim to define targets for therapy. Chapter 9 describes current concepts in PSC biology and strategies for new therapy.

1.7 Autoimmune Sclerosing Cholangitis/IgG4 Cholangiopathy

Autoimmune sclerosing cholangitis/IgG4 cholangiopathy, also designated IgG4-associated cholangitis (IAC), is the biliary manifestation of immunoglobulin G4-related disease which is characterized by inflammatory lesions and can involve a number of organs [27]. IAC is frequently associated with IgG4 disease of the

pancreas (autoimmune pancreatitis). The diagnosis of IAC and autoimmune pancreatitis is based on the HISORt criteria (a combination of histology, imaging, serology, other organ involvement, and response to therapy) [28]. The histological findings in IAC are characterized by dense lymphoplasmacytic infiltrates (>10 IgG4-positive cells per high-power field), storiform fibrosis, and obliterative phlebitis. IAC is also associated with elevated serum IgG4 levels, but levels may be normal in up to 20% of patients [28]. IAC presents with features similar to those of PSC, and the two conditions may be difficult to differentiate [29]. IAC may also be difficult to differentiate from malignant conditions of the pancreas and biliary tract. Elevated serum IgG4 levels were present in 9% among 127 PSC patients [30], and 15.6% of 122 PSC liver explants showed marked hilar IgG4 lymphoplasmacytic infiltration [31].

Age at presentation of IAC varies, but these patients are generally older at diagnosis than patients with classic PSC. In similarity with PSC, there is a male predominance in IAC, whereas concomitant IBD is more uncommon. IAC typically responds to corticosteroid therapy [29] and is therefore important to recognize.

Data on incidence and prevalence of IgG4-related disease are limited. Most reports on IAC and autoimmune pancreatitis have been published from Japan and the Far East. In a nationwide epidemiological survey of autoimmune pancreatitis in Japan in 2007, the estimated annual incidence rate was 0.9 per 100,000 inhabitants, with an overall prevalence rate of 2.2 per 100,000 [32]. With more clinical focus on these conditions, they are increasingly being recognized also in the Western world. Among 3482 cases of IgG4-related disease reported up to March 2014, 2621 originated from Asia, 470 from Europe, and 452 from North America [33]. No separate epidemiological data on IAC are available as of yet.

The IgG4-related biliary disease is further described in Chapter 12.

1.8 Cholangiocarcinoma

CCAs are classified as (1) intrahepatic CCA, (2) perihilar (Klatskin tumor) CCA, and distal CCA, the two latter collectively being defined as extrahepatic CCA. The majority (60–70%) of tumors are hilar, 20–30% are distal extrahepatic, whereas intrahepatic CCA accounts for 5–10% of cases [34, 35]. Intrahepatic and extrahepatic CCA differ regarding epidemiology, potential risk factors, clinical features, therapeutic options, and prognosis. CCAs constitute about 3% of all gastrointestinal cancers. Intrahepatic CCA is the second most common primary liver cancer after hepatocellular carcinoma and accounts for approximately 10% of primary liver cancers [36]. Hepatobiliary malignancies cause around 13% of cancer-related deaths globally, and 10–20% among these are due to CCA [37]. Most CCAs are sporadic, but a proportion (<30%) of cases develops on the background of established risk factors [35, 38]. Except for tumors developing in patients with PSC, CCA is rare before the age of 40, and the peak age is in the seventh decade [36]. Men are affected from 1.3 to 3.3 times more than women.

CCA typically presents at a late stage when curative therapeutic options are limited, and less than 1/3 of patients are resectable at diagnosis. Overall, the 5-year survival rate for CCA patients has not improved over the last decades and is only about 10% [39]. Due to the dismal prognosis of this malignancy, mortality and incidence rates are similar [40].

Over the last decades, several studies have reported increasing incidence and mortality rates for intrahepatic CCA, along with stable or decreasing rates for extrahepatic CCA on an international basis [34, 35, 38, 41]. Changes in the International Classification of Diseases for Oncology (ICD-O) may have influenced these observations since coding misclassifications of hilar CCA as an intrahepatic rather than extrahepatic malignancy have been likely [34]. The adoption of new editions of ICD-O at different time points in different countries may also have contributed to varying incidence rates for intra- and extrahepatic CCA [34]. Although the separate trends for intra- and extrahepatic tumors should be interpreted with caution, there appears to be an overall rising incidence of CCA.

There is a wide variation in the incidence of intrahepatic CCA in different parts of the world, most likely associated with the variable distribution of risk factors. Asians are affected almost twice more frequently than whites and blacks [36]. Low incidence rates have been reported from Australia with 0.2 and 0.1 per 100,000 in men and women, respectively [36], whereas Northeast Thailand has the highest incidence rate of intrahepatic CCA worldwide, with numbers up to 113 per 100,000 person-years in men and 50 per 100,000 in women [37]. This high risk of CCA in Thailand is related to the high prevalence of hepatobiliary fluke infestations (*Opisthorchis viverrini* and *Clonorchis sinensis*) [35, 42].

From a population-based study in the USA using data from the cancer registries of the Surveillance, Epidemiology, and End Results (SEER) program, the age-adjusted incidence rate of intrahepatic CCA increased by 165% from 0.32 per 100,000 in 1975–1979 to 0.85 per 100,000 in 1995–1999, with most of the increase occurring after 1985 [43]. The incidence of extrahepatic CCA declined by 14% from 1.08 per 100,000 in 1979 to 0.82 per 100,000 in 1998 [36]. An updated review of CCA incidence rates between 1992 and 2007 in the USA supported the previously reported increase in incidence of intrahepatic CCA and a relatively stable incidence of extrahepatic CCA up to 2000. However, when the investigation was extended to 2007, the incidence of intrahepatic CCA remained overall stable with only slight fluctuations, whereas the incidence of extrahepatic tumors had increased after 2000 [44]. During 2004–2007, the age-adjusted incidence rate for intrahepatic and extrahepatic CCA was 0.89 (95% CI 0.87–0.91) and 0.99 (95% CI 0.96–1.01) per 100,000, respectively. Misclassification of hilar CCAs did not appear to significantly affect these trends. These findings differ from those in a study of CCA in England and Wales between 1990 and 2008, where age-standardized incidence rates rose for intrahepatic CCA and fell for extrahepatic CCA [34]. A marked increase of intrahepatic CCA (from 0.87 to 1.62 per 100,000) and a decrease of extrahepatic CCA (from 0.55 to 0.47 per 100,000) remained in a reanalysis after transferral of

Klatskin tumors from the intrahepatic to the extrahepatic group for the period 1995–2008. Similar trends have been observed in some countries (Japan, Italy, Germany), whereas a study from Denmark found a fall in incidence of both intrahepatic and extrahepatic CCA between 1978 and 2002, and a report from France concluded with stable incidences of both tumor groups [42, 45].

A number of molecular alterations involved in the pathogenesis of CCA have now been identified. These are potential biomarkers for early diagnosis as well as representing possible targets for therapy. Several studies on targeted therapies in CCA are currently ongoing [46], as discussed in Chapter 10.

1.9 Gallbladder Cancer

Gallbladder cancer is the most common malignancy of the biliary tree, accounting for 80–95% of cases in autopsy studies [47]. It is among the five to six most common cancers of the gastrointestinal tract [48]. Overall, gallbladder cancer accounts for less than 1% of all cancer deaths [49]. Symptoms and signs of gallbladder cancer are nonspecific, and the condition is usually diagnosed at an advanced clinical stage when only 10–30% of patients are candidates for potential radical cholecystectomy [48]. The prognosis is dismal, with 5-year survival rates of only 0–12% [39, 49]. Gallbladder carcinoma is discovered in 1–2% of patients who undergo cholecystectomy for anticipated benign disease [48]. Several risk factors have been associated with the development of gallbladder carcinoma, the most important being a history of gallstone disease [48, 50].

The risk of gallbladder cancer increases with age [47]. Among 10,301 patients with a diagnosis of gallbladder carcinoma in the SEER program in the USA during the years 1973–2002, the median age at diagnosis was 73 years [49]. During the last decade, there was a significant decrease in the incidence of gallbladder carcinoma in patients older than 50 years, along with a slight increase in the incidence among younger individuals. Gallbladder cancer affects women more commonly (two to six times) than men [47, 50]. In another report from the SEER registry, the incidence of gallbladder cancer declined by 42% from 1979 to 1997 and was then stable through 2004. The death rate decreased by 48% from 1979 to 2004. The mortality rate was 6.8 times higher in the age group ≥65 years compared with those being 45–64 years.

There is a wide variation in the worldwide incidence of gallbladder carcinoma, and the incidence also varies among ethnic groups within countries [47]. In a search of cancer registries worldwide, Randi et al. [50] identified two major groups of high-risk populations, in Latin America and Asia, respectively. The highest overall incidence rates had been reported for women in Delhi, India (21.5 per 100,000), followed by Karachi South, Pakistan (13.8 per 100,000), and Quito, Ecuador (12.9 per 100,000). High incidence rates were also noted in the Far East Asia (Korea and Japan) and in some countries in Eastern Europe (Slovakia, Poland, Czech Republic,

and Yugoslavia) and South America (Columbia). Gallbladder cancer has even been reported to be the main cause of death from cancer among women in some areas of South America [50]. Low incidences of gallbladder cancer (<3 per 100,000 women and 1.5 per 100,000 men) were identified in most cancer registries from Northern Europe and from the USA (SEER registry) and Canada. In the USA, the incidence rate is higher among Hispanic women in California and New Mexico than in any other ethnic groups [47]. Age-adjusted mortality rates are around 50% higher in African Americans than in whites [39].

1.10 Gallstone Disease

Gallstone disease is one of the most common digestive disorders and represents a major health burden in developed countries, regarding morbidity as well as costs [51]. It is the most common cause of biliary tract disorder in adults. Cross-sectional gallbladder ultrasonography surveys have contributed to the estimation of prevalence rates. Cholelithiasis is a complex disease in which genetic components as well as multiple individual and shared environmental factors contribute to the pathogenesis [52], and these factors are likely to influence on the varied distribution of gallbladder stones worldwide. Gallbladder stones are more common in European countries and North and South America than in Asia and Africa [51, 52]. Prevalence of gallstones in Europe and the USA is generally in the range 6–22%, however, with higher frequencies in some ethnic subgroups [51, 52]. Intermediate prevalences, in the range 3–15%, are seen in Asian populations and lower numbers in Africa. Cholesterol gallstones dominate in Europe and North and South America, whereas bilirubin gallstones are more common in Asia and Africa.

From a national population-based survey comprising more than 14,000 persons in the USA, the prevalence of gallstones or previous cholecystectomy was 14.3% [53]. The prevalence was higher in women (16.6%) than in men (7.9%). Age-standardized prevalence was higher in Mexican Americans (males 8.9%, females 26.7%) and non-Hispanic white (men 8.6%, women 16.6%) than in non-Hispanic blacks (men 5.3%, women 13.9%). Gallbladder disease was estimated to affect more that 20 million persons (6.3 million men and 14.2 million women) aged 20–74 years [53]. A high proportion (30.4% and 48.2% of men and women, respectively) of persons with gallbladder disease had undergone cholecystectomy. The highest prevalence occurs in North American Indians, being up to 73% in Pima Indians older than 30 years [51]. High prevalence is also seen in Indians in South America.

In a report on digestive and liver disease statistics in the USA, gallstone disease was the most common principal gastrointestinal inpatient diagnosis in the year 2000, amounting to 262,411 hospitalizations and a median inpatient charge of USD 11,584 [54]. In addition, there were an estimated 778,632 outpatient visits.

Gallstone disease is an important health problem that requires continuing attention, and the current scientific understanding and controversies are further discussed in Chapter 11.

References

1. Kelly D. Pediatric cholestatic syndromes. In: Boyer TD, Manns MP, Sanyal AJ, editors. Zakim and Boyer's hepatology: a textbook of liver disease. 6th ed. Philadelphia: Elsevier Saunders; 2012. p. 1223–56.
2. Tiao MM, Tsai SS, Kuo HW, Chen CL, Yang CY. Epidemiological features of biliary atresia in Taiwan, a national study 1996-2003. J Gastroenterol Hepatol. 2008;23(1):62–6.
3. Hirschfield GM. Genetic determinants of cholestasis. Clin Liver Dis. 2013;17(2):147–59.
4. Zollner G, Trauner M. Mechanisms of cholestasis. Clin Liver Dis. 2008;12(1):1–26.
5. Jacquemin E. Progressive familial intrahepatic cholestasis. Clin Res Hepatol Gastroenterol. 2012;36(Suppl 1):S26–35.
6. Bjornsson ES, Jonasson JG. Drug-induced cholestasis. Clin Liver Dis. 2013;17(2):191–209.
7. Chalasani N, Bonkovsky HL, Fontana R, Lee W, Stolz A, Talwalkar J, et al. Features and outcomes of 899 patients with drug-induced liver injury: the DILIN prospective study. Gastroenterology. 2015;148(7):1340–52.
8. Sgro C, Clinard F, Ouazir K, Chanay H, Allard C, Guilleminet C, et al. Incidence of drug-induced hepatic injuries: a French population-based study. Hepatology. 2002;36(2):451–5.
9. Andrade RJ, Lucena MI, Fernandez MC, Pelaez G, Pachkoria K, Garcia-Ruiz E, et al. Drug-induced liver injury: an analysis of 461 incidences submitted to the Spanish registry over a 10-year period. Gastroenterology. 2005;129(2):512–21.
10. Bjornsson ES, Bergmann OM, Bjornsson HK, Kvaran RB, Olafsson S. Incidence, presentation, and outcomes in patients with drug-induced liver injury in the general population of Iceland. Gastroenterology. 2013;144(7):1419–25.
11. Kaplan MM, Gershwin ME. Primary biliary cirrhosis. N Engl J Med. 2005;353(12):1261–73.
12. Lindor KD, Gershwin ME, Poupon R, Kaplan M, Bergasa NV, Heathcote EJ. Primary biliary cirrhosis. Hepatology. 2009;50(1):291–308.
13. Carey EJ, Ali AH, Lindor KD. Primary biliary cirrhosis. Lancet. 2015;386:1565–75.
14. Trivedi PJ, Lammers WJ, van Buuren HR, Pares A, Floreani A, Janssen HL, et al. Stratification of hepatocellular carcinoma risk in primary biliary cirrhosis: a multicentre international study. Gut. 2016;65:321–9.
15. Boonstra K, Beuers U, Ponsioen CY. Epidemiology of primary sclerosing cholangitis and primary biliary cirrhosis: a systematic review. J Hepatol. 2012;56(5):1181–8.
16. Metcalf JV, Bhopal RS, Gray J, Howel D, James OF. Incidence and prevalence of primary biliary cirrhosis in the city of Newcastle upon Tyne. England Int J Epidemiol. 1997;26(4):830–6.
17. Kim WR, Lindor KD, Locke III GR, Therneau TM, Homburger HA, Batts KP, et al. Epidemiology and natural history of primary biliary cirrhosis in a US community. Gastroenterology. 2000;119(6):1631–6.
18. Boonstra K, Kunst AE, Stadhouders PH, Tuynman HA, Poen AC, van Nieuwkerk KM, et al. Rising incidence and prevalence of primary biliary cirrhosis: a large population-based study. Liver Int. 2014;34(6):e31–8.
19. Griffiths L, Dyson JK, Jones DE. The new epidemiology of primary biliary cirrhosis. Semin Liver Dis. 2014;34(3):318–28.
20. Karlsen TH, Boberg KM, Schrumpf E. Primary sclerosing cholangitis. In: Boyer TD, Manns MP, Sanyal AJ, editors. Zakim and Boyer's hepatology: a textbook of liver disease. 6th ed. Philadelphia: Elsevier Saunders; 2012. p. 754–81.
21. Gregorio GV, Portmann B, Karani J, Harrison P, Donaldson PT, Vergani D, et al. Autoimmune hepatitis/sclerosing cholangitis overlap syndrome in childhood: a 16-year prospective study. Hepatology. 2001;33(3):544–53.
22. Boonstra K, Weersma RK, van Erpecum KJ, Rauws EA, Spanier BW, Poen AC, et al. Population-based epidemiology, malignancy risk, and outcome of primary sclerosing cholangitis. Hepatology. 2013;58(6):2045–55.
23. Karlsen TH, Schrumpf E, Boberg KM. Primary sclerosing cholangitis. Best Pract Res Clin Gastroenterol. 2010;24(5):655–66.

24. Bergquist A, Ekbom A, Olsson R, Kornfeldt D, Loof L, Danielsson A, et al. Hepatic and extrahepatic malignancies in primary sclerosing cholangitis. J Hepatol. 2002;36(3):321–7.
25. Molodecky NA, Kareemi H, Parab R, Barkema HW, Quan H, Myers RP, et al. Incidence of primary sclerosing cholangitis: a systematic review and meta-analysis. Hepatology. 2011;53(5):1590–9.
26. Kaplan GG, Laupland KB, Butzner D, Urbanski SJ, Lee SS. The burden of large and small duct primary sclerosing cholangitis in adults and children: a population-based analysis. Am J Gastroenterol. 2007;102(5):1042–9.
27. Hubers LM, Maillette de Buy Wenniger LJ, Doorenspleet ME, Klarenbeek PL, Verheij J, Rauws EA, et al. IgG4-associated cholangitis: a comprehensive review. Clin Rev Allergy Immunol. 2015;48(2–3):198–206.
28. Joshi D, Webster GJ. Immunoglobulin G4-related sclerosing cholangitis. Hepatology. 2015;61(4):1432–4.
29. Bjornsson E, Chari ST, Smyrk TC, Lindor K. Immunoglobulin G4 associated cholangitis: description of an emerging clinical entity based on review of the literature. Hepatology. 2007;45(6):1547–54.
30. Mendes FD, Jorgensen R, Keach J, Katzmann JA, Smyrk T, Donlinger J, et al. Elevated serum IgG4 concentration in patients with primary sclerosing cholangitis. Am J Gastroenterol. 2006;101(9):2070–5.
31. Fischer S, Trivedi PJ, Ward S, Greig PD, Therapondos G, Hirschfield GM. Frequency and significance of IgG4 immunohistochemical staining in liver explants from patients with primary sclerosing cholangitis. Int J Exp Pathol. 2014;95(3):209–15.
32. Kanno A, Nishimori I, Masamune A, Kikuta K, Hirota M, Kuriyama S, et al. Nationwide epidemiological survey of autoimmune pancreatitis in Japan. Pancreas. 2012;41(6):835–9.
33. Brito-Zeron P, Ramos-Casals M, Bosch X, Stone JH. The clinical spectrum of IgG4-related disease. Autoimmun Rev. 2014;13(12):1203–10.
34. Khan SA, Emadossadaty S, Ladep NG, Thomas HC, Elliott P, Taylor-Robinson SD, et al. Rising trends in cholangiocarcinoma: is the ICD classification system misleading us? J Hepatol. 2012;56(4):848–54.
35. Razumilava N, Gores GJ. Classification, diagnosis, and management of cholangiocarcinoma. Clin Gastroenterol Hepatol. 2013;11(1):13–21.
36. Shaib Y, El-Serag HB. The epidemiology of cholangiocarcinoma. Semin Liver Dis. 2004;24(2):115–25.
37. Rizvi S, Gores GJ. Pathogenesis, diagnosis, and management of cholangiocarcinoma. Gastroenterology. 2013;145(6):1215–29.
38. Khan SA, Davidson BR, Goldin RD, Heaton N, Karani J, Pereira SP, et al. Guidelines for the diagnosis and treatment of cholangiocarcinoma: an update. Gut. 2012;61(12):1657–69.
39. Everhart JE, Ruhl CE. Burden of digestive diseases in the United States part III: liver, biliary tract, and pancreas. Gastroenterology. 2009;136(4):1134–44.
40. Khan SA, Toledano MB, Taylor-Robinson SD. Epidemiology, risk factors, and pathogenesis of cholangiocarcinoma. HPB Off J Int Hepato Pancreato Biliary Assoc. 2008;10(2):77–82.
41. Lazaridis KN, Gores GJ. Cholangiocarcinoma. Gastroenterology. 2005;128(6):1655–67.
42. Bergquist A, von Seth E. Epidemiology of cholangiocarcinoma. Best Pract Res Clin Gastroenterol. 2015;29(2):221–32.
43. Shaib YH, Davila JA, McGlynn K, El-Serag HB. Rising incidence of intrahepatic cholangiocarcinoma in the United States: a true increase? J Hepatol. 2004;40(3):472–7.
44. Tyson GL, Ilyas JA, Duan Z, Green LK, Younes M, El-Serag HB, et al. Secular trends in the incidence of cholangiocarcinoma in the USA and the impact of misclassification. Dig Dis Sci. 2014;59(12):3103–10.
45. Lepage C, Cottet V, Chauvenet M, Phelip JM, Bedenne L, Faivre J, et al. Trends in the incidence and management of biliary tract cancer: a French population-based study. J Hepatol. 2011;54(2):306–10.
46. Schweitzer N, Vogel A. Systemic therapy of cholangiocarcinoma: from chemotherapy to targeted therapies. Best Pract Res Clin Gastroenterol. 2015;29(2):345–53.

47. Gourgiotis S, Kocher HM, Solaini L, Yarollahi A, Tsiambas E, Salemis NS. Gallbladder cancer. Am J Surg. 2008;196(2):252–64.
48. Misra S, Chaturvedi A, Misra NC, Sharma ID. Carcinoma of the gallbladder. Lancet Oncol. 2003;4(3):167–76.
49. Kiran RP, Pokala N, Dudrick SJ. Incidence pattern and survival for gallbladder cancer over three decades – an analysis of 10301 patients. Ann Surg Oncol. 2007;14(2):827–32.
50. Randi G, Franceschi S, La Vecchia C. Gallbladder cancer worldwide: geographical distribution and risk factors. Int J Cancer. 2006;118(7):1591–602.
51. Shaffer EA. Gallstone disease: epidemiology of gallbladder stone disease. Best Pract Res Clin Gastroenterol. 2006;20(6):981–96.
52. Lammert F, Sauerbruch T. Mechanisms of disease: the genetic epidemiology of gallbladder stones. Nat Clin Pract Gastroenterol Hepatol. 2005;2(9):423–33.
53. Everhart JE, Khare M, Hill M, Maurer KR. Prevalence and ethnic differences in gallbladder disease in the United States. Gastroenterology. 1999;117(3):632–9.
54. Russo MW, Wei JT, Thiny MT, Gangarosa LM, Brown A, Ringel Y, et al. Digestive and liver diseases statistics, 2004. Gastroenterology. 2004;126(5):1448–53.

The Healthy Biliary Tree: Cellular and Immune Biology

2

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Abstract

The biliary tree is an arborizing system of intra- and extrahepatic conduits connecting the liver to the intestine. The biliary tree has a complex tridimensional structure, encompassing bile ducts of different sizes, morphologies, and functions. The most studied function of biliary epithelial cells (cholangiocytes) is to regulate the hydration and alkalinity of the primary bile secreted by hepatocytes. An increasing number of evidence highlight the ability of cholangiocyte to undergo changes in phenotype, proliferation, and secretory activity in response to liver damage. Cholangiocytes are involved in biliary innate immunity; altered

Financial Support Cariplo grant 2014–1099 to MC; Telethon grant GGP09189 to LF; NIH Grants RO1DK096096, RO1DK-079,005-07, DK034989 Silvio O. Conte Digestive Diseases Research Core Center, and projects FFC 24/2015, and Telethon grant GGP12133 to MS.

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G. Hirschfield et al. (eds.), *Biliary Disease*, DOI 10.1007/978-3-319-50168-0_2

biliary innate immunity plays a role in a number of biliary diseases, including genetic cholangiopathies, such as cystic fibrosis-related liver disease. In addition, cholangiocytes may behave as antigen-presenting cells and secrete immunoglobulins as well as several antimicrobial peptides. Thus, cholangiocytes, by participating actively to the immune and inflammatory responses, represent a first defense line against liver injury from different causes. In fact, cholangiocytes possess a number of sensing receptors for pathogen-associated molecular patterns (PAMPs), such as Toll-like receptors (TLRs), which modulate their proinflammatory behavior. Derangements of the signals controlling these mechanisms are at the basis of the pathogenesis of different cholangiopathies, often extending beyond the classically recognized immune-mediated (primary biliary cirrhosis, primary sclerosing cholangitis), as in cystic fibrosis liver disease.

Take-Home Points

- The main function of the biliary epithelium is the transport of bile to the gallbladder and intestine and modification of the primary bile secreted by hepatocytes.
- Cholangiocytes, the cells lining the biliary epithelium, are active players in innate immunity and inflammation.
- They are able to secrete IgA (sIgA) into bile and a variety of peptides with antimicrobial properties.
- Upon injury, reactive cholangiocytes are able to secrete a number of proinflammatory and profibrotic cytokines, chemokines, and growth factors.
- They express HLA class I molecules and HLA class II in inflammation, but not co-stimulatory molecules CD80 and CD86; thus, they are unlikely to play a direct role in the activation of T cells during hepatic inflammation.

2.1 The Normal Biliary Tree

The biliary tree is a complex tridimensional network of epithelial conduits laying into the hepatic parenchyma and extending outside the liver up to the Vater's papilla. The intrahepatic and extrahepatic sections of the biliary tree have different functions. Ductal structures are lined by biliary epithelial cells (BECs), also termed as cholangiocytes. Although representing less than 5% of the total liver cells, cholangiocytes are involved in several physiological functions as well as in many pathological responses. Within the liver, the intrahepatic bile ducts start from the Canals of Hering (CoH), the interface structure composed partly by cholangiocytes and partly by hepatocytes. CoH are localized at the periphery of the portal tract where they link the bile canaliculi, formed by two juxtaposed hepatocytes, to the smallest ramifications of the bile duct system, entirely lined by BECs (bile ductules). Then the bile ducts gradually enlarge through different levels of organization, including interlobular, septal, areal, and, finally, segmental ducts, the largest intrahepatic

biliary structure. Then segmental ducts coalesce in the two main hepatic ducts, which merge at the level of the hepatic hilum to form the common hepatic duct, and, after receiving the cystic duct, they form the common bile duct that connects the liver and the gallbladder to the intestine. The intra- and extrahepatic portions of the biliary tree have different embryologic origins and differ in terms of functions, pathologic processes, and oncologic aspects.

The biliary tree collects the primary bile produced by hepatocytes and modifies its composition by hydration and alkalinization and then delivers the bile to the intestine where it is essential for fat absorption. Besides bile transport/modifications, the biliary epithelium is intimately involved in liver inflammation and in the regenerative/reparative responses to liver injury. The interlobular bile ducts are a preferred target in auto- and alloimmune diseases, while the smallest ducts are mainly involved in liver repair as they proliferate and expand in close relationship with the activation of the stem cell compartment. A growing body of evidence indicates an important role of cholangiocytes in liver innate immunity.

2.2 Development of the Bile Duct Epithelium

Phenotypic diversity of intrahepatic and extrahepatic bile ducts reflects their different embryological origin. While the extrahepatic bile ducts originate from the caudal part of the ventral foregut [14, 106] from a $Hex^{-}/Pdx\text{-}1^{+}/Sox17^{+}$ primordial cell population [124], the intrahepatic system is derived from the hepatoblasts, bipotent parenchymal cells present in the fetal liver bud, characterized by a $Hex^{+}/Pdx\text{-}1^{-}/Sox17^{-}/AFP^{+}$ phenotype. From the 8th gestational week (GW) onward, hepatoblasts surrounding the mesenchyme of the nascent portal space start to differentiate toward a biliary phenotype, leading to a cell monolayer called ductal plate (DP) [114], expressing phenotypic markers of both hepatocellular (keratin 8 (K8) and K18) and biliary lineages (K19) [117]. From the 12th to the 16th GW, DP duplicates to form luminal structures where the hepatocyte markers are progressively downregulated, while other biliary markers, including K7, are neo-expressed [114, 117]. Residual non-duplicating DP is deleted by apoptosis [113], although a back-transdifferentiation of the DP remnants to periportal hepatocytes has been recently hypothesized [17]. In the meanwhile, biliary structures with a ductal configuration gradually migrate to be incorporated into the portal mesenchyme [30, 32]. Notably, intrahepatic bile duct maturation develops in strict conjunction with the formation of both the arterial vasculature and the peribiliary plexus to provide them with the blood nourishment. The fine synchronization of this maturation process depends upon the balanced expression of a range of angiogenic growth factors, mainly VEGF-A and the angiopoietins, Ang-1 and Ang-2, and of the corresponding receptors, VEGFR1, VEGFR2, and Tie-2, by both DP cells and endothelial cells [32, 116, 121]. The cell fate specification toward a biliary phenotype and the correct assembly of biliary cells in a ductal configuration are regulated by a complex and finely tuned interplay of growth factors, transcription factors, morphogens, and miRNAs. They include members of the transforming growth factor (TGF), hepatocyte nuclear

factor (HNF), and sex-determining region Y(SRY)-related HMG box transcription factor (SOX) families, as well as effectors of Notch and WNT signaling, derived from the surrounding microenvironment [61, 124].

2.2.1 Morphogens and Transcription Factors Involved in Biliary Lineage Specification and Bile Duct Formation

Studies on zebra fish [96] and mouse [69, 123] embryos further demonstrated the role of Notch signaling in biliary development. Initially, Jagged1 (Jag1) expressed by the nascent portal mesenchyme induces the transdifferentiation to the biliary phenotype of the Notch2-expressing hepatoblasts localized in close contact to the mesenchyme. Following this interaction, the hairy and enhancer of split-1 (Hes1), a downstream effector of the Notch receptor, is activated and binds to the nuclear transcription factor recombinant signal binding protein for immunoglobulin kappa J (RBP-Jk) to stimulate the activation of the cholangiocyte-specific transcription factors HNF1β and Sox9 [7, 36]. In this phase, expression of the neural cell adhesion molecule (NCAM) in conjunction with the antiapoptotic protein Bcl-2 is an early signature of the developing ductal plate [29]. These primordial ductal structures have an asymmetrical configuration ("transient asymmetry"), as they are formed on the portal side by cells with a biliary phenotype and on the parenchymal side by hepatoblasts [61]. In the following phase, the neo-formed DP cells start to express Jag1, which, in turn, induces the transdifferentiation of a second layer of hepatoblasts to DP cells, thus acquiring a symmetrical configuration [7, 88]. In their maturation to symmetrical ducts entirely lined by cells with a biliary phenotype, DP cells progressively loose NCAM and Bcl-2 [29], gaining in turn E-cadherin expression [7], a prerequisite for the correct alignment of the biliary structures, dependent on the "planar cell polarity" (PCP) mechanism. In a recent study, Poncy and colleagues [87] show that Sox9 cooperates with Sox4 to maintain the apical-basal polarity of cholangiocytes, and the correct formation of their primary cilia, to allow the proper ramification of the biliary duct system. A defect in Sox9 has been also related to a delayed biliary differentiation of hepatoblasts lining the asymmetric ducts leading to biliary dysgenesis [87]. Interestingly, biliary dysgenesis is also induced by the inactivation of Notch2 and/or Hes1, although these defects are not sufficient by themselves to completely block the biliary tree formation [40, 57, 67, 112]. These data highlight intensive cross-talk mechanisms among the different morphogens and likely a redundancy of the signaling regulating biliary tree structuring. These data are further corroborated by the studies on Alagille syndrome, a liver disease due to genetic defects in Jag1 and/or Notch2 featuring a hypoplasia of the intrahepatic bile ducts that fail to elongate during fetal development [31, 67]. Another morphogen relevant in liver development is Hedgehog (Hh). Following binding to its receptors, including Patched (Ptc), Hh acts through its downstream effectors, such as Smoothened (Smo), Glioblastoma (Gli), Fused (Fu), Costal2 (Cos2), and others. During the earliest phases of embryonic development, Hh is transiently expressed by the endodermal cells of the ventral foregut and, together

with Gli1, by hepatic progenitor cells (HPCs). Although the function of Hh signaling in bile duct specification and elongation is not completely elucidated, recent data support an important role of Hh in bile duct morphogenesis. Meckel syndrome, a rare autosomal recessive inherited disease, characterized by ductal plate malformation, showed a dysregulation of the Hh signaling [119] likely dependent upon the lack of the primary cilium expressing Ptc, Smo, and Gli [28]. Moreover, Hh signaling is involved in the correct assembly of the liver architecture following partial hepatectomy (PH). In C57BL/6 mice undergoing PH, treatment with cyclopamine, a Smo inhibitor, reduced the proliferation of both hepatocytes and cholangiocytes and inhibited the activation of HPC [82]. Noteworthy, both Notch and Hh signal pathways can modulate Sox9, and their dysregulation led to an altered Sox9 expression phenotypically evolving to liver fibrosis and cancer [54, 78].

TGF-β signaling is another fundamental determinant of biliary morphogenesis since the early stages. High expression of TGF-β is detected around the nascent periportal area, then gradually diminishing through the parenchyma [24]; on the other way, its cognate receptor, TGF-β type II-R, is selectively expressed by the DP cells, albeit transiently. The TGF-β gradient is regulated by several checkpoints provided by HNF6 and OC-2 expressed by DP cells and by miRNA 23b expressed in the parenchymal area [90]. All the members of the TGF-β family (1, 2, and 3) are able to promote the hepatoblast-cholangiocyte shift and to induce the expression of several biliary markers on the DP cells [7, 24]. Furthermore, TGF-β may interact with Notch by modulating the expression of some common targets, such as the transcription factors Hes1 and Hey1, or by upregulating the expression of Jag1 [47].

Another signaling critically involved in several stages of liver development is the Wnt/β-catenin pathway. It governs the proliferation of hepatoblasts and their biliary commitment and also the elongation and the correct tridimensional shaping of the mature biliary structure, through the mechanism of the PCP [39, 77]. The canonical activation of this pathway stimulates the proliferation of hepatoblasts and the expression by DP cells of phenotypic biliary markers, such as K19 and Sox9, in the earliest GWs [76]. In contrast, the noncanonical activation of Wnt/β-catenin signaling stimulates in vitro the formation of duct-like structures with a biliary phenotype in embryonic liver cells challenged with Wnt-3A [52].

As exemplified by TGF-β and Notch, all the abovementioned signaling may cross talk each other and act in concert to allow a correct tridimensional arrangement of the normal biliary tree and to maintain its physical and functional integrity.

Recently, miRNAs have been proposed as novel players of bile duct development, mainly acting as negative regulators. In particular, using a genome-wide approach on *zebra fish*, Hand and colleagues [48] showed that 38 miRNAs changed their levels of expression over the fetal liver development, with miR-30a and miR-30c being those specifically expressed by DP cells. Moreover, Rogler [90] demonstrated that miR-23b expressed in the developing liver by parenchymal but not portal mesenchymal cells is able, in vitro, to repress the cholangiocyte commitment of a fetal liver cell line by interfering with the TGF-β signaling at the level of its small mother against decapentaplegic (SMAD) downstream effectors.

2.3 Biological Properties Enabling Cholangiocytes to Respond to Liver Damage

Cholangiocytes are pivotal players in the liver reaction to damage by displaying a wide variety of fundamental biological activities, from proliferation, to regulation of both innate and adaptive immunity responses, to the ability to orchestrate the multicellular response to biliary damage.

2.3.1 Reactive Ductular Cells: The Main Element of the Hepatic Response to Damage

In the healthy adult liver, cholangiocytes usually stand in a quiescent state. However, following a liver insult, cholangiocytes start to proliferate and set in motion the "hepatic reparative complex." This histologic lesion is common to many forms of liver damage targeting either the parenchymal or the ductal compartment and is characterized by the appearance of reactive ductular cells (RDCs). RDCs are biliary-like cells with a less differentiated phenotype, most often organized in highly ramified structures, without a clearly recognizable lumen. RDCs are thought to originate from the expansion of the hepatic progenitor cell (HPC) compartment, a bipotent cell type residing in a niche strictly adjacent to the CoH. HPC can transdifferentiate into cholangiocytes and hepatocytes depending upon the nature of liver injury [91]. HPCs are single cells, characterized by an oval- or sometimes spindle-shaped morphology with a big nucleus and scant cytoplasm and by a mixed immunophenotype, expressing both biliary (K7, K19) and hepatocellular (K8, K18, alpha-fetoprotein) together with neuroendocrine (chromogranin A, NCAM) and staminal markers (c-Kit, CD34). Studies on viral hepatitis [25], chronic liver diseases [68], metabolic liver diseases [89], and genetic cholangiopathies [31] showed a positive correlation between the extent of RDC and HPC and the development of liver fibrosis ultimately leading to progression to cirrhosis. The nature of this functional correlation relies on the rich secretory functions of both RDC and HPC. They include a range of cytokines and chemokines (i.e., TNF-α, TGF-β2, IL6, MCP-1, IL8, CINC) [60, 104], growth factors (i.e., VEGFs, PDGFs, CTGF, angiopoietins) [30, 32, 84, 86], and other proinflammatory mediators (i.e., NO, reactive oxygen species) [23, 60, 81, 99] directing the recruitment, proliferation, and activation of the multiple cell types populating the hepatic reparative system, such as fibroblasts, inflammatory cells, and endothelial cells.

2.3.2 Cholangiocyte Proliferation

Cholangiocyte proliferation is a critical mechanism for RDC generation that depends on a number of factors released locally at the site of injury by the damaged liver epithelial cells and by the inflammatory infiltrate as well as on

circulating hormones. Among the pro-proliferative stimuli, a fundamental role is covered several by growth factors, including hepatocyte growth factor (HGF) [55], insulin-like growth factor-1 (IGF-1) [4], epidermal growth factor (EGF) [59], and vascular endothelial growth factor A (VEGF-A) [30]. In addition, members of the IL-6 family (IL-6, oncostatin M) [72, 109], gastrointestinal hormones (secretin) [45], and sex hormones (estrogen, testosterone, prolactin, and FSH) [71] play important roles. In contrast, gastrin, melatonin, somatostatin, serotonin, and GABA have negative effects on cholangiocyte proliferation [60, 104]. Some genetic diseases of the biliary epithelium may be paradigmatic of the role played by some of these growth factors in stimulating cholangiocyte proliferation. Liver disease related to autosomal dominant polycystic kidney disease (ADPKD) is a genetic cholangiopathy due to mutations in the ciliary proteins polycystin 1 or 2, featuring an abnormal hyperproliferation of the cholangiocytes leading to multiple and often large fluid-filled cystic malformations of the intrahepatic bile ducts. Based on this mechanism, liver cyst formation in ADPKD has been related to a sort of "neoplasia in disguise." In this disease, mutations in PC2 induce an increased Ca^{2+} influx into cholangiocytes which activates protein kinase A (PKA), leading in turn to the phosphorylation of ERK1/ERK2, one of the main determinants of cholangiocyte proliferation. Once phosphorylated, ERK1/ERK2 upregulates HIF1-α, a potent inducer of VEGF-A production by cholangiocytes. Through a positive autocrine loop, VEGF-A phosphorylates VEGFR2, which further sustains cell proliferation by activating the Raf/MEK/ERK1/ERK2 pathway [100]. Moreover, PKA activation inhibits tuberin, a repressor of mammalian target of rapamycin (mTOR); mTOR is a downstream effector of the IGF-1R that, following activation by its ligand IGF-1, stimulates cyclins to promote cholangiocyte proliferation [42, 101].

The balance between proliferation and apoptosis is severely perturbed in cholangiocarcinoma (CCA), a rare but aggressive liver malignancy originating from the intra- or extrahepatic bile ducts. In this epithelial cancer, several proliferative and survival pathways are dysregulated, resulting in the synergism of different co-stimulatory effects exerted by growth factors and cytokines, such as EGF, VEGF, HGF, prostaglandin E (PGE), and IL-6 [95]. In CCA, IL-6 is hypersecreted by tumoral cholangiocytes, whereby autocrine IL-6R phosphorylation induces survival responses through JAK1 and STAT3 activation, upstream effectors of the PI3K/AKT signaling, ultimately leading to escape from cell death [85]. In addition, the aberrant phosphorylation of EGFR, similar to the activation of Met, the HGF receptor, activates both the PI3K/AKT pathway, and the RAS/RAF/MEK/ERK cascade, together with the p38 MAPK [6, 95, 103]. VEGFR2 activation, as reported also in ADPKD, results in the activation of the p42/44 MAPK signaling which may contribute to the neoplastic growth of tumoral ducts [30, 95, 100]. Recent interest has been drawn on the modulatory effects of specific miRNA in CCA proliferation; among the different miRNAs found to be dysregulated in CCA, miR-21 promotes CCA cell proliferation by repressing the expression of PTEN, an inhibitor of the PI3K activation [118].

2.3.3 Cholangiocyte Role in Bile Secretion

The main function of the biliary epithelium has been considered to transport the bile to the gallbladder and the intestine and modify the primary bile secreted by hepatocytes (also termed canalicular bile) according to the digestive needs. The ductal component accounts for the 40% of the total bile produced daily by the human liver. Bile production is modulated by digestive hormones and by a paracrine cross talk between hepatocytes and cholangiocytes. This system is modulated by the balance between pro-secretory signals: acetylcholine [3], bombesin [20], glucagon [63], secretin [63], vasointestinal peptide (VIP) [21], and antisecretory factors: dopamine [43], endothelin-1 (ET-1) [16], gastrin [44], insulin [65], and somatostatin [46] (Fig. 2.1). In fact, biliary ion and water secretion requires the coordinated activity of several membrane carriers and channels expressed on either the apical or the basolateral side of both epithelial cell types. Cholangiocytes are able to reabsorb water, glucose, glutathione, bile acids, and electrolytes and to excrete water and anions, in particular chloride (Cl^-) and bicarbonate (HCO_3^-) [15] (Fig. 2.2). All the stimulatory and inhibitory signals are

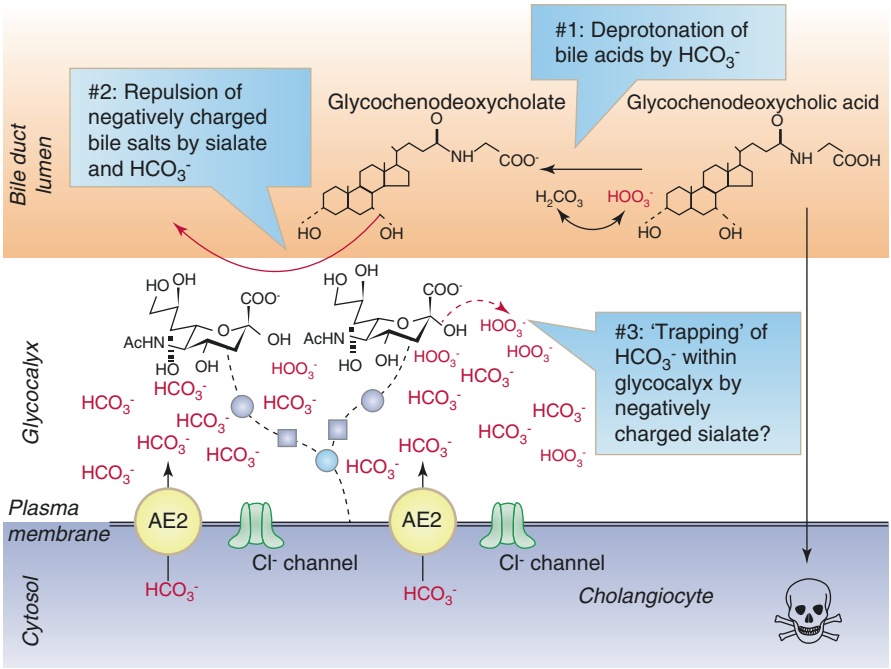

Fig. 2.1 The cholangiocyte glycocalyx forms a biliary surface microclimate pH regulatory system stabilizing the "biliary HCO_3^- umbrella." The 20–40 nm glycocalyx on the apical membrane of a cholangiocyte may trap HCO_3^- molecules in order to create an alkaline milieu which keeps bile acids in a negatively loaded state (bile salts) and, thereby, prevents carrier-independent invasion of apolar hydrophobic bile acids into cholangiocytes (and hepatocytes) (Figure modified after Maillette de Buy Wenniger et al. [70])

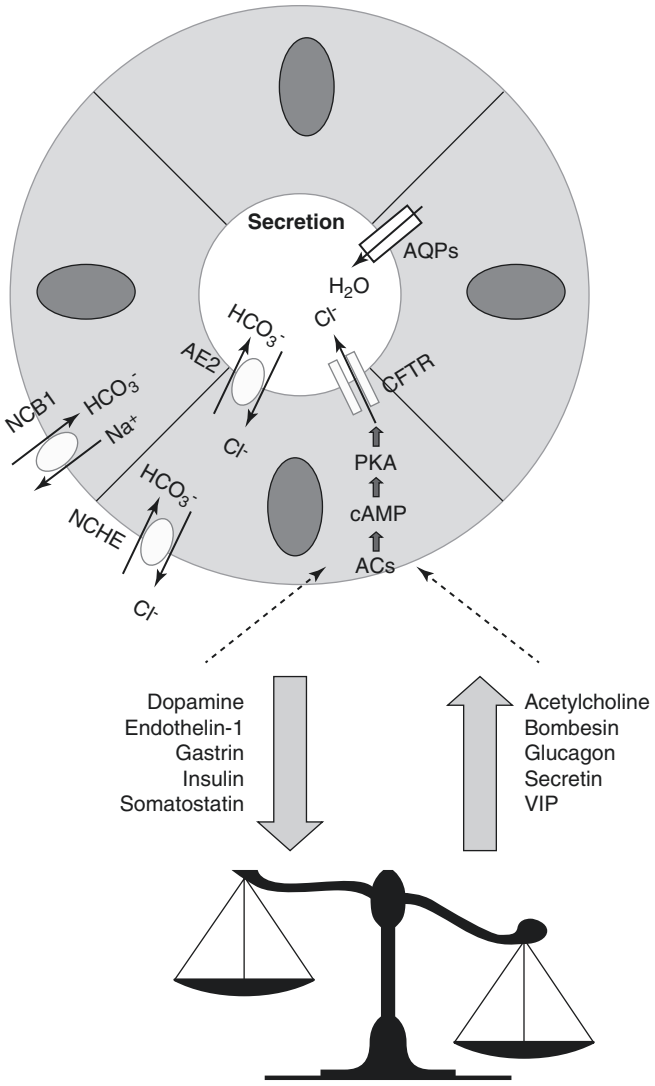

Fig. 2.2 Regulatory mechanisms of bile ductal secretion. Modification of the primary bile secreted by hepatocytes (canalicular bile) is a finely tuned mechanism operated by cholangiocytes, under the control of pro- (acetylcholine, bombesin, glucagon, secretin, VIP) and antisecretory (dopamine, endothelin-1, gastrin, insulin, somatostatin) signals of hormonal and cellular origin released in the peribiliary microenvironment. These peptides regulate the expression and activation of several ACs expressed by cholangiocytes that modulate the intracellular concentration of cAMP, a second messenger acting on PKA. Once activated, PKA stimulates the secretion of Cl^- into the luminal area of the bile duct through the cAMP-dependent channel CFTR. This leads to the extrusion of HCO_3^- and the reabsorption of Cl^- by the AE2 antiport, thus providing the alkalinization of the bile and its fluidification, in concert with AQPs, which extrude water into the lumen

coordinated and integrated at the cholangiocyte level by different adenylyl cyclase (AC) isoforms, in particular the AC4, AC5, AC6, AC7, AC8, and AC9 all located at the cell membrane, and by the soluble AC [105]. In normal cholangiocytes, a pro-secretory stimulus induced by hormones, such as secretin, leads to the activation of ACs, in particular AC8 and AC9, leading to an increase in the intracellular levels of cyclic adenosine monophosphate (cAMP). The increased cAMP concentration activates the PKA [105] that induces the extrusion of Cl^- into the ductal lumen through the cystic fibrosis transmembrane conductance regulator (CFTR) channel [75]. The chloride flux into the luminal area of the bile ducts generates the driving force responsible for the biliary alkalinization, by inducing the HCO_3^- extrusion from the apical side of cholangiocytes operated by the Na^+-independent Cl^-/HCO_3^- antiport (AE2) (Fig. 2.1). Moreover, the negative potential generated by the Cl^- efflux to the luminal surface of ductal epithelium induces the water flow through the action of aquaporin-1 and aquaporin-4, which hydrate and fluidificate the bile (Fig. 2.2). To maintain the homeostasis of the secreting system, the intracellular import of HCO_3^- is provided by the Na^+-dependent Cl^-/HCO_3^- exchanger (NCHE) and by the Na^+/HCO_3^- cotransporter (NCB1) expressed on the basal aspect of the cholangiocytes. Finally, the correct difference of potential between the intra- and extracellular domains of the biliary epithelium is maintained by two Na^+ transport systems: the Na^+/K^+ adenosine triphosphate (ATP)-ase and the $Na^+/K^+/2Cl^-$ cotransporter (NKCC1) [104].

Genetically determined defects in some of these transport systems are associated with specific disease conditions. For instance, mutations in the *CFTR* gene inherited as an autosomal recessive trait are responsible for cystic fibrosis (CF). In CF, the gene defect leads to the mislocalization or the loss of function of the CFTR channel. The CF liver disease (CFLD) is a progressive chronic cholangiopathy characterized by focal necroinflammation, vanishing of the bile ducts and portal fibrosis leading to secondary biliary cirrhosis. Derangement of a correct Cl^-/HCO_3^- excretion in the bile duct lumen due to the CFTR loss of function is responsible for the reduced alkalinity of the bile and, subsequently, for the formation of bile plugs into the biliary tree due to its insufficient hydration. The altered biochemical properties of the bile make it more susceptible to bacterial infections (Figs. 2.3 and 2.4). Recent studies showing that CFTR regulates innate immune responses in the biliary tree have significantly changed the interpretation of the pathogenesis of CF-associated cholangiopathy (see below).

2.3.4 Cholangiocytes in Inflammation

As previously outlined, cholangiocytes lining the smallest portions of the biliary tree are, together with HPC, the first site of reaction to liver injury, regardless of its origin. They acquire a reactive phenotype (RDC) characterized by de novo secretion of a number of proinflammatory and profibrotic cytokines (IL-1β, IL-6, and TGF-β, among others), chemokines, and nitric oxide. Thus, cholangiocytes and RDC are active players in the inflammatory reaction leading on one hand to liver repair, on the other to liver fibrosis, and, eventually, cancer [33, 66, 104]. In cholangiopathies,

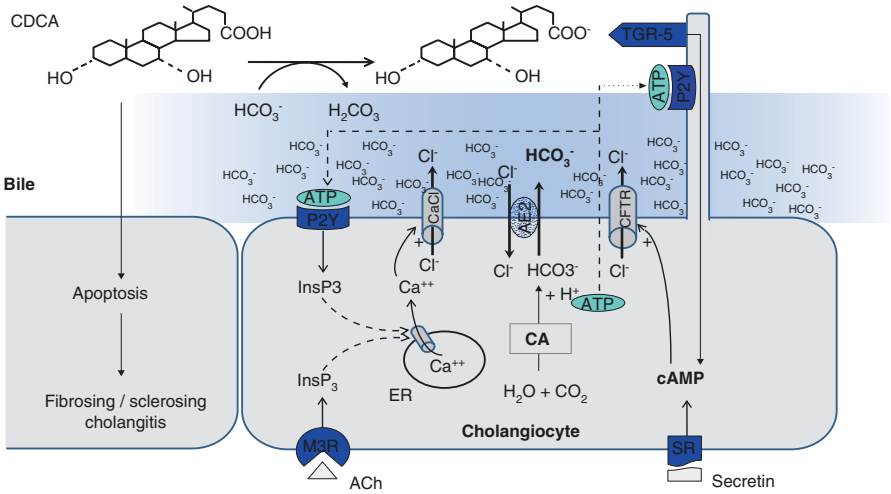

Fig. 2.3 The "biliary HCO_3^- umbrella" hypothesis. It is hypothesized that cholangiocytes protect their apical surface against protonated apolar hydrophobic bile acid monomers by maintaining an alkaline pH above the apical membrane. Dysfunction of any of the elements involved in HCO_3^- formation might weaken the biliary HCO_3^- umbrella and contribute to fibrosing/sclerosing cholangitis. Therapeutic agents such as UDCA or *nor*UDCA stabilize the biliary HCO_3^- umbrella. *ACh* acetylcholine, *ATP* adenosine trisphosphate, *CA* carbonic anhydrase, *CaCl* Ca^{++}-dependent chloride channel, *cAMP* cyclic adenosine monophosphate, *CDCA* chenodeoxycholic acid, *CFTR* cystic fibrosis transmembrane conductance regulator, *ER* endoplasmic reticulum, *P2Y* purinergic P2Y receptors, *SR* secretin receptor, *TGR5* G protein-coupled bile acid receptor (The figure is cited from Beuers et al. [13])

the biliary epithelium is exposed to cytokines and inflammatory mediators produced by infiltrating lymphocytes, macrophages, and hepatocytes as well. These inflammatory cytokines stimulate cholangiocyte proliferative responses, activate fibrogenic processes, and induce histocompatibility antigen expression. In a first study published in 2001, we showed that IL-1β, IL-6, TNF-α, and IFN-γ caused ductular cholestasis by interfering with cAMP-dependent ion transport mechanisms [98]. In a further study, we addressed the role of nitric oxide (NO) in biliary inflammation. NO has many effects depending on the NOS isoforms involved and the pathophysiologic contest. After induction of inducible NOS (iNOS or NOS2), the high concentrations of NO produced may result in the formation of reactive nitrogen oxide species (RNOS) responsible for a number of pathobiological effects. A strong reactivity for iNOS was reported in cholangiocytes of lipopolysaccharide (LPS)-treated rats, a model of sepsis-associated cholestasis. We have shown that LPS, TNF-α, and IFN-γ (proinflammatory cytokines potentially involved in inflammatory cholangiopathies) stimulate NO production in cultured cholangiocytes through an increase in iNOS gene and protein expression [99]. We also showed that micromolar concentrations of NO inhibit AC activity, cAMP-dependent fluid secretion, as well as cAMP-dependent Cl^- and HCO_3^- transport mediated by CFTR and by AE2, respectively [74]. Interestingly, the cholestatic effects of NO and of proinflammatory

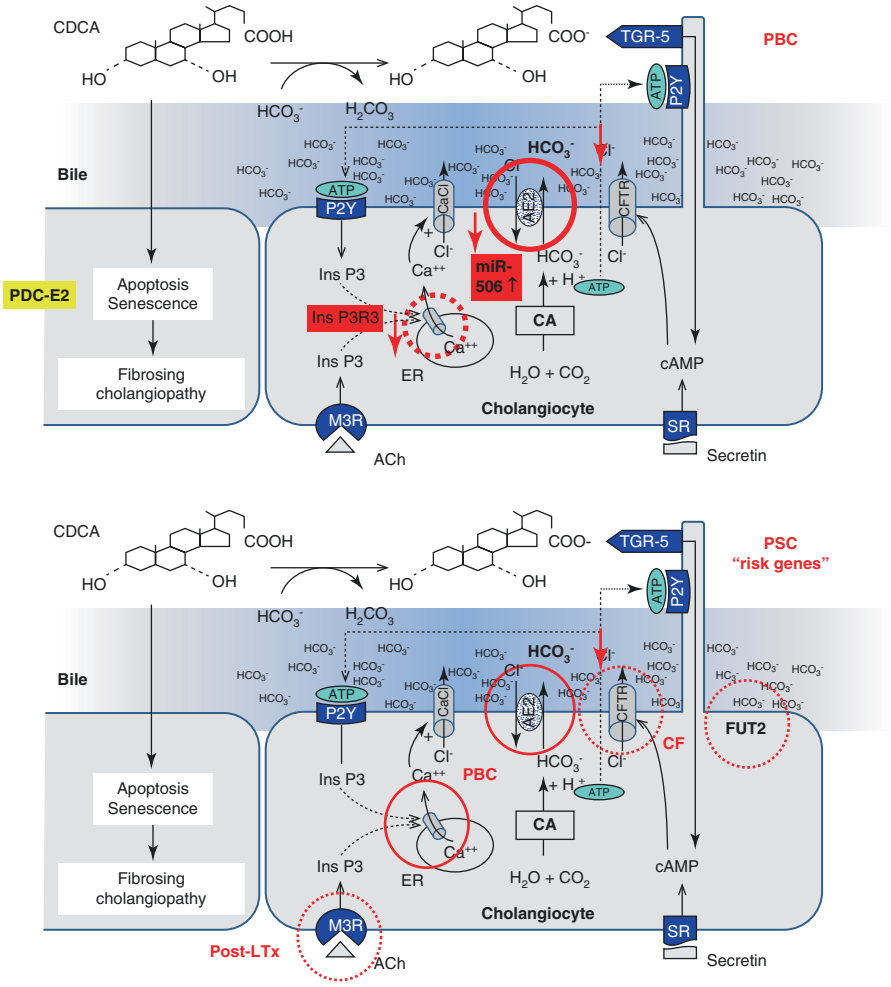

Fig. 2.4 Defects of the "biliary HCO_3^- umbrella" in various human cholangiopathies? (**a**) miR-506 is upregulated in cholangiocytes from PBC patients and downregulates AE2 and $InsP_3R3$ leading to impaired biliary secretory functions. (**b**) A defective biliary HCO_3^- umbrella could contribute to explain the enigmatic pathogenesis of various other fibrosing cholangiopathies. Genome screening of patients with PSC has disclosed *GPBAR-1/TGR5* as a susceptibility gene that when defective may affect the biliary HCO_3^- umbrella. TGR5 is expressed on cilia of intrahepatic and extrahepatic bile ducts, the site where bile duct alterations in PSC are observed (The figure is cited from Banales et al. [11] and Anathanarayanan [5])

cytokines are prevented by iNOS inhibitors and by agents able to block the formation of RNOS [99]. By immunohistochemistry, iNOS expression has been detected in the biliary epithelium and RDC in a series of chronic liver diseases, indicating that these pathophysiologic events may actually occur in vivo [99].

In PSC, biliary structures, including interlobular and septal ducts, strongly express iNOS. The finding that the inhibitory effects of proinflammatory cytokines

on cholangiocyte secretion and cAMP production are mediated by iNOS induction and NO production explains the histological findings of the "cholangitis lenta" typical of sepsis, but also links chronic inflammation with the cholestatic manifestation of cholangiopathies prior to the ductopenic phase. Proinflammatory cytokines would induce iNOS expression on cholangiocytes, with production of micromolar concentrations of NO and generation of RNOS. RNOS, interfering with cAMP-mediated fluid and electrolyte transport, may cause a reduction in bile hydration and alkalinity that would further increase bile duct and hepatocellular damage. Positive nitrotyrosine immunoreactivity in PSC samples confirms the potential role of RNOS and protein nitrosylation in vivo [99]. Given the harmful effects of NO on cholangiocyte secretory functions and on DNA repair mechanisms, selective inhibition of iNOS expression is of potential clinical relevance. Furthermore, these data provided the first functional proof of the presence in cholangiocytes of Toll-like receptors responsive to endotoxins. These findings are even more relevant now in the light of the hypothesized changes in innate immunity and in gut microbiome in PSC.

Production of inflammatory mediators in epithelial cells may also be triggered by a primary, genetically determined dysfunction of epithelial cells. Two examples relevant for human diseases are congenital hepatic fibrosis and cystic fibrosis-related cholangiopathy (see Sect. 2.3.5). Congenital hepatic fibrosis (CHF) is a dysgenetic cholangiopathy caused by a genetic defect in fibrocystin, a ciliary protein of unknown function expressed on ductal epithelia [122]. In CHF a low level of inflammatory reaction can be greeted by secretion of proinflammatory cytokines in conditions of cell dysfunction; we have shown that fibrocystin-defective cholangiocytes are able to release chemokines (CXCL1, CXCL10, CXCL12) in a way dependent on a perturbation of the β-catenin signaling ([102]; Strazzabosco M. and Fabris L. personal data). These secretory functions enable cholangiocytes to recruit macrophages to the peribiliary region. Another example of this mechanism (see below) is the secretion of proinflammatory cytokines by CFTR-defective cholangiocytes in CF. These observations support the concept that a genetically determined epithelial cell dysfunction may trigger a persistent and long-lasting, smoldering inflammation unable to resolve liver injury, which may ultimately lead to organ scarring, a process termed as "parainflammation" by Medzhitov [73] or auto-inflammation. We hypothesize that this phenomenon may be relevant in PSC.

2.3.5 Cholangiocytes and Innate Immunity

Because of its central anatomical localization, and the direct vascular connections with the intestine, the liver is constantly exposed to high amounts of gut-derived microorganisms and their toxic products. Cholangiocytes were originally thought to participate to the immune response only by secreting immunoglobulin (Ig) A into the bile [62, 79]. However, an increasing number of studies indicate that the role of biliary tree involvement in immune defenses is much more complex and driven by a number of sensors [22, 107]. Perturbation of these immune responses could lead to the development of severe chronic diseases, such as CFLD [36, 93].

In healthy liver, cholangiocytes cooperate to the self-protection against gut-derived pathogens and toxins, a function driven by several redundant signals, including those mediated by Toll-like receptors (TLRs) [19], nuclear receptors (NRs) [38], and antimicrobial peptides, like defensin and cathelicidin [26, 34]. Cholangiocytes can sense and react to pathogens and endotoxins present in the bile and clear them, by recognizing structural elements of bacterial origin, such as lipopolysaccharides (LPSs), DNA or RNA fragments, and flagellin. Collectively, these bacterial components are known as pathogen-associated molecular patterns (PAMPs) and are detected by the TLRs [10, 53, 94].

2.3.5.1 TLR Signaling

TLRs are a family of nine transmembrane receptors formed by an extracellular domain, rich in leucine and cysteine residues, responsible for the PAMP recognition, and an intracellular domain, Toll/interleukin-1 receptors (TIR), which are able to bind different adaptor molecules (MyD88, Mal, TRIF, and TRAM) [10, 12, 53, 94]. Cholangiocytes constitutively express TLR2, TLR3, TLR4, TLR5, and TLR9 [108, 110]. In cholangiocytes, the pathway activated by TLR4 in response to PAMPs is the best understood, while other TLR-mediated signaling are less characterized together with their ligands. TLR2 is a receptor for the lipoteichoic acid (LTA), a component of the bacterial membrane, and, together with TLR4, it is involved in the protection from *C. parvum* [83]. TLR3 is a sensor for viral dsRNA and is upregulated in several chronic inflammatory cholangiopathies, such as primary biliary cirrhosis and biliary atresia [50, 80]. TLR9 is a receptor for CpG DNA, short-stranded DNA typical of bacteria, reported to be upregulated in PSC [56]. TLR4 is the main sensor for LPS; following LPS stimulation, TLR4 dimerizes and activates an intracellular signal cascade mediated by transforming growth factor-beta-activated kinase (TAK)1, ultimately resulting in the NF-κB nuclear import. Usually, the NEMO/IKKα/IKKβ complex is bound to the NF-κB subunits p50/p65 and retains them in a cytoplasmic inactive complex. Upon TAK1 activation, one of the components of this complex, the inhibitory protein kappa-Bα (IκBα), is phosphorylated and degraded, allowing the translocation of the NF-κB subunit p65 into the nucleus [8]. Once into the nucleus, p65 stimulates the secretion of a wide range of proinflammatory cytokines and chemokines, such as tumor necrosis factor alpha (TNF-α), IL-1, IL-6, IL-8, IL-12, G-CSF, and LIX [2, 94, 108]. Alternative to NF-κB, TLR4 may also activate the MAPK signaling stimulating secretion of proinflammatory mediators via the activator protein 1 (AP-1) [120]. Notably, in healthy conditions, the liver is equipped with fine, self-limiting regulatory mechanisms that negatively regulate TLR4 or its co-receptors, such as MD-2, thus preventing the initiation of an inflammatory response potentially induced by the presence of PAMPs [1]. Among them, cholangiocytes express high levels of interleukin-1 receptor-associated kinase M (IRAK-M) that negatively regulates TLR4 expression. In contrast, TLR4 is further upregulated in response to inflammatory mediators secreted via NF-κB stimulation. Additional mechanisms of natural tolerance aimed at modulating TLR4 expression through a negative regulatory loop are represented by miRNA. Upregulation of miR-146 occurs in response to LPS stimulation of

TLR4, thus providing a partially inhibitory mechanism on the TAK/TRAF complex, which contrasts the activation of the TLR4-induced NF-κB-mediated cytokine secretion [97] (Fig. 2.5). Derangement of one or more of these checkpoints regulating the immune tolerance in cholangiocytes may elicit exaggerated inflammatory responses when pathogens or endotoxins are present in the bile.

Recent studies suggest that, when the apical Cl-channel CFTR is defective, TLR4-dependent inflammatory responses are upregulated. In CFTR-KO mice, experimental colitis induced by dextran sodium sulfate stimulates a brisk inflammatory response centered on the bile ducts that is not observed in WT littermates. This response is characterized by an abundant peribiliary accumulation of $CD45^+$ inflammatory cells mainly encompassing $F4/80^+$ macrophages and $NIMP\text{-}R14^+$ neutrophils, resulting from a dysregulation of the TLR4/NF-κB axis [35] due to an aberrant

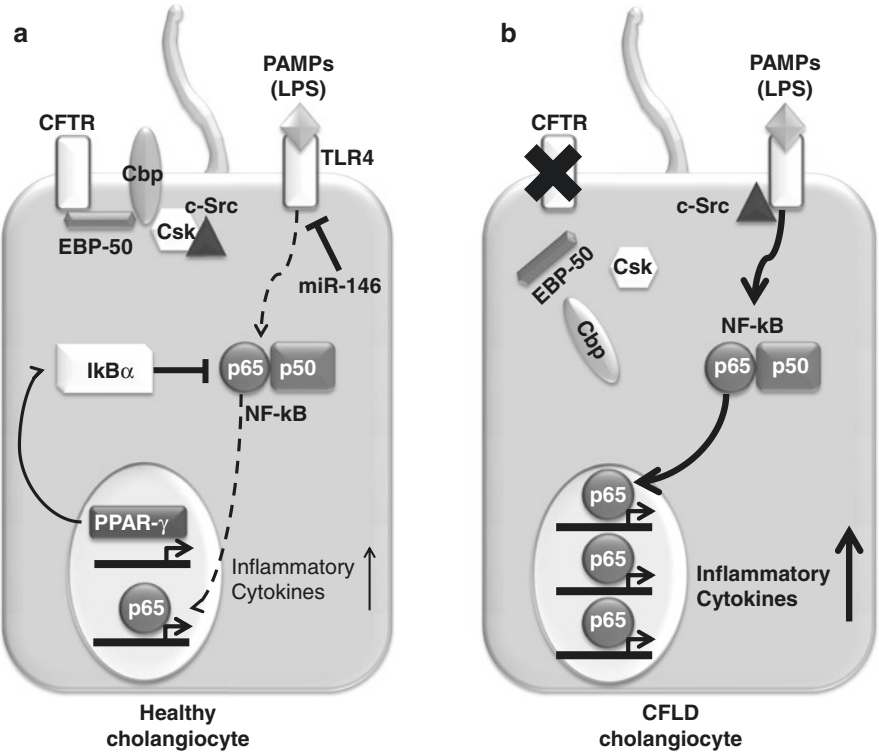

Fig. 2.5 TLR and CFTR modulation of proinflammatory cytokine secretion by cholangiocytes. (**a**) In normal condition, following activation by PAMPs (i.e., LPS), TLRs stimulate the secretion of a range of NF-κB-dependent proinflammatory cytokines, which mount and sustain the immune response. This reaction is negatively regulated by several factors, in particular by PPAR-γ, which stimulates the expression of IκBα, a protein that prevents p65 nuclear import by retaining it in the cytoplasm, and by miR-146, a TLR inhibitor. Moreover, c-Src, a TLR activator, is kept inactive by a multiprotein complex including CFTR and other bridge proteins. (**b**) In CFLD, the defective CFTR induces a disaggregation of this complex and allows the phosphorylation of c-Src that, by activating TLR, stimulates cholangiocyte secretion of proinflammatory cytokines

phosphorylation of c-Src [37]. In normal conditions, c-Src is sequestered and maintained in an unphosphorylated state by a multiprotein complex, composed by CFTR together with the bridge proteins EPB-50, Cbp, and Csk. Mutations in the CFTR gene lead to the degradation of this complex. Therefore, in CF patients, disaggregation of this complex allows an aberrant, persistent phosphorylation of c-Src that, in turn, activates TLR4, further stimulating the hypersecretion of proinflammatory cytokines in response to PAMPs (Fiorotto R. personal data). This mechanism has great translational potential. In a mouse model of CFLD (CFTR-KO mice), our group has recently shown that the treatment with PPAR-γ agonists, such as pioglitazone and rosiglitazone, deactivation of the TLR4 signaling, is able to partially abrogate the proinflammatory cytokine production by CFTR-KO cholangiocytes. In CFTR-KO DSS-treated mice, this approach led to a significant reduction in the periductal inflammatory infiltrate, in association with an amelioration of the CFLD phenotype [93] (Fig. 2.5).

These observations may be extended to the treatment of other inflammatory cholangiopathies. Nuclear receptors (NRs) are a wide group of transcription factors containing two highly conserved domains: a central DNA-binding domain and a carboxy-terminal ligand-binding domain. Among the different receptors belonging to this superfamily, the glucocorticoid receptor (GR), the retinoic acid receptor (RAR), the vitamin D receptor (VDR), the liver X receptors (LXRs), and the peroxisome proliferator-activated receptors (PPARs) are those most extensively studied and characterized. All of them share the strong ability to bind the retinoic X receptor (RXR) that is critical for exerting the transcriptional activity [38]. NRs regulate several functions, including cell proliferation and apoptosis, cell homeostasis, carbohydrate and lipid metabolism, and cell interactions involved in tissue repair [111]. In the biliary epithelium, NRs control a range of fundamental physiological functions, from detoxification of bile acids (VDR, FXR) to bile secretion (GR, FXR) [38]. Moreover, VDR, LXRs, and PPARs, acting as negative regulators, are able to transrepress the proinflammatory signals stimulated by PAMPs. PPARs are particularly relevant, since all the isoforms can suppress the pathways activated by the PAMPs-TLRs interaction. PPAR is a family of NRs composed by three isoforms: PPAR-α, PPAR-β/δ, and PPAR-γ, all expressed, at different levels, in normal cholangiocytes [93]. Among them, PPAR-γ is a known modulator of the LPS-induced proinflammatory response, by either directly blocking the production of proinflammatory cytokines or interfering with the activation of NF-κB, STAT1, and AP-1. PPAR-γ may interfere with NF-κB at different levels, by binding the p65/p50 complex, thus inhibiting the p65 nuclearization, or by inducing IκBα expression, which retains p65 in the cytoplasm. PPAR-γ may also inhibit MAPK activation induced by the phosphorylation of c-Jun N-terminal kinase (JNK), thereby preventing AP-1 stimulation.

2.3.5.2 Cholangiocyte Secretion of IgA and Antimicrobial Peptides

In addition to secreting secretory IgA (sIgA) into the bile, cholangiocytes may secrete a variety of peptides with antimicrobial properties. Among them, defensin and cathelicidin provide the first line of the immune defense against Gram+ and Gram- bacteria, fungi, and other pathogens by disrupting their cell membranes (Fig. 2.6). β-defensins (hBD1 and hBD3) are cationic peptides widely expressed

by healthy cholangiocytes, in contrast with hBD2, which is undetectable in normal conditions. Antimicrobial activity of hBD1 is particularly effective against *P. aeruginosa* and Gram- bacteria. Instead, hBD2 biliary expression is upregulated in infectious cholangiopathies, dependent on a TLR-2/TLR-4 mechanism leading to the activation of NF-κB, as shown in acute cholangitis from *C. parvum* [19]. Moreover, hBD2 expression on cultured cholangiocytes can be induced by stimulation with TNF-α and IL-1β, whose release in the portal tract is increased in several chronic cholangiopathies [49]. These data highlight the concept that whereas hBD1 and hBD3 represent an innate protective mechanism of the healthy bile ducts, hBD2 is involved in specific disease conditions of infectious origin as well as in chronic cholangiopathies (Fig. 2.6). Cathelicidins are another family of antimicrobial cationic peptides, active on a broad spectrum of pathogens, protozoa, and fungi [58]. Cathelicidins are particularly sensitive to PAMPs derived from *E. coli* [41]. Their expression is stimulated by the interaction of chenodeoxycholic and ursodeoxycholic acids with the nuclear receptors FXR and VDR. Notably, in experimental models of biliary obstruction in rats (bile duct ligation), hBD1 but

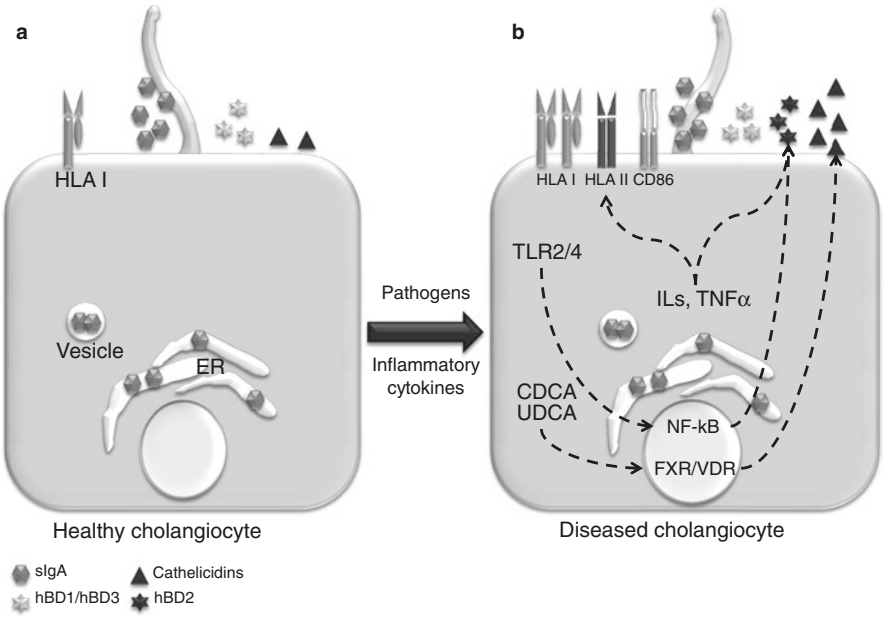

Fig. 2.6 Cholangiocyte involvement in innate and adaptive immunity. Bile ducts act as a first defense barrier against a variety of gut-derived pathogens and toxic products. (**a**) In normal conditions, cholangiocytes may secrete a variety of immunologically active factors, including sIgA, cathelicidins, hBD1, and hBD3 and express low levels of HLA-I. These factors cooperate to hinder toxins, fungi, and bacteria eventually present in the bile. (**b**) Following liver damage of different origin (immune-mediated or infections), cholangiocytes react by secreting de novo antimicrobial peptides, such as hBD2 (in a TLR2/4-NF-κB dependent mechanism), and by expressing de novo HLA-II and CD86, which enable them to behave as APCs. Therapeutic agents acting on nuclear receptors, i.e., FXR and VDR stimulated by ursodeoxycholic (*UDCA*) and chenodeoxycholic acid (*CDCA*), respectively, can modulate secretion of cathelicidins

not cathelicidin expression was induced [51], thus confirming their involvement in specific disease settings (Fig. 2.6).

2.3.6 Cholangiocyte Involvement in Antigen Presentation

The ability to behave as antigen-presenting cells (APCs) is an important immunobiological function played by cholangiocytes. In normal conditions, cholangiocytes express only low levels of human leucocyte antigen (HLA) class I, while class II, necessary for antigen presentation to $CD4^+$ T cells, is not detectable [27]. HLA class II expression on the biliary epithelium has been detected in PSC biopsy specimens and in some PBC cases [9, 18]. In vitro experiments in cultured cholangiocytes showed that infection with cytomegalovirus [92] or treatment with proinflammatory cytokines (IL-1, IFN-γ, TNF-α) significantly increased the expression of HLA class I and stimulated the neo-expression of HLA class II. Of note, the membrane receptors CD80 and CD86, which cooperate with HLA class II for the activation, proliferation, and cytokine secretion of T cells, are not constitutively expressed on normal cholangiocytes. However, unlike HLA class II, in vitro expression of CD80 and CD86 has not been induced by cytokine treatment [64], suggesting that cholangiocyte's ability to act as APC needs a close interaction with other inflammatory cells in order to start or sustain the immunological response that can lead to T cell activation. Immunohistochemical analysis of tissue specimens from patients with PSC and PBC has revealed however CD86 expression in bile ducts, likely indicating a more direct role of cholangiocytes in promoting the necroinflammatory damage featuring these two immune-mediated cholangiopathies [115] (Fig. 2.6).

Conclusions

In addition to the functions classically recognized to normal bile ducts, including bile alkalization, fluidification, and transport, a growing body of data support the involvement of cholangiocytes in inflammation and innate immunity. This is not surprising, considering the capabilities of cholangiocytes to mount a reparative response to many forms of liver damage. Reactive cholangiocytes have the ability to produce a wide range of soluble factors involved in inflammation, such as cytokines, chemokines, growth factors, sIgA, and antimicrobial peptides, along with the ability to express HLA class I and II. The imbalance of signals and pathways orchestrating the immunobiological competence of cholangiocytes is emerging as a new pathogenetic mechanism in specific biliary disease settings. CFLD is paradigmatic of this concept. In spite of the recent advances, a thorough knowledge of the complex immunobiological functions played by cholangiocytes, both in normal and diseased conditions, is still far to be achieved, and further studies are needed.

Acknowledgments We would like to thank Professor Ulrich Beuers who produced three figures for this chapter.

References

1. Abreu MT, Fukata M, Arditi M. TLR signaling in the gut in health and disease. J Immunol. 2005;174:4453–60.
2. Akira S, Takeda K, Kaisho T. Toll-like receptors: critical proteins linking innate and acquired immunity. Nat Immunol. 2001;2:675–80.
3. Alvaro D, Alpini G, Jezequel AM, Bassotti C, Francia C, Fraioli F, Romeo R, Marucci L, Le Sage G, Glaser SS, Benedetti A. Role and mechanisms of action of acetylcholine in the regulation of rat cholangiocyte secretory functions. J Clin Invest. 1997;100:1349–62.
4. Alvaro D, Metalli VD, Alpini G, Onori P, Franchitto A, Barbaro B, Glaser SS, Francis H, Cantafora A, Blotta I, Attili AF, Gaudio E. The intrahepatic biliary epithelium is a target of the growth hormone/insulin-like growth factor 1 axis. J Hepatol. 2005;43:875–83.
5. Ananthanarayanan M, Banales JM, Guerra MT, Spirli C, Munoz-Garrido P, Mitchell-Richards K, Tafur D, Saez E, Nathanson MH. Post-translational regulation of the type III inositol 1,4,5-trisphosphate receptor by miRNA-506. J Biol Chem. 2015;290:184–96.
6. Andersen JB, Spee B, Blechacz BR, Avital I, Komuta M, Barbour A, Conner EA, Gillen MC, Roskams T, Roberts LR, Factor VM, Thorgeirsson SS. Genomic and genetic characterization of cholangiocarcinoma identifies therapeutic targets for tyrosine kinase inhibitors. Gastroenterology. 2012;142:1021–1031.e15.
7. Antoniou A, Raynaud P, Cordi S, Zong Y, Tronche F, Stanger BZ, Jacquemin P, Pierreux CE, Clotman F, Lemaigre FP. Intrahepatic bile ducts develop according to a new mode of tubulogenesis regulated by the transcription factor SOX9. Gastroenterology. 2009;136:2325–33.
8. Baeuerle PA, Baltimore D. NF-kappa B: ten years after. Cell. 1996;87:13–20.
9. Ballardini G, Bianchi FB, Mirakian R, Fallani M, Pisi E, Bottazzo GF. HLA-A,B,C, HLA-D/DR and HLA-D/DQ expression on unfixed liver biopsy sections from patients with chronic liver disease. Clin Exp Immunol. 1987;70:35–46.
10. Barton GM, Medzhitov R. Toll-like receptors and their ligands. Curr Top Microbiol Immunol. 2002;270:81–92.
11. Banales JM, Sáez E, Uriz M, Sarvide S, Urribarri AD, Splinter P, Tietz Bogert PS, Bujanda L, Prieto J, Medina JF, LaRusso NF. Up-regulation of microRNA 506 leads to decreased Cl-/HCO3- anion exchanger 2 expression in biliary epithelium of patients with primary biliary cirrhosis. Hepatology. 2012;56:687–97.
12. Bell JK, Mullen GE, Leifer CA, Mazzoni A, Davies DR, Segal DM. Leucine-rich repeats and pathogen recognition in Toll-like receptors. Trends Immunol. 2003;24:528–33.
13. Beuers U, Hohenester S, de Buy Wenniger LJ, Kremer AE, Jansen PL, Elferink RP. The biliary HCO(3)(-) umbrella: a unifying hypothesis on pathogenetic and therapeutic aspects of fibrosing cholangiopathies. Hepatology. 2010;52:1489–96.
14. Bezerra JA. Liver development: a paradigm for hepatobiliary disease in later life. Semin Liver Dis. 1998;18:203–16.
15. Boyer JL. Bile formation and secretion. Compr Physiol. 2013;3:1035–78.
16. Caligiuri A, Glaser S, Rodgers RE, Phinizy JL, Robertson W, Papa E, Pinzani M, Alpini G. Endothelin-1 inhibits secretin-stimulated ductal secretion by interacting with ETA receptors on large cholangiocytes. Am J Phys. 1998;275:G835–46.
17. Carpentier R, Suñer RE, van Hul N, Kopp JL, Beaudry JB, Cordi S, Antoniou A, Raynaud P, Lepreux S, Jacquemin P, Leclercq IA, Sander M, Lemaigre FP. Embryonic ductal plate cells give rise to cholangiocytes, periportal hepatocytes, and adult liver progenitor cells. Gastroenterology. 2011;141:1432–8.
18. Chapman RW, Kelly PM, Heryet A, Jewell DP, Fleming KA. Expression of HLA-DR antigens on bile duct epithelium in primary sclerosing cholangitis. Gut. 1988;29:422–7.
19. Chen XM, O'Hara SP, Nelson JB, Splinter PL, Small AJ, Tietz PS, Limper AH, LaRusso NF. Multiple TLRs are expressed in human cholangiocytes and mediate host epithelial defense responses to *Cryptosporidium parvum* via activation of NF-kappaB. J Immunol. 2005;175:7447–56.

20. Cho WK, Boyer JL. Characterization of ion transport mechanisms involved in bombesin-stimulated biliary secretion in rat cholangiocytes. J Hepatol. 1997a;30:1045–51.
21. Cho WK, Boyer JL. Vasoactive intestinal polypeptide is a potent regulator of bile secretion from rat cholangiocytes. Gastroenterology. 1997b;117:420–8.
22. Chuang YH, Lan RY, Gershwin ME. The immunopathology of human biliary cell epithelium. Semin Immunopathol. 2009;31:323–31.
23. Clemens MG. Nitric oxide in liver injury. Hepatology. 1999;30:1–5.
24. Clotman F, Jacquemin P, Plumb-Rudewiez N, Pierreux CE, Van der Smissen P, Dietz HC, Courtoy PJ, Rousseau GG, Lemaigre FP. Control of liver cell fate decision by a gradient of TGF beta signaling modulated by Onecut transcription factors. Genes Dev. 2005;19:1849–54.
25. Clouston AD, Powell EE, Walsh MJ, Richardson MM, Demetris AJ, Jonsson JR. Fibrosis correlates with a ductular reaction in hepatitis C: roles of impaired replication, progenitor cells and steatosis. Hepatology. 2005;41:809–18.
26. D'Aldebert E, Biyeyeme Bi Mve MJ, Mergey M, Wendum D, Firrincieli D, Coilly A, Fouassier L, Corpechot C, Poupon R, Housset C, Chignard N. Bile salts control the antimicrobial peptide cathelicidin through nuclear receptors in the human biliary epithelium. Gastroenterology. 2009;136:1435–43.
27. Dillon PW, Belchis D, Minnick K, Tracy T. Differential expression of the major histocompatibility antigens and ICAM-1 on bile duct epithelial cells in biliary atresia. Tohoku J Exp Med. 1997;181:33–40.
28. Dowdle WE, Robinson JF, Kneist A, Sirerol-Piquer MS, Frints SG, Corbit KC, Zaghloul NA, van Lijnschoten G, Mulders L, Verver DE, Zerres K, Reed RR, Attié-Bitach T, Johnson CA, García-Verdugo JM, Katsanis N, Bergmann C, Reiter JF. Disruption of a ciliary B9 protein complex causes Meckel syndrome. Am J Hum Genet. 2011;89:94–110.
29. Fabris L, Strazzabosco M, Crosby HA, Ballardini G, Hubscher SG, Kelly DA, Neuberger JM, Strain AJ, Joplin R. Characterization and isolation of ductular cells coexpressing neural cell adhesion molecule and Bcl-2 from primary cholangiopathies and ductal plate malformations. Am J Pathol. 2000;156:1599–612.
30. Fabris L, Cadamuro M, Fiorotto R, Roskams T, Spirlì C, Melero S, Sonzogni A, Joplin RE, Okolicsanyi L, Strazzabosco M. Effects of angiogenic factor overexpression by human and rodent cholangiocytes in polycystic liver diseases. Hepatology. 2006;43:1001–12.
31. Fabris L, Cadamuro M, Guido M, Spirli C, Fiorotto R, Colledan M, Torre G, Alberti D, Sonzogni A, Okolicsanyi L, Strazzabosco M. Analysis of liver repair mechanisms in Alagille syndrome and biliary atresia reveals a role for notch signaling. Am J Pathol. 2007;171:641–53.
32. Fabris L, Cadamuro M, Libbrecht L, Raynaud P, Spirlì C, Fiorotto R, Okolicsanyi L, Lemaigre F, Strazzabosco M, Roskams T. Epithelial expression of angiogenic growth factors modulate arterial vasculogenesis in human liver development. Hepatology. 2008;47:719–28.
33. Fabris L, Strazzabosco M. Epithelial-mesenchymal interactions in biliary diseases. Semin Liver Dis. 2011;31:11–32.
34. Fellermann K, Stange EF. Defensins – innate immunity at the epithelial frontier. Eur J Gastroenterol Hepatol. 2001;13:771–6.
35. Fiorotto R, Scirpo R, Trauner M, Fabris L, Hoque R, Spirli C, Strazzabosco M (2011) Loss of CFTR affects biliary epithelium innate immunity and causes TLR4-NF-κB-mediated inflammatory response in mice. Gastroenterology 141:1498–508, 1508.e1–5.
36. Fiorotto R, Raizner A, Morell CM, Torsello B, Scirpo R, Fabris L, Spirli C, Strazzabosco M. Notch signaling regulates tubular morphogenesis during repair from biliary damage in mice. J Hepatol. 2013;59:124–30.
37. Fiorotto R, Villani A, Kourtidis A, Scirpo R, Spirli C, Anastasiadis PZ, Strazzabosco M. The Cystic Fibrosis Conductance Regulator (CFTR) controls c-Src tyrosine kinase signaling and regulates innate immunity and epithelial polarity in cholangiocytes. Hepatology. 2014;60(Suppl 1):299A.
38. Firrincieli D, Zuniga S, Poupon R, Housset C, Chignard N. Role of nuclear receptors in the biliary epithelium. Dig Dis. 2011;29:52–7.

39. Fischer E, Legue E, Doyen A, Nato F, Nicolas JF, Torres V, Yaniv M, Pontoglio M. Defective planar cell polarity in polycystic kidney disease. Nat Genet. 2006;38:21–3.
40. Flynn DM, Nijjar S, Hubscher SG, de Goyet JV, Kelly DA, Strain AJ, Crosby HA. The role of Notch receptor expression in bile duct development and disease. J Pathol. 2004;204:55–64.
41. Gallo RL, Kim KJ, Bernfield M, Kozak CA, Zanetti M, Merluzzi L, Gennaro R. Identification of CRAMP, a cathelin-related antimicrobial peptide expressed in the embryonic and adult mouse. J Biol Chem. 1997;272:13088–93.
42. Gatto M, Drudi-Metalli V, Torrice A, Alpini G, Cantafora A, Blotta I, Alvaro D. Insulin-like growth factor-1 isoforms in rat hepatocytes and cholangiocytes and their involvement in protection against cholestatic injury. Lab Invest. 2008;88:986–94.
43. Glaser S, Alvaro D, Roskams T, Phinizy JL, Stoica G, Francis H, Ueno Y, Barbaro B, Marzioni M, Mauldin J, Rashid S, Mancino MG, LeSage G, Alpini G. Dopaminergic inhibition of secretin-stimulated choleresis by increased PKC-gamma expression and decrease of PKA activity. Am J Physiol Gastrointest Liver Physiol. 2003a;284:G683–94.
44. Glaser S, Alvaro D, Ueno Y, Francis H, Marzioni M, Phinizy JL, Baumann B, Mancino MG, Venter J, LeSage G, Alpini G. Gastrin reverses established cholangiocyte proliferation and enhanced secretin-stimulated ductal secretion of BDL rats by activation of apoptosis through increased expression of Ca2+− dependent PKC isoforms. Liver Int. 2003b;23:78–88.
45. Glaser S, Lam IP, Franchitto A, Gaudio E, Onori P, Chow BK, Wise C, Kopriva S, Venter J, White M, Ueno Y, Dostal D, Carpino G, Mancinelli R, Butler W, Chiasson V, DeMorrow S, Francis H, Alpini G. Knockout of secretin receptor reduces large cholangiocyte hyperplasia in mice with extrahepatic cholestasis induced by bile duct ligation. Hepatology. 2010;52:204–14.
46. Gong AY, Tietz PS, Muff MA, Splinter PL, Huebert RC, Strowski MZ, Chen XM, LaRusso NF. Somatostatin stimulates ductal bile absorption and inhibits ductal bile secretion in mice via SSTR2 on cholangiocytes. Am J Physiol Cell Physiol. 2003;284:C1205–14.
47. Guo X, Wang XF. Signaling cross-talk between TGFbeta/BMP and other pathways. Cell Res. 2009;19:71–88.
48. Hand NJ, Master ZR, Eauclaire SF, Weinblatt DE, Matthews RP, Friedman JR. The microRNA-30 family is required for vertebrate hepatobiliary development. Gastroenterology. 2009;136:1081–90.
49. Harada K, Ohba K, Ozaki S, Isse K, Hirayama T, Wada A, Nakanuma Y. Peptide antibiotic human beta-defensin-1 and -2 contribute to antimicrobial defense of the intrahepatic biliary tree. Hepatology. 2004;40:925–32.
50. Harada K, Sato Y, Itatsu K, Isse K, Ikeda H, Yasoshima M, Zen Y, Matsui A, Nakanuma Y. Innate immune response to double-stranded RNA in biliary epithelial cells is associated with the pathogenesis of biliary atresia. Hepatology. 2007;46:1146–54.
51. Huang YH, Wang PW, Tiao MM, Chou MH, Du YY, Huang CC, Chuang JH. Glucocorticoid modulates high-mobility group box 1 expression and Toll-like receptor activation in obstructive jaundice. J Surg Res. 2011;170:e47–55.
52. Hussain SZ, Sneddon T, Tan X, Micsenyi A, Michalopoulos GK, Monga SP. Wnt impacts growth and differentiation in ex vivo liver development. Exp Cell Res. 2004;292:157–69.
53. Iwasaki A, Medzhitov R. Toll-like receptor control of the adaptive immune responses. Nat Immunol. 2004;5:987–95.
54. Jo A, Denduluri S, Zhang B, Wang Z, Yin L, Yan Z, Kang R, Shi LL, Mok J, Lee MJ, Haydon RC. The versatile functions of Sox9 in development, stem cells, and human diseases. Genes Dis. 2014;1:149–61.
55. Joplin R, Hishida T, Tsubouchi H, Daikuhara Y, Ayres R, Neuberger JM, Strain AJ. Human intrahepatic biliary epithelial cells proliferate in vitro in response to human hepatocyte growth factor. J Clin Invest. 1992;90:1284–9.
56. Karrar A, Broomé U, Södergren T, Jaksch M, Bergquist A, Björnstedt M, Sumitran-Holgersson S. Biliary epithelial cell antibodies link adaptive and innate immune responses in primary sclerosing cholangitis. Gastroenterology. 2007;132:1504–14.
57. Kodama Y, Hijikata M, Kageyama R, Shimotohno K, Chiba T. The role of notch signaling in the development of intrahepatic bile ducts. Gastroenterology. 2004;127:1775–86.

58. Kościuczuk EM, Lisowski P, Jarczak J, Strzałkowska N, Jóźwik A, Horbańczuk J, Krzyżewski J, Zwierzchowski L, Bagnicka E. Cathelicidins: family of antimicrobial peptides. A review. Mol Biol Rep. 2012;39:10957–70.
59. LaRusso NF, Ishii M, Vroman BT. The ins and outs of membrane movement in biliary epithelia. Trans Am Clin Climatol Assoc. 1991;102:245–58.
60. Lazaridis KN, Strazzabosco M, Larusso NF. The cholangiopathies: disorders of biliary epithelia. Gastroenterology. 2004;127:1565–77.
61. Lemaigre FP. Mechanisms of liver development: concepts for understanding liver disorders and design of novel therapies. Gastroenterology. 2009;137:62–79.
62. Lemaître-Coelho I, Jackson GD, Vaerman JP. Rat bile as a convenient source of secretory IgA and free secretory component. Eur J Immunol. 1977;7:588–90.
63. Lenzen R, Elster J, Behrend C, Hampel KE, Bechstein WO, Neuhaus P. Bile acid-independent bile flow is differently regulated by glucagon and secretin in humans after orthotopic liver transplantation. Hepatology. 1997;26:1272–81.
64. Leon MP, Bassendine MF, Wilson JL, Ali S, Thick M, Kirby JA. Immunogenicity of biliary epithelium: investigation of antigen presentation to CD4+ T cells. Hepatology. 1996;24:561–7.
65. LeSage GD, Marucci L, Alvaro D, Glaser SS, Benedetti A, Marzioni M, Patel T, Francis H, Phinizy JL, Alpini G. Insulin inhibits secretin-induced ductal secretion by activation of PKC alpha and inhibition of PKA activity. Hepatology. 2002;36:641–51.
66. Li MK, Crawford JM. The pathology of cholestasis. Semin Liver Dis. 2004;24:21–42.
67. Libbrecht L, Spinner NB, Moore EC, Cassiman D, Van Damme-Lombaerts R, Roskams T. Peripheral bile duct paucity and cholestasis in the liver of a patient with Alagille syndrome: further evidence supporting a lack of postnatal bile duct branching and elongation. Am J Surg Pathol. 2005;29:820–6.
68. Lowes KN, Brennan BA, Yeoh GC, Olynyk JK. Oval cell numbers in human chronic liver diseases are directly related to disease severity. Am J Pathol. 1999;154:537–41.
69. Lozier J, McCright B, Gridley T. Notch signaling regulates bile duct morphogenesis in mice. PLoS One. 2008;3:e1851.
70. Maillette de Buy Wenniger LJ, Hohenester S, Maroni L, van Vliet SJ, Oude Elferink RP, Beuers U. The cholangiocyte glycocalyx stabilizes the 'biliary HCO3- umbrella': an integrated line of defense against toxic bile acids. Dig Dis. 2015;33:397
71. Mancinelli R, Onori P, Demorrow S, Francis H, Glaser S, Franchitto A, Carpino G, Alpini G, Gaudio E. Role of sex hormones in the modulation of cholangiocyte function. World J Gastrointest Pathophysiol. 2010;1:50–62.
72. Matsumoto K, Fujii H, Michalopoulos G, Fung JJ, Demetris AJ. Human biliary epithelial cells secrete and respond to cytokines and hepatocyte growth factors in vitro: interleukin-6, hepatocyte growth factor and epidermal growth factor promote DNA synthesis in vitro. Hepatology. 1994;20:376–82.
73. Medzhitov R. Origin and physiological roles of inflammation. Nature. 2008;454:428–35.
74. Melero S, Spirlì C, Zsembery A, Medina JF, Joplin RE, Duner E, Zuin M, Neuberger JM, Prieto J, Strazzabosco M. Defective regulation of cholangiocyte Cl−/HCO3(−) and Na+/H+ exchanger activities in primary biliary cirrhosis. Hepatology. 2002;35:1513–21.
75. Mennone A, Biemesderfer D, Negoianu D, Yang CL, Abbiati T, Schultheis PJ, Shull GE, Aronson PS, Boyer JL. Role of sodium/hydrogen exchanger isoform NHE3 in fluid secretion and absorption in mouse and rat cholangiocytes. Am J Physiol Gastrointest Liver Physiol. 2001;280:G247–54.
76. Micsenyi A, Tan X, Sneddon T, Luo JH, Michalopoulos GK, Monga SP. Beta-catenin is temporally regulated during normal liver development. Gastroenterology. 2004;126:1134–46.
77. Montcouquiol M, Crenshaw 3rd EB, Kelley MW. Noncanonical Wnt signaling and neural polarity. Annu Rev Neurosci. 2006;29:363–86.
78. Morell CM, Strazzabosco M. Notch signaling and new therapeutic options in liver disease. J Hepatol. 2014;60:885–90.

79. Nagura H, Smith PD, Nakane PK, Brown WR. IGA in human bile and liver. J Immunol. 1981;126:587–95.
80. Nakamura M, Funami K, Komori A, Yokoyama T, Aiba Y, Araki A, Takii Y, Ito M, Matsuyama M, Koyabu M, Migita K, Taniguchi K, Fujioka H, Yatsuhashi H, Matsumoto M, Ishibashi H, Seya T. Increased expression of Toll-like receptor 3 in intrahepatic biliary epithelial cells at sites of ductular reaction in diseased livers. Hepatol Int. 2008;2:222–30.
81. Novo E, Parola M. Redox mechanisms in hepatic chronic wound healing and fibrogenesis. Fibrogenesis Tissue Repair. 2008;1:5.
82. Ochoa B, Syn WK, Delgado I, Karaca GF, Jung Y, Wang J, Zubiaga AM, Fresnedo O, Omenetti A, Zdanowicz M, Choi SS, Diehl AM. Hedgehog signaling is critical for normal liver regeneration after partial hepatectomy in mice. Hepatology. 2010;51:1712–23.
83. O'Hara SP, Bogert PS, Trussoni CE, Chen X, LaRusso NF. TLR4 promotes *Cryptosporidium parvum* clearance in a mouse model of biliary cryptosporidiosis. J Parasitol. 2011;97:813–21.
84. Omenetti A, Popov Y, Jung Y, Choi SS, Witek RP, Yang L, Brown KD, Schuppan D, Diehl AM. The hedgehog pathway regulates remodelling responses to biliary obstruction in rats. Gut. 2008;57:1275–82.
85. Park J, Tadlock L, Gores GJ, Patel T. Inhibition of interleukin 6-mediated mitogen-activated protein kinase activation attenuates growth of a cholangiocarcinoma cell line. Hepatology. 1999;30:1128–33.
86. Pi L, Robinson PM, Jorgensen M, Oh SH, Brown AR, Weinreb PH, Trinh TL, Yianni P, Liu C, Leask A, Violette SM, Scott EW, Schultz GS, Petersen BE. Connective tissue growth factor and integrin αvβ6: a new pair of regulators critical for ductular reaction and biliary fibrosis in mice. Hepatology. 2015;61:678–91.
87. Poncy A, Antoniou A, Cordi S, Pierreux CE, Jacquemin P, Lemaigre FP. Transcription factors SOX4 and SOX9 cooperatively control development of bile ducts. Dev Biol. 2015;404:136–48.
88. Raynaud P, Carpentier R, Antoniou A, Lemaigre FP. Biliary differentiation and bile duct morphogenesis in development and disease. Int J Biochem Cell Biol. 2011;43:245–56.
89. Richardson MM, Jonsson JR, Powell EE, Brunt EM, Neuschwander-Tetri BA, Bhathal PS, Dixon JB, Weltman MD, Tilg H, Moschen AR, Purdie DM, Demetris AJ, Clouston AD. Progressive fibrosis in nonalcoholic steatohepatitis: association with altered regeneration and a ductular reaction. Gastroenterology. 2007;133:80–90.
90. Rogler CE, Levoci L, Ader T, Massimi A, Tchaikovskaya T, Norel R, Rogler LE. MicroRNA-23b cluster microRNAs regulate transforming growth factor-beta/bone morphogenetic protein signaling and liver stem cell differentiation by targeting Smads. Hepatology. 2009;50:575–84.
91. Roskams TA, Libbrecht L, Desmet VJ. Progenitor cells in diseased human liver. Semin Liver Dis. 2003;23:385–296.
92. Scholz M, Cinatl J, Blaheta RA, Kornhuber B, Markus BH, Doerr HW. Expression of human leukocyte antigens class I and class II on cultured biliary epithelial cells after cytomegalovirus infection. Tissue Antigens. 1997;49:640–3.
93. Scirpo R, Fiorotto R, Villani A, Amenduni M, Spirli C, Strazzabosco M (2015) Stimulation of nuclear receptor peroxisome proliferator-activated receptor-γ limits NF-κB-dependent inflammation in mouse cystic fibrosis biliary epithelium. Hepatology. 2015;62:1551–62.
94. Seki E, Brenner DA. Toll-like receptors and adaptor molecules in liver disease: update. Hepatology. 2008;48:322–35.
95. Sia D, Tovar V, Moeini A, Llovet JM. Intrahepatic cholangiocarcinoma: pathogenesis and rationale for molecular therapies. Oncogene. 2013;32:4861–70.
96. Simons M, Gloy J, Ganner A, Bullerkotte A, Bashkurov M, Krönig C, Schermer B, Benzing T, Cabello OA, Jenny A, Mlodzik M, Polok B, Driever W, Obara T, Walz G. Inversin, the gene product mutated in nephronophthisis type II, functions as a molecular switch between Wnt signaling pathways. Nat Genet. 2005;37:537–43.

97. Soares JB, Pimentel-Nunes P, Roncon-Albuquerque R, Leite-Moreira A. The role of lipopolysaccharide/toll-like receptor 4 signaling in chronic liver diseases. Hepatol Int. 2010;4:659–72.
98. Spirlì C, Nathanson MH, Fiorotto R, Duner E, Denson LA, Sanz JM, Di Virgilio F, Okolicsanyi L, Casagrande F, Strazzabosco M. Pro-inflammatory cytokines inhibit secretion in rat bile duct epithelium. Gastroenterology. 2001;121:156–69.
99. Spirlì C, Fabris L, Duner E, Fiorotto R, Ballardini G, Roskams T, Larusso NF, Sonzogni A, Okolicsanyi L, Strazzabosco M. Cytokine-stimulated nitric oxide production inhibits adenylyl cyclase and cAMP-dependent secretion in cholangiocytes. Gastroenterology. 2003;124:737–53.
100. Spirli C, Okolicsanyi S, Fiorotto R, Fabris L, Cadamuro M, Lecchi S, Tian X, Somlo S, Strazzabosco M. ERK1/2-dependent vascular endothelial growth factor signaling sustains cyst growth in polycystin-2 defective mice. Gastroenterology. 2010a;138:360–71.e7.
101. Spirli C, Okolicsanyi S, Fiorotto R, Fabris L, Cadamuro M, Lecchi S, Tian X, Somlo S, Strazzabosco M. Mammalian target of rapamycin regulates vascular endothelial growth factor-dependent liver cyst growth in polycystin-2-defective mice. Hepatology. 2010b;51:1778–188.
102. Spirli C, Locatelli L, Morell CM, Fiorotto R, Morton SD, Cadamuro M, Fabris L, Strazzabosco M. Protein kinase A-dependent pSer(675) -β-catenin, a novel signaling defect in a mouse model of congenital hepatic fibrosis. Hepatology. 2013;58:1713–23.
103. Steelman LS, Chappell WH, Abrams SL, Kempf RC, Long J, Laidler P, Mijatovic S, Maksimovic-Ivanic D, Stivala F, Mazzarino MC, Donia M, Fagone P, Malaponte G, Nicoletti F, Libra M, Milella M, Tafuri A, Bonati A, Bäsecke J, Cocco L, Evangelisti C, Martelli AM, Montalto G, Cervello M, McCubrey JA. Roles of the Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR pathways in controlling growth and sensitivity to therapy-implications for cancer and aging. Aging. 2011;3:192–222.
104. Strazzabosco M, Fabris L, Spirli C. Pathophysiology of cholangiopathies. J Clin Gastroenterol. 2005;39:S90–S102.
105. Strazzabosco M, Fiorotto R, Melero S, Glaser S, Francis H, Spirli C, Alpini G. Differentially expressed adenylyl cyclase isoforms mediate secretory functions in cholangiocyte subpopulation. Hepatology. 2009;50:244–52.
106. Strazzabosco M, Fabris L. Development of the bile ducts: essentials for the clinical hepatologist. J Hepatol. 2012;56:1159–70.
107. Syal G, Fausther M, Dranoff JA. Advances in cholangiocyte immunobiology. Am J Physiol Gastrointest Liver Physiol. 2012;303:G1077–86.
108. Szabo G, Dolganiuc A, Mandrekar P. Pattern recognition receptors: a contemporary view on liver diseases. Hepatology. 2006;44:287–98.
109. Taga T, Kishimoto T. Gp130 and the interleukin-6 family of cytokines. Annu Rev Immunol. 1997;15:797–819.
110. Takeda K, Kaisho T, Akira S. Toll-like receptors. Annu Rev Immunol. 2003;21:335–76.
111. Tata JR. Signalling through nuclear receptors. Nat Rev Mol Cell Biol. 2002;3:702–10.
112. Tchorz JS, Kinter J, Müller M, Tornillo L, Heim MH, Bettler B. Notch2 signaling promotes biliary epithelial cell fate specification and tubulogenesis during bile duct development in mice. Hepatology. 2009;50:871–9.
113. Terada T, Nakanuma Y. Detection of apoptosis and expression of apoptosis-related proteins during human intrahepatic bile duct development. Am J Pathol. 1995;146:67–74.
114. Terada T, Kitamura Y, Nakanuma Y. Normal and abnormal development of the human intrahepatic biliary system: a review. Tohoku J Exp Med. 1997;181:19–32.
115. Tsuneyama K, Harada K, Yasoshima M, Kaji K, Gershwin ME, Nakanuma Y. Expression of co-stimulatory factor B7-2 on the intrahepatic bile ducts in primary biliary cirrhosis and primary sclerosing cholangitis: an immunohistochemical study. J Pathol. 1998;186:126–30.
116. Tufro A, Norwood VF, Carey RM, Gomez RA. Vascular endothelial growth factor induces nephrogenesis and vasculogenesis. J Am Soc Nephrol. 1999;10:2125–34.

117. Van Eyken P, Sciot R, Callea F, Van der Steen K, Moerman P, Desmet VJ. The development of the intrahepatic bile ducts in man: a keratin-immunohistochemical study. Hepatology. 1988;8:1586–95.
118. Wang LJ, He CC, Sui X, Cai MJ, Zhou CY, Ma JL, Wu L, Wang H, Han SX, Zhu Q. MiR-21 promotes intrahepatic cholangiocarcinoma proliferation and growth in vitro and in vivo by targeting PTPN14 and PTEN. Oncotarget. 2015;6:5932–46.
119. Weatherbee SD, Niswander LA, Anderson KV. A mouse model for Meckel syndrome reveals Mks1 is required for ciliogenesis and Hedgehog signaling. Hum Mol Genet. 2009;18: 4565–75.
120. Whitmarsh AJ, Davis RJ. Transcription factor AP-1 regulation by mitogen-activated protein kinase signal transduction pathways. J Mol Med. 1996;74:589–607.
121. Yancopoulos GD, Davis S, Gale NW, Rudge JS, Wiegand SJ, Holash J. Vascular-specific growth factors and blood vessel formation. Nature. 2000;407:242–8.
122. Zhang MZ, Mai W, Li C, Cho SY, Hao C, Moeckel G, Zhao R, Kim I, Wang J, Xiong H, Wang H, Sato Y, Wu Y, Nakanuma Y, Lilova M, Pei Y, Harris RC, Li S, Coffey RJ, Sun L, Wu D, Chen XZ, Breyer MD, Zhao ZJ, McKanna JA, Wu G. PKHD1 protein encoded by the gene for autosomal recessive polycystic kidney disease associates with basal bodies and primary cilia in renal epithelial cells. Proc Natl Acad Sci U S A. 2004;101:2311–6.
123. Zong Y, Panikkar A, Xu J, Antoniou A, Raynaud P, Lemaigre F, Stanger BZ. Notch signaling controls liver development by regulating biliary differentiation. Development. 2009;136: 1727–39.
124. Zong Y, Stanger BZ. Molecular mechanisms of bile duct development. Int J Biochem Cell Biol. 2011;43:257–64.

Pathological Features of Biliary Disease in Children and Adults

3

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Abstract

Many of the histological features of biliary disease are similar in the child and in the adult. The differential diagnosis also overlaps, but there are special considerations in the paediatric and particularly in the neonatal setting. Biliary histology can be a manifestation of a broad range of diseases. This chapter will provide an overview of the main pathological features that are seen in biliary diseases as a whole, with a more detailed consideration of the most common causes of chronic biliary disease in children and adults. The main emphasis will be on a practical diagnostic approach to the histological assessment of biliary disease – this will include considering the changing role of liver biopsy in the diagnosis and management of patients with biliary disease.

Take-Home Points

- Liver biopsy is no longer required for the routine diagnosis of primary biliary cholangitis (PBC) or primary sclerosing cholangitis (PSC) but remains important for diagnosing cases with atypical features (e.g. AMA-negative PBC, small duct PSC).

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G. Hirschfield et al. (eds.), *Biliary Disease*, DOI 10.1007/978-3-319-50168-0_3

- During the early stages of chronic biliary disease, clinical and histological changes are often mild, and the diagnosis may be overlooked. Subtle features of chronic cholestasis, such as periportal deposition of copper-associated protein (CAP), are a pointer to the presence of biliary disease and should prompt further relevant investigations.
- Biliary changes in a biopsy are often accompanied by portal inflammation. This might be wrongly interpreted as the dominant disease process or taken as evidence of an additional disease process.
- In children PBC is vanishingly rare, and a biliary picture is therefore more likely to be due to PSC. However, before accepting a diagnosis of PSC, secondary causes of sclerosing cholangitis should be considered.
- Histological assessment of disease severity, such as grading inflammatory activity and staging features indicative of disease progression (e.g. fibrosis, bile duct loss and periportal copper-associated protein deposits), may have clinical implications for prognosis and treatment.

3.1 What Are the Pathological Features of Biliary Disease?

A summary of the main histological features that should be assessed in liver biopsies obtained from patients suspected to have chronic biliary diseases is presented in Table 3.1 [1].

3.1.1 Bile Duct Lesions

Most chronic biliary diseases are associated with damage to intrahepatic bile ducts, in many cases, resulting in bile duct loss. A summary of the main diseases, which may be associated with duct loss, is presented in Table 3.2 [2]. Classical diagnostic bile duct lesions such as inflammatory/granulomatous bile duct destruction in PBC and concentric "onion skin" periductal fibrosis/sclerosing duct lesions in PSC are patchy in distribution and should be considered a "bonus" when seen in core liver biopsies. In most cases, liver biopsy identifies features compatible with chronic biliary disease, and the final diagnosis requires correlation with other clinical, biochemical, immunological and radiological findings.

3.1.2 Ductular Reaction

Ductular reaction probably occurs as a compensatory mechanism, allowing resorption and intrahepatic recycling of bile acids, when the normal enterohepatic pathway is impaired as a result of bile duct loss. It is typically most prominent at the periphery of portal tracts and is often accompanied by inflammatory cells, mostly neutrophils ("cholangiolitis"), and by the development of periportal fibrosis. It

Table 3.1 Summary of the main histological features that should be assessed in liver biopsies obtained from patients with chronic biliary disease

Histological feature	Examples/comments
Bile ducts	Bile duct injury • e.g. lymphocytic/granulomatous cholangitis in PBC, fibrosing cholangiopathy in PSC Bile duct loss
Ductular reaction	Mainly at periphery of portal tracts (marginal ductular reaction) Compensatory mechanism for duct loss Role in pathogenesis of periportal fibrosis (biliary interface activity)
Cholestasis	Bilirubinostasis (bile pigment in hepatocytes or biliary canaliculi) • Occurs late in most chronic biliary diseases Cholate stasis (related to toxic effects of retained bile acids) • Usually reflects prolonged cholestasis • Mainly affects periportal hepatocytes • Changes seen include feathery degeneration and ballooning of hepatocytes, Mallory-Denk bodies • Frequently associated with deposition of copper-associated protein
Inflammation	Mainly involves portal tracts and periportal regions (interface hepatitis) Presence and severity of interface hepatitis important in the pathogenesis of fibrosis and defining "overlap syndromes" with autoimmune hepatitis Lobular inflammatory changes typically absent or mild
Fibrosis	Initially periportal (related to interface hepatitis & ductular reaction) Later progresses to bridging fibrosis and nodule formation (cirrhosis) Biliary fibrosis is often patchy and may not progress to true cirrhosis

Table 3.2 Diseases associated with damage to intrahepatic bile ducts, which may result in bile duct loss

Aetiology	Main examples
Developmental/genetic	Extrahepatic biliary atresia Paucity of intrahepatic bile ducts (syndromic and non-syndromic) Progressive familial intrahepatic cholestasis (including MDR3 deficiency[a]) Alpha-1 antitrypsin deficiency Fibropolycystic liver disease
Immune mediated	Primary biliary cholangitis (PBC) Primary sclerosing cholangitis (PSC) IgG4-associated cholangitis[a] Sarcoidosis Liver allograft rejection Graft versus host disease
Vascular	Ischaemic cholangiopathy[a], e.g. hepatic artery thrombosis, radiation injury, intraarterial chemotherapy "Sclerosing cholangitis in critically ill patients" (SC-CIP) Portal vein occlusion[a] ("portal biliopathy")
Infective	Cryptosporidia and/or CMV (AIDS-associated or other immunodeficiency-related sclerosing cholangitis[a]) Recurrent pyogenic cholangitis[a] Septic shock[a] Hydatid cyst infection of biliary tract

Table 3.2 (continued)

Aetiology	Main examples
Drugs	Phenothiazines, augmentin, tricyclic antidepressants, carbamazepine, phenytoin
Neoplastic diseases	Langerhans cell histiocytosis (histiocytosis X)[a] Hodgkin's lymphoma Systemic mastocytosis
Unknown	"Idiopathic" adult ductopenia Hypereosinophilic syndrome[a]

Adapted from Hubscher [2]

[a]A number of the diseases listed here may also be associated with histological and/or radiological features resembling those seen in PSC.

should be noted that ductules are distinct from the bile duct proper. The bile ducts are typically located in the centre of portal tracts, have a clearly identifiable lumen and lie close to a hepatic artery branch of a similar diameter. Ductules are peripherally located, are smaller in size and typically have slit-like lumens.

3.1.3 Cholestasis

The two main histological manifestations of cholestasis are (1) the presence of bile pigment in the cytoplasm of hepatocytes or in the lumen of biliary canaliculi *(*"bilirubinostasis") and (2) changes related to the toxic effects of retained bile acids in periportal hepatocytes *(*"cholate stasis").

3.1.3.1 Bilirubinostasis

Bilirubinostasis may be an early feature of some biliary diseases associated with biliary obstruction (e.g. biliary atresia, PSC with a dominant stricture) but tends to occur as a late event in other chronic biliary diseases such as PBC.

3.1.3.2 Cholate Stasis

Cholate stasis usually occurs as a consequence of prolonged cholestasis. Histological features include hepatocyte ballooning, feathery degeneration and cytoplasmic deposits of Mallory-Denk bodies. An important early manifestation of chronic cholestasis, which usually precedes other features of cholate stasis, is the accumulation of copper-associated protein (CAP) in periportal hepatocytes (Fig. 3.1). CAP is normally excreted in bile, and the presence of even small amounts in a biopsy without evidence of advanced fibrosis is strongly suggestive of a biliary problem. Another useful early pointer to a biliary problem is aberrant expression of keratin 7 (K7) in periportal hepatocytes. In the normal liver, K7 expression is limited to biliary epithelial cells, and the expression of K7 in periportal hepatocytes may represent a protective response to an increased concentration of bile acids ("biliary metaplasia"), which precedes the development of a more obvious ductular reaction (Fig. 3.1).

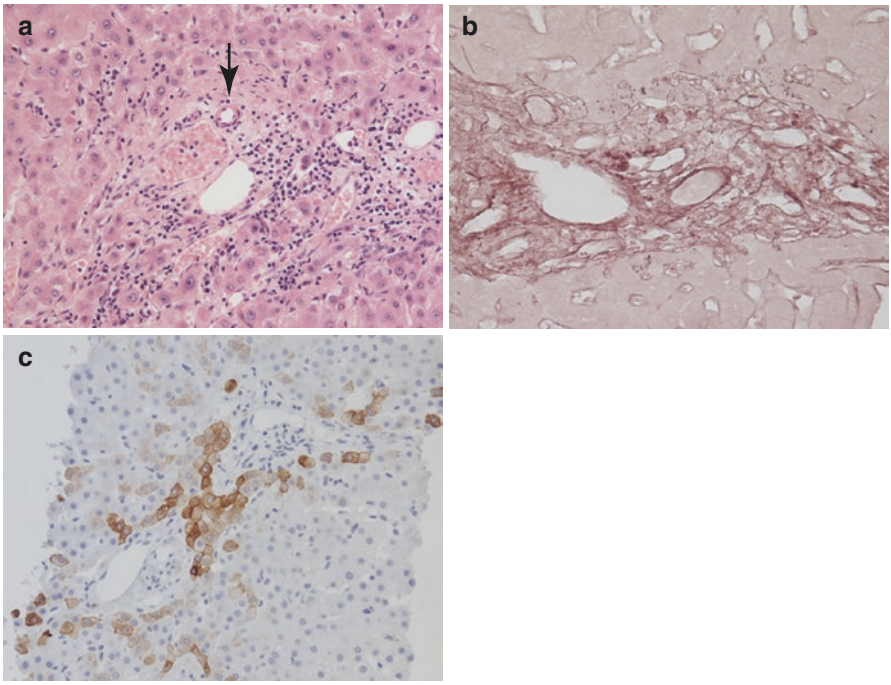

Fig. 3.1 Liver biopsy from a woman with early PBC, showing subtle features of chronic cholestasis. She presented with abnormal liver biochemistry and a negative autoantibody screen, and the biopsy had initially been reported as showing features of chronic hepatitis, cause unknown. (**a**) Portal tract contains a lymphocytic infiltrate associated with mild interface hepatitis, supporting a diagnosis of chronic hepatitis. Ductular reaction is not conspicuous. The presence of an arterial branch (*arrow*) without an accompanying bile duct is a clue to the presence of a biliary problem. Additional findings of (**b**) periportal deposits of copper-associated protein and (**c**) keratin 7 positive periportal cells with an intermediate hepatobiliary phenotype further support a diagnosis of chronic biliary disease. Repeat autoantibody testing revealed the presence of anti-mitochondrial antibodies. (**b** = orcein, **c** = keratin 7 immunohistochemistry)

3.1.4 Inflammation

Varying degrees of inflammation, mainly involving portal and periportal regions, can be seen as part of the normal spectrum of many chronic biliary diseases, notably PBC and PSC. In some cases of early PBC or PSC in which "biliary features" are not yet prominent, these portal inflammatory changes may be mistakenly interpreted as the dominant pathological process (Fig. 3.1). The presence and severity of interface hepatitis have been shown to predict adverse outcomes in patients with PBC and are consequently important components in the diagnosis of "overlap syndromes" involving PBC/PSC and autoimmune hepatitis (discussed later). Minor degrees of lobular inflammation can also be seen in chronic biliary diseases. More severe lobular necroinflammatory changes such as confluent or bridging necrosis

are not typically seen and should point to the possibility of an additional or alternative cause of liver disease.

3.1.5 Fibrosis

Most chronic biliary diseases are associated with the development of fibrosis. This typically begins as a periportal lesion, related to interface hepatitis and/or ductular reaction. As fibrosis progresses there is formation of fibrous septa with bridging fibrosis, in some cases, ultimately leading to the development of cirrhosis. Fibrosis in most chronic biliary diseases (paediatric and adult) is often patchy in distribution leading to sampling variability in liver biopsies. Furthermore, some patients with chronic biliary disease, especially PBC, progress to end-stage disease clinically, without developing true cirrhosis. This is one of the main reasons for the recent proposal to change the term primary biliary cirrhosis to primary biliary cholangitis [3].

3.1.6 Assessment of Disease Severity in Chronic Biliary Disease: Grading and Staging

In addition to establishing or supporting a diagnosis of chronic biliary disease, liver biopsies may also be used to assess disease severity. The term "grading" is used to describe features of ongoing/active liver injury (e.g. inflammation), which may lead to the development of chronic (irreversible) liver damage but are still potentially treatable. By contrast, the term "staging" is used to describe features of progressive liver injury, which may lead to end-stage liver disease and are less readily reversible. Histological features relevant to staging chronic biliary diseases include fibrosis, bile duct loss and CAP deposits [4].

A number of semi-quantitative scoring systems have been proposed for refining the assessment of histological grading and staging. The two staging systems, which have been most widely used in assessing disease severity in PBC, are those described by Scheuer in 1967 [5] and Ludwig in 1978 [6]. The Ludwig system has also been used for assessing disease severity in PSC [7]. Both systems recognise four stages, which are subdivided on the basis of various combinations of portal/periportal inflammation, ductular reaction and fibrosis (stage 4 = cirrhosis). A more recent staging system for PBC proposed by Nakanuma in 2010 incorporates three features thought to be important in disease progression – fibrosis, bile duct loss and orcein-positive granules [4]. Subsequent studies have suggested that the Nakanuma system is more useful than previously described staging systems in predicting adverse outcomes in patients with PBC [8, 9] and may also be helpful in predicting treatment responses [10]. Similar observations have been made in one study carried out in patients with PSC [11]. Problems with sampling variability apply to all of the histological staging systems that have been used in patients with chronic biliary disease, which limit the utility of liver biopsy to assess disease severity in routine clinical practice. Histological assessments may still have a role in the context of clinical

trials where liver biopsies have been used for risk stratification and as a surrogate marker of treatment outcomes [12, 13].

3.2 Histological Changes in Specific Diseases

3.2.1 Developmental/Genetic Diseases

3.2.1.1 Biliary Atresia

In biliary atresia diagnostic biopsy is no longer the norm [14]. Jaundiced neonates with acholic stool and atretic gallbladder on ultrasound proceed to operative cholangiogram. If atresia is confirmed, which can affect varying portions of the extrahepatic biliary tree, Kasai portoenterostomy is performed. The removed hilar plate shows fibro-inflammatory obliteration of the ducts, and multiple small duct profiles are often seen. Biopsy is only carried out in atypical cases, and the peripheral liver shows the manifestations of large bile duct obstruction often with prominent ductular bile plugging (Fig. 3.2). In early biopsies appropriately sized bile ducts are usually identifiable, whereas children who come to transplantation often have a paucity of intrahepatic bile ducts, a presumed manifestation of ongoing large duct obstruction despite Kasai procedure. It should be noted that one of the manifestations of alpha-1 antitrypsin deficiency (A1ATd) in a neonatal biopsy is a biliary picture mimicking large duct obstruction. Other biopsy manifestations in A1ATd include either bile duct paucity or a neonatal hepatitis-like pattern and/or periportal steatosis.

It is occasionally observed that babies with suspected biliary atresia (clinically and histologically) have an operative cholangiogram showing patent but thin and tortuous large bile ducts. This has been termed "neonatal sclerosing cholangitis". This condition is believed to be rare and is poorly defined, with no specific histological clues in a peripheral liver biopsy. Familial occurrence has suggested an autosomal recessive inheritance. There can be extrahepatic manifestations, notably in

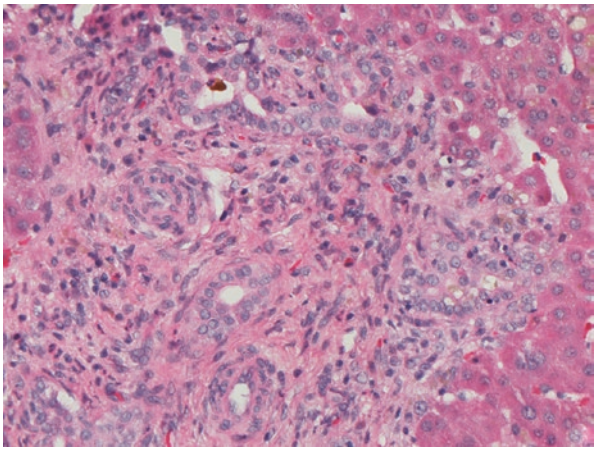

Fig. 3.2 Centrally in this portal tract, a round bile duct proper is seen of a similar size to the artery. Ductules at the edge of the portal tract contain bile plugs typical of large duct obstruction. Specimen taken at the time of Kasai procedure for biliary atresia

the skin in "neonatal ichthyosis sclerosing cholangitis syndrome" related to mutations in the *claudin 1* gene [15]. The long-term course is likely to be a progression to biliary cirrhosis.

3.2.1.2 Syndromic and Non-syndromic Paucity of Intrahepatic Bile Ducts

In these conditions the portal tracts lack an appropriately sized bile duct, i.e. a duct of similar calibre to, and in close proximity to, the hepatic artery branch. It is always important to distinguish the bile duct proper from marginal ductules (Fig. 3.2). In normal subjects the ratio of the bile ducts to portal tracts is 0.9–1.8. Paucity is said to be present if the ratio is less than 0.4 [16].

Syndromic Paucity of Intrahepatic Bile Ducts (Alagille Syndrome)

Alagille syndrome (arteriohepatic dysplasia) is characterised by cholestasis with variable additional features of posterior embryotoxon, butterfly-like vertebral arch defects, triangular facies and peripheral pulmonary artery hypoplasia either isolated or associated with complex cardiovascular abnormalities [16]. Alagille syndrome is an autosomal dominant condition with complete penetrance but variable expression. Approximately 95% of cases are caused by mutations in the *JAG* 1 gene. This encodes jagged-1, a ligand in the Notch signalling pathway. The remaining cases have mutations in *NOTCH2*. The latter group are more likely to have renal manifestations [17]. Histologically, bile duct loss can be patchy across the liver, and the loss is typically not accompanied by marginal ductular reaction or fibrosis.

Non-syndromic Paucity of Intrahepatic Bile Ducts

The non-syndromic group represents a wide variety of conditions, and in many cases the cause remains unknown. A1AT deficiency and CMV infection have been cited as causes along with Zellweger cerebrohepatorenal syndrome, coprostanic acidemia and cystic fibrosis [18]. Down syndrome, other trisomies, hypopituitarism, syndromes associated with ductal plate malformation, mitochondrial DNA depletion and Niemann-Pick type C disease have also been described as causes albeit in small numbers [19, 20]. Parenteral nutrition may also damage the bile ducts (see below).

3.2.1.3 Diseases Associated with Ductal Plate Malformation

Ductal plate malformation is a generic term encompassing a group of phenotypically and genetically heterogeneous conditions under the umbrella term of *fibropolycystic syndromes*. Cysts are variably present in the liver and kidneys. Congenital hepatic fibrosis (CHF) is the classical example in the liver. The ductal plate malformation (DPM) arises when the hepatoblasts, which condense around the primitive portal tract to form the embryonic ductal plate, subsequently fail to undergo the normal sequence of events involved in remodelling leading to the formation of a single duct. The persisting ductal plate structures have a distinctive appearance in a liver biopsy. They are typically located at the periphery of portal tracts and have complex profiles with an incomplete circular arrangement. They may also be dilated

and plugged with bile. The ductular reaction, which occurs in many chronic biliary diseases, has a similar anatomical location to DPM, and distinction between these two processes may sometimes be difficult. Ductular reaction is characterised by smaller biliary structures, which are more tightly aggregated and have a narrow or inconspicuous lumen. CHF is often also associated with loss of portal veins – affected portal tracts consequently have a sclerotic appearance due to the absence of portal vein branches, which are normally larger than the bile ducts and hepatic arteries. The parenchyma, in uncomplicated cases, is strikingly normal.

3.2.1.4 Cystic Fibrosis

In cystic fibrosis**,** as management of the respiratory manifestations improves, liver disease is emerging as an important cause of morbidity. The effects on the liver are very patchy and referred to as "focal biliary fibrosis". Biopsies are unlikely to be representative of the liver as a whole but may show portal biliary features, and the ductules classically contain inspissated eosinophilic secretions associated with an infiltrate of neutrophil polymorphs. Cystic fibrosis (CF) is caused by mutations in the *cystic fibrosis transmembrane conductance regulator* (CFTR) gene. Mutations causing CF do not seem to be a cause of PSC, but polymorphisms in the CFTR gene have been investigated, and those associated with reduction or non-function of the CFTR protein product confer protection against PSC when compared with non-diseased controls [21].

3.2.1.5 Progressive Familial Intrahepatic Cholestasis (PFIC)

PFIC is a term describing a group of conditions, usually diagnosed in infancy, which, as the name implies, can be progressive and can be considered in the differential diagnosis of chronic biliary tract disease in childhood. Underlying genetic defects are known and should ideally be used for the nomenclature of these conditions [17, 22]. Types "1" and "2" present in infancy, and, importantly, affected babies have a low gamma glutamyl transferase (GGT) despite severe cholestasis. Type 1 (*ATP8B1*/ATP8B1FIC1) can have extrahepatic manifestations, whilst type 2 (*ABCB11*/ABCB11 bile salt export pump, BSEP) is confined to the liver. Histologically, type 1 shows a bland canalicular cholestasis and type 2 the non-specific pattern of "neonatal hepatitis" where cholestasis is accompanied by giant cell change of hepatocytes and extramedullary haemopoiesis. The bile in type 1 has a characteristic ultrastructural appearance, but the diagnosis is best established by genetic testing. Type 3 (*ABCB4*/ABCB4 MDR3 – functional interaction with ATP8B1) is more likely to have a post-infantile presentation, and GGT is raised here. Histologically there is a progressive biliary fibrosis without distinguishing features. Duct paucity and sclerosing bile duct lesions are not typically seen in PFIC but have been recognised as an occasional finding in adults with MDR-3 deficiency [23]. Less pathogenic mutations in the genes mentioned here can cause intermittent cholestatic episodes (benign recurrent intrahepatic cholestasis, BRIC). Cholestasis might also be precipitated by drug exposure – oral contraceptives in teenage girls, for example. Biopsies in this setting typically show a bland bilirubinostasis.

3.2.2 Immune-Mediated Biliary Diseases

3.2.2.1 Primary Biliary Cholangitis (PBC)

PBC is a disease characterised by inflammatory damage to small (interlobular) bile ducts. The classical florid duct lesion involves lymphocytic or granulomatous destruction of the bile ducts resulting in bile duct loss. The presence of florid destructive cholangitis has a high diagnostic specificity for PBC – however, these lesions are patchy in distribution and are only seen in approximately 30–50% of liver biopsies [24, 25]. More frequently, as discussed earlier, liver biopsies show features compatible with a diagnosis of chronic biliary disease, and the final diagnosis of PBC thus requires correlation with other relevant findings.

It is now increasingly accepted that PBC can be diagnosed on the basis of the clinical, biochemical and immunological findings, and liver biopsy is thus no longer required for the routine diagnosis of PBC [26, 27]. Liver biopsy continues to play an important diagnostic role in atypical cases (e.g. AMA-negative PBC) and in cases where there may be a dual pathology (e.g. PBC and fatty liver disease). Liver biopsy is also recommended in the assessment of patients suspected to have an "overlap syndrome" with autoimmune hepatitis. In addition to showing histological features compatible with PBC, the diagnosis of PBC-AIH "overlap syndrome" requires the presence of at least moderate interface hepatitis [26, 28]. A recent study suggested that classical histological features of AIH such as hepatocyte rosettes, emperipolesis and lobular hepatitis are less frequent and/or severe in PBC patients with interface hepatitis than in people with "pure" AIH [29]. The severity of inflammatory activity has been shown to predict subsequent progression to cirrhosis and liver failure, and there is some evidence to suggest that PBC patients with unusually prominent "hepatic features" may consequently benefit from treatment with immunosuppressive therapy [30, 31].

3.2.2.2 Primary Sclerosing Cholangitis (PSC)

PSC is a disease that affects bile ducts of all sizes (intrahepatic and extrahepatic). There is emerging evidence to suggest that PSC may have a number of different subtypes, which differentially involve ducts of different sizes – amongst adults with PSC, approximately 5–15% appear to have disease confined to small ducts, whilst 10% have disease only or predominantly involving large ducts.

The classical fibrosing duct lesions seen in PSC mainly involve medium-sized (septal) bile ducts and are thus seen infrequently (<20% of cases) in liver biopsies, which mainly sample smaller ducts [24]. The early stages are characterised by foci of loose concentric periductal fibrosis ("onion skin"-like) often accompanied by varying numbers of periductal inflammatory cells. As the disease progresses, there is formation of more dense acellular fibrosis associated with progressive obliteration of the bile duct lumen, eventually resulting in complete replacement of the original duct by a nodule of fibrous tissue (nodular scar). Features of fibrosing cholangiopathy can also be seen in a number of diseases where sclerosing cholangitis occurs as a secondary phenomenon. Some examples of diseases associated with secondary sclerosing cholangitis are included in Table 3.2. Small (interlobular) ducts may occasionally show periductal fibrosis, but more typically disappear

without trace ("vanishing bile duct syndrome"). A recent study has suggested that basement membrane thickening in interlobular ducts (demonstrated by PAS-diastase staining) may be a useful diagnostic feature of PSC [32]. Large bile ducts are typically dilated, ulcerated and inflamed – resulting in the characteristic beading appearance seen radiologically. Biopsies are rarely obtained from large bile ducts, except in the investigation of cases suspected to have developed hilar cholangiocarcinoma. A detailed discussion of the approaches used to establish a tissue diagnosis of cholangiocarcinoma is beyond the scope of this article. A recent study of PSC hepatectomy specimens obtained at liver transplantation has suggested that hyperplasia of peribiliary glands may be important in the pathogenesis, both of periductal fibrosis and of neoplastic transformation in large bile ducts [33]. Two other studies have identified arterial abnormalities in PSC, including enlargement, mural thickening and fibrointimal hyperplasia, although the functional significance of these findings is uncertain [34, 35].

According to current clinical practice guidelines, liver biopsy is no longer required for the routine diagnosis of PSC but continues to play a role in the diagnosis of atypical cases (e.g. small duct PSC) and in the assessment of suspected "overlap syndromes" with autoimmune hepatitis [26, 36, 37].

Although liver biopsy is essential for a diagnosis of small duct PSC, the histological changes required to make the diagnosis have not been clearly defined or uniformly applied. In the largest study providing detailed histological findings, only 4/25 (16%) cases had fibrosing duct lesions, with the remainder having features of chronic biliary disease compatible with PSC [38]. Studies of the natural history and HLA genotype of patients diagnosed with small duct PSC suggest that a relatively small proportion (20–30%) represent the early stages or mild form of large duct PSC, typically occurring in association with inflammatory bowel disease (IBD), whereas the remaining cases, usually occurring without IBD and failing to progress to large duct PSC, may represent a different entity [39, 40].

Liver biopsy is also recommended for the diagnosis of PSC-AIH overlap syndrome, although histological criteria for the hepatic features required to diagnose the AIH component of the syndrome are less clearly defined than those used to diagnose the PBC-AIH overlap syndrome [26, 28, 31, 36, 37]. Some cases have a sequential presentation, initially presenting with typical clinical, biochemical, immunological and histological features of AIH before developing features of PSC after an interval of up to 11 years. This pattern of presentation is most frequently seen in children. Studies using routine cholangiography in children presenting with features of AIH have shown that up to 50% have cholangiographic changes compatible with PSC – the term "autoimmune sclerosing cholangitis" (ASC) has been used to describe such cases (discussed further below) [41]. Liver biopsies obtained from patients with a suspected clinical diagnosis of AIH, particularly in the paediatric population, should therefore be examined carefully for biliary features, which might raise the possibility of PSC – the presence of such features provides an indication for cholangiography if this has not already been done.

PSC should also be regarded as a diagnosis of exclusion in childhood, and relevant causes of "secondary" sclerosing cholangitis should therefore be actively

sought before applying this label. A proportion of cases presenting with a purely biliary phenotype, usually in teenagers and labelled as PSC, might represent late-stage ASC (see below). Dominant inflammatory changes in a biopsy might prompt immunosuppressive treatment in this setting, but the biliary disease is likely to be relentlessly progressive and unresponsive to immunosuppression. Although histological confirmation is not theoretically required to establish a diagnosis of PSC in children, liver biopsies are more frequently obtained in the paediatric population compared with adults. This is mainly because liver biopsy helps to define children with ASC, who may benefit from immunosuppression, and to exclude some causes of secondary sclerosing cholangitis. Biopsy is also essential in defining "small duct" PSC in children.

3.2.2.3 Autoimmune Sclerosing Cholangitis (ASC)

The term "autoimmune sclerosing cholangitis" arose from the observation that a significant proportion of children with presumed autoimmune hepatitis had, or developed, biliary tract disease reminiscent of primary sclerosing cholangitis [41]. The biliary phenotype tends to become more prominent over time and confers a worse outlook than the presence of autoimmune hepatitis alone [41–44]. The term should not be confused with "autoimmune cholangitis" which has been used to denote antimitochondrial antibody-negative primary biliary cholangitis. Children with ASC are more likely to have inflammatory bowel disease (prevalence 44–75%) than children with AIH (prevalence 5–18%) [41, 42].

The biliary component of ASC is defined by the presence of an abnormal cholangiogram showing strictures and dilatations of the biliary tree. Not all children with autoimmune sclerosing cholangitis will have biliary features on biopsy at the time of presentation. The King's College Hospital studies described biliary histological features in 65% of those ultimately categorised as "ASC", but it should be noted that 28% of biopsies from children ultimately categorised as autoimmune hepatitis type I and 6% in autoimmune hepatitis type II had some biliary histological features [41, 43, 45] . Cholangiography is therefore advocated in all children presenting with autoimmune hepatitis to define this cohort. Histologically, there may be a progression from a predominantly inflammatory to a predominantly biliary picture, possibly reflecting successful treatment of the AIH component with immunosuppression [44]. In the assessment of paediatric liver biopsies, it is important for the pathologist to keep an open mind and think in terms of "autoimmune liver disease" as an umbrella term rather than attempting sub-classification before all details, serological, biochemical and radiological, are brought together. Knowledge of the patterns present in a biopsy, inflammatory and/or biliary, the relative dominance of one pattern over another and assessments of the severity of all features present, does help to guide management.

3.2.2.4 IgG4-Associated Sclerosing Cholangitis

IgG4-associated sclerosing cholangitis (IgG4-SC) occurs as part of the spectrum of systemic IgG4-associated disease and is associated with a range of lesions involving the bile ducts (extra- and intrahepatic). Most cases of IgG4-SC (around 90%) occur in association with IgG4-associated disease involving the pancreas (type 1 autoimmune

pancreatitis). Occasional cases present without obvious pancreatic disease and may be difficult to distinguish from PSC. The disease mostly affects extrahepatic bile ducts, where histological features resemble those seen in autoimmune pancreatitis. Characteristic findings include (1) lymphoplasmacytic infiltrates with storiform fibrosis, (2) obliterative phlebitis and (3) increased numbers of IgG4-postive plasma cells with a high IgG4/IgG ratio [46]. Inflammatory infiltrates typically involve deeper layers of the duct wall with relative sparing of the mucosa, contrasting with PSC where inflammation tends to be most prominent superficially and is usually associated with ulceration of the mucosa. Histological manifestations of intrahepatic IgG4-SC are less well characterised and may be difficult to distinguish from PSC. Features favouring a diagnosis of IgG4-SC in a liver biopsy specimen include plasma cell-rich fibro-inflammatory nodules, which may be confined to portal tracts (sometimes surrounding the bile ducts) or efface the liver parenchyma, the presence of more than 10 IgG4+ plasma cells per high-power field and an IgG4/IgG ratio of more than 40%. By contrast, periductal "onion skin" fibrosis and ductopenia are rarely seen in IgG4-SC and favour a diagnosis of PSC. Other intrahepatic manifestations of IgG4 disease, which may be observed in liver biopsy specimens, include plasma cell-rich portal inflammatory infiltrates, interface hepatitis and lobular hepatitis.

The relationship between PSC and IgG4-SC remains incompletely understood. Approximately 10–30% of otherwise typical PSC cases have elevated serum IgG4 levels and/or tissue infiltrates rich in IgG4+ plasma cells, without fulfilling other diagnostic criteria for IgG4 disease. There is a suggestion that such cases may represent a distinct subset of PSC, with untreated cases having a tendency to behave in a more aggressive fashion than typical IgG4-negative PSC [2, 47]. Whether such cases benefit from immunosuppressive treatment is currently uncertain. The possibility of IgG4-positive subtype of AIH has also been postulated, but further studies are required for confirmation [48].

3.2.2.5 Sarcoidosis

The granulomas that occur in hepatic sarcoidosis mainly involve portal tracts. They are often large with a mulitinodular arrangement and are frequently associated with fibrous scarring. Some patients with hepatic sarcoidosis develop features of chronic cholestasis associated with ductopenia. It is likely that bile duct loss in hepatic sarcoidosis reflects a "bystander injury" occurring as a consequence of bile ducts lying in close proximity to the characteristic portal-based fibro-inflammatory lesions in sarcoidosis. Such cases may have a resemblance to PBC, which is also associated with ductopenia and portal granulomas. However, the granulomas seen in PBC are typically smaller, fewer in number and not associated with prominent fibrous scarring.

3.2.2.6 Transplant-Related Bile Duct Diseases

Small (interlobular) bile ducts are important targets for immune-mediated injury in *liver allograft rejection* and in *hepatic graft* versus *host disease,* usually occurring as a complication of haemopoietic stem cell transplantation. In both of these instances, bile duct injury may sometimes lead to the development of progressive bile duct loss [49]. A detailed discussion of these two diseases is beyond the scope of this review.

3.2.3 Vascular Diseases Associated with Bile Duct Injury

3.2.3.1 Hepatic Arterial Occlusion ("Ischaemic Cholangiopathy")

In contrast to hepatocytes, which have a dual blood supply from portal vein and hepatic artery, bile ducts have a single blood supply from the hepatic artery only and are consequently susceptible to conditions which result in reduced hepatic arterial blood flow – examples include hepatic artery thrombosis, radiation injury and intra-arterial chemotherapy. Occlusion of the main hepatic artery may produce a spectrum of lesions, which resemble those seen in PSC radiologically and histologically. Ischaemic necrosis of large bile ducts has distinctive features histologically, with necrosis and bile staining often extending into surrounding tissues.

3.2.3.2 "Sclerosing Cholangitis in Critically Ill Patients" (SC-CIP)

A combination of ischemia and infection has been postulated as the mechanism for a form of sclerosing cholangitis that can occur in critically ill patients. Most cases occur as a complication of long-term treatment in intensive care units and are associated with episodes of severe arterial hypotension requiring inotrope support. Early changes involve ischaemic injury of large bile ducts with formation of biliary casts and subsequent bacterial infection. Liver biopsies obtained after the initial injury have shown degenerative changes in smaller bile ducts and features of biliary obstruction such as portal oedema, ductular reaction, cholestasis and bile infarcts [50]. Some cases have developed progressive fibrosis leading to biliary cirrhosis. Prognosis is generally poor, with liver transplantation being the only curative treatment option [51].

3.2.3.3 Portal Vein Occlusion ("Portal Hypertensive Biliopathy")

Portal hypertensive biliopathy is mainly seen as a complication of extrahepatic portal vein obstruction, usually by thrombosis. In some cases this results in the formation of dilated shunt vessels ("cavernomatous transformation" of the portal vein), which surround and compress the extrahepatic bile duct (choledochal varices) resulting in changes resembling those in PSC. Biliary features may also be seen less frequently in patients who have obliteration of small intrahepatic veins as part of the syndrome of so-called "idiopathic non-cirrhotic portal hypertension" (also known as "hepatoportal sclerosis" or "obliterative portal venopathy" [52]). The pathogenesis of biliary injury in this setting is uncertain, but one suggested mechanism is that vascular abnormalities involving small portal vein branches may also impair the hepatic arterial blood supply to the bile ducts via the peribiliary vascular plexus.

3.2.4 Infective Causes of Biliary Disease

A number of infective agents have been identified as possible causes of secondary sclerosing cholangitis. The best described example occurs in the setting of immunodeficiency syndromes – *HIV-associated or other immunodeficiency-related*

sclerosing cholangitis. In HIV-infected individuals, sclerosing cholangitis occurs as a complication of advanced immunosuppression associated with low CD4 counts and is less frequently seen now as a result of improvements in antiretroviral therapy. The commonest infection seen in AIDS-associated cholangitis is cryptosporidiosis, in which the causative organisms directly colonise the biliary tree and adhere to the luminal surface of the bile ducts.

Other postulated infectious causes of secondary sclerosing cholangitis include r*ecurrent pyogenic cholangitis* [53] and *septic shock.*

3.2.5 Drugs and Iatrogenic Causes of Biliary Disease

3.2.5.1 Drug-Induced Cholestasis

Drugs are a common cause of acute cholestasis and may sometimes progress to chronic biliary disease. There are three main patterns of injury – "pure" (bland) cholestasis, cholestatic hepatitis and chronic cholestasis with ductopenia. These three patterns have overlapping features in terms of causative agents, clinical presentation and pathological features.

Pure intrahepatic cholestasis is characterised by the presence of bile pigment (bilirubinostasis), most prominent in perivenular hepatocytes, with no significant inflammation or features to suggest large duct obstruction. Common causes include the oral contraceptive pill, anabolic steroids and some antibiotics. The differential diagnosis of bland intrahepatic cholestasis includes bile transporter defects (e.g. BRIC, PFIC), occult malignancy (e.g. Hodgkin's lymphoma), sepsis and early large duct obstruction, but this pattern of injury is most frequently drug related.

Histological features of *acute cholestatic hepatitis* include a combination of bilirubinostasis and lobular hepatitis. Numerous causative agents have been identified including non-steroidal anti-inflammatory drugs, anticonvulsants, anti-infective agents (e.g. penicillins, amoxicillin, cephalosporins) and statins. Other causes of acute cholestatic hepatitis to consider in the differential diagnosis are viral hepatitis, autoimmune hepatitis and seronegative hepatitis. Histological features favouring a drug reaction in this setting include disproportionately severe bilirubinostasis, with only mild inflammation, sharply circumscribed areas of centrilobular necrosis and the presence of numerous eosinophils or of granulomas.

Some acute cholestatic drug reactions may progress to *chronic cholestasis with ductopenia.* This possibility should be considered in a patient where cholestasis fails to resolve clinically after the suspected causative agent has been discontinued. Histological features include ductopenia (sometimes preceded by inflammatory duct lesions), ductular reaction and features of chronic cholestasis in some cases leading to the development of biliary fibrosis or cirrhosis. Drugs recognised to cause bile duct loss and chronic cholestasis include phenothiazines, some antibiotics (e.g. augmentin, penicillins) and carbamazepine. The differential diagnosis includes autoimmune biliary diseases such as PBC or PSC. Portal inflammation and fibrosis tend to be less prominent in drug-induced chronic cholestasis.

3.2.5.2 Intestinal Failure-Associated Liver Disease (IFALD)

Intestinal failure-associated liver disease is the preferred term for the liver damage seen in children who require parenteral nutrition secondary either to a short or dysfunctional gut. It recognises the multifactorial nature of the process rather than simply labelling it "parenteral nutrition-associated liver disease". In the paediatric liver biopsy, the manifestations often include biliary features with the development of biliary fibrosis. The changes seen can mimic large duct obstruction. Bile duct paucity can occur, particularly in young infants [54] . Steatosis is rarely seen. There is often conspicuous ceroid pigment in macrophages seen on a DPAS stain, and sometimes intracellular inclusions comprising altered glycogen are observed [55].

3.2.6 Neoplastic Diseases Associated with Cholestasis or Bile Duct Injury

3.2.6.1 Langerhans Cell Histiocytosis

An important cause of secondary sclerosing cholangitis, mainly seen in the paediatric population, is liver involvement by *Langerhans cell histiocytosis* (previously called histiocytosis X) [56]. This disseminated neoplastic proliferation of S100- and CD1a-positive antigen presenting cells involves the liver in 18% of cases. Hepatic involvement may manifest as tumour-like masses or as more diffuse infiltration of portal tracts by characteristic Langerhans cells, which have abundant eosinophilic cytoplasm and coffee bean-shaped nuclei, accompanied by other inflammatory cells usually including several eosinophils. Langerhans cells typically surround and infiltrate small- and medium-sized bile ducts leading to bile duct loss. Concentric periductal fibrosis is also a common finding producing changes resembling those seen in sclerosing cholangitis.

3.2.6.2 Lymphoproliferative Diseases

Intrahepatic cholestasis may be a feature of various lymphoproliferative diseases involving the liver, including Hodgkin's lymphoma. Some patients with Hodgkin's lymphoma have developed an apparently paraneoplastic syndrome of bland intrahepatic cholestasis associated with ductopenia, not obviously related to hepatic infiltration by lymphoma [57].

3.2.7 Miscellaneous/Unknown Causes of Biliary Disease

The term "idiopathic adult ductopenia" was coined by Ludwig and colleagues to describe cases of chronic cholestatic liver disease, which resembled PBC or PSC, histologically, but lacked other diagnostic features [58]. The number of cases for which this term can be applied has diminished with time, most likely reflecting improved recognition of entities such as AMA-negative PBC and small duct PSC. Some cases may represent late-onset non-syndromic paucity of intrahepatic

bile ducts [59]. A small number of cases remain with no identifiable cause – there is a suggestion that some of these may have a familial tendency. Subtypes with mild disease (type 1) and severe progressive disease (type 2) have been recognised [1].

References

1. Nakanuma Y, Zen Y, Portmann BC. Diseases of the bile ducts. In: Burt AD, Portmann BC, Ferrell LD, editors. MacSween's pathology of the liver. 6th ed. Edinburgh: Churchill Livingstone; 2012. p. 491–562.
2. Hubscher SG. Role of liver biopsy in autoimmune liver disease. Diagn Histopathol. 2014;20(3):109–18.
3. Beuers U, Gershwin ME, Gish RG, Invernizzi P, Jones DE, Lindor K, et al. Changing nomenclature for PBC: from 'cirrhosis' to 'cholangitis'. J Hepatol. 2015 Nov;63(5):1285–7.
4. Nakanuma Y, Zen Y, Harada K, Sasaki M, Nonomura A, Uehara T, et al. Application of a new histological staging and grading system for primary biliary cirrhosis to liver biopsy specimens: Interobserver agreement. Pathol Int. 2010;60(3):167–74.
5. Scheuer P. Primary biliary cirrhosis. Proc R Soc Med. 1967 Dec;60(12):1257–60.
6. Ludwig J, Dickson ER, McDonald GS. Staging of chronic nonsuppurative destructive cholangitis (syndrome of primary biliary cirrhosis). Virchows Arch A Pathol Anat Histol. 1978;379(2):103–12.
7. Ludwig J, Barham SS, LaRusso NF, Elveback LR, Wiesner RH, McCall JT. Morphologic features of chronic hepatitis associated with primary sclerosing cholangitis and chronic ulcerative colitis. Hepatology. 1981;1(6):632–40.
8. Kakuda Y, Harada K, Sawada-Kitamura S, Ikeda H, Sato Y, Sasaki M, et al. Evaluation of a new histologic staging and grading system for primary biliary cirrhosis in comparison with classical systems. Hum Pathol. 2013;44(6):1107–17.
9. Chan AW, Chan RC, Wong GL, Wong VW, Choi PC, Chan HL, et al. Evaluation of histological staging systems for primary biliary cirrhosis: correlation with clinical and biochemical factors and significance of pathological parameters in prognostication. Histopathology. 2014;65(2):174–86.
10. Nakamura M, Kondo H, Tanaka A, Komori A, Ito M, Yamamoto K, et al. Autoantibody status and histological variables influence biochemical response to treatment and long-term outcomes in Japanese patients with primary biliary cirrhosis. Hepatol Res. 2015;45(8):846–55.
11. de Vries EM, Verheij J, Hubscher SG, Leeflang MM, Boonstra K, Beuers U, et al. Applicability and prognostic value of histologic scoring systems in primary sclerosing cholangitis. J Hepatol. 2015;63(5):1212–9.
12. Trivedi PJ, Corpechot C, Pares A, Hirschfield GM. Risk stratification in autoimmune cholestatic liver diseases: opportunities for clinicians and trialists. Hepatology. 2016;63(2):644–59.
13. Ponsioen CY, Chapman RW, Chazouilleres O, Hirschfield GM, Karlsen TH, Lohse AW, et al. Surrogate endpoints for clinical trials in primary sclerosing cholangitis; review and results from an International PSC Study Group consensus process. Hepatology. 2016;63:1357–67.
14. Hartley JL, Davenport M, Kelly DA. Biliary atresia. Lancet. 2009;374(9702):1704–13.
15. Girard M, Franchi-Abella S, Lacaille F, Debray D. Specificities of sclerosing cholangitis in childhood. Clin Res Hepatol Gastroenterol. 2012;36(6):530–5.
16. Alagille D, Estrada A, Hadchouel M, Gautier M, Odievre M, Dommergues JP. Syndromic paucity of interlobular bile ducts (Alagille syndrome or arteriohepatic dysplasia): review of 80 cases. J Pediatr. 1987;110(2):195–200.
17. Hartley JL, Gissen P, Kelly DA. Alagille syndrome and other hereditary causes of cholestasis. Clin Liver Dis. 2013;17(2):279–300.
18. Jevon GP, Dimmick JE. Histopathologic approach to metabolic liver disease: part 2. Pediatr Dev Pathol. 1998;1(4):261–9.

19. Yehezkely-Schildkraut V, Munichor M, Mandel H, Berkowitz D, Hartman C, Eshach-Adiv O, et al. Nonsyndromic paucity of interlobular bile ducts: report of 10 patients. J Pediatr Gastroenterol Nutr. 2003 Nov;37(5):546–9.
20. Kahn E, Daum F, Markowitz J, Teichberg S, Duffy L, Harper R, et al. Nonsyndromatic paucity of interlobular bile ducts: light and electron microscopic evaluation of sequential liver biopsies in early childhood. Hepatology. 1986;6(5):890–901.
21. Henckaerts L, Jaspers M, Van Steenbergen W, Vliegen L, Fevery J, Nuytten H, et al. Cystic fibrosis transmembrane conductance regulator gene polymorphisms in patients with primary sclerosing cholangitis. J Hepatol. 2009;50(1):150–7.
22. Stapelbroek JM, van Erpecum KJ, Klomp LW, Houwen RH. Liver disease associated with canalicular transport defects: current and future therapies. J Hepatol. 2010;52(2):258–71.
23. Wendum D, Barbu V, Rosmorduc O, Arrive L, Flejou JF, Poupon R. Aspects of liver pathology in adult patients with MDR3/ABCB4 gene mutations. Virchows Arch. 2012;460(3):291–8.
24. Wiesner RH, LaRusso NF, Ludwig J, Dickson ER. Comparison of the clinicopathologic features of primary sclerosing cholangitis and primary biliary cirrhosis. Gastroenterology. 1985;88(1 Pt 1):108–14.
25. Drebber U, Mueller JJ, Klein E, Kasper HU, Schulze F, Schardt K, et al. Liver biopsy in primary biliary cirrhosis: clinicopathological data and stage. Pathol Int. 2009;59(8):546–54.
26. European Association for the Study of the Liver. EASL clinical practice guidelines: management of cholestatic liver diseases. J Hepatol [Practice Guideline]. 2009;51(2):237–67.
27. Lindor KD, Gershwin ME, Poupon R, Kaplan M, Bergasa NV, Heathcote EJ. Primary biliary cirrhosis. Hepatology. 2009;50(1):291–308.
28. Boberg KM, Chapman RW, Hirschfield GM, Lohse AW, Manns MP, Schrumpf E. Overlap syndromes: the International Autoimmune Hepatitis Group (IAIHG) position statement on a controversial issue. J Hepatol. 2011;54(2):374–85.
29. Kobayashi M, Kakuda Y, Harada K, Sato Y, Sasaki M, Ikeda H, et al. Clinicopathological study of primary biliary cirrhosis with interface hepatitis compared to autoimmune hepatitis. World J Gastroenterol. 2014;20(13):3597–608.
30. Trivedi PJ, Hirschfield GM. Review article: overlap syndromes and autoimmune liver disease. Aliment Pharmacol Ther. 2012;36(6):517–33.
31. European Association for the Study of the Liver. EASL clinical practice guidelines: autoimmune hepatitis. J Hepatol. 2015;63(4):971–1004.
32. Colling R, Verrill C, Fryer E, Kartsonaki C, Wang LM, Chapman R, et al. Bile duct basement membrane thickening in primary sclerosing cholangitis. Histopathology. 2016;68:819–24.
33. Carpino G, Cardinale V, Renzi A, Hov JR, Berloco PB, Rossi M, et al. Activation of biliary tree stem cells within peribiliary glands in primary sclerosing cholangitis. J Hepatol. 2015;63(5):1220–8.
34. Carrasco-Avino G, Schiano TD, Ward SC, Thung SN, Fiel MI. Primary sclerosing cholangitis: detailed histologic assessment and integration using bioinformatics highlights arterial fibrointimal hyperplasia as a novel feature. Am J Clin Pathol. 2015;143(4):505–13.
35. Fiel MI, Sima HR, Azarian A, Schiano TD. A morphometric study of the hepatic arterioles in end-stage primary sclerosing cholangitis. Virchows Arch. 2015;466(2):143–9.
36. Chapman R, Fevery J, Kalloo A, Nagorney DM, Boberg KM, Shneider B, et al. Diagnosis and management of primary sclerosing cholangitis. Hepatology. 2010;51(2):660–78.
37. Lindor KD, Kowdley KV, Harrison ME. ACG clinical guideline: primary sclerosing cholangitis. Am J Gastroenterol. 2015;110(5):646–59; quiz 60.
38. Singal AK, Stanca CM, Clark V, Dixon L, Levy C, Odin JA, et al. Natural history of small duct primary sclerosing cholangitis: a case series with review of the literature. Hepatol Int. 2011;5(3):808–13.
39. Bjornsson E, Olsson R, Bergquist A, Lindgren S, Braden B, Chapman RW, et al. The natural history of small-duct primary sclerosing cholangitis. Gastroenterology. 2008;134(4):975–80.
40. Naess S, Bjornsson E, Anmarkrud JA, Al Mamari S, Juran BD, Lazaridis KN, et al. Small duct primary sclerosing cholangitis without inflammatory bowel disease is genetically different from large duct disease. Liver Int. 2014;34(10):1488–95.

41. Gregorio GV, Portmann B, Karani J, Harrison P, Donaldson PT, Vergani D, et al. Autoimmune hepatitis/sclerosing cholangitis overlap syndrome in childhood: a 16-year prospective study. Hepatology. 2001;33(3):544–53.
42. Deneau M, Jensen MK, Holmen J, Williams MS, Book LS, Guthery SL. Primary sclerosing cholangitis, autoimmune hepatitis, and overlap in utah children: epidemiology and natural history. Hepatology. 2013;58(4):1392–400.
43. Mieli-Vergani G, Vergani D. Paediatric autoimmune liver disease. Arch Dis Child. 2013;98(12):1012–7.
44. Mieli-Vergani G, Heller S, Jara P, Vergani D, Chang MH, Fujisawa T, et al. Autoimmune hepatitis. J Pediatr Gastroenterol Nutr. 2009;49(2):158–64.
45. Gregorio GV, Portmann B, Reid F, Donaldson PT, Doherty DG, McCartney M, et al. Autoimmune hepatitis in childhood: a 20-year experience. Hepatology. 1997;25(3):541–7.
46. Deshpande V. IgG4-related disease of the gastrointestinal tract: a 21st century chameleon. Arch Pathol Lab Med. 2015;139(6):742–9.
47. Berntsen NL, Klingenberg O, Juran BD, Benito de Valle M, Lindkvist B, Lazaridis KN, et al. Association between HLA haplotypes and increased serum levels of IgG4 in patients with primary sclerosing cholangitis. Gastroenterology. 2015;148(5):924–7.e2.
48. Yada N, Kudo M, Chung H, Watanabe T. Autoimmune hepatitis and immunoglobulin G4-associated autoimmune hepatitis. Dig Dis. 2013;31(5–6):415–20.
49. Hubscher SG, Clouston AD. Transplantation pathology. In: Burt AD, Portmann BC, Ferrell LD, editors. MacSween's pathology of the liver. 6th ed. Edinburgh: Churchill Livingstone; 2012. p. 853–933.
50. Esposito I, Kubisova A, Stiehl A, Kulaksiz H, Schirmacher P. Secondary sclerosing cholangitis after intensive care unit treatment: clues to the histopathological differential diagnosis. Virchows Arch. 2008;453(4):339–45.
51. Leonhardt S, Veltzke-Schlieker W, Adler A, Schott E, Eurich D, Faber W, et al. Secondary sclerosing cholangitis in critically ill patients: clinical presentation, cholangiographic features, natural history, and outcome: a series of 16 cases. Medicine (Baltimore). 2015;94(49):e2188.
52. Hubscher SG. Pathology of non-cirrhotic portal hypertension and incomplete septal cirrhosis. Diagn Histopathol. 2011;17(12):530–8.
53. Gholami S, Wood L, Berry G, Triadafilopoulos G, Visser BC, Dua MM. Recurrent pyogenic cholangitis: got stones? Dig Dis Sci. 2016;61:3147–50.
54. Naini BV, Lassman CR. Total parenteral nutrition therapy and liver injury: a histopathologic study with clinical correlation. Hum Pathol. 2012;43(6):826–33.
55. Brown RM, Gray G, Poulton K, Wassmer E, Gupte G. Ground glass hepatocellular inclusions caused by disturbed glycogen metabolism in three children on parenteral nutrition. Pediatr Dev Pathol. 2009;12(1):79–80.
56. Kaplan KJ, Goodman ZD, Ishak KG. Liver involvement in Langerhans' cell histiocytosis: a study of nine cases. Mod Pathol. 1999;12(4):370–8.
57. Hubscher SG, Lumley MA, Elias E. Vanishing bile duct syndrome: a possible mechanism for intrahepatic cholestasis in Hodgkin's lymphoma. Hepatology. 1993;17(1):70–7.
58. Ludwig J, Wiesner RH, LaRusso NF. Idiopathic adulthood ductopenia. A cause of chronic cholestatic liver disease and biliary cirrhosis. J Hepatol. 1988;7(2):193–9.
59. Ludwig J. Idiopathic adulthood ductopenia: an update. Mayo Clin Proc. 1998;73(3):285–91.

Animal Models of Biliary Disease: Current Approaches and Limitations

4

Marion J. Pollheimer and Peter Fickert

Abstract

Biliary diseases represent an important group of inborn and acquired diseases of the intra- and extrahepatic bile ducts with severe morbidity and mortality due to the development of biliary type of liver fibrosis, liver cirrhosis, and eventually cholangiocarcinoma [1]. The spectrum of cholangiopathies is heterogeneous with respect to underlying mechanisms, clinical course, and presentation. However, these liver diseases share a common target: the cholangiocyte. These diseases include immune-mediated, idiopathic cholangiopathies, such as primary biliary cholangitis (previously known as primary biliary cirrhosis) and primary sclerosing cholangitis, biliary atresia, as well as graft-versus-host disease. The difficulties in studying the complex nature of cholangiocyte injury in humans as well as the currently limited treatment options stress the need for reliable, well-defined, and reproducible animal models in order to gain insights into the pathophysiology and to test novel therapies. The aim of this chapter is to critically discuss the characteristics and limitations of rodent models of biliary diseases for primary biliary cholangitis, primary sclerosing cholangitis, biliary atresia, graft-versus-host disease, as well as cholangiocarcinoma.

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G. Hirschfield et al. (eds.), *Biliary Disease*, DOI 10.1007/978-3-319-50168-0_4

Take-Home Points

- The term biliary disease includes a diverse spectrum of liver diseases, which however share the same primary pathogenetic target: the cholangiocyte.
- The etiology of biliary diseases is unknown, and the use of rodent models is valuable to understand their pathogenesis as well as for testing new drugs.
- A major limitation of the use of animal models is the species differences between rodents and humans.
- Several animal models exhibit comparable characteristics of different biliary diseases.
- There is no "perfect model" that mirrors human biliary disease characteristics making the task of understanding the underlying pathogenetic mechanisms hard. Various aspects of different models might be required to study particular pathogenetic steps.

4.1 Introduction

Biliary disease represents an umbrella term for numerous complex liver diseases which share the primary pathogenetic target within extra- or intrahepatic bile ducts, including primary biliary cholangitis (PBC) (previously known as primary biliary cirrhosis), primary sclerosing cholangitis (PSC), biliary atresia (BA), graft-versus-host disease (GvHD), and cholangiocarcinoma (CCA). However, they differ significantly in regard to their etiopathogenesis, symptoms and clinical course, liver phenotype, gender predominance, and concomitant diseases [1]. The etiology of these diseases still remains only partly understood, but their pathogenesis has become somehow clearer [1–3].

The main advantages for the use of rodent (especially mouse) models are – compared to other mammalians – low costs, the ability for high-throughput studies, ease of handling and breeding, and the possibility of genetic manipulation in mice. Mouse models enable us also to test new drugs and genetic or surgical manipulation within reasonable time frames because of their low life span [4]. However, despite undoubtedly significant research progress, each animal model still harbors its own and important limitations [4]. Such limitations include substantial species differences between rodents and humans in regard to (1) immune and inflammatory responses [5], (2) hepatic and intestinal nuclear receptor expression [6], (3) bile acid metabolism and pool composition [7], and (4) gut microbiota [8] to name only few. In addition, some models exhibit comparable characteristics of different biliary diseases with underlying shared mechanisms of immune-mediated cholangiocyte injury and biliary type of liver fibrosis, and one specific model may consequently mirror different aspects of several cholangiopathies [9]. However, for clarity reasons we would like to provide the characteristics for each model discussed. Due to

limitations of space, we focus exclusively on available mouse models for PBC, PSC, BA, GvHD, and CCA. We would also ask our colleges for pardon, since due to the limitation of references, numerous important publications in this research area could not be cited adequately.

4.2 Primary Biliary Cholangitis (PBC)

The identification of anti-mitochondrial antibodies (AMA) directed against the major mitochondrial antigen pyruvate dehydrogenase complex E2 (PDC-E2) detectable in over 95% of patients leading to consecutive destruction of interlobular bile ducts was a milestone in understanding the autoimmune-mediated nature of PBC [10–12]. Accordingly, PBC represents a classical autoimmune disorder with clear female predominance. Histologically, PBC is characterized by chronic nonsuppurative inflammation with so-called florid duct lesions consisting of epithelioid cell granulomas surrounding small bile ducts eventually progressing to segmental vanishing of bile ducts and ultimately to the biliary type of liver fibrosis and in some patients to liver cirrhosis. Thus, the main attributes of a candidate PBC model include a clear female predominance, presence of AMA in more than 90% and presence of antinuclear antibodies (ANA) in 50–80%, chronic inflammation of small bile ducts with focal duct obliteration and epithelioid cell granuloma formation, chronicity and slow progression of disease with vanishing of bile ducts, biliary type of liver fibrosis with CD4 T cells in liver and hilar lymph nodes, as well as PDC-E2-specific autoreactive CD8 T cells in liver [2].

Animal models for PBC can be subdivided into (1) spontaneous models utilizing genetic modification in mice, (2) neonatally thymectomized mice, (3) inducible models with the use of xenobiotics harboring structural similarities to PDC-E2, and (4) infectious-induced PBC-like phenotype. A list of animal models for PBC is given in Table 4.1. Due to limitations of space, we will focus here on genetically modified mice.

4.2.1 dnTGFβRII Mice

Transforming growth factor-beta (TGF-β) has pleiotropic effects on cell growth and immunological control with a promoting effect on the development of the regulatory T-cell compartment [13]. Overexpression of a dominant negative form of the TGF-β promoter leads to development of PBC-like features with lymphoid cell infiltration of portal fields as well as colitis with 100% AMA positivity [14, 15]. The adoptive transfer of CD8+ cells from these animals into immunodeficient *Rag1−/−* mice underlined the importance of CD8+ cells, since these mice developed similar histopathology to human PBC; however, CD4+ T-cell transfer had no effect on the liver phenotype but worsened colitis [16]. Further studies with an anti-CD20 antibody in young dnTGFβRII showed complete loss of serum AMA positivity and

Table 4.1 Mouse models of PBC

Mouse model	Presence of AMA	Liver phenotype	Limitations/Comments	Reference
Spontaneous models: genetically modified mice				
dnTGFβRII mice	+	Lymphoid cell infiltration of portal tracts, bile duct injury, liver fibrosis	No information on large duct disease; development of colitis	[15–17]
dnTGFβRII IL-12p35−/− mice	+	Lymphoid cell infiltration of portal tracts, bile duct injury, liver fibrosis	No information on large duct disease	[19]
IL-2Rα−/− mice	+	Lymphoid cell infiltration of portal tracts, bile duct injury, large duct disease	No information on fibrosis development, large duct disease; development of colitis	[21]
IL-2Rα$^{-/-}$ IL12-p40$^{-/-}$ mice	n.d.	Lymphoid cell infiltration of portal tracts, bile duct injury	No information on fibrosis development or large duct disease; development of colitis	[22]
NOD.c3c4 mice	+	Lymphoid cell infiltration of portal tracts, bile duct injury, large duct disease	No development of liver fibrosis; large duct disease	[23]
Ae2a,b−/− mice	+	Lymphoid cell infiltration of portal tracts, bile duct injury, liver fibrosis	No information on large duct disease	[28]
Scurfy mice	+	Lymphoid cell infiltration of portal tracts, bile duct injury	No development of liver fibrosis, no information on large duct disease	[30]
MRL/lpr mice	+	Lymphoid cell infiltration of portal tracts	No development of liver fibrosis, no bile duct destruction	[31]
Neonatally thymectomized mice	n.d.	Lymphoid cell infiltration of portal tracts, bile duct injury	No information on large duct disease or liver fibrosis	[120]
Xenobiotic-immunized/infectious-induced models				
2-OA-immunized mice	+	Lymphoid cell infiltration of portal tracts, bile duct injury	No information on large duct disease or live fibrosis	[121]
2OA-BSA-immunized mice + co-treatment with poly I:C	+	Lymphoid cell infiltration of portal tracts, bile duct injury, liver fibrosis	No information on large duct disease	[122]

Mice immunized with LPS + PDH + Freund's adjuvant	n.d.	Lymphoid cell infiltration of portal tracts, bile duct injury, liver fibrosis	No information on large duct disease	[123]
Novosphingobium aromaticivorans-immunized mice	+	Lymphoid cell infiltration of portal tracts, bile duct injury	No development of liver fibrosis, no information on large duct disease	[124]
Escherichia coli-immunized (NOD).B6	+	Lymphoid cell infiltration of portal tracts, bile duct injury, liver fibrosis	No information on liver fibrosis or large duct disease	[125]

Abbreviation: *2-OA* 2-octynoic acid, *Ae2* anion exchanger 2, *AMA* anti-mitochondrial antibodies, *IL* interleukin, *LPS* lipopolysaccharide, *MRL* magnetic resonance imaging, *n.d.* not determined, *NOD* nonobese diabetic, *PDH* pyruvate dehydrogenase, *poly I:C* polyinosinic-polycytidylic acid, *TGF* transforming growth factor

decreased liver inflammation, but were ineffective when initiated in mice with established disease [17]. A central role for natural killer T (NKT) cells in PBC pathogenesis is supported by the generation of CD1d$^{-/-}$-dnTGFβRII mice, in which reduced NKT function caused ameliorated inflammation, bile duct damage, mild ductopenia, cholestasis, and biliary fibrosis [18]. IL-12, consisting of a p40 and a p35 subunit, was studied by generating an *IL-12p35−/−* and *IL-12p40−/−* mouse strain on the dnTGFβRII background [19]. Whereas the *IL-12p40−/−*mice were protected from liver inflammation, in *IL-12p35−/−* mice, liver inflammation with similar severity but delayed onset compared to the parental dnTGFβRII mice was detected [19]. In addition, the deletion of IL-12p35 subunit from dnTGFβRII mice leads to frequent development of liver fibrosis with numerous immunological and histological features similar to human PBC [19]. To further characterize this interesting and promising mouse model, it will be crucial to study the effect of the different cytokines, including IL-12, -23, and -35 on liver phenotypes and on fibrotic changes via cytokine administration or cytokine-neutralizing antibodies [20].

4.2.2 IL-2Rα$^{-/-}$ Mice

Mice with genetic IL-2 receptor deficiency show 100% AMA positivity, lymphocytic portal inflammation, as well as CD4+ and CD8+ lymphocytes infiltrating the bile duct epithelium of intralobular bile ducts [21]. Interestingly, these animals show concomitant severe intestinal inflammation, which is usually not seen in PBC but PSC patients. No hepatic granuloma formation is seen in this mouse model [21]. In addition, there is no information on serum markers for cholestasis and whether also large bile ducts are involved.

Questioning the role of IL-12 in PBC triggered the generation of double knockouts via crossing *IL-2Rα−/−* and *IL-12p40−/−* mice [22]. *IL-2Rα−/−IL-12p40−/−* double-knockout mice show exacerbated autoimmune cholangitis, higher degree of liver fibrosis, and ameliorated colitis compared to *IL-2Rα−/−* single-knockout mice [22]. For more detailed characterization of cholestasis in this interesting mouse model, serum bile acid and alkaline phosphatase levels are awaited [22]. In addition, it would also be important to know whether *IL-2Rα−/− IL-12p-40−/−* mice develop large duct disease.

4.2.3 NOD.c3c4 Mice

The introgression of large genetic intervals on chromosomes 3 and 4 in nonobese diabetic (NOD) mouse strain leads to the development of NOD.c3c4 mice [23, 24]. On histological examination, in a high percentage, eosinophilic infiltration of bile ducts and autoreactivity against the PDC-E2 component are seen. To lower extend destructive cholangitis and granuloma formation can be observed. Whereas these animals show high seropositivity for AMA and ANA (80–90%), unfortunately, we do not have any information on cholestasis parameters of these animals. Intriguingly,

extrahepatic bile duct disease is observed in NOD.c3c4 mice – a feature that would better fit to PSC rather than PBC – with development of cystic dilations of bile ducts, partial exfoliation of the biliary epithelium, and dense neutrophil-granulocytic infiltration [23]. The underlying mechanisms, however, for this peculiar phenotype is not clear and deserves detailed time-course studies (e.g., cholangiography or bile duct plastination for better characterization of large duct disease, characterization of the inflammatory infiltrate). The pronounced neutrophil-granulocytic infiltration of bile ducts could be, at least in part, a secondary phenomenon due to dilatation and secondary ascending cholangitis. Consequently bile culture studies should also be of interest. Interestingly however, treatment of NOD.c3c4 mice with a monoclonal antibody directed against CD3 protected these mice from cholangitis [23]. In general, due to the complex morphological changes in NOD.c3c4 mice, this mouse model may serve as a model for different cholangiopathies, including also several important aspects of PSC pathogenesis.

4.2.4 Ae2a,b$^{-/-}$ Mice

The observations that anion exchanger 2 (AE2) is downregulated in the liver and lymphocytes of PBC patients and that ursodeoxycholic acid restores AE2 expression and stimulates biliary bicarbonate secretion partially by activation of hepatic AE2 [25–27] were the trigger to generate Ae2a,b$^{-/-}$ mice. This mouse model shares some immunologic and hepatobiliary features with PBC [28]. Histologically, mild to severe portal inflammation with high interindividual variations in regard to the liver phenotype is observed. In addition, the defective Treg cell function and CD8+ T-cell expansion seen in these mice could be due to the AE2 dysfunction, which seems to be critically involved also in the homeostasis of the immune system. However, so far a detailed characterization of this model in regard to investigation of large ducts and in regard to potential biliary fibrosis has been not performed. One major limitation of the model may lie within the fact that this mouse strain seems to be very difficult to breed (personal communication Juan Medina, Pamplona, Spain).

4.2.5 Scurfy Mice

Scurfy mice with a selective loss of the transcription factor Fox-P3 (forkhead box P3, also known as scurfin) resulting in a functional deficiency of Treg cells show serological and morphological features of immune-mediated cholangitis, including severe bile duct injury [29, 30]. However, serum bile acid and alkaline phosphatase levels are not reported in these mice. Findings in scurfy mice underline the potential importance of Treg cells for the pathogenesis of PBC. One of the major limitations of this model is based on an extremely short life span of these mice of about 4 weeks, which seriously limits their use for longitudinal studies (e.g., disease progress, drug testing).

4.2.6 MRL/lpr Mice

MRL/lpr mice with the lymphoproliferative gene lpr (also known as MRL/MP-lpr/lpr) spontaneously develop severe autoimmune-mediated disorders, such as vasculitis, glomerulonephritis, inflammation of salivary glands, interstitial pneumonia and plasma-cellular infiltration of portal fields with biliary injury, and development of AMA [31]. The relatively low percentage, about 50% of mice showing PBC-like features, critically limits the usefulness of these mice as a PBC model.

Currently no "ideal PBC model" exists among the available mouse models. Although an enormous progress has been achieved in the last decades in the generation of different model systems that show astonishing similarities with human PBC, concerning immunological and histological characteristics, each model harbors still its specific limitations. As PBC represents a chronic cholangiopathy with slow progression to biliary fibrosis and cirrhosis, long-term studies with detailed characterization of the cholestatic phenotype would be of major interest and urgent need for these models.

4.3 Primary Sclerosing Cholangitis (PSC)

PSC leads to irregular scarring of the biliary tree causing bile duct strictures and dilatation-affecting intra- and extrahepatic bile ducts and may finally lead to biliary cirrhosis and liver failure. PSC primarily affects young men and is frequently associated with inflammatory bowel disease with specific clinical features including rectal sparing, right-sided disease, and backwash ileitis (i.e., PSC-IBD) [32]. The main attributes of an ideal PSC model therefore include the following clinicopathological features: male predominance, progressive fibrous-obliterative cholangitis of medium-sized and large bile ducts, onionskin-type-like periductal fibrosis, biliary type of liver fibrosis, concomitant predominantly right-sided mild colitis or pancolitis, and the high risk for CCA.

Animal models for (primary) sclerosing cholangitis [(P)SC] arbitrarily can be clustered into six different groups [33]: chemically induced cholangitis, knockout 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. Subtypes of models, their respective characteristics, and according references are summarized in Table 4.2. Due to limitations of space, we have to focus on only a few of them.

4.3.1 Mdr2 (Abcb4) Gene Knockout Mice

Mdr2−/− mice show key features of human SC with development of cholangitis and onionskin-type periductal fibrosis similar to human PSC with strictures and dilatations of bile ducts and biliary type of liver fibrosis. Pathogenetically, the lack of biliary phospholipid secretion and increased concentration of free

Table 4.2 Animal models of sclerosing cholangitis

Mouse model	Mice	Liver phenotype	Limitations	Ref.
Chemically induced cholangitis				
DDC	Swiss albino mice PDX-1 knockout mice	Pericholangitis; periductal fibrosis; ductular proliferation; biliary type of fibrosis	No characteristic BD strictures and dilatation on plastination	[34–37]
LCA	Swiss albino mice	Bile infarcts; destructive cholangitis; periductal fibrosis	No tolerable long-term protocol established	[38]
Knockout mouse models				
Abcb4$^{-/-}$	FVB/N	Cholangitis; pericholangitis; periductal fibrosis; biliary type of fibrosis	No colitis or CCA but liver cell tumors	[39–46]
Cftr$^{-/-}$	C57BL/6J	Focal cholangitis; ductular proliferation	High risk for intestinal obstruction, weak spontaneous phenotype (without DSS)	[47–51]
fch/fch	BALB/c	Cholangitis; ductular proliferation; biliary type of fibrosis	Extrahepatic BD not studied so far	[35, 36]
Infectious agents				
Cryptosporidium parvum	BALB/c nu/nu, BALB/c SCID, C57BL76-SCID, NIH-III nu/nu CD40$^{-/-}$, IFNγ$^{-/-}$, CD154$^{-/-}$, CD40-CD154$^{-/-}$, Tnfsf5$^{-/-}$, Tnfrsf1a$^{-/-}$, Tnfrsf1b$^{-/-}$, Tnfrsf1a/1b$^{-/-}$, Tnfsf5-Tnfrsf1a$^{-/-}$, Tnfsf5-Tnfrsf1b$^{-/-}$, Tnfsf5-Tnfrsf1a/1b$^{-/-}$,CD40-Tnfrsf1a/1b$^{-/-}$	Strongly depending on genetic background: cholangitis; pericholangitis; periductal fibrosis; biliary type of fibrosis	Complex models, phenotype so far not well characterized	[52–55]
Helicobacter hepaticus	A/JCr, C3H/HeNCr, C57BL/6NCr, A/J	Cholangitis; pericholangitis	Complex models	[56, 57]

(continued)

Table 4.2 (continued)

Mouse model	Mice	Liver phenotype	Limitations	Ref.
Common bile duct ligation	C57BL/6 J	Bile infarcts; cholangitis; pericholangitis; periductal fibrosis; biliary type of fibrosis	Technical pitfalls	[58, 59]
Models of biliary epithelial and endothelial cell injury				
Experimental GVHD	BALB/c	Cholangitis; pericholangitis; periductal fibrosis; biliary type of fibrosis	Low fibrotic response	[60]

Abbreviation: *CCA* cholangiocellular carcinoma, *Cftr* cystic fibrosis transmembrane conductance regulator, *DDC* 3,5-diethoxycarbonyl-1,4-dihydrocollidine, *DSS* dextran sodium sulfate, *fch* ferrochelatase, *GVHD* graft-versus-host disease, *IBD* inflammatory bowel disease, *LCA* lithocholic acid, *Mdr2* multidrug resistance protein-2

non-micellar-bound bile acids cause damage of bile duct epithelial cells [61] due to regurgitation of bile into the portal tracts leading to inflammation and fibrosis [58, 62]. However, the pathogenetic cause of disease still has to be determined in more detail, especially in regard to the specific role of bile acids. The *Mdr2−/−*mouse model proved to be useful to test novel treatment strategies for (P)SC and liver fibrosis of the biliary type. Hence, this model is increasingly used [39–43, 58, 63–66]. Since the fibrotic response is strongly influenced by the genetic background and varies, it will be interesting to determine the potential effects of mouse genetic background on liver fibrosis degree in *Mdr2−/−* mice. Only male mice should be used for modeling PSC, since female *Mdr2−/−* mice develop gall stone disease already at young age, which is not a common feature in PSC patients and would also lead to significant variations in the cholestatic phenotype of animals [67]. One of the major limitations of this model, however, is the fact that there is insufficient evidence for the impact of MDR3 mutations/dysfunction or low biliary phospholipid output in PSC pathogenesis [68]. In addition, *Mdr2−/−* mice do not develop colitis (at least in the genetic backgrounds tested already) or CCA but hepatocellular neoplastic nodules, which is unusual in PSC patients [69].

4.3.2 Mice Harboring a Mutation of Exon 10 of the Cystic Fibrosis (CF) Transmembrane Conductance Regulator Gene Knockout Mice (*Cftr*$^{-/-}$ Mice)

Cftr−/− mice develop focal cholangitis with inspissated bile and bile duct proliferation, resulting in biliary cirrhosis. Since CFTR gene mutations may play a pathogenetic role in PSC [70], *Cftr*$^{-/-}$ mice proved useful in the study of PSC development, since CFTR gene mutations may play a pathogenetic role in PSC although being not entirely clear so far [47]. A major limitation of this specific mouse model is that the genetic background strongly determines liver and/or intestinal phenotype [48–51].

4.3.3 Mice with a Point Mutation in the Ferrochelatase Gene (fch/fch) and Mice Fed the Porphyrinogenic Substance 3,5-Dietoxycarbonyl-1,4-Dihydrocollidine (DDC)

Both mice show sclerosing cholangitis and pronounced biliary fibrosis paralleled by ductular proliferation and portoportal bridging within weeks [34–36]. However, neither strictures nor dilations of the large duct system despite showing definite histological features of typical periductal fibrosis in PSC are seen which takes 4–8 weeks after DDC feeding depending on DDC-diet concentration and the mouse strain used [34]. The pathogenetic cause of disease is most likely linked to the biliary excreted DDC metabolite protoporphyrin IX and resulting ductal porphyrin plugs [34]. In addition, a link between DDC feeding and interference with biliary phospholipid secretion has been described [37]. The main advantages of this model include high reproducibility, high suitability for pathophysiological studies on the

mechanisms of cholangitis, ductular reaction, and biliary type of liver fibrosis. However, the use for testing of treatment strategies for SC is limited due to the fixed liver phenotype and possible drug-drug interactions.

Taken together similar to PBC, currently there is no "ideal PSC model" available [63, 71, 72]. Since PSC represents a long-standing disease with complex underlying pathogenetic mechanisms, in which endogenous and exogenous factors are involved, it seems not likely that one single model will perfectly mirror PSC, but we will rather need various aspects of different models to study particular pathogenetic steps of PSC.

4.4 Graft-Versus-Host Disease (GvHD)

Bile ducts are major targets in acute and chronic GvHD representing a common complication and limiting factor of an allogeneic tissue and bone marrow transplantation. In humans, acute GvHD occurs within 100 days of transplant, and chronic GvHD (cGvHD) typically develops 100 days after transplantation. In mice, this temporal classification is not necessarily seen, since disease manifestation can differ in time of onset and is mainly defined by the clinical phenotype. Thus, chronic GvHD develops within weeks after transplantation in most mouse models [73]. Pathogenetically, cholangiocytes of small- to medium-caliber bile ducts are the major targets of T-cell-mediated destruction, causing apoptotic cell death and ultimately ductopenia [74]. So far, the detailed pathomechanism of GvHD is not clear.

In mice, GvHD across minor histocompatibility antigens can be induced experimentally by injection of spleen and bone marrow cells of congenic B10.D2 mice into sublethally irradiated BALB/c mice [60]. Bile ducts develop severe cholangitis with predominate lymphocytic inflammatory infiltrates 2–3 weeks after transplantation, and later on periductal fibrosis is observed. The major limitations of this mouse model are that neither loss of intrahepatic small bile ducts nor progression to liver cirrhosis during an observation period of 14 month is observed [60]. Generally speaking, factors confounding the translation of findings in mouse models to the human disease lie behind the fact that in humans acute GvHD typically precedes the chronic form, although in some cases chronic GvHD can occur without the occurrence of clinically obvious acute GvHD [73]. In addition, most patients are given immunosuppressive therapy to prevent acute GvHD influencing the development of chronic GvHD and further complicating modeling human GvHD in animals.

4.5 Biliary Atresia (BA)

BA is the most frequent identifiable cause of neonatal cholestasis, and most patients require early liver transplantation [75]. To date, the underlying pathophysiological mechanisms are unknown, although a pivotal role for a dysregulation of cellular and humoral immunity, viral, toxic, and genetic factors are considered [75]. To date, different model systems for BA have been established, including young lambs and calves

[76], sea lampreys [77, 78], zebrafish [79] and mice [79–83]. In newborn BALB/c mice, infection with rhesus rotavirus type A (RRV) in the first 2 days of life leads to liver disease with development of hepatobiliary injury and cholestasis within 1 week of infection [82, 83] mimicking human BA in several aspects [82–84]. Intriguingly, this mouse model shares major clinicopathological features with the human disease, including a time-restricted susceptibility of bile duct injury to the early postnatal period, acholic stools, bile duct proliferation, and portal inflammation as well as type 1 rich inflammatory infiltrate in the liver and bile ducts [84–90]. However, one of the main limitations of this mouse model is the high mortality rate of mice.

4.6 Cholangiocarcinoma (CCA)

CCA is an epithelial biliary malignancy that originates from oncogenic transformation of cholangiocytes. Depending on the anatomic site, they may originate from different cell types, including intrahepatic biliary epithelial cells, hepatic progenitor cells, or mucin-producing cuboidal cholangiocytes of the extrahepatic biliary epithelium and peribiliary glands [91]. The identification of cellular origin in different subtypes may represent a prerequisite for effective therapy, but its impact on prognosis remains uncertain. CCA carcinogenesis is not entirely clear; however, well-known risk factors include the presence of PSC, liver fluke infections, hepatolithiasis or chronic hepatitis C, cirrhosis and toxins sharing induction of chronic cholestasis, and biliary and/or liver inflammation [92–95]. In the last years, several rodent models of CCA have been developed, including mice with xenograft and orthotopic tumors [96–102], genetically modified CCA models [103–105], and carcinogen-induced CCA models [106, 107]. Although these models provide adequate tools to gain insights into the pathophysiology of CCA development and to test new potential therapeutic agents in a preclinical setting, they harbor important limitations and difficulties discussed below (summarized in Table 4.3).

4.6.1 Xenograft and Orthotopic Models

In xenograft models, CCA cell lines are implanted into nude or severe combined immunodeficiency (SCID) mice. In 1985, the first study of CCA was developed by injecting a cell line derived from a human CCA metastasis subcutaneously into the flank of nude mice [108]. This model has not only been used for performing time-course and pathophysiological studies in regard to tumor growth, but also a high number of potential antitumor agents, including caffeic acid, tamoxifen, melatonin, and clobenpropit, have been tested [110–112]. Currently, although being not entirely clear so far, there is increasing evidence for a pivotal role of MicroRNAs (miRNAs) in cholangiocarcinogenesis with miR-26a and miR-494 known to promote tumor growth via targeting Wnt signaling pathway or modulation of the cell cycle [100, 113]. The informative value of studying interactions between cancer and peritumoral stroma cells in these mice, however, is limited, since tumor growth strongly

Table 4.3 Mouse models of CCA

Model	Mouse	Latency for tumor development	Limitations	Reference
Xenograft	Nude mouse	2 weeks	No metastases	[108]
p53 knock out mouse + CCl_4	$p53^{-/-}$ C57B16 mouse CCl_4 ip at the age of 6 months	$p53^{-/-}$: 29 weeks *p53+/−*: 53 weeks	No information on metastases	[109]
Smad4-Pten knock out mouse	Cre-mediated deletion of Pten and Smad 4	4–7 months	Absence of chronic liver injury and inflammation, no metastases, concomitant of the salivary glands tumors	[104]
DEN + left median bile duct ligation	ip injection of DEN in young Balb/c + left median bile duct ligation + oral gavage of DEN	28 weeks	Model complexity	[107]

Abbreviation: *CCl_4* carbon tetrachloride, *DEN* diethylnitrosamine, *ip* intraperitoneal, *pten* phosphatase and tensin, *Smad 4* SMAD family member

depends on the species-specific microenvironment which is critically different from the tumor developing within the liver [91, 114]. Alternatively, CCA cells may be directly implanted into the bile duct of rodents that enables the study of organotypical interactions between tumor cells and surrounding stroma [115, 116]. In general, it has been shown that orthotopic CCA models are better predictors of drug efficacy and of potentially higher clinical relevance than xenograft models [114]. However, this approach has obvious limitations in the bile duct size of mice being far more time-consuming and technically difficult and therefore more expensive than conventional xenograft models [114].

4.7 Genetically Engineered Mice

4.7.1 p53 Knockout Mice Fed with Carbon Tetrachloride (CCl_4)

Four months administration of CCl_4 in mice harboring a deletion in the p53 gene leads to development of progressive liver injury and fibrosis paralleled by bile duct proliferation [109]. In accordance with the human CCA, p53 gene mutations are frequently observed [117]. Over time, these mice show tumors mimicking human CCA with atypical, infiltrating keratin 19-positive ducts and tubules with a dense collagenous stroma [109]. Similar to the human situation, this mouse model shows CCA development based on a combination of genetic susceptibility with a toxic chronic liver injury [114]. However, this mouse model is not very practicable; since the duration of time needed for tumor development is quite long (29–52 weeks) [114].

4.7.2 Smad4-Pten Knockout Mice

Smad4-Pten knockout mice, harboring a Cre-mediated deletion of phosphatase and tensin homolog (Pten) and SMAD family member 4 (Smad4), a tumor suppressor gene frequently altered in CCA [118] develop tumors histologically similar to intrahepatic CCA [104]. The authors crossbred mice carrying the Smad4 conditional allele (Smad4 Co) and/or the Pten conditional allele (Pten Co) which were crossed with albumin-Cre mice (Alb-Cre). After 2–3 months of age, biliary epithelial hyperplasia is observed, and at 4–7 months, mice show CCAs in all the animals followed by a progressive increase of tumoral intrahepatic nodules. This model is of major relevance for the understanding of the genetic and molecular mechanisms underlying disease development. In accordance with the human situation, PTEN loss has been linked to human CCA development by activation of the pro-proliferative and antiapoptotic PI3K pathway [93, 119]. Although this mouse model enables the investigation of intrahepatic CCA similar to the human situation already at 4–5 months of age, without any necessary further manipulation, limitations of this model include the absence of chronic liver injury and inflammation, the absence of metastases (even in older animals), and the concomitant development of tumors of the salivary glands [93].

4.7.3 The "DEN-Left Median Bile Duct Ligation" Model

Repeated intraperitoneal injection of diethyl-nitrosamine (DEN) in young Balb/c mice, following left median bile duct ligation and oral gavage of DEN, leads to the development of CCA [93, 107]. After 8 weeks, livers showed multifocal cystic hyperplasia of the intrahepatic bile ducts and multifocal cyst formation. At week 12, the biliary epithelium of the hyperplastic foci and the epithelium lining the cysts showed elongated nuclei. After 16 weeks bile duct tumors develop with full development of CCA in these areas at week 28. Despite its complexity requiring technically demanding bile duct surgery and long-term challenge with DEN, it represents the only known non-engineered mouse for CCA development [93].

Over the last few years, a number of HCC and CCA rodent models have been developed, many of them representing valuable tools for investigating some pathophysiological aspects of cancer development. However, due to the complexity of carcinogenesis per se, it is difficult to determine to what extent a single mouse model reproduces the human disease.

Conclusions

Biliary diseases are complex liver diseases in which extra- or intrahepatic bile ducts are affected. Significant research progress has been made the last years to develop animal models that will help understand their pathogenesis. Several limitations however exist which result in no "perfect model" that mirrors human

disease. The use of various aspects of different models might be the way forward to study particular pathogenetic steps in biliary disease.

References

1. Lazaridis KN, LaRusso NF. The Cholangiopathies. Mayo Clin Proc. 2015;90(6):791–800.
2. Hirschfield GM, Gershwin ME. The immunobiology and pathophysiology of primary biliary cirrhosis. Ann Rev Phathol. 2013;24(8):303–30.
3. Hirschfield GM, Karlsen TH, Lindor KD, Adams DH. Primary sclerosing cholangitis. Lancet. 2013;382(9904):1587–99.
4. Fickert P, Pollheimer MJ, Beuers U, Lackner C, Hirschfield G, Housset C, Keitel V, Schramm C, Marschall HU, Karlsen TH, Melum E, Kaser A, Eksteen B, Strazzabosco M, Manns M, Trauner M, International PSC Study Group (IPSCSG). Characterization of animal models for primary sclerosing cholangitis (PSC). J Hepatol. 2014;60(6):1290–303.
5. Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W, Richards DR, McDonald-Smith GP, et al.; Inflammation and Host Response to Injury, Large Scale Collaborative Research Program. Genomic responses in mouse models poorly mimic human inflammatory diseases. Proc Natl Acad Sci U S A 2013;110(9):3507–12.
6. Goodwin B, Watson MA, Kim H, Miao J, Kemper JK, Kliewer SA. Differential regulation of rat and human CYP7A1 by the nuclear oxysterol receptor liver X receptor-alpha. Mol Endocrinol. 2003;17(3):386–94.
7. Chiang JY. Bile acids: regulation of synthesis. J Lipid Res. 2009;50(10):1955–66.
8. Nguyen TL, Vieira-Silva S, Liston A, Raes J. How informative is the mouse for human gut microbiota research? Dis Model Mech. 2015;8(1):1–16.
9. Liu Y, Meyer C, Xu C, Weng H, Hellerbrand C, ten Dijke P, Dooley S. Animal models of chronic liver diseases. Am J Physiol Gastrointest Liver Physiol. 2013;304(5):G449–68.
10. Leung PS, Chuang DT, Wynn RM, Cha S, Danner DJ, Ansari A, Coppel RL, Gershwin ME. Autoantibodies to BCOADC-E2 in patients with primary biliary cirrhosis recognize a conformational epitope. Hepatology. 1995;22(2):505–13.
11. Moteki S, Leung PS, Dickson ER, Van Thiel DH, Galperin C, Buch T, Alarcon-Segovia D, Kershenobich D, Kawano K, Coppel RL, et al. Epitope mapping and reactivity of autoantibodies to the E2 component of 2-oxoglutarate dehydrogenase complex in primary biliary cirrhosis using recombinant 2-oxoglutarate dehydrogenase complex. Hepatology. 1996;23(3):436–44.
12. Van de Water J, Ansari A, Prindiville T, Coppel RL, Ricalton N, Kotzin BL, Liu S, Roche TE, Krams SM, Munoz S, Gershwin ME. Heterogeneity of autoreactive T cell clones specific for the E2 component of the pyruvate dehydrogenase complex in primary biliary cirrhosis. J Exp Med. 1995;181(2):723–33.
13. Massagué J. TGF-beta signal transduction. Annu Rev Biochem. 1998;67:753–91.
14. Tsuneyama K, Moritoki Y, Kikuchi K, Nakanuma Y. Pathological features of new animal models for primary biliary cirrhosis. Int J Hepatol. 2012:403954.
15. Oertelt S, Lian ZX, Cheng CM, Chuang YH, Padgett KA, He XS, Ridgway WM, Ansari AA, Coppel RL, Li MO, Flavell RA, Kronenberg M, Mackay IR, Gershwin ME. Anti-mitochondrial antibodies and primary biliary cirrhosis in TGF-beta receptor II dominant-negative mice. J Immunol. 2006;177(3):1655–60.
16. Yang GX, Lian ZX, Chuang YH, et al. Adoptive transfer of CD8(+) T cells from transforming growth factor beta receptor type II (dominant negative form) induces autoimmune cholangitis in mice. Hepatology. 2008;47:1974–82.
17. Moritoki Y, Lian ZX, Lindor K, Tuscano J, Tsuneyama K, Zhang W, Ueno Y, Dunn R, Kehry M, Coppel RL, Mackay IR, Gershwin ME. B-cell depletion with anti-CD20 ameliorates autoimmune cholangitis but exacerbates colitis in transforming growth factor-beta receptor II dominant negative mice. Hepatology. 2009;50(6):1893–903.

18. Chuang YH, Lian ZX, Yang GX, et al. Natural killer T cells exacerbate liver injury in a transforming growth factor beta receptor II dominant-negative mouse model of primary biliary cirrhosis. Hepatology. 2008;47:571–80.
19. Tsuda M, Zhang W, Yang GX, Tsuneyama K, Ando Y, Kawata K, et al. Deletion of interleukin (IL)-12p35 induces liver fibrosis in dominant-negative TGFβ receptor type II mice. Hepatology. 2013;57:806–16.
20. Popov Y. Mouse model of primary biliary cirrhosis with progressive fibrosis: are we there yet? Hepatology. 2013;57(2):429–31.
21. Wakabayashi K, Lian ZX, Moritoki Y, et al. IL-2 receptor alpha(−/−) mice and the development of primary biliary cirrhosis. Hepatology. 2006;44:1240–9.
22. Yao Y, Yang W, Yang YQ, Ma HD, Lu FT, Li L, Tao YY, Tsuneyama K, Zhang W, Friedman S, Gershwin ME, Lian ZX. Distinct from its canonical effects, deletion of IL-12p40 induces cholangitis and fibrosis in interleukin-2Rα(−/−) mice. J Autoimmun. 2014;51:99–108.
23. Irie J, Wu Y, Wicker LS, Rainbow D, Nalesnik MA, Hirsch R, Peterson LB, Leung PS, Cheng C, Mackay IR, Gershwin ME, Ridgway WM. NOD.c3c4 congenic mice develop autoimmune biliary disease that serologically and pathogenetically models human primary biliary cirrhosis. J Exp Med. 2006;203(5):1209–19.
24. Koarada S, Wu Y, Fertig N, Sass DA, Nalesnik M, Todd JA, Lyons PA, Fenyk-Melody J, Rainbow DB, Wicker LS, Peterson LB, Ridgway WM. Genetic control of autoimmunity: protection from diabetes, but spontaneous autoimmune biliary disease in a nonobese diabetic congenic strain. J Immunol. 2004;173(4):2315–23.
25. Melero S, Spirlì C, Zsembery A, Medina JF, Joplin RE, Duner E, et al. Defective regulation of cholangiocyte Cl−/HCO3(−) and Na+/H+ exchanger activities in primary biliary cirrhosis. Hepatology. 2002;35:1513–21.
26. Prieto J, García N, Martí-Climent JM, Peñuelas I, Richter JA, Medina JF. Assessment of biliary bicarbonate secretion in humans by positron emission tomography. Gastroenterology. 1999;117:167–72.
27. Medina JF, Martínez-Ansó, Vazquez JJ, Prieto J. Decreased anion exchanger 2 immunoreactivity in the liver of patients with primary biliary cirrhosis. Hepatology. 1997; 25:12–7.
28. Salas JT, Banales JM, Sarvide S, Recalde S, Ferrer A, Uriarte I, et al. Ae2a,b-deficient mice develop antimitochondrial antibodies and other features resembling primary biliary cirrhosis. Gastroenterology. 2008;134:1482–93.
29. Brunkow ME, Jeffery EW, Hjerrild KA, Paeper B, Clark LB, Yasayko SA, Wilkinson JE, Galas D, Ziegler SF, Ramsdell F. Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. Nat Genet. 2001;27(1):68–73.
30. Zhang W, Sharma R, Ju ST, He XS, Tao Y, Tsuneyama K, et al. Deficiency in regulatory T cells results in development of antimitochondrial antibodies and autoimmune cholangitis. Hepatology. 2009;49:545–52.
31. Ohba K, Omagari K, Murase K, Hazama H, Masuda J, Kinoshita H, et al. A possible mouse model for spontaneous cholangitis: serological and histological characteristics of MRL/lpr mice. Pathology. 2002;34:250–6.
32. Loftus Jr EV, Harewood GC, Loftus CG, Tremaine WJ, Harmsen WS, Zinsmeister AR, Jewell DA, Sandborn WJ. PSC-IBD: a unique form of inflammatory bowel disease associated with primary sclerosing cholangitis. Gut. 2005;54(1):91–6.
33. Pollheimer MJ, Halilbasic E, Fickert P, Trauner M. Pathogenesis of primary sclerosing cholangitis. Best Pract Res Clin Gastroenterol. 2011;25(6):727–39.
34. Fickert P, Stöger U, Fuchsbichler A, Moustafa T, Marschall HU, Weiglein AH, Tsybrovskyy O, Jaeschke H, Zatloukal K, Denk H, Trauner M. A new xenobiotic-induced mouse model of sclerosing cholangitis and biliary fibrosis. Am J Pathol. 2007;171(2):525–36.
35. Meerman L, Koopen NR, Bloks V, Van Goor H, Havinga R, Wolthers BG, Kramer W, Stengelin S, Müller M, Kuipers F, Jansen PL. Biliary fibrosis associated with altered bile composition in a mouse model of erythropoietic protoporphyria. Gastroenterology. 1999; 117(3):696–705.

36. Libbrecht L, Meerman L, Kuipers F, Roskams T, Desmet V, Jansen P. Liver pathology and hepatocarcinogenesis in a long-term mouse model of erythropoietic protoporphyria. J Pathol. 2003;199(2):191–200.
37. Lyoumi S, Abitbol M, Rainteau D, Karim Z, Bernex F, Oustric V, Millot S, Lettéron P, Heming N, Guillmot L, Montagutelli X, Berdeaux G, Gouya L, Poupon R, Deybach JC, Beaumont C, Puy H. Protoporphyrin retention in hepatocytes and Kupffer cells prevents sclerosing cholangitis in erythropoietic protoporphyria mouse model. Gastroenterology. 2011;141(4):1509–19, 1519.e1–3.
38. Fickert P, Fuchsbichler A, Marschall HU, Wagner M, Zollner G, Krause R, et al. Lithocholic acid feeding induces segmental BD obstruction and destructive cholangitis in mice. Am J Pathol. 2006;168:410–22.
39. Halilbasic E, Fiorotto R, Fickert P, Marschall HU, Moustafa T, Spirli C, et al. Side chain structure determines unique physiologic and therapeutic properties of norursodeoxycholic acid in Mdr2−/− mice. Hepatology. 2009;49:1972–81.
40. Baghdasaryan A, Claudel T, Kosters A, Gumhold J, Silbert D, Thüringer A, Leski K, Fickert P, Karpen SJ, Trauner M. Curcumin improves sclerosing cholangitis in Mdr2−/− mice by inhibition of cholangiocyte inflammatory response and portal myofibroblast proliferation. Gut. 2010;59(4):521–30.
41. Baghdasaryan A, Claudel T, Gumhold J, Silbert D, Adorini L, Roda A, et al. Dual farnesoid X receptor/TGR5 agonist INT-767 reduces liver injury in the Mdr2-/- (Abcb4-/-) mouse cholangiopathy model by promoting biliary HCO-3 output. Hepatology. 2011;54:1303–12
42. Moustafa T, Fickert P, Magnes C, Guelly C, Thueringer A, Frank S, Kratky D, Sattler W, Reicher H, Sinner F, Gumhold J, Silbert D, Fauler G, Höfler G, Lass A, Zechner R, Trauner M. Alterations in lipid metabolism mediate inflammation, fibrosis, and proliferation in a mouse model of chronic cholestatic liver injury. Gastroenterology. 2012;142(1):140–51.e12.
43. Ehlken H, Kondylis V, Heinrichsdorff J, Ochoa-Callejero L, Roskams T, Pasparakis M. Hepatocyte IKK2 protects Mdr2−/− mice from chronic liver failure. PLoS One. 2011;6(10):e25942.
44. Fickert P, Zollner G, Fuchsbichler A, Stumptner C, Weiglein AH, Lammert F, et al. Ursodeoxycholic acid aggravates bile infarcts in BD-ligated and Mdr2 knockout mice via disruption of cholangioles. Gastroenterology. 2002;123:1238–51.
45. Fickert P, Fuchsbichler A, Wagner M, Zollner G, Kaser A, Tilg H, et al. Regurgitation of bile acids from leaky BDs causes sclerosing cholangitis in Mdr2 (Abcb4) knockout mice. Gastroenterology. 2004;127:261–74.
46. Popov Y, Patsenker E, Fickert P, Trauner M, Schuppan D, et al. Mdr2 (Abcb4)−/− mice spontaneously develop severe biliary fibrosis via massive dysregulation of pro- and antifibrogenic genes. J Hepatol. 2005;43:1045–54.
47. Henckaerts L, Jaspers M, Van Steenbergen W, Vliegen L, Fevery J, Nuytten H, Roskams T, Rutgeerts P, Cassiman JJ, Vermeire S, Cuppens H. Cystic fibrosis transmembrane conductance regulator gene polymorphisms in patients with primary sclerosing cholangitis. J Hepatol. 2009;50(1):150–7.
48. Snouwaert JN, Brigman KK, Latour AM, Malouf NN, Boucher RC, Smithies O, Koller BH. An animal model for cystic fibrosis made by gene targeting. Science. 1992;257(5073):1083–8.
49. Dorin JR, Dickinson P, Alton EW, Smith SN, Geddes DM, Stevenson BJ, Kimber WL, Fleming S, Clarke AR, Hooper ML, et al. Cystic fibrosis in the mouse by targeted insertional mutagenesis. Nature. 1992;359(6392):211–5.
50. Colledge WH, Abella BS, Southern KW, Ratcliff R, Jiang C, Cheng SH, MacVinish LJ, Anderson JR, Cuthbert AW, Evans MJ. Generation and characterization of a delta F508 cystic fibrosis mouse model. Nat Genet. 1995;10(4):445–52.
51. Zeiher BG, Eichwald E, Zabner J, Smith JJ, Puga AP, McCray Jr PB, Capecchi MR, Welsh MJ, Thomas KR. A mouse model for the delta F508 allele of cystic fibrosis. J Clin Invest. 1995;96(4):2051–64.

52. Stephens J, Cosyns M, Jones M, Hayward A. Liver and bile duct pathology following Cryptosporidium parvum infection of immunodeficient mice. Hepatology. 1999;30:27–35.
53. Ungar BL, Burris JA, Quinn CA, Finkelman FD. New mouse models for chronic Cryptosporidium infection in immunodeficient hosts. Infect Immun. 1990;58:961–9.
54. Mead JR, Arrowood MJ, Sidwell RW, Healey MC. Chronic Cryptosporidium parvum infections in congenitally immunodeficient SCID and nude mice. J Infect Dis. 1991;163:1297–304.
55. Ponnuraj EM, Hayward AR. Requirement for TNF-Tnfrsf1 signalling for sclerosing cholangitis in mice chronically infected by Cryptosporidium parvum. Clin Exp Immunol. 2002;128:416–20.
56. Ward JM, Anver MR, Haines DC, Benveniste RE. Chronic active hepatitis in mice caused by Helicobacter hepaticus. Am J Pathol. 1994;145:959–68.
57. Avenaud P, Le Bail B, Mayo K, Marais A, Fawaz R, Bioulac-Sage P, et al. Natural history of Helicobacter hepaticus infection in conventional A/J mice, with special reference to liver involvement. Infect Immun. 2003;71:3667–72.
58. Fickert P, Wagner M, Marschall HU, Fuchsbichler A, Zollner G, Tsybrovskyy O, Zatloukal K, Liu J, Waalkes MP, Cover C, Denk H, Hofmann AF, Jaeschke H, Trauner M. 24-norUrsodeoxycholic acid is superior to ursodeoxycholic acid in the treatment of sclerosing cholangitis in Mdr2 (Abcb4) knockout mice. Gastroenterology. 2006;130(2):465–81.
59. Georgiev P, Jochum W, Heinrich S, Jang JH, Nocito A, Dahm F, et al. Characterization of time-related changes after experimental bile duct ligation. Br J Surg. 2008;95:646–56.
60. Nonomura A, Kono N, Minato H, Nakanuma Y. Diffuse biliary tract involvement mimicking primary sclerosing cholangitis in an experimental model of chronic graft-versus-host disease in mice. Pathol Int. 1998;48(6):421–7.
61. Smit JJ, Schinkel AH, Oude Elferink RP, Groen AK, Wagenaar E, van Deemter L, et al. Homozygous disruption of the murine mdr2 P-glycoprotein gene leads to a complete absence of phospholipid from bile and to liver disease. Cell. 1993;75:451–62.
62. Ernst TM, Schwinge D, Raabe N, Daubmann A, Kaul MG, Adam G, Schramm C, Ittrich H. Imaging of the murine biliopancreatic tract at 7 Tesla: technique and results in a model of primary sclerosing cholangitis. J Magn Reson Imaging. 2014;40(6):1355–64.
63. Trauner M, Fickert P, Baghdasaryan A, Claudel T, Halilbasic E, Moustafa T, Wagner M, Zollner G. New insights into autoimmune cholangitis through animal models. Dig Dis. 2010;28(1):99–104.
64. Barikbin R, Neureiter D, Wirth J, Erhardt A, Schwinge D, Kluwe J, Schramm C, Tiegs G, Sass G. Induction of heme oxygenase 1 prevents progression of liver fibrosis in Mdr2 knockout mice. Hepatology. 2012;55(2):553–62.
65. Strack I, Schulte S, Varnholt H, Schievenbusch S, Töx U, Wendland K, Steffen HM, Drebber U, Dienes HP, Odenthal M. β-Adrenoceptor blockade in sclerosing cholangitis of Mdr2 knockout mice: antifibrotic effects in a model of nonsinusoidal fibrosis. Lab Investig. 2011;91(2):252–61.
66. Blaas L, Kornfeld JW, Schramek D, Musteanu M, Zollner G, Gumhold J, van Zijl F, Schneller D, Esterbauer H, Egger G, Mair M, Kenner L, Mikulits W, Eferl R, Moriggl R, Penninger J, Trauner M, Casanova E. Disruption of the growth hormone--signal transducer and activator of transcription 5 – insulinlike growth factor 1 axis severely aggravates liver fibrosis in a mouse model of cholestasis. Hepatology. 2010;51(4):1319–26.
67. Lammert F, Wang DQ, Hillebrandt S, Geier A, Fickert P, Trauner M, Matern S, Paigen B, Carey MC. Spontaneous cholecysto- and hepatolithiasis in Mdr2−/− mice: a model for low phospholipid-associated cholelithiasis. Hepatology. 2004;39(1):117–28.
68. Trauner M, Fickert P, Wagner M. MDR3 (ABCB4) defects: a paradigm for the genetics of adult cholestatic syndromes. Semin Liver Dis. 2007;27(1):77–98.
69. Katzenellenbogen M, Mizrahi L, Pappo O, Klopstock N, Olam D, Jacob-Hirsch J, Amariglio N, Rechavi G, Domany E, Galun E, Goldenberg D. Molecular mechanisms of liver carcinogenesis in the mdr2-knockout mice. Mol Cancer Res. 2007;5(11):1159–70.

70. Pollheimer MJ, Trauner M, Fickert P. Will we ever model PSC? – "it's hard to be a PSC model!". Clin Res Hepatol Gastroenterol. 2011;35(12):792–804.
71. Vierling JM. Animal models for primary sclerosing cholangitis. Best Pract Res Clin Gastroenterol. 2001;15(4):591–610.
72. Pollheimer MJ, Fickert P. Animal models in primary biliary cirrhosis and primary sclerosing cholangitis. Clin Rev Allergy Immunol. 2015;48(2–3):207–17.
73. Schroeder MA, DiPersio JF. Mouse models of graft-versus-host disease: advances and limitations. Dis Model Mech. 2011;4(3):318–33.
74. Vierling JM, Hreha G, Wang H, Braun M. The role of biliary epithelial cells in the immunopathogenesis of non-suppurative destructive cholangitis in murine hepatic graft-versus-host disease. Trans Am Clin Climatol Assoc. 2011;122:326–35.
75. Mack CL. What causes biliary atresia? Unique aspects of the neonatal immune system provide clues to disease pathogenesis. Cell Mol Gastroenterol Hepatol. 2015;1(3): 267–74.
76. Harper P, Plant JW, Unger DB. Congenital biliary atresia and jaundice in lambs and calves. Aust Vet J. 1990;67(1):18–22.
77. Cai SY, Lionarons DA, Hagey L, Soroka CJ, Mennone A, Boyer JL. Adult sea lamprey tolerates biliary atresia by altering bile salt composition and renal excretion. Hepatology. 2013;57(6):2418–26.
78. Yeoh EJ, Ross ME, Shurtleff SA, Williams WK, Patel D, Mahfouz R, Behm FG, Raimondi SC, Relling MV, Patel A, Cheng C, Campana D, Wilkins D, Zhou X, Li J, Liu H, Pui CH, Evans WE, Naeve C, Wong L, Downing JR. Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. Cancer Cell. 2002;1(2):133–43.
79. Bangaru B, Morecki R, Glaser JH, Gartner LM, Horwitz MS. Comparative studies of biliary atresia in the human newborn and reovirus-induced cholangitis in weanling mice. Lab Invest. 1980;43(5):456–62.
80. Szavay PO, Leonhardt J, Czech-Schmidt G, Petersen C. The role of reovirus type 3 infection in an established murine model for biliary atresia. Eur J Pediatr Surg. 2002;12(4):248–50.
81. Wilson GA, Morrison LA, Fields BN. Association of the reovirus S1 gene with serotype 3-induced biliary atresia in mice. J Virol. 1994;68(10):6458–65.
82. Petersen C, Biermanns D, Kuske M, Schäkel K, Meyer-Junghänel L, Mildenberger H. New aspects in a murine model for extrahepatic biliary atresia. J Pediatr Surg. 1997;32(8):1190–5.
83. Riepenhoff-Talty M, Schaekel K, Clark HF, Mueller W, Uhnoo I, Rossi T, Fisher J, Ogra PL. Group A rotaviruses produce extrahepatic biliary obstruction in orally inoculated newborn mice. Pediatr Res. 1993;33(4 Pt 1):394–9.
84. Asai A, Miethke A, Bezerra JA. Pathogenesis of biliary atresia: defining biology to understand clinical phenotypes. Nat Rev Gastroenterol Hepatol. 2015;12(6):342–52.
85. Shivakumar P, Campbell KM, Sabla GE, Miethke A, Tiao G, McNeal MM, Ward RL, Bezerra JA. Obstruction of extrahepatic bile ducts by lymphocytes is regulated by IFN-gamma in experimental biliary atresia. J Clin Invest. 2004;114(3):322–9.
86. Carvalho E, Liu C, Shivakumar P, Sabla G, Aronow B, Bezerra JA. Analysis of the biliary transcriptome in experimental biliary atresia. Gastroenterology. 2005;129(2):713–7.
87. Leonhardt J, Stanulla M, von Wasielewski R, Skokowa J, Kübler J, Ure BM, Petersen C. Gene expression profile of the infective murine model for biliary atresia. Pediatr Surg Int. 2006;22(1):84–9.
88. Mack CL, Tucker RM, Sokol RJ, Kotzin BL. Armed CD4+ Th1 effector cells and activated macrophages participate in bile duct injury in murine biliary atresia. Clin Immunol. 2005;115(2):200–9.
89. Shivakumar P, Sabla GE, Whitington P, Chougnet CA, Bezerra JA. Neonatal NK cells target the mouse duct epithelium via Nkg2d and drive tissue-specific injury in experimental biliary atresia. J Clin Invest. 2009;119(8):2281–90.

90. Shivakumar P, Sabla G, Mohanty S, McNeal M, Ward R, Stringer K, Caldwell C, Chougnet C, Bezerra JA. Effector role of neonatal hepatic CD8+ lymphocytes in epithelial injury and autoimmunity in experimental biliary atresia. Gastroenterology. 2007;133(1):268–77.
91. Rizvi S, Gores GJ. Pathogenesis, diagnosis, and management of cholangiocarcinoma. Gastroenterology. 2013;145(6):1215–29.
92. Yamamoto S, Kubo S, Hai S, Uenishi T, Yamamoto T, Shuto T, Takemura S, Tanaka H, Yamazaki O, Hirohashi K, Tanaka T. Hepatitis C virus infection as a likely etiology of intrahepatic cholangiocarcinoma. Cancer Sci. 2004;95(7):592–5.
93. De Minicis S, Kisseleva T, Francis H, Baroni GS, Benedetti A, Brenner D, Alvaro D, Alpini G, Marzioni M. Liver carcinogenesis: rodent models of hepatocarcinoma and cholangiocarcinoma. Dig Liver Dis. 2013;45(6):450–9.
94. Khan SA, Thomas HC, Davidson BR, Taylor-Robinson SD. Cholangiocarcinoma. Lancet. 2005;366(9493):1303–14.
95. Blechacz B, Gores GJ. Cholangiocarcinoma: advances in pathogenesis, diagnosis, and treatment. Hepatology. 2008;48(1):308–21.
96. Meng F, Yamagiwa Y, Ueno Y, Patel T. Over-expression of interleukin-6 enhances cell survival and transformed cell growth in human malignant cholangiocytes. J Hepatol. 2006;44(6):1055–65.
97. Fava G, Marucci L, Glaser S, Francis H, De Morrow S, Benedetti A, Alvaro D, Venter J, Meininger C, Patel T, Taffetani S, Marzioni M, Summers R, Reichenbach R, Alpini G. gamma-Aminobutyric acid inhibits cholangiocarcinoma growth by cyclic AMP-dependent regulation of the protein kinase A/extracellular signal-regulated kinase 1/2 pathway. Cancer Res. 2005;65(24):11437–46.
98. Pawar P, Ma L, Byon CH, Liu H, Ahn EY, Jhala N, Arnoletti JP, McDonald JM, Chen Y. Molecular mechanisms of tamoxifen therapy for cholangiocarcinoma: role of calmodulin. Clin Cancer Res. 2009;15(4):1288–96.
99. Tang T, Zheng JW, Chen B, Li H, Li X, Xue KY, Ai X, Zou SQ. Effects of targeting magnetic drug nanoparticles on human cholangiocarcinoma xenografts in nude mice. Hepatobiliary Pancreat Dis Int. 2007;6(3):303–7.
100. Zhang J, Han C, Wu T. MicroRNA-26a promotes cholangiocarcinoma growth by activating β-catenin. Gastroenterology. 2012;143(1):246–56.e8.
101. Olaru AV, Ghiaur G, Yamanaka S, Luvsanjav D, An F, Popescu I, Alexandrescu S, Allen S, Pawlik TM, Torbenson M, Georgiades C, Roberts LR, Gores GJ, Ferguson-Smith A, Almeida MI, Calin GA, Mezey E, Selaru FM. MicroRNA down-regulated in human cholangiocarcinoma control cell cycle through multiple targets involved in the G1/S checkpoint. Hepatology. 2011;54(6):2089–98.
102. Zhang K, Chen D, Wang X, Zhang S, Wang J, Gao Y, Yan B. RNA interference targeting slug increases cholangiocarcinoma cell sensitivity to cisplatin via upregulating PUMA. Int J Mol Sci. 2011;12(1):385–400.
103. Zender S, Nickeleit I, Wuestefeld T, Sörensen I, Dauch D, Bozko P, El-Khatib M, Geffers R, Bektas H, Manns MP, Gossler A, Wilkens L, Plentz R, Zender L, Malek NP. A critical role for notch signaling in the formation of cholangiocellular carcinomas. Cancer Cell. 2013;23(6):784–95.
104. Xu X, Kobayashi S, Qiao W, Li C, Xiao C, Radaeva S, Stiles B, Wang RH, Ohara N, Yoshino T, LeRoith D, Torbenson MS, Gores GJ, Wu H, Gao B, Deng CX. Induction of intrahepatic cholangiocellular carcinoma by liver-specific disruption of Smad4 and Pten in mice. J Clin Invest. 2006;116(7):1843–52.
105. Farazi PA, Zeisberg M, Glickman J, Zhang Y, Kalluri R, DePinho RA. Chronic bile duct injury associated with fibrotic matrix microenvironment provokes cholangiocarcinoma in p53-deficient mice. Cancer Res. 2006;66(13):6622–7.
106. Fava G, Alpini G, Rychlicki C, Saccomanno S, DeMorrow S, Trozzi L, Candelaresi C, Venter J, Di Sario A, Marzioni M, Bearzi I, Glaser S, Alvaro D, Marucci L, Francis H, Svegliati-Baroni G, Benedetti A. Leptin enhances cholangiocarcinoma cell growth. Cancer Res. 2008;68(16):6752–61.

107. Yang H, Li TW, Peng J, Tang X, Ko KS, Xia M, Aller MA. A mouse model of cholestasis-associated cholangiocarcinoma and transcription factors involved in progression. Gastroenterology. 2011;141(1):378–88, 388.e1–4.
108. Hudd C, Euhus DM, LaRegina MC, Herbold DR, Palmer DC, Johnson FE. Effect of cholecystokinin on human cholangiocarcinoma xenografted into nude mice. Cancer Res. 1985;45(3):1372–7.
109. Farazi PA, Zeisberg M, Glickman J, Zhang Y, Kalluri R, DePinho RA. Chronic bile duct injury associated with fibrotic matrix microenvironment provokes cholangiocarcinoma in p53-deficient mice. Cancer Res. 2006;66(13):6622–7.
110. Wang M, Xiao J, Shen M, Yahong Y, Tian R, Zhu F, Jiang J, Du Z, Hu J, Liu W, Qin R. Isolation and characterization of tumorigenic extrahepatic cholangiocarcinoma cells with stem cell-like properties. Int J Cancer. 2011;128(1):72–81.
111. Lu D, Han C, Wu T. Microsomal prostaglandin E synthase-1 inhibits PTEN and promotes experimental cholangiocarcinogenesis and tumor progression. Gastroenterology. 2011;140(7):2084–94.
112. Jing G, Yuan K, Turk AN, Jhala NC, Arnoletti JP, Zhang K, McDonald JM, Chen Y. Tamoxifen enhances therapeutic effects of gemcitabine on cholangiocarcinoma tumorigenesis. Lab Invest. 2011;91(6):896–904.
113. Yamanaka S, Campbell NR, An F, Kuo SC, Potter JJ, Mezey E, Maitra A, Selaru FM. Coordinated effects of microRNA-494 induce G_2/M arrest in human cholangiocarcinoma. Cell Cycle. 2012;11(14):2729–38.
114. Ko KS, Peng J, Yang H. Animal models of cholangiocarcinoma. Curr Opin Gastroenterol. 2013;29(3):312–8.
115. Campbell DJ, Dumur CI, Lamour NF, Dewitt JL, Sirica AE. Novel organotypic culture model of cholangiocarcinoma progression. Hepatol Res. 2012;42(11):1119–30.
116. Noda T, Shimoda M, Ortiz V, Sirica AE, Wands JR. Immunization with aspartate-β-hydroxylase-loaded dendritic cells produces antitumor effects in a rat model of intrahepatic cholangiocarcinoma. Hepatology. 2012;55(1):86–97.
117. Furubo S, Harada K, Shimonishi T, Katayanagi K, Tsui W, Nakanuma Y. Protein expression and genetic alterations of p53 and ras in intrahepatic cholangiocarcinoma. Histopathology. 1999;35(3):230–40.
118. Kang YK, Kim WH, Jang JJ. Expression of G1-S modulators (p53, p16, p27, cyclin D, Rb) and Smad4/Dpc4 in intrahepatic cholangiocarcinoma. Hum Pathol. 2002;33(9):877–83.
119. Tanno S, Yanagawa N, Habiro A, Koizumi K, Nakano Y, Osanai M, Mizukami Y, Okumura T, Testa JR, Kohgo Y. Serine/threonine kinase AKT is frequently activated in human bile duct cancer and is associated with increased radioresistance. Cancer Res. 2004;64(10):3486–90.
120. Aisaka Y, Watanabe Y, Kamiyasu M, Masanaga T, Tsuji K, Nakanishi T, Kajiyama G, Gershwin ME. Immune-mediated cholangiohepatitis in neonatally thymectomized mice: the role of T cells and analysis of T-cell receptor Vbeta usage. J Autoimmun. 2000;14:239–46.
121. Wakabayashi K, Yoshida K, Leung PS, et al. Induction of autoimmune cholangitis in non-obese diabetic (NOD).1101 mice following a chemical xenobiotic immunization. Clin Exp Immunol. 2008;155:577–86.
122. Ambrosini YM, Yang GX, Zhang W, Tsuda M, Shu S, Tsuneyama K, et al. The multi-hit hypothesis of primary biliary cirrhosis: polyinosinic-polycytidylic acid (poly I:C) and murine autoimmune cholangitis. Clin Exp Immunol. 2011;166:110–20.
123. Ide T, Sata M, Suzuki H, Uchimura Y, Murashima S, Shirachi M, et al. An experimental animal model of primary biliary cirrhosis induced by lipopolysaccharide and pyruvate dehydrogenase. Kurume Med J. 43:185–8.
124. Mattner J, Savage PB, Leung P, Oertelt SS, Wang V, Trivedi O, et al. Liver autoimmunity triggered by microbial activation of natural killer T cells. Cell Host Microbe. 2008;3:304–15.
125. Wang JJ, Yang GX, Zhang WC, Lu L, Tsuneyama K, Kronenberg M, Véla JL, Lopez-Hoyos M, He XS, Ridgway WM, Leung PS, Gershwin ME. Escherichia coli infection induces autoimmune cholangitis and anti-mitochondrial antibodies in non-obese diabetic (NOD).B6 (Idd10/Idd18) mice. Clin Exp Immunol. 2014;175(2):192–201.

The Microbiome and Human Disease: A New Organ of Interest in Biliary Disease

5

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Abstract

The composition of the gut microbiome is different in patients and controls in many conditions including some biliary diseases. The liver's close relationship with the gut makes it the primary recipient of degradation products and metabolites originating from the gut. The gut microbiome is therefore important for liver health. The microbiome also has a major influence on bile acid homeostasis, making it particularly relevant for the bile ducts. To what degree it is active in the pathogenesis or progress of biliary diseases is however not known. Still, there is a clinical potential as biomarkers of diagnosis, disease stage or activity. Furthermore, gut microbiome interventions could theoretically be one means of adjusting the biliary environment. This research field is advancing rapidly and provides hopes of new treatment options in the era of personalized medicine.

Take-Home Points

- The gut microbiome is an integrated part of the human immune system and metabolism but varies extensively between individuals.
- The liver is continuously exposed to portal blood from the gut making the microbiome an important element in all liver diseases.

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G. Hirschfield et al. (eds.), *Biliary Disease*, DOI 10.1007/978-3-319-50168-0_5

- The gut microbiome has a key role in bile acid homeostasis due to bile acid transformations and can influence the biliary environment in bile duct diseases.
- Gut microbial profiles or metabolites originating from the microbiome may become important in the diagnosis and categorization of diseases.
- Gut microbiome-targeted treatment may both prove a role of the microbiome in a disease and represent new therapeutic options within the foreseeable future.

5.1 Introduction

Recently there has been an increasing interest in the role of the microbiome, i.e. the normal microflora covering the epithelial surfaces of the body, in human health and disease. The microbiome varies between different body locations in part reflecting different environment and functions. The human gut contains about 100 trillion (10^{14}) microbes, ten times more than the number of cells in the human body. Not surprisingly, this large organ has a strong impact on the host metabolism and immune system and possibly behaviour. Alterations in the gut microbiome in patients, i.e. dysbiosis, have been observed in multiple metabolic and inflammatory conditions including obesity and type 2 diabetes, atherosclerosis, inflammatory bowel diseases and various autoimmune disorders.

A pathogenetic link between the liver and gut is well established. In advanced liver disease, there is a long history of treating complications with microbiome modulation; hepatic encephalopathy is in part alleviated by lactulose, which is a prebiotic, and more recently by rifaximin, an antibiotic acting solely in the gut, representing good evidence that dysbiosis is relevant in end-stage liver disease. More generally, the liver is continuously exposed to nutrients and bacterial products from blood it receives from the gut via the portal vein. These are not passively absorbed but are translated into metabolic and immunologic outcomes [1]. Importantly, the gut microbiome may be considered an integral part of the human metabolism, and the gut-liver axis therefore goes far beyond a simple concept of leakage of toxic bacterial products [2]. Rather, there are bidirectional effects where host factors can induce changes in the gut microbiome that, in return, affect host biology, making the gut microbiome a part of chronic liver disease independent of aetiology.

Biliary disease as a group represents a diverse collection of conditions ranging from classic monogenic diseases to polygenic conditions strongly influenced by lifestyle and other environmental factors. A shared feature of these conditions is however the importance of the biliary environment, highlighting the gut microbiome and its impact on bile acid homeostasis as particularly relevant. Since microbial colonization of bile ducts is a frequent phenomenon in biliary diseases, the possible effects of the biliary microbiome should also be considered.

This chapter will give a brief overview of the microbiome and its possible effects in biliary disease. The future role and importance of microbiome medicine is not clearly defined, but there is already strong data suggesting that treatment specifically targeting the gut microbiome may be effective in other conditions, both within and outside the gastrointestinal tract. In addition, gut microbiome alterations are easily detected by modern technology and may have diagnostic or prognostic value. While there is an urgent need to understand the disease mechanisms, the potential of the microbiome in treatment and diagnosis suggests that microbiome medicine may have clinical implications even before these mechanisms are fully elucidated.

5.2 The Study of the Healthy Gut Microbiome

We are generally considered to be sterile in utero, although the presence of a foetal microbiome has not been ruled out [3]. Colonization of the epithelial surfaces starts at birth with bacteria from the birth canal or from the skin if delivered by caesarean section. Clinical implications of these widely different starting points in life are so far not properly defined. The microbiome gradually develops until a grown-up-like composition at about 2–5 years of age, particularly driven by major life events like dietary changes (from breast-feeding to solid foods) and illness or drug treatment [3]. Genetic factors have a definite influence on the microbiome, but the measureable effects of genetics on the microbial profile in adults are modest [4]. The early developments of the gut microbiome suggest that early environmental influences and interventions may be of particular importance for later health [3]. This is an area of intense research, and indeed, in experimental models, early antibiotics have profound effects on later metabolism and immunity [5]. Age itself is an influential factor, together with gender and geography [6]. However, the long-term dietary pattern seems to be major driver of the gut microbiome [7]. The adult gut microbiome is highly variable between individuals [8, 9]. With some day-to-day variation, it is generally fairly stable over time (with some drift) but sensitive to major interventions like diet changes and antibiotics.

Modern microbiome research has been made possible in part because of technical advances and new analytical methods originating from genetics [10]. Rather than relying on culture technics from classic microbiology, high-throughput sequencing-based analyses of all microbial DNA and RNA present in different environments have profoundly increased our knowledge on the contents and diversity of the microbiomes of the body [8, 9]. Depending on the purpose, it is possible to exploit the diversity of a single bacterial gene between species (*16S rRNA*), to provide a profile of microbial content. This profile will both include measures of diversity, i.e. the richness of species and evenness of their prevalence. It is also possible to acquire a more complete overview of the functional content of the microbiome by sequencing all bacterial genes or the expression levels of these genes. Of interest, the available data suggest that while the gut microbiomes may vary widely between individuals on a species level, the functional content of the different microbiomes are much more similar [9], as would be expected in a human organ.

In experimental research, the use of gnotobiotic animals has made it possible to study the mechanistic role of the microbiome in health and disease. In gnotobiotic animals, the complete microbial colonization status is known; typically they are germ-free or colonized with one or a few bacterial strains. Germ-free animals have provided strong evidence of an instrumental role of the gut microbiome in models of, e.g. obesity and colitis [11, 12]. This was, e.g. shown by faecal transplantation from mice with genetically induced obesity to germ-free mice without the gene defect, but still inducing obesity, illustrating both the effect of the gut microbiome itself and also the bidirectional effects between host and gut. Gut microbes produce a large number of small molecules, peptides and proteins that may be involved in physiological processes, making integrated analyses of the metabolome and microbiome increasingly important [13]. Finally, the biggest scientific advances have come in the study of bacteria, but fungi and viruses are also important members of the microbiome with possible clinical implications.

5.3 Pathogenetic Mechanisms of the Microbiome in Biliary Disease

5.3.1 Microbial Bile Acid Transformations

As evident from Chap. 4, "bile acid signalling and the current understanding of cholestasis: new opportunities for treatments", targeting bile acid homeostasis may be important in biliary disease. A complete understanding of bile acid homeostasis is only possible by including the gut microbiome. While primary bile acids cholic acid and chenodeoxycholic acid are synthesized in the liver from cholesterol, conjugated to taurin or glycine and released into the intestine, gut bacteria actively modify the bile acid pool through deconjugation (e.g. turning glycocholic acid into cholic acid, an ability exhibited by many bacteria) and 7-α dehydroxylation (performed by a minority of species), generating secondary bile acids deoxycholic acid and lithocholic acid [14]. The microbial modifications influence the properties of the individual bile acids as signalling molecules, directly regulating bile acid homeostasis and other metabolic pathways, significantly expanding the effects of therapies affecting the gut microbiome. Reversely, bile acids have antimicrobial properties and may inhibit bacterial growth, although some bacteria are specifically stimulated by certain bile acids [15].

The role of the gut microbiome in bile acid homeostasis was dramatically shown in a study in germ-free mice, where no secondary bile acids were observed and only conjugated primary bile acids were excreted in the faeces [16]. The bile acid pool was also significantly reduced in conventionally raised mice compared with germ-free, and there were major differences in expression of genes involved in bile acid synthesis, conjugation and reabsorption shown to be dependent of the degree of negative feedback on the bile acid synthesis from intestine where bile acids are reabsorbed. In humans, treatment with vancomycin (but not amoxicillin) reduced the ability of the gut microbiome to dehydroxylate bile acids, causing reduced

faecal excretion and serum levels of secondary bile acids. In addition, bile acid synthesis was increased probably caused by reduced negative feedback from the intestine [17].

Reversely, when no bile acids entered the intestine in a mouse model of acute cholestasis-induced bile duct ligation, intestinal bacterial overgrowth rapidly developed but without altering the relative microbial profile [18]. However, in parallel there was a fast increase in bacterial translocation and a reduction in intestinal barrier proteins.

5.3.2 Other Metabolic Effects of the Gut Microbiome

Besides bile acid transformations, the gut microbiome influence many other aspects of the metabolism. These include the well-known role in vitamin synthesis, but also the generation of short-chain fatty acids from dietary fibre, metabolism of lipids, indole derivatives (e.g. tryptophan) and many others [13]. Several of these can be linked to important physiological functions and disease states, but for the vast majority the role and mechanisms of action are unknown. In theory, microbial metabolites may be actively involved in several human disease states. A milestone in this field was the identification of trimethylamine-N-oxide (TMAO) as a biomarker in cardiovascular disease, as well as a possible mediator in atherosclerosis. The interesting characteristic of TMAO is that it is synthesized in the liver from trimethylamine, which may only be generated in the gut by bacterial metabolism of dietary choline and carnitine. Overall, this creates a direct link between diet, the gut microbiome and a human disease, which could be speculated to have parallels in other conditions, not the least in the liver and bile ducts with the particular exposure of portal blood from the gut.

5.3.3 The Gut Microbiome and the Immune System

Several biliary diseases involve a dysregulated immune system. The intestine has a dual and almost contradictory role of being tolerant to food antigens and the commensal bacteria while keeping the ability to protect against pathogens [19]. While translocation of bacterial products (endotoxin and other pathogen-associated microbial patterns) may activate the immune system and cause liver inflammation, it is also evident that an intact innate immune system responding to such patterns are necessary for intestinal homeostasis [1].

The barrier itself consists of thin epithelium as well as two protective mucus layers (in the colon) [19]. In germ-free mice, the number of mucus-producing cells is reduced and the mucus layer underdeveloped, showing that stimulation by the gut microbiome is required for its development. The gut microbiome also influences the development and regulation of several components of the immune system. While lymphoid structures are initiated during foetal development, the normal gut-associated lymphoid tissue requires microbial colonization after birth to develop

properly. The microbiome also modulates the differentiation of lymphocytes. In experimental systems, it has been observed both increases of anti-inflammatory T regulatory cells were driven by, e.g. *Bacteroides fragilis* and certain *Clostridium* species [20, 21], but also pro-inflammatory T cells induced by segmented filamentous bacteria [22]. Such changes are not only confined to the intestine but may influence the systemic lymphocyte populations and inflammatory disease outside the gut. These lessons come from gnotobiotic systems, and to what extent modulation of a complex adult-like gut microbiome in adults may influence immune activation in human disease is not known.

5.4 The Microbiome in Biliary Disease

Direct evidence linking the microbiome of the gut or biliary tree to the development of human biliary disease is weak but several observations suggest that it has an impact. Based on studies using classical microbiological methods, the biliary tract is generally considered sterile. In contrast, several biliary disorders like primary sclerosing cholangitis (PSC), choledocholithiasis and malignant obstructive conditions are often associated with bacteriobilia and cholangitis. These are probably secondary phenomena but could influence outcome as shown by an association between colonization of bile and poor outcome in PSC.

Only a few studies have so far been performed in biliary disease applying sequencing-based methods. Gallstone disease has been investigated in a few studies, but these are small and with mostly nonoverlapping methods and results [23, 24]. Several bacterial taxa had different abundances in patients and controls; the most consistent being a reduction of faecal *Roseburia* in gallstone patients, while a reduced diversity of the gut microbiome in gallstone patients has not been consistently reported. The microbiome of the bile and the stones may also turn out to be of importance in this condition.

In primary biliary cirrhosis, the strongest link to the gut microbiome is the observation that the key autoantibodies antimitochondrial antibodies may cross-react with proteins in *E.coli* as well as other bacteria, suggesting that an immune response against the microbiome may be involved in the pathogenesis [1]. A similar observation has been made in PSC. The most common autoantibodies in PSC, perinuclear anti-neutrophil cytoplasmic antibodies, may cross-react with bacterial proteins. PSC has a priori the strongest connection to the gut microbiome, classically considered an extra-intestinal manifestation of inflammatory bowel disease (IBD), which affects up to 80% of the patients. The first treatment trials in PSC used antibiotics, and both metronidazole and vancomycin have been shown to improve liver function tests. Thus, while effects on clinical endpoints have not been shown, the disease process seems to be sensitive to manipulation of the gut microbiome.

The first studies of the gut microbiome in intestinal mucosa [25] and stool (own data) in PSC show a reduced diversity of microbes, similar to what has been seen in many inflammatory conditions. Several bacterial taxa have different prevalence in PSC patients, IBD patients without PSC as well as healthy controls, but whether

these findings can be linked to the disease process or truly are IBD independent is so far uncertain. Also, a variant in the fucosyl transferase 2 gene (*FUT2*), a genetic risk factor in PSC and IBD, has been shown to influence the microbiota both in the gut and bile [26]. This may relate to reduced availability of fucosylated glycans as growth substrates or adhesion molecules for bacteria. An important hypothesis also suggests that the biliary inflammation in PSC could be caused by T cells primed in the gut [27], which could fit both with the observed cross reactivity of autoantibodies and the altered gut microbiome. An alternative link between intestinal inflammation and biliary disease has been observed in animal models of small bowel bacterial overgrowth, mediated by leakage of bacterial products [28].

Experimental evidence can also shed light on the effects of biliary disease. In cholangiocarcinoma, mouse models of infection with the carcinogenic liver fluke *Opisthorchis viverrini* modifies both the intestinal and biliary microbiome, which may contribute to the inflammatory carcinogenic process [29]. In mice where the multidrug resistance 2 gene (*Mdr2−/−*) is knocked out, a commonly used mouse model of PSC, the hepatobiliary disease markedly worsened in a germ-free environment [30]. In contrast, several models of other diseases driven by primary immune mechanisms are alleviated in a germ-free state. In vitro data suggested that the effect in *Mdr2−/−* in part could be explained by the absence of commensal microbial metabolites, e.g. secondary bile acids. Beyond providing a proof of concept that biliary disease may be influenced by the microbiome, it also suggests that individuals with human *ABCB4* (the human *Mdr2* ortholog)-related biliary phenotypes, i.e. progressive familial cholestasis type 3 or subgroups of PSC patients or other unclassified cholestatic syndromes with *ABCB4* defects, may be sensitive to microbiome manipulation.

5.5 Clinical Opportunities in Biliary Disease

Clinical translation is an important aim of microbiome research. While clinical translation of advances in human genomics has taken time, there is justified hope that the gut microbiome will be part of individualized treatment plans in several diseases soon, both during a diagnostic workup and as part of therapy. The intense research activity and the use of experimental models mean that new treatment can be developed and tested long time before we have full mechanistic insight.

5.5.1 Predictive Power of the Microbiome

In cross-sectional studies, the gut microbiota profiles in many patient populations are quite different from healthy individuals. Irrespective of these being primary or secondary changes, simple indices calculated from the abundance of only a minor number of bacteria in stool or gut mucosa exhibit diagnostic value in diseases like type 2 diabetes and Crohn's disease [31]. We have made similar observations in PSC (Fig. 5.1). A diagnostic tool separating biliary disease from perfect health

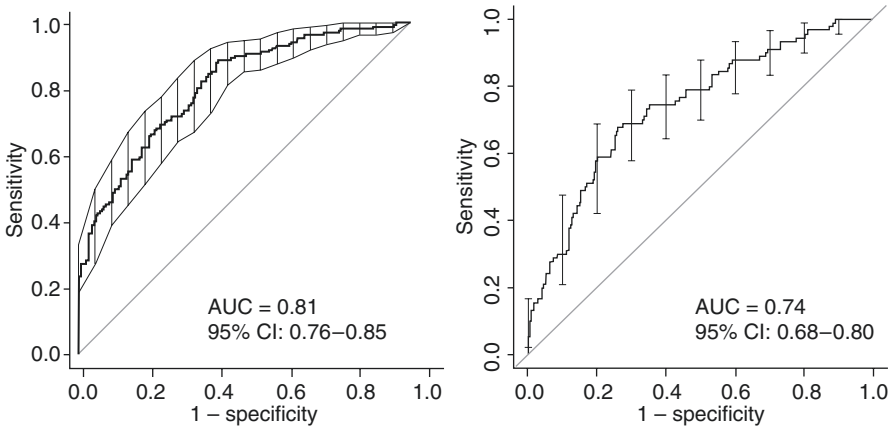

Fig. 5.1 Diagnostic value of microbiome indices in type 2 diabetes and primary sclerosing cholangitis. Area under the receiver operating characteristic curve to identify type 2 diabetes (*left*) and primary sclerosing cholangitis (*right*) from healthy controls based on n~50 bacterial gene markers (*left*) and a simple index based the on abundance of 8 bacterial taxa (*right*). The *left panel* is reprinted by permission from (Macmillan Publishers Ltd.: *Nature* [36] © 2012. The *right panel* is printed by courtesy of Martin Kummen)

may not sound clinically valuable, but conceptually this opens the door for studies aiming to diagnose useful categories. In mice, liver diseases of different aetiology have been associated with different microbiome profiles [18], suggesting that a gut profile could help diagnosing a specific biliary disease (e.g. PSC vs. PBC) or relevant subgroups or complications in a biliary disease (e.g. cholangiocarcinoma in PSC). Since the gut microbiome influences biliary physiology, characteristics of the microbiome may also have value as a staging parameter, prognosticator or as treatment guide. This is so far best shown in studies in IBD [31, 32], where the microbial profiles of mucosal biopsies were predictors of disease activity at 6 months and response to anti-TNF antibodies, respectively. Importantly, the microbiome may not only be assessed by genetic methods in the gut. There is also a large potential in known, or so far unknown, products of microbial metabolism measured in blood or stool, exemplified by the role of TMAO in cardiovascular disease described above. Dedicated studies are therefore needed to address the diagnostic and prognostic values of the microbiome and its metabolites in biliary disease.

5.5.2 Gut Microbiome as Therapeutic Target

The gut microbiome is particularly attractive as a novel treatment target. An interesting starting point is faecal microbiota transplantation (FMT), which could be considered an external reset of the system. FMT has a long history dating back to the fourth century and treatment for abdominal conditions in traditional Chinese medicine. In Western medicine it has been used for many years in individual cases of recurrent *C. difficile* infections. More recently the potent effect of

FMT for this condition has been documented in randomized controlled trials, and its use is increasing.

Following the interest in the microbiome in many areas of medicine, FMT is tested in a wide variety of conditions, within or outside clinical trials. Besides providing a new treatment option, well-designed FMT trials also provide the strongest evidence for a role of the gut microbiome in the development or progression of human disease. A landmark study from the Netherlands, the randomized controlled FAT-LOSE trial, provided such evidence in metabolic diseases by showing that allogenic FMT from lean people to obese people improved insulin sensitivity [33]. No studies of FMT have so far been published in biliary diseases, but based on the close relationship with IBD, the first trials are likely to be performed in PSC.

A critical question when changing focus from life-threatening conditions like *C. difficile* to lifestyle diseases is FMT-related risk. There has been a strong focus on pretreatment donor screening for contagious diseases, but the possible major long-term effects on susceptibility to metabolic or inflammatory conditions are rarely discussed. A series of technical issues also need to be resolved like choice of administration route, how many treatments are needed and how often and, importantly, choice of donor. After all, if the microbiome is really that important, it implies that all donors are not as useful and that all recipients should not receive the same treatment.

For chronic conditions, a drug-like agent targeting the gut microbiome is probably more attractive. Possible agents include probiotics, prebiotics and antibiotics. Probiotics are defined as live microorganisms that are not constituents of the host microbiome but confer a health benefit to the host [34]. The bacterial strains currently available are of limited value, and, in line with this, no effect has been observed in small trials in PSC. The prospects do however include new and more effective bacterial cocktails designed on the basis of effects observed in FMT trials or experimental studies, or even bacterial strain genetically engineered to produce beneficial metabolites. Prebiotics are nondigestible food components (typically dietary fibre) that are selectively metabolized by beneficial members of the gut microbial community and therefore act to stimulate their growth [34]. Prebiotics have been shown to alter the gut microbiome, but only modest clinical effects have so far been observed in, e.g. type 2 diabetes. Choice of prebiotic in treatment trials could be motivated by the microbiome alterations, e.g. intending to increase microbiome diversity in the gut. Antibiotics have strong impact on the gut microbiome and may influence the disease process in, e.g. PSC (see above). While still not ruled out as a possible drug option in PSC, the major impact on the microbiome (with unknown consequences for host physiology) and risk of antibiotic resistance make chronic antibiotic treatment unattractive long term.

5.5.3 Lifestyle Advice in Biliary Disease

The importance of diet in the shaping of the gut microbiome opens the door for more efficient disease preventive lifestyle advice on the population level (“eat yourself healthy”) but also protective or disease-targeted diets in patients. Extraordinary

examples of diets causing microbiome-related disease include an experimental model of obesity-driven hepatocellular carcinoma, which was shown to be caused by increased deoxycholic acid levels produced by increased numbers of gram-positive bacteria able to generate secondary bile acids [35], and a milk fat-driven model of colitis caused by diet-related increases of taurin-conjugated bile acids with subsequent increases of the bacterium *Bilophila wadsworthia* [15]. In biliary diseases, with a possible exception for gallstone disease, little is known about disease-associated and healthy diets. Importantly, the same diet is not necessarily advisable for everyone, and gut microbial profile or other biomarkers could be speculated to provide guidance. Needless to say, there is a lot of work undone in this field.

5.5.4 Pharmacomicrobiomics

Slightly on the side is the increasing awareness that common drugs modulate or are being modulated by the microbiome. This modulation could contribute to the effect but also to the lack of effect in some individuals. One classical example is digoxin, which is inactivated in more than one out of ten by strains of the gut bacterium *Eggerthella lenta*. Given the actions of bile acids on microbes, drugs like ursodeoxycholic acid and synthetic bile acid analogues could be speculated to act in part via the gut microbiome. Pharmacomicrobiomics will therefore be a part of personalized microbiome medicine.

Conclusions

The gut microbiome is important in health and disease and may be relevant in several diseases in the foreseeable future as part of the individualized diagnostic workup or as therapeutic target. This field is so far largely uncharted in biliary disease, but there is justified hope that it may provide new clinical opportunities.

References

1. Henao-Mejia J, Elinav E, Thaiss CA, Licona-Limon P, Flavell RA. Role of the intestinal microbiome in liver disease. J Autoimmun. 2013;46:66–73.
2. Schnabl B, Brenner DA. Interactions between the intestinal microbiome and liver diseases. Gastroenterology. 2014;146:1513–24.
3. Rodriguez JM, Murphy K, Stanton C, Ross RP, Kober OI, Juge N, Avershina E, et al. The composition of the gut microbiota throughout life, with an emphasis on early life. Microb Ecol Health Dis. 2015;26:26050.
4. Goodrich JK, Waters JL, Poole AC, Sutter JL, Koren O, Blekhman R, Beaumont M, et al. Human genetics shape the gut microbiome. Cell. 2014;159:789–99.
5. Cox LM, Yamanishi S, Sohn J, Alekseyenko AV, Leung JM, Cho I, Kim SG, et al. Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. Cell. 2014;158:705–21.
6. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, Magris M, et al. Human gut microbiome viewed across age and geography. Nature. 2012;486:222–7.

7. Xu Z, Knight R. Dietary effects on human gut microbiome diversity. Br J Nutr. 2015;113(Suppl):S1–5.
8. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010; 464:59–65.
9. The Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature. 2012;486:207–14.
10. Kuczynski J, Lauber CL, Walters WA, Parfrey LW, Clemente JC, Gevers D, Knight R. Experimental and analytical tools for studying the human microbiome. Nat Rev Genet. 2012;13:47–58.
11. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006;444:1027–31.
12. Garrett WS, Lord GM, Punit S, Lugo-Villarino G, Mazmanian SK, Ito S, Glickman JN, et al. Communicable ulcerative colitis induced by T-bet deficiency in the innate immune system. Cell. 2007;131:33–45.
13. Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, Pettersson S. Host-gut microbiota metabolic interactions. Science. 2012;336:1262–7.
14. Midtvedt T. Microbial bile acid transformation. Am J Clin Nutr. 1974;27:1341–7.
15. Devkota S, Wang Y, Musch MW, Leone V, Fehlner-Peach H, Nadimpalli A, Antonopoulos DA, et al. Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10−/− mice. Nature. 2012;487:104–8.
16. Sayin SI, Wahlstrom A, Felin J, Jantti S, Marschall HU, Bamberg K, Angelin B, et al. Gut microbiota regulates bile acid metabolism by reducing the levels of Tauro-beta-muricholic acid, a naturally occurring FXR antagonist. Cell Metab. 2013;17:225–35.
17. Vrieze A, Out C, Fuentes S, Jonker L, Reuling I, Kootte RS, van Nood E, et al. Impact of oral vancomycin on gut microbiota, bile acid metabolism, and insulin sensitivity. J Hepatol. 2014; 60:824–31.
18. Fouts DE, Torralba M, Nelson KE, Brenner DA, Schnabl B. Bacterial translocation and changes in the intestinal microbiome in mouse models of liver disease. J Hepatol. 2012;56:1283–92.
19. Sommer F, Backhed F. The gut microbiota--masters of host development and physiology. Nat Rev Microbiol. 2013;11:227–38.
20. Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, Fukuda S, et al. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. Nature. 2013;500:232–6.
21. Round JL, Lee SM, Li J, Tran G, Jabri B, Chatila TA, Mazmanian SK. The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. Science. 2011;332:974–7.
22. Wu HJ, Ivanov II, Darce J, Hattori K, Shima T, Umesaki Y, Littman DR, et al. Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. Immunity. 2010;32:815–27.
23. Wu T, Zhang Z, Liu B, Hou D, Liang Y, Zhang J, Shi P. Gut microbiota dysbiosis and bacterial community assembly associated with cholesterol gallstones in large-scale study. BMC Genomics. 2013;14:669.
24. Keren N, Konikoff FM, Paitan Y, Gabay G, Reshef L, Naftali T, Gophna U. Interactions between the intestinal microbiota and bile-acids in gallstones patients. Environ Microbiol Rep. 2015;7:874–80.
25. Rossen NG, Fuentes S, Boonstra K, D'Haens GR, Heilig HG, Zoetendal EG, de Vos WM, et al. The mucosa-associated microbiota of PSC patients is characterized by low diversity and low abundance of uncultured Clostridiales II. J Crohns Colitis. 2015;9:342–8.
26. Folseraas T, Melum E, Rausch P, Juran BD, Ellinghaus E, Shiryaev A, Laerdahl JK, et al. Extended analysis of a genome-wide association study in primary sclerosing cholangitis detects multiple novel risk loci. J Hepatol. 2012;57:366–75.

27. Adams DH, Eksteen B. Aberrant homing of mucosal T cells and extra-intestinal manifestations of inflammatory bowel disease. Nat Rev Immunol. 2006;6:244–51.
28. Lichtman SN, Keku J, Clark RL, Schwab JH, Sartor RB. Biliary tract disease in rats with experimental small bowel bacterial overgrowth. Hepatology. 1991;13:766–72.
29. Plieskatt JL, Deenonpoe R, Mulvenna JP, Krause L, Sripa B, Bethony JM, Brindley PJ. Infection with the carcinogenic liver fluke *Opisthorchis viverrini* modifies intestinal and biliary microbiome. FASEB J. 2013;27:4572–84.
30. Tabibian JH, O'Hara SP, Trussoni CE, Tietz PS, Splinter PL, Mounajjed T, Hagey LR, et al. Absence of the intestinal microbiota exacerbates hepatobiliary disease in a murine model of primary sclerosing cholangitis in mice. Hepatology. 2016;63:185–96.
31. Gevers D, Kugathasan S, Denson LA, Vazquez-Baeza Y, Van Treuren W, Ren B, Schwager E, et al. The treatment-naive microbiome in new-onset Crohn's disease. Cell Host Microbe. 2014;15:382–92.
32. Kolho KL, Korpela K, Jaakkola T, Pichai MV, Zoetendal EG, Salonen A, de Vos WM. Fecal microbiota in pediatric inflammatory bowel disease and its relation to inflammation. Am J Gastroenterol. 2015;110:921–30.
33. Vrieze A, van Nood E, Holleman F, Salojarvi J, Kootte RS, Bartelsman JF, Dallinga-Thie GM, et al. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in subjects with metabolic syndrome. Gastroenterology. 2012;143:913–6.
34. Backhed F, Fraser CM, Ringel Y, Sanders ME, Sartor RB, Sherman PM, Versalovic J, et al. Defining a healthy human gut microbiome: current concepts, future directions, and clinical applications. Cell Host Microbe. 2012;12:611–22.
35. Yoshimoto S, Loo TM, Atarashi K, Kanda H, Sato S, Oyadomari S, Iwakura Y, et al. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. Nature. 2013;499:97–101.
36. Qin J et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature. 2012;490:55–60.

Biliary Atresia: From Pathology to Treatment

6

Jane Hartley and Deirdre Kelly

Abstract

Biliary atresia is a rare disease of infancy with an estimated incidence in Europe of 1 in 15,000–19,000. The disease onset is confined to the neonatal period, and there is no equivalent liver disease outside of this period. It affects all races and ethnic groups, although black mothers are 2.5 times more likely to have a baby with biliary atresia than other ethnic groups. The pathogenesis of biliary atresia is unknown; and research to further understand the underlying mechanisms of its development is disadvantaged by the rarity of disease and lack of correlation between human and animal models. A remarkable medical achievement has been the Kasai portoenterostomy surgical procedure, which along with liver transplantation (when necessary) has increased survival into adulthood with a good quality of life.

Take-Home Points

- Biliary atresia is a rare disease of the liver and bile ducts that affects infants. Its pathogenesis is unknown; however, multifactorial causes have been reported, with the end phenotype being progressive fibrosis with obliteration of the biliary tree.
- Jaundice and pale stools are indicative of biliary atresia. Operative cholangiogram is however the gold standard and definitive test for its detection.
- The development of the Kasai portoenterostomy procedure and liver transplantation has increased survival of patients.

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G. Hirschfield et al. (eds.), *Biliary Disease*, DOI 10.1007/978-3-319-50168-0_6

- Despite a successful Kasai, fibrosis may develop resulting in portal hypertension and potential need for liver transplantation in childhood or adulthood.
- Further research on molecular pathogenesis of biliary atresia and development of fibrosis is vital for development of novel therapies.

6.1 Introduction

Biliary atresia is a rare disease of infancy with an estimated incidence in Europe of 1 in 15,000–19,000 [1, 2]. The disease onset is confined to the neonatal period, and there is no equivalent liver disease outside of this period. Over the past 30 years, biliary atresia has changed from being universally fatal in infancy to one in which a palliative surgical procedure (Kasai portoenterostomy) and liver transplantation, if necessary, facilitates survival into adulthood with a good quality of life.

Biliary atresia can be divided into three variants [3]:

- Isolated biliary atresia in which there are no other developmental anomalies.
- Biliary atresia splenic malformation syndrome (BASM) in which biliary atresia is associated with some or all of the following features:
  - Pre-duodenal portal vein
  - Polysplenia or asplenia
  - Interrupted inferior vena cava
  - Situs inversus and malrotation
  - Cardiac anomalies
- Other random congenital anomalies which are associated with biliary atresia are anorectal malformations, oesophageal atresia and jejunal atresia. Cat eye syndrome with coloboma and anorectal anomalies may also have biliary atresia, and this is due to chromosome 22 aneuploidy.

Cystic type biliary atresia or non-visualisation of the foetal gall bladder may provide antenatal suspicion of biliary atresia, which improves postnatal confirmation and planning timely surgery [4]. Interestingly, non-visualisation of the gall bladder with low amniotic gamma-glutamyl transpeptidase (GGT) levels is highly indicative of biliary atresia [5].

The exact cause of biliary atresia remains elusive despite much research which suggests either a multifactorial cause or a number of different pathological processes with the end phenotype being that of biliary atresia.

6.2 Epidemiology and Registries

Biliary atresia affects all races and ethnic groups, but the incidence and susceptibility to the disease vary across the world. The highest reported incidence is in Taiwan with an incidence of 1 in 5000 [6]. Estimates in the USA are 1:15,000 with black

mothers being 2.5 times more likely to have a baby with biliary atresia than other ethnic groups [7]. There appears to be no seasonal or gender predominance [8]. Due to the rarity of the disease, registries have been developed in many countries to increase the quality of data for study. Individual European countries such as The Netherlands and France have their own registries with the UK being the first to collate nationwide data. For 5 years, 60 centres from 19 different European countries collaborated to form EBAR (European Biliary Atresia Registry) and collected data from over 500 patients with biliary atresia. The North American registry, ChiLDREN (Childhood Liver Disease Research and Education Network), not only collects data but also has a central repository for biological samples.

6.3 Pathogenesis

There are many different hypotheses regarding the pathogenesis of biliary atresia but none that can be applied to all cases, suggesting there are multifactorial causes with the end phenotype being progressive fibrosis with obliteration of the biliary tree. There is no association between seasonal clustering, smoking, maternal age, education, income, alcohol or folic acid intake, gravidity, preterm birth or infant weight. When bile acids were measured in healthy newborn babies (using the Guthrie card), 77% of those with biliary atresia could be identified suggesting that biliary atresia is established prior to birth and is not a postnatal event [9].

Research is hampered by the rarity of the disease and lack of correlation between human and animal models.

6.3.1 Animal Models

Recently the sea lamprey has been suggested to be a good animal model to study the pathogenesis and to identify treatment options as during its development it undergoes a transient period of biliary atresia with cholestasis and fibrosis. Understanding the molecular process of this may provide further insight into human biliary atresia [10].

The murine model in which biliary atresia develops following intraperitoneal injection of reovirus has been shown to have similar features of fibrosis to those of human biliary atresia and therefore is a good model for use in studying biliary atresia [11]. Following inoculation of the intraperitoneum with rotavirus, the genes which are subsequently upregulated within the liver are involved in immune biological processes. Six genes have been identified to predominate, CCL3, CXCL5, CXCL13, CXCR2, CCL5 and CCL6, and may prove to be useful targets for treatment in the future [12].

6.3.2 Immunity

The inflammatory response with a periductal infiltrate of mononuclear cells is the likely mechanism of bile duct damage in biliary atresia, although the trigger for this remains unknown. There is increased expression of HLA-DR and adhesion

molecules (ICAM1 and E-selectin) and increased soluble inflammatory molecules and cytokines (interleukin 2, interleukin 18 and TNF**α**) [13, 14].

P-selectin (CD-62P) is an adhesion molecule in which recruitment and accumulation of inflammatory cells are dependent. Compared to other neonatal cholestatic conditions, the frequency of P-selectin expressed in the endothelium, platelets and bile duct epithelium is significantly higher in those with biliary atresia, suggesting that adhesion molecules may play a role in the inflammatory process of biliary atresia [15]. Additional studies have found other inflammatory cells such as natural killer cells and their activity to be important in the continuing inflammation and progression to fibrosis.

6.3.3 Genetic

Biliary atresia is not inherited in a Mendelian pattern. Even in identical twins, there is discordance of symptoms. The syndromic type of biliary atresia suggests there is an event crucial in the developmental pathway, but this has yet to be elucidated. The *INV* mouse model for biliary atresia develops situs inversus and biliary atresia with pathological changes in the ductal plate [16]. However the effect of mutations in the *INV* gene is species specific, and humans with BASM do not have mutations in *INV*. In humans with mutations in *INV*, situs inversus occurs, but biliary atresia has not been associated [17].

Numerous genome-wide association studies have investigated potential genetic susceptibility. No two studies have identified the same susceptibility genes; however, all studies have identified genes which when knocked down in the zebrafish result in reduced bile flow; they are associated with organogenesis (glypican 1 or GPC1 [18] and adenosine diphosphate ribosylation factor 6 or ARF6 [19]) or have a greater expression in the neonatal liver than in adults (adducin 3 or ADD3 [20, 21]). The changes in these genes are unlikely to be sufficient to cause biliary atresia on their own but may cause susceptibility, with additional influences being other genetic variants or extraneous triggers [22].

6.3.4 Toxins

An outbreak of biliary atresia has occurred in lambs born to ewes that had ingested the plant isoflavonoid, biliatresone. Biliatresone causes destruction of the extrahepatic biliary tree but not the intrahepatic. Host genetic susceptibility may also be important. The toxin also affects cilia and disrupts cell polarity that may account for the phenotype of biliary atresia splenic malformation syndrome and hence makes toxins an attractive aetiological factor in biliary atresia development. The evidence for biliatresone implication in human biliary atresia is yet to be studied [23].

6.3.5 Viral Infection

Viruses as a cause of biliary atresia have been an attractive hypothesis and have been extensively investigated; however, no conclusive evidence for infection has been found. Initial studies have shown that mice inoculated with rotavirus strains RRV and SA11-FM developed jaundice and histological findings similar to that of biliary atresia in humans [24]. In a study of 74 liver biopsies from children at diagnosis of biliary atresia, in only a third of cases, a virus was identified, and the detection rate increased with age suggesting that the viruses are acquired. On long-term follow-up, those who had viruses identified had similar outcome to those who did not [25]. The frequency of viruses in the population and the rarity of biliary atresia also reduced the likelihood that it is the sole cause. It may however be the instigator for immune dysregulation.

6.3.6 Cilia

Primary cilia are important for the flow of bile within bile ducts as well as normal placement of abdominal situs. The association of laterality abnormalities in BASM suggests cilia may be affected. Indeed biliary cilia from infants with biliary atresia are reduced in number and are abnormal in morphology compared to normal liver or other liver pathology [26, 27].

6.3.7 Alloimmune Response

The identification of maternal CD8+ T cells in the bile ducts of babies with biliary atresia suggests that there may be an alloimmune process akin to graft-vs-host disease, occurring in utero [28].

6.4 Screening for Biliary Atresia

To enable the Kasai procedure to be carried out at the optimal time, it is imperative to diagnose biliary atresia early, and hence screening of infants in the newborn period may facilitate this. Screening cards of stool colour were given to mothers at their infants' 1-month health check, over a 19-year period of time in Japan. The cards were completed by the mothers and returned to the paediatrician or obstetrician, and infants with pale stools underwent further investigations. In this time period, 313,230 babies were born, and 34 were identified as having biliary atresia. The mean time to Kasai was 59.7 days which was no different to before the implementation of the stool colour charts; however, the long-term survival with the native liver was improved. The long-term survival with the native liver showed a large

improvement with the probability of the native liver survival at 5, 10 and 15 years being 87.6%, 76.9% and 48.5% [29]. Nationwide screening using stool colour cards has been adopted by Taiwan. Five years after the introduction of the stool colour card, the rate of successful Kasai has increased from 35% to 61% and the 5-year survival with the native liver from 27% to 64% [6, 30]. Canada has also published on their experience with stool colour cards. Colour cards were given to mothers antenatally who were asked to return the card within 30 days of birth. The improvement in earlier referral with biliary atresia and hence Kasai success has shown that it is potentially effective. Using Markov modelling, it was also shown to be a highly cost-effective method of screening [31].

In England the measurement of conjugated bile acids in the infant blood spot screening card (Guthrie card) has been able to identify babies with biliary atresia in 77% of cases [9]. Other screening techniques which have been studied include serum Apo CII and CIII proteins, urinary sulphated bile acids and faecal conjugated bilirubin. A study of conjugated bilirubin in liquid blood used for neonatal screening tests at 6–10 days of age in 10,079 neonates identified babies with significant liver disease with a sensitivity of 100% and specificity of 99.5%. However to be practical the identification of conjugate bilirubin in the dried blood spot needs to be developed [32]. Nevertheless these techniques require laboratory expertise and are more expensive compared to the stool colour chart.

6.5 Clinical Presentation

Babies are usually born at term (it is rare in preterm infants, but when it does occur, it tends to become evident with pale stools and conjugated jaundice from around the expected due date) with a normal birth weight. Jaundice is usually not evident at birth but develops over the first few days. There may be confusion with physiological jaundice as this is common but also apparent in the first few days of life. The hyperbilirubinemia is unconjugated and resolved by day 14. Therefore if a baby continues to be jaundiced beyond this time, a split bilirubin should be measured to identify conjugated jaundice which indicates liver pathology and hence referral to a paediatric liver centre for further investigation to confirm biliary atresia and exclude other causes [33].

The stool is pale due to the lack of bile pigment. The loss of pigment becomes more obvious with time and is acholic by 6 weeks. The urine is generally dark due to the water-soluble bilirubin conjugates (in nappies the dark urine may cover the stool and be falsely reassuring).

Most babies are born with a normal birth weight and gain adequate weight in the first few weeks. However they are usually hyperphagic due to the poor absorption of long-chain fats, and failure to thrive will occur without medium-chain triglyceride feed supplementation. Breast-fed infants retain their growth better than bottle-fed babies because of the modified fat in breast milk.

On examination most children with biliary atresia have few clinical signs except for jaundice and a slightly enlarged liver. Splenomegaly and ascites are late features suggesting a delayed diagnosis and established fibrosis or cirrhosis. It is important to identify comorbidities such as congenital heart disease and any cardiac murmur or signs that require a detailed cardiology assessment [3].

The differential diagnosis of a jaundiced infant with a raised GGT is shown in Table 6.1. When severe, alpha-1 antitrypsin deficiency is the most likely condition to mimic biliary atresia with raised bilirubin, high GGT and pale stools, it should be excluded by blood alpha-1 antitrypsin levels and protein phenotype before considering laparotomy for biliary atresia.

Table 6.1 Differential diagnosis of infants with neonatal liver disease

Differential diagnosis	Investigation	Results
Alpha-1 antitrypsin deficiency	Level and protein phenotype	Low alpha-1 antitrypsin level PiZZ phenotype
Hypothyroidism	TFT's	Raised TSH Low T4
Hypopituitarism	TFT, cortisol, glucose	Low TSH, cortisol hypoglycaemia
Galactosaemia	Urine-reducing substances Plasma Gal-1-PUT	Positive-reducing substances Absent or reduced Gal-1-PUT detected
Tyrosinaemia	Urine succinylacetone DNA	High succinylacetone Mutations in *FAH*
Alagille syndrome	ECHO Thoracic vertebrae X-ray Slit lamp examination DNA	Peripheral pulmonary stenosis, butterfly shaped thoracic vertebrae, posterior embryotoxon *JAG1* or *NOTCH2* mutations
Congenital infection	Serology urine and blood, PCR-CMV, toxoplasma	Positive testing
Progressive familial intrahepatic cholestasis 3	Gamma-glutamyl transpeptidase (GGT) Liver biopsy DNA	High GGT cholestasis Specific findings on histology Mutation ABCB4
Storage disease, e.g. Niemann-Pick C	Liver biopsy Bone marrow biopsy Filipin staining DNA	Storage cells on bone marrow and liver biopsy (can be difficult to see in young children), positive filipin staining of fibroblasts Mutation in *NPC1 & 2*
Citrin deficiency	Plasma and urine amino acids DNA	Increased plasma and urine citrulline and arginine Mutation in *SLC25A13*
Peroxisomal disorders	Plasma very long-chain fatty acids DNA	High levels of very long-chain fatty acids Mutation in *PEX* genes
Intestinal failure-associated liver disease	Liver biopsy	Specific findings on liver biopsy

Table 6.2 The typical biochemistry of a child with biliary atresia

	Typical concentration at presentation	Normal range
Conjugated bilirubin (μmol/L)	>100	<20
Alkaline phosphatase (IU/L)	>600	<500
γ-Glutamyl transferase (IU/L)	>100	20–40
Aspartate aminotransferase (U/L)	80–200	15–40
Alanine aminotransferase (U/L)	80–200	10–55
Albumin (g/L)	Normal at presentation	37–56
Prothrombin time (seconds)	Normal at presentation	9–13

6.6 Investigations

6.6.1 Blood Investigations

Typical biochemical variables are shown in Table 6.2.

Pitfalls:

- The conjugated bilirubin is typically >100 μmol/L; however, lower levels can be seen and should not be falsely reassuring.
- With the commencement of ursodeoxycholic acid and adequate nutrition, the bilirubin may fall, but this is not sustained and should not be falsely reassuring [34].

Synthetic function (albumin and prothrombin time) are usually normal at presentation unless there is vitamin K deficiency or a late presentation with cirrhosis. Cholesterol may be raised, but triglycerides are usually normal.

6.6.2 Bile Investigations

The Japanese and Chinese have reported continuous attempts to aspirate bile from the third part of the duodenum using a nasoduodenal tube. If there are no bile secretions over a 24 h period, then this is strongly suggestive of biliary atresia [35].

6.6.3 Metabolomics

Research looking into the metabolomics profile of infants with biliary atresia has identified a different profile as compared to other children with neonatal cholestasis. This is not currently in clinical practice but may be a useful investigation in the future [36].

6.6.4 Imaging

Ultrasounds: most infants will have an absent or atretic gall bladder on a fasting ultrasound, although in a small number of infants the gall bladder may be identified

(up to 20%). To allow the gall bladder, if present, to be seen, a 4 h fast prior to the scan is recommended. Some centres also use the triangular cord sign (a thick tubular or triangular echogenic density (fibrous ductal remnant) along the anterior aspect of the portal vein at its bifurcation into the right and left branches) and have found this to be a sensitive marker (49–73%) for identifying biliary atresia; however, it is operator dependent and not always reproducible [37].

Radionucleotide excretion scan: (TBIDA or HIDA) may be useful if there is uncertainty of stool colour. Historically it was a standard test for bile excretion when biliary atresia was suspected; however, if the stool is visually pale, then it adds little to the diagnostic investigations. The injected isotope is taken up by the liver, but the usual excretion pattern into the intestine is not seen in biliary atresia by 24 h. However, in infants with severe cholestasis from any cause, there may be no excretion within 24 h either. This scan is a sensitive test for biliary atresia, but it is not specific. To aid uptake of the radionucleotide, infants should be given phenobarbitone for 3 days prior to the scan; in a recent study, priming with phenobarbitone or ursodeoxycholic acid, however, did not change the excretion pattern of the test compared with no drug augmentation [38].

Endoscopic retrograde cholangiopancreatography (ERCP): can be used to visualise the biliary tract if diagnosis is uncertain. It is safe and feasible in young infants and may avoid an operative cholangiogram [39].

Magnetic resonance cholangiopancreatography (MRCP): currently this technology may not be detailed enough to identify the luminal patency of infant biliary tract that may only be 1 mm in diameter. It may evolve with technical advances to become a useful test [40].

Operative cholangiogram: this is the gold standard and definitive test. Dye is injected into the biliary tree under direct vision at laparotomy or laparoscopically, and the patency of the bile ducts is assessed.

6.6.5 Liver Histology

A percutaneous liver biopsy provides information regarding extrahepatic biliary obstruction. Typically the findings are those of:

- Portal tract fibrosis
- Oedema
- Ductular proliferation
- Cholestasis

The findings occur in any large duct obstruction and are not specific for biliary atresia; however, it may be useful for cases in which there is diagnostic uncertainty to exclude other causes of jaundice. The histological features also develop over time and may not be typical if sampled early [41]. Although histological confirmation prior to operative cholangiogram may be desirable, an infant with jaundice and pale stools with no other diagnosis will always require this definitive test. A liver biopsy is typically taken at the time of Kasai to provide information on the extent of fibrosis.

Table 6.3 Recommended initial drug doses for fat-soluble vitamins and ursodeoxycholic acid

Vitamin A	5000 units/day
Vitamin D: ergocalciferol	3000 units/day
Vitamin E: alpha tocopheryl acetate or	50 mg/day
vitamin K	1 mg/day
Ursodeoxycholic acid	10 mg/kg twice daily

6.7 Management

6.7.1 Medication

The infant should receive fat-soluble vitamin supplementation and ursodeoxycholic acid before surgery and until clearance of jaundice following the operation. Fat-soluble vitamins are absorbed along with the long-chain fats which require bile salt micelles. In biliary atresia vitamin deficiency is possible as bile is not present in the intestine. Table 6.3 provides recommended initial drug doses for vitamins and ursodeoxycholic acid.

6.7.2 Nutrition

Poor absorption of long-chain triglycerides (LCT) occurs due to reduced or absent bile salt micelles. A change of feed from primarily LCT to one containing 60–65% medium-chain triglycerides (MCT) enables absorption of fats without the need for bile salt micelles [42]. This means that the infant will absorb more calories and weight gain will be more effective. For those infants who are breast-fed, an MCT supplement can be provided alongside the breast-feeding which should be continued if possible. If an infant is not feeding well, then early instigation of nocturnal nasogastric tube feeding will enable good nutrition to continue.

6.7.3 Surgery

Without a Kasai portoenterostomy and liver transplant, if necessary, biliary atresia is fatal within the first 2 years of life.

The Kasai portoenterostomy operative procedure was first described by Morio Kasai in the 1950s and revolutionised the management of biliary atresia.

The procedure consists of complete excision of the extrahepatic biliary tree with transection of the porta hepatitis at the liver capsule to expose the microscopic ductules. A jejunal loop (roux loop) is anastomosed to the cut surface to facilitate the drainage of bile from these ductules into the intestine.

A successful Kasai is defined as normalisation of bilirubin levels within 6 months of the procedure. The success of the procedure is dependent on the age at operation, extent of liver damage (fibrosis at time of Kasai, ongoing inflammation and episodes of cholangitis) and experience of the centre [43]. Those infants who have

concomitant CMV infection have a poorer outcome with reduced clearance of jaundice, native liver survival and increased mortality [44].

Fibrosis and cirrhosis are more likely to develop with long-standing obstruction; hence, the hypothesis that the earlier the Kasai, the better the outcome.

This is indeed true for those with biliary atresia splenic malformation (BASM) or rarer forms of biliary atresia. However, in isolated biliary atresia cases, even having a Kasai at 100 days can lead to a 45% 5-year survival with the native liver [45].

A UK study of 93 cases of biliary atresia in 15 centres showed a significant difference in success rate in those centres that operated on five or more cases each year (61% vs 14% 5-year survival with native liver) and led to centralisation of the service [1]. Re-auditing after 4 years showed a 51% 4-year survival with the native liver and 89% survival overall [46]. Ten years following centralisation, the median age at Kasai was 54 days with 55% undergoing a successful Kasai. Survival with the native liver at 5 years was 46% and 40% at 10 years. The overall patient survival however continued to be 89% at 10 years [47].

A systematic review of 153 infants showed that those who received post-operative steroids had an improvement in clearance of jaundice. This, however, has not been seen in other smaller studies [48].

6.8 Management Following an Unsuccessful Kasai

In infants in whom the Kasai is unsuccessful (jaundice has not cleared within 6 months of surgery), liver transplant is usually indicated within 6 months to 2 years of age. Living-related transplant is a good option for these infants.

Indications for listing for transplant include:

- Poor synthetic function – the albumin concentration usually starts to fall initially, whilst the prothrombin time is maintained.
- Poor nutrition despite adequate calorie intake (nasogastric tube feeding) leading to failure to thrive.
- Recurrent cholangitis leading to biliary cirrhosis.

Some infants who initially clear their jaundice following surgery become jaundiced again. Increasing inflammation and fibrosis, possibly due to subclinical cholangitis, may be the cause. This scenario can be extremely disappointing for families.

Optimal nutrition is extremely important in infants who continue to be cholestatic, and most will require nasogastric feeding either as bolus feeds during the day or as a continuous overnight feed. If despite these measures poor weight gain occurs, then whilst awaiting transplant, parenteral nutrition may be required.

It is important to monitor the fat-soluble vitamin serum levels to ensure they are within the normal range, and adjustments to doses should be made if necessary.

There is a small group of patients who remain mildly jaundiced but continue to have good synthetic function. Close monitoring is essential to enable optimal timing for transplantation as necessary.

6.9 Management Following Successful Kasai

Thirty per cent of children who have a successful Kasai will have near or complete normalisation of their liver biochemistry. Most, however, will have abnormal transaminases, and despite timely surgery and a successful Kasai portoenterostomy, there is progression of fibrosis with a risk of hepatic decompensation, portal hypertension and malignancy throughout life [49].

It has not proved entirely possible to predict at an early stage which patients will have long-term survival with the native liver and which will require liver transplant. Many different scoring systems such as the APRI (which looks at AST to platelet ratio) have been developed based on the bilirubin levels, AST, age at Kasai and episodes of cholangitis [50, 51].

6.9.1 Fibrosis

Despite a successful Kasai, fibrosis continues to develop in many children with biliary atresia resulting in the development of portal hypertension and potential need for liver transplant later in childhood or adulthood. The reason for the progressive fibrosis is not known but thought to be related to ongoing inflammation. The protein prohibitin is markedly reduced in children with biliary atresia. Prohibitin negatively interacts with histone deacetylase 4 in the presence of bile acids resulting in epigenetic changes leading to fibrosis. Reducing histone deacetylase 4 results in a reduction in fibrosis in animal models of biliary obstruction which is an exciting potential treatment for the future [52].

The cytokine profile of infants with biliary atresia despite a successful Kasai and clearance of jaundice is different than those of children without biliary atresia, with increased profibrotic cytokines IFN-γ and IL-10 [53].

A genetic polymorphism that increases susceptibility to the development of fibrosis may be the reason why fibrosis continues to occur in some but not others. Polymorphisms in genes such as *CFC1*, *ICAM1*, macrophage migration inhibitory factor gene, CD14 endotoxin receptor gene and hepcidin have been suggested. Abnormalities in apoptosis due to a specific antigenic stimulation may also occur as there is an upregulation of Kupffer cells, natural killer cells, CD3+ and CD8+ T cells and increased CXCR3+ cells [54].

The fibrosis of biliary atresia may be non-invasively monitored using transient elastography (fibroscan). This will help in the counselling of families and establishing prognosis [55, 56].

6.9.2 Portal Hypertension

Portal hypertension with splenomegaly and/or hypersplenism is prevalent in up to 50% of children after 5 years following Kasai; hence, vigilance for the detection and management of varices is important. Varices ~~at high risk of bleeding in childhood~~

are more likely to occur in children who have portal hypertension within the first 18 months of life and (?) continue to have an abnormal bilirubin level [57].

Prediction for oesophageal varices Three calculations based on spleen size, platelet count and albumin may help to stratify patients with biliary atresia into those who may require pre-emptive intervention with endoscopic treatment of varices [58]:

- Clinical prediction rule (CPR) = (0.75 × platelets)/(platelet count to spleen size ratio + 5)+(2.5 × albumin).
- AST to platelet ratio index (APRI).
- Varices prediction rule (albumin × platelets/1000).

Acoustic radiation focus impulse (ARFI) elastography of the spleen at the time of Kasai correlates with the diameter of the portal vein, spleen diameter and the development of collateral vessels. Those who underwent subsequent liver transplant had a higher SS score at the time of Kasai.

Occult bleeding may occur due to varices in the roux loop. This may occur with or without transplant. MR angiography may be useful for detecting the exact site [59].

6.9.3 Nutrition

Optimal nutrition is extremely important in infants despite a successful Kasai, and some will require nasogastric feeding supplements. With increasing weight gain and time from surgery, the need for supplemental nutrition decreases.

It is important to monitor the fat-soluble vitamin serum levels to ensure that they are within the normal range and adjustments to doses should be made if necessary.

6.9.4 Cholangitis

Cholangitis can cause decompensation of liver function with falling albumin levels and coagulopathy. Following adequate treatment, the liver synthetic function may improve although a high level of vigilance is required to identify those who require listing for transplantation.

Cholangitis presents with acholic stools, abdominal pain and symptoms of sepsis. Treatment is with intravenous antibiotics for at least 10–14 days. Bacteria may be cultured from the blood or from a liver biopsy specimen. To prevent cholangitis, some centres use low-dose rotating oral antibiotics for the first year following Kasai (e.g. 12 weeks each of amoxicillin, cephalexin and trimethoprim); oral probiotics may also be beneficial. Ursodeoxycholic acid may also be used to aid bile flow. The development of hepatic bile lakes may occur at any time following Kasai and are sources of recurrent infection [60, 61].

Cholangitis is a significant detrimental event post-Kasai. It is an independent predictor of poor outcome, for early failure of the Kasai and also survival with the native liver at 3 years following successful Kasai [62].

It is thought that cholangitis occurs more commonly in the first 2 years following Kasai; however, long-term studies have shown that 63% of cases had a diagnosis of cholangitis 5 years following Kasai, and hence vigilance for cholangitis should continue into adulthood. Recurrent or late cholangitis may suggest an obstruction in the roux loop requiring surgical reconstruction [63].

6.9.5 Metabolic Bone Disease

Bone fractures are more common in children with biliary atresia with their native liver, occurring in up to 15% of patients. This is 6× higher than in the general population. Vitamin D deficiency is common and requires monitoring and supplementation.

6.9.6 Pruritus

In children with a successful Kasai, pruritus may be difficult to control but generally improves with age. Table 6.4 provides recommended therapies which may be beneficial for pruritus.

6.9.7 Hepatopulmonary Syndrome

This occurs due to abnormal shunting in the pulmonary vascular bed and may lead to hypoxia. It is an indication for transplantation and is reversible post-transplantation [64].

6.9.8 Malignancy

A study looking at biliary atresia patients with a successful Kasai, over an 18-year period, identified that 8% of infants develop liver tumours. Seventy-seven per cent of the tumours were benign (focal nodular hyperplasia, regenerative nodule,

Table 6.4 Drug therapies which may be beneficial for the management of pruritus in children

Drug	Dose
Cholestyramine	1–4 g daily
Phenobarbitone	3–5 mg/kg/day
Rifampicin	5–10 mg/kg/day
Ondansetron	2–4 mg twice daily
Naltrexone	6–20 mg/kg/day
Antihistamines	These are ineffective but may cause sedation at night to provide symptomatic relief

adenoma), whilst there were three malignant tumours identified (two hepatocellular carcinomas and one cholangiocarcinoma). It is important to be vigilant for tumours using the tumour marker, alpha-fetoprotein and serial scans [49].

6.9.9 Indications for Liver Transplant Following a Successful Kasai

- Chronic liver failure with poor synthetic function
- Variceal bleeding which is refractory to endoscopic and medical management
- Hepatopulmonary syndrome
- Poor quality of life and growth failure
- Hepatocellular carcinoma

6.10 Long-Term Follow-Up

The conditional survival probability is the calculated probability of survival after a period of time. In 244 Chinese patients, the median survival with the native liver was 41.2 months, and the 1-year, 3-year and 5-year survival with the native liver was 85.4%, 61.1% and 43.3%, respectively. The probability of surviving to 5 years with the native liver given survival to 1, 2, 3 and 4 years was 50%, 56%, 73% and 93%, respectively. This shows that with time the long-term prognosis of survival with the native liver improves [65].

With children surviving into adulthood, it is important to consider quality of life. From the Netherlands Study group on Biliary Atresia Registry, in young adults with biliary atresia, their health-related quality of life was comparable to the reference group except for females with their native liver. This group may require more intensive support [66]. Despite 98% of patients continuing to have health concerns, over 50% of cases had a positive HRQOL assessment [63, 67].

A large multicentre North American review of 219 patients with biliary atresia who were 5–18 years following a successful Kasai showed that the majority of patients achieved normal growth and 75% had normal liver synthetic function. However, only 2% of patients had no liver disease with no signs of portal hypertension, normal liver biochemistry and normal synthetic function. In the same patients, health-related quality of life (HRQOL) was comparable to those who had been transplanted but significantly worse than healthy adults.

Conclusions

The change in survival from biliary atresia being universally fatal to one with greater than 85% survival with a good quality of life is a remarkable medical achievement. The greatest change has been the development of the Kasai procedure and the availability of liver transplantation. The lack of knowledge of the pathophysiology of biliary atresia, despite extensive research, is hampering the development of targeted therapy and will continue to be an area in which research

is essential. In those who have had a successful Kasai, morbidity is associated with the development of progressive fibrosis leading to portal hypertension. This is an important area in which more research is required. Modulation of the fibrotic process will greatly improve the quality of life in children with biliary atresia.

6.11 Future Considerations

- Collaboration between countries, including the collection of biological specimens, will aid in collating of large data sets.
- Screening for biliary atresia by stool cards is an effective method of detecting the disease and should be implemented in clinical practice. Alternatively, laboratory techniques that detect conjugated bilirubin in dried blood spots may improve early diagnosis.
- Continuing research into the molecular pathogenesis of biliary atresia and the long-term development of fibrosis is essential to aid further understanding and development of novel therapies.
- With children now surviving with their native livers into adulthood, a greater understanding of the long-term outcome will be gained which is important for counselling families in the future.

References

1. McKiernan PJ, Baker AJ, Kelly DA. The frequency and outcome of biliary atresia in the UK and Ireland. Lancet. 2000;355:25–9.
2. Chardot C, Carton M, Spire-Bendelac N, Le Pommelet C, Golmard JL, Auvert B. Epidemiology of biliary atresia in France: a national study 1986–96. J Hepatol. 1999;31:1006–13.
3. Schwarz KB, Haber BH, Rosenthal P, Mack CL, Moore J, Bove K, Bezerra JA, Karpen SJ, Kerkar N, Shneider BL, Turmelle YP, Whitington PF, Molleston JP, Murray KF, Ng VL, Romero R, Wang KS, Sokol RJ, Magee JC, Childhood Liver Disease Research and Education Network. Extrahepatic anomalies in infants with biliary atresia: results of a large prospective North American multicenter study. Hepatology. 2013;58(5):1724–31.
4. Chalouhi GE, Muller F, Dreux S, Ville Y, Chardot C. Prenatal non-visualization of fetal gallbladder: beware of biliary atresia! Ultrasound Obstet Gynecol. 2011;38(2):237–8.
5. Bardin R, Ashwal E, Davidov B, Danon D, Shohat M, Meizner I. Nonvisualization of the fetal gallbladder: can levels of γ-glutamyl transpeptidase in amniotic fluid predict fetal prognosis? Fetal Diagn Ther. 2016;39(1):50–5.
6. Hsiao CH, Chang MH, Chen HL, Lee HC, Wu TC, Lin CC, Yang YJ, Chen AC, Tiao MM, Lau BH, Chu CH, Lai MW, Taiwan Infant Stool Color Card Study Group. Universal screening for biliary atresia using an infant stool color card in Taiwan. Hepatology. 2008;47(4):1233–40.
7. Yoon PW, Bresee JS, Olney RS, James LM, Khoury MJ. Epidemiology of biliary atresia: a population-based study. Pediatrics. 1997;99(3):376–82.
8. Wada H, Muraji T, Yokoi A, Okamoto T, Sato S, Takamizawa S, Tsugawa J, Nishijima E. Insignificant seasonal and geographical variation in incidence of biliary atresia in Japan: a regional survey of over 20 years. J Pediatr Surg. 2007;42(12):2090–2.

9. Mushtaq I, Logan S, Morris M, Johnson AW, Wade AM, Kelly D, Clayton PT. Screening of newborn infants for cholestatic hepatobiliary disease with tandem mass spectrometry. BMJ. 1999;319(7208):471–7.
10. Chung-Davidson YW, Yeh CY, Li W. The sea lamprey as an etiological model for biliary atresia. Biomed Res Int. 2015;2015:832943.
11. Keyzer-Dekker CM, Lind RC, Kuebler JF, Offerhaus GJ, Ten Kate FJ, Morsink FH, Verkade HJ, Petersen C, Hulscher JB. Liver fibrosis during the development of biliary atresia: proof of principle in the murine model. J Pediatr Surg. 2015;50(8):1304–9.
12. You Z, Wen J, Cheng L, Ye H, Li B. Screening of targeted genes in extrahepatic bile ducts of mice with experimental biliary atresia. Mol Med Rep. 2015;12(3):4326–31.
13. Davenport M, Gonde C, Redkar R, Koukoulis G, Tredger M, Mieli-Vergani G, Portmann B, Howard ER. Immunohistochemistry of the liver and biliary tree in extrahepatic biliary atresia. J Pediatr Surg. 2001;36(7):1017–25.
14. Narayanaswamy B, Gonde C, Tredger JM, Hussain M, Vergani D, Davenport M. Serial circulating markers of inflammation in biliary atresia – evolution of the post-operative inflammatory process. Hepatology. 2007;46(1):180–7.
15. Sira MM, Sira AM, Ehsan NA, Mosbeh A. P-selectin (CD62P) expression in liver tissue of biliary atresia: a new perspective in etiopathogenesis. J Pediatr Gastroenterol Nutr. 2015;61(5):561–7. doi:10.1097/MPG.0000000000000875.
16. Shimadera S, Iwai N, Deguchi E, Kimura O, Fumino S, Yokoyama T. The inv mouse as an experimental model of biliary atresia. J Pediatr Surg. 2007;42(9):1555–60.
17. Schön P, Tsuchiya K, Lenoir D, Mochizuki T, Guichard C, Takai S, Maiti AK, Nihei H, Weil J, Yokoyama T, Bouvagnet P. Identification, genomic organization, chromosomal mapping and mutation analysis of the human INV gene, the ortholog of a murine gene implicated in left-right axis development and biliary atresia. Hum Genet. 2002;110(2):157–65.
18. Cui S, Leyya-Vega M, Tsai EA, EauClaire SF, Glessner JT, Hakonarson H, Devoto M, Haber BA, Spinner NB, Matthews RP. Evidence from human and zebrafish that GPC1 is a biliary atresia susceptibility gene. Gastroenterology. 2013;144:1107–15.
19. Suzuki T, Kanai Y, Hara T, Sasaki J, Sasaki T, Kohara M, Maehama T, Taya C, Shitar H, Yonekawa H, et al. Crucial role of the small GTPase ARF6 in hepatic cord formation during liver development. Mol Cell Biol. 2006;26:6149–56.
20. Cheng G, Tang CS, Wong EH, Cheng WW, So MT, Miao X, Zhang R, Cui L, Liu X, Ngan ES, et al. Common genetic variants regulating ADD3 gene expression alter biliary atresia risk. J Hepatol. 2013;59:1285–91.
21. Tsai EA, Grochowski CM, Loomes KM, Bessho K, Hakonarson H, Bezerra JA, Russo PA, Haber BA, Spinner NB, Devoto M. Replication of a GWAS signal in a Caucasian population implicates ADD3 in susceptibility to biliary atresia. Hum Genet. 2014;133:235–43.
22. Ningappa M, Min J, Higgs BW, Ashokkumar C, Ranganathan S, Sindhi R. Genome-wide association studies in biliary atresia. Wiley Interdiscip Rev Syst Biol Med. 2015;7(5):267–73.
23. Lorent K, Gong W, Koo KA, Waisbourd-Zinman O, Karjoo S, Zhao X, Sealy I, Kettleborough RN, Stemple DL, Windsor PA, Whittaker SJ, Porter JR, Wells RG, Pack M. Identification of a plant isoflavonoid that causes biliary atresia. Sci Transl Med. 2015;7(286):286ra67.
24. Riepenhoff-Talty M, Schaekel K, Clark HF, Mueller W, Uhnoo I, Rossi T, Fisher J, Ogra PL. Group A rotaviruses produce extrahepatic biliary obstruction in orally inoculated newborn mice. Pediatr Res. 1993;33(4 Pt 1):394–9.
25. Rauschenfels S, Krassmann M, Al-Masri AN, Verhagen W, Leonhardt J, Kuebler JF, Petersen C. Incidence of hepatotropic viruses in biliary atresia. Eur J Pediatr. 2009;168(4):469–76. doi:10.1007/s00431-008-0774-2. Epub 2008 Jun 17.
26. Hartley JL, O'Callaghan C, Rossetti S, Consugar M, Ward CJ, Kelly DA, Harris PC. Investigation of primary cilia in the pathogenesis of biliary atresia. J Pediatr Gastroenterol Nutr. 2011;52(4):485–8.
27. Karjoo S, Hand NJ, Loarca L, Russo PA, Friedman JR, Wells RG. Extrahepatic cholangiocyte cilia are abnormal in biliary atresia. J Pediatr Gastroenterol Nutr. 2013;57(1):96–101.

28. Muraji T. Maternal microchimerism in biliary atresia: are maternal cells effector cells, targets, or just bystanders? Chimerism. 2014;5(1):1–5.
29. Gu YH, Yokoyama K, Mizuta K, Tsuchioka T, Kudo T, Sasaki H, Nio M, Tang J, Ohkubo T, Matsui A. Stool color card screening for early detection of biliary atresia and long-term native liver survival: a 19-year cohort study in Japan. J Pediatr. 2015;166(4):897–902.
30. Tseng JJ, Lai MS, Lin MC, Fu YC. Stool color card screening for biliary atresia. Pediatrics. 2011;128(5):e1209–15.
31. Powell JE, Keffler S, Kelly DA, Green A. Population screening for neonatal liver disease: potential for a community-based programme. J Med Screen. 2003;10(3):112–6.
32. National Institute for Health and Clinical Excellence. Neonatal Jaundice. (Clinical Guideline 98) 2010. www.nice.org.uk/CG98.
33. Schreiber RA, Masucci L, Kaczorowski J, Collet JP, Lutley P, Espinosa V, Bryan S. Home based screening for biliary atresia using stool colour cards: a large-scale prospective cohort study and cost effectiveness analysis. J Med Screen. 2014;21(3):126–32.
34. Terui K, Higashimoto Y, Saito E, Saito T, Mitsuanga T, Nakata M, Iwai J, Yoshida H. Diagnosis of biliary atresia can not be excluded by declining trend of serum direct bilirubin. Pediatr Rep. 2013;5(4):e17.
35. Larrosa-Haro A, Caro-López AM, Coello-Ramírez P, Zavala-Ocampo J, Vázquez-Camacho G. Duodenal tube test in the diagnosis of biliary atresia. J Pediatr Gastroenterol Nutr. 2001;32(3):311–5.
36. Zhou K, Xie G, Wang J, Zhao A, Liu J, Su M, Ni Y, Zhou Y, Pan W, Che Y, Zhang T, Xiao Y, Wang Y, Wen J, Jia W, Cai W. Metabonomics reveals metabolite changes in biliary atresia infants. J Proteome Res. 2015;14(6):2569–74.
37. Zhou LY, Wang W, Shan QY, Liu BX, Zheng YL, Xu ZF, Xu M, Pan FS, Lu M, Xie XY. Optimizing the US diagnosis of biliary atresia with a modified triangular cord thickness and gallbladder classification. Radiol. 2015;14(6):2569–74.
38. Malik D, Khan SH, Ali SW, Rather TA, Pakala R, Hassan MU, Yadav N, Pasupula M. Comparison of phenobarbitone and ursodeoxycholic acid in drug-augmented hepatobiliary scintigraphy for excluding the diagnosis of obstructive cholestasis in neonatal cholestasis syndrome. Nucl Med Commun. 2015;36(8):827–32. doi:10.1097/MNM.0000000000000322.
39. Shteyer E, Wengrower D, Benuri-Silbiger I, Gozal D, Wilschanski M, Goldin E. Endoscopic retrograde cholangiopancreatography in neonatal cholestasis. J Pediatr Gastroenterol Nutr. 2012;55(2):142–5.
40. Takaya J, Nakano S, Imai Y, Fujii Y, Kaneko K. Usefulness of magnetic resonance cholangiopancreatography in biliary structures in infants: a four-case report. Eur J Pediatr. 2007;166(3):211–4. Epub 2006 Sep 8.
41. Azar G, Beneck D, Lane B, Markowitz J, Daum F, Kahn E. Atypical morphologic presentation of biliary atresia and value of serial liver biopsies. J Pediatr Gastroenterol Nutr. 2002;34(2):212–5.
42. Beath SV, Booth IW, Kelly DA. Nutritional support in liver disease. Arch Dis Child. 1993;69(5):545–7.
43. Zhen C, Guoliang Q, Lishuang M, Zhen Z, Chen W, Jun Z, Shuli L, Kaoping G, Chao L, Xuan Y, Long L. Design and validation of an early scoring system for predicting early outcomes of type III biliary atresia after Kasai's operation. Pediatr Surg Int. 2015;31(6):535–42.
44. Zani A, Quaglia A, Hadzić N, Zuckerman M, Davenport M. Cytomegalovirus-associated biliary atresia: an aetiological and prognostic subgroup. J Pediatr Surg. 2015;50(10):1739–45.
45. Davenport M, Caponcelli E, Livesey E, Hadzic N, Howard E. Surgical outcome in biliary atresia: etiology affects the influence of age at surgery. Ann Surg. 2008;247(4):694–8.
46. McKiernan PJ, Baker AJ, Lloyd C, Mieli-Vergani G, Kelly DA. British paediatric surveillance unit study of biliary atresia: outcome at 13 years. J Pediatr Gastroenterol Nutr. 2009;48(1):78–81.
47. Davenport M, Ong E, Sharif K, Alizai N, McClean P, Hadzic N, Kelly DA. Biliary atresia in England and Wales: results of centralization and new benchmark. J Pediatr Surg. 2011;46(9):1689–94.

48. Davenport M, Parsons C, Tizzard S, Hadzic N. Steroids in biliary atresia: single surgeon, single centre, prospective study. J Hepatol. 2013;59(5):1054–8.
49. Yoon HJ, Jeon TY, Yoo SY, Kim JH, Eo H, Lee SK, Kim JS. Hepatic tumours in children with biliary atresia: single-centre experience in 13 cases and review of the literature. Clin Radiol. 2014;69(3):e113–9.
50. Suominen JS, Lampela H, Heikkilä P, Lohi J, Jalanko H, Pakarinen MP. APRi predicts native liver survival by reflecting portal fibrogenesis and hepatic neovascularization at the time of portoenterostomy in biliary atresia. J Pediatr Surg. 2015;50(9):1528–31.
51. Ohhama Y, Shinkai M, Fujita S, Nishi T, Yamamoto H. Early prediction of long-term survival and the timing of liver transplantation after the Kasai operation. J Pediatr Surg. 2000;35(7):1031–4.
52. Barbier-Torres L, Beraza N, Fernández-Tussy P, Lopitz-Otsoa F, Fernández-Ramos D, Zubiete-Franco I, Varela-Rey M, Delgado TC, Gutiérrez V, Anguita J, Pares A, Banales JM, Villa E, Caballería J, Alvarez L, Lu SC, Mato JM, Martínez-Chantar ML. Histone Deacetylase 4 promotes cholestatic liver injury in the absence of Prohibitin-1. Hepatology. 2015;62(4):1237–48.
53. Jian ZH, Wang LC, Lin CC, Wang J. The correlation between plasma cytokine levels in jaundice-free children with biliary atresia. World J Pediatr. 2015;11(4):352–7.
54. Squires JE, Shivakumar P, Mourya R, Bessho K, Walters S, Bezerra JA. Natural killer cells promote long-term hepatobiliary inflammation in a low-dose rotavirus model of experimental biliary atresia. PLoS One. 2015;10(5):e0127191.
55. Shen QL, Chen YJ, Wang ZM, Zhang TC, Pang WB, Shu J, Peng CH. Assessment of liver fibrosis by Fibroscan as compared to liver biopsy in biliary atresia. World J Gastroenterol. 2015;21(22):6931–6.
56. Uchida H, Sakamoto S, Kobayashi M, Shigeta T, Matsunami M, Sasaki K, Kanazawa H, Fukuda A, Kanamori Y, Miyasaka M, Nosaka S, Kawagishi N, Kasahara M. The degree of spleen stiffness measured on acoustic radiation force impulse elastography predicts the severity of portal hypertension in patients with biliary atresia after portoenterostomy. J Pediatr Surg. 2015;50(4):559–64.
57. Duché M, Ducot B, Ackermann O, Jacquemin E, Bernard O. Progression to high-risk gastroesophageal varices in children with biliary atresia with low-risk signs at first endoscopy. J Pediatr Gastroenterol Nutr. 2015;60(5):664–8.
58. Yang LY, Fu J, Peng XF, Pang SY, Gao KK, Chen ZR, He LJ, Wen Z, Wang H, Li L, Wang FH, Yu JK, Xu Y, Gong ST, Xia HM, Liu HY. Validation of aspartate aminotransferase to platelet ratio for diagnosis of liver fibrosis and prediction of postoperative prognosis in infants with biliary atresia. World J Gastroenterol. 2015;21(19):5893–900.
59. Jimbo K, Suzuki M, Fujii T, Ohtsuka Y, Sugo H, Kawasaki S, Shimizu T. Usefulness of magnetic resonance angiography for the evaluation of varices at hepaticojejunostomy after liver transplantation. Acta Radiol Open. 2015;4(5):2058460115578600.
60. Wu ET, Chen HL, Ni YH, Lee PI, Hsu HY, Lai HS, Chang MH. Bacterial cholangitis in patients with biliary atresia: impact on short-term outcome. Pediatr Surg Int. 2001;17(5–6):390–5.
61. Lien TH, Bu LN, Wu JF, Chen HL, Chen AC, Lai MW, Shih HH, Lee IH, Hsu HY, Ni YH, Chang MH. Use of lactobacillus casei rhamnosus to prevent cholangitis in biliary atresia after kasai operation. J Pediatr Gastroenterol Nutr. 2015;60(5):654–8.
62. Chung PH, Wong KK, Tam PK. Predictors for failure after Kasai operation. J Pediatr Surg. 2015;50(2):293–6.
63. Ng VL, Haber BH, Magee JC, Miethke A, Murray KF, Michail S, Karpen SJ, Kerkar N, Molleston JP, Romero R, Rosenthal P, Schwarz KB, Shneider BL, Turmelle YP, Alonso EM, Sherker AH, Sokol RJ, Childhood Liver Disease Research and Education Network (CHiLDREN). Medical status of 219 children with biliary atresia surviving long-term with their native livers: results from a North American multicenter consortium. J Pediatr. 2014;165(3):539–46.

64. Park TJ, Ahn KS, Kim YH, Kim H, Park UJ, Kim HT, Cho WH, Park WH, Kang KJ. Improved severe hepatopulmonary syndrome after liver transplantation in an adolescent with end-stage liver disease secondary to biliary atresia. Clin Mol Hepatol. 2014;20(1):76–80.
65. Qiao G, Li L, Cheng W, Zhang Z, Ge J, Wang C Conditional probability of survival in patients with biliary atresia after Kasai portoenterostomy: a Chinese population-based study. J Pediatr Surg. 2015;50(8):1310–5.
66. de Vries W, Lind RC, Sze YK, van der Steeg AF, Sieders E, Porte RJ, Verkade HJ, Hulscher JB, Hoekstra-Weeber JE. Overall quality of life in adult biliary atresia survivors with or without liver transplantation: results from a National Cohort. Eur J Pediatr Surg. 2016;26(4):345–56.
67. Sundaram SS, Alonso EM, Haber B, Magee JC, Fredericks E, Kamath B, Kerkar N, Rosenthal P, Shepherd R, Limbers C, Varni JW, Robuck P, Sokol RJ, Childhood Liver Disease Research and Education Network (ChiLDREN). Health related quality of life in patients with biliary atresia surviving with their native liver. J Pediatr. 2013;163(4):1052–7.

Drug-Induced Cholestasis: Mechanisms and Importance

7

Guruprasad P. Aithal and Ann K. Daly

Abstract

Drug-induced liver injury (DILI) is classified as cholestatic in 25% of cases based on the pattern of liver enzyme elevation in the absence of evidence of biliary obstruction. Even when the drug responsible for the injury has been discontinued, 7.8% of those with drug-induced cholestasis die; when recovery occurs, it is typically slower in this form of DILI than in hepatocellular cases. Chronicity of cholestasis is reflected by ductopenia on histology and is associated with poor quality of life. Although animal and in vitro experiments investigating drug-induced cholestasis have focused on the mechanisms and transporters involved in the secretion of bile from hepatocytes into the canaliculi, human studies investigating genetic susceptibility to DILI have revealed the importance of adaptive immune system in its pathogenesis. The first genome-wide association study (GWAS) performed for any form of DILI demonstrated a very strong association between flucloxacillin DILI and the class I HLA allele *B*57:01,* although the majority of these reactions do not exhibit obvious features of hypersensitivity. Another GWAS confirmed a previously described association between co-amoxiclav-induced DILI and the *DRB1*15:01*; this study also described another novel association involving *HLA-A*02:01*. A candidate gene study from Japan involving cases of ticlopidine-induced DILI, the majority of which had cholestasis, found an association with the HLA class I allele *A*33:03*. Activation of T-cells requires drug gaining protein reactivity and presentation of the drug-protein adduct by antigen-presenting cells expressing specific HLA; strong local T-cell responses within the biliary system would result in predominantly biliary injury.

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G. Hirschfield et al. (eds.), *Biliary Disease*, DOI 10.1007/978-3-319-50168-0_7

Take-Home Points

- DILI is classified as cholestatic in 25% of cases, and 7.8% of those with drug-induced cholestasis will die, even when the drug responsible for the injury has been discontinued.
- The role of adaptive immunity in the pathogenesis of DILI has been highlighted via genetic studies.
- Co-amoxiclav-related DILI has been associated with HLA class II DRB1*15:01 and HLA-A*02:01.
- Flucloxacillin DILI has been associated with HLA-B*57:01. However, only 1 in every 500–1000 patients carrying HLA-B*57:01 will develop the reaction when exposed to the drug.
- Additional non-HLA and non-genetic factors may contribute to risk of developing DILI.

7.1 Introduction

Idiosyncratic drug-induced liver injury (DILI) is best described as an adverse hepatic reaction that is unexpected on the basis of the pharmacological action of the drug administered; this is distinct and different from liver injury secondary to drug overdose [1]. Although adverse hepatic reaction to medications has a wide range of manifestations including drug-associated chronic liver diseases, the term DILI refers to events that are acute in onset. Diagnosis of DILI relies upon "causality assessment," a clinical evaluation involving a number of considerations including the temporal relationship between the drug therapy and the clinical manifestation as well as exclusion of alternative aetiology explaining the manifestation. The broad grouping of DILI into its three main patterns of presentations is based on the earliest identified liver enzyme estimations. The biochemical abnormalities associated with DILI are expressed as the ratio of alanine transaminase (ALT) elevation times upper limit of normal (ULN) to alkaline phosphatase (ALP) elevation times ULN. The pattern of DILI is classified as "hepatocellular" when there is a fivefold elevation above the upper limit of normal (ULN) for (ALT) (or ratio of ALT elevation times ULN to alkaline phosphatase (ALP) elevation time ULN is ≥ 5), "cholestatic" when there is a twofold elevation for alkaline phosphatase (or ratio of ALT elevation times ULN to ALP elevation times ULN is ≤ 2), or "mixed" when this ratio of ALT to ALP elevation times ULN is between 4.9 and 2.1.

7.2 Burden and Impact

A large number of drugs undergo hepatic metabolism and/or biliary excretion; therefore, hepatotoxicity is a potential complication of a vast majority of medications. Owing to their relatively low incidence, hepatotoxicity may become apparent

only when a large number of people have been exposed to the drug; for this reason, hepatotoxicity has been the second most common reason for withdrawal of drugs from the market worldwide, accounting for 32% of such cases between 1975 and 2007 [36].

In routine clinical practice, DILI due to new and old drugs continues to have an impact. A recent prospective population-based study from Iceland estimated the crude annual incidence of DILI of all patterns to be 19 per 100,000 population [8]. The incidence of acute serious liver injury requiring hospitalization has been estimated to be 0.7–1 per 100,000 population per year. In a recent audit from the UK involving 881 consecutive patients presenting with jaundice in whom biliary obstruction had been ruled out by imaging [14], DILI was the underlying aetiology in 15% of cases and the second most common cause of jaundice after alcoholic liver disease (which accounted for 25% of cases). Among 885 cases (considered definite, probable, or possible DILI based on causality assessment) from a large international DILI consortium (iDILIC) cohort, 26% had cholestatic and 27% mixed pattern of DILI. Although the majority of DILI cases resolve when the offending agent has been withdrawn, 10% of severe cases of DILI require liver transplantation or result in death; mortality is lower in cholestatic (7.8%) or mixed (2.4%) pattern of cases when compared with the hepatocellular (12.7%) form of DILI [2, 3, 10]. Cholestasis due to drugs typically resolves more slowly than the hepatocellular pattern of DILI taking up to 6 months; cases with elevation of liver enzymes after 6 months are classified as persistent DILI with the term chronic DILI being reserved for cases where there is evidence of persistent liver injury more than 1 year after the onset of DILI.

In a recent study, those with elevation of liver enzymes at and over 12 months following initial DILI were more likely to have a cholestatic DILI at the onset (54% vs. 20%, $P < 0.01$) compared with those where DILI resolved [17]. Chronicity was associated with significantly poorer SF-36 physical summary scores throughout the period starting from the onset of DILI to the point of last follow-up compared with DILI without chronicity ($P < 0.01$). Among 12 patients where serial liver biopsy specimens were available, 8 demonstrated fibrosis progression of at least one Ishak stage over a mean time interval of 397 days; all of these patients had cholestatic DILI including 6 where there was evidence of ductopenia.

7.3 Mechanisms Underlying Drug-Induced Cholestasis

Synthesis of bile salts includes multiple steps and several enzymatic reactions within the hepatocytes. Bile salt flow across hepatocytes into canaliculi is governed by a number of transporters and regulators as any excess accumulation of bile salts within hepatocyte can be cytotoxic. From canaliculi, bile enters the biliary tree and then the duodenum. In a process called enterohepatic circulation, 90% of the bile salts are reabsorbed in the small intestine to be transported back to the liver via portal circulation and are taken up by the hepatocytes from the sinusoidal blood plasma. Experiments using animal models and in vitro methods exploring the mechanisms that contribute to the development of drug-induced cholestasis have primarily focused on biliary secretion from hepatocytes into the canaliculi. A summary of such

studies and the putative mechanisms of cholestasis induced by a range of drugs described by these studies are summarized in Table 7.1. It is however unclear as to whether these mechanisms contribute to drug-induced cholestasis in humans. It is interesting to note that in contrast to the findings from the experimental model-based investigations, human studies investigating genetic factors underlying DILI have highlighted the prominent role of adaptive immunity in the pathogenesis.

Table 7.1 Putative mechanisms underlying drug-induced cholestasis

Drugs	Putative mechanism of DILI	Genetic factor associated with susceptibility to DILI
Androgenic and anabolic steroids	17-alkyl-substituted steroids are associated with disaggregation and/or detachment of microfilaments from the bile canalicular membranes interfering with its function Impaired bile salt excretion due to internalization of BSEP through a Ca^{2+}-dependent protein kinase C-mediated mechanism [32]	One of two patients was heterozygous for the mutation c.2093G>A in ABCB11 (protein consequence R698H), a predictably deleterious substitution at a conserved site [16]
Bosentan Dual endothelin receptor antagonist	Competitive inhibition of bile salt export pump, BSEP (ABCB11), and trans-inhibition which depends upon functional multidrug resistance protein MRP2 (ABCC2) [31]	
Cholestasis due to variety of drugs	Reduced expression of BSEP Variation in multidrug resistance protein, MDR3, which transports phospholipids	Polymorphism in exon 13 of *ABCB11* (V444A) more frequent in drug-induced cholestasis. *ABCB4* polymorphism in a case of risperidone-induced cholestasis [24]
Chlorpromazine	Drug, its 7, 8-dihydroxy and 7-hydroxy metabolites interfere with bile acid secretion via disruption of canalicular membrane fluidity and impaired transmembrane transport function due to $(Na^+ + K^+)$-dependent adenosine triphosphatase [34]	Inherited defect in sulfoxidation [41]
Cyclosporin	Competitive inhibition of bile salt export pump, BSEP (ABCB11) [4]	
Estradiol-17β-D-glucuronide	Trans-inhibits BSEP via MRP2 [37].	
Ethinyl estradiol	Reduction in bile flow by reduction in a number of ATP-dependent bile salt carriers at the canalicular membrane including BSEP and MRP2 [9]	
Rifampicin and isoniazid co-treatment	Accumulation of protoporphyrin IX, endogenous hepatotoxin, in the liver via a pregnane X receptor-mediated alteration of heme biosynthesis pathway associated mixed pattern of liver injury with bile plug formation [25]	

7.3.1 Genetic Susceptibility: HLA Associations and Possible Role for Non-HLA Genes

It is increasingly clear that genetic factors can predict susceptibility to drug-induced cholestasis. Advances in gene cloning and development of PCR-based genotyping methods during the 1980s and 1990s have enabled specific genetic associations to be investigated in detail. Initially this involved a candidate gene approach where genetic polymorphisms in biologically plausible gene candidates were analyzed in liver injury cases and frequencies compared with those in healthy controls. Candidate genes examined included mainly human leukocyte antigen (HLA) and genes encoding enzymes relevant to drug metabolism and transport. The main limitation with genetic studies on all forms of drug-induced liver injury (DILI) is that case finding is difficult; so most studies have been small which limits the power to detect associations reliably. Replicating associations has also been difficult. The development of genome-wide association studies (GWAS) where the contribution of all genes in the human genome to disease susceptibility can be investigated [12] has been a useful development in understanding the genetics of DILI better, but lack of large numbers of cases continues to be a limitation.

7.3.2 HLA Genes as Risk Factors

The key advances on genetic susceptibility relate to cholestasis induced by the two widely prescribed antimicrobials, flucloxacillin and co-amoxiclav. The main genes showing associations are the HLA genes. The proteins encoded by HLA genes are important in presenting foreign peptides, usually derived from invading pathogens, to T lymphocytes. HLA genes are located in a large gene cluster on chromosome 6 in an area of the genome referred to as the major histocompatibility complex (MHC). More detailed descriptions of HLA genes and their biological roles are widely available [6, 27]. HLA genes are divided into two types, class I and II. The proteins encoded by these gene classes have overlapping but slightly different functions in the immune response. Class I genes tend to be expressed in most cells in the body and have a role in presenting peptides to cytotoxic T-cells which may cause local damage. On the other hand, class II genes tend to be expressed mainly by immune system cells and have a more important role in stimulating T helper cells to produce cytokines which may induce inflammation. HLA genes are subject to a large amount of genetic polymorphism. This probably arose originally to help protect the host from pathogens, but increasingly alternative forms of HLA genes (alleles) are considered to be risk factors for autoimmune disease (e.g., multiple sclerosis and primary biliary cirrhosis) and also for adverse drug reactions. There is increasing evidence that many cholestatic DILI reactions involve an inappropriate immune response to a drug, possibly after a covalent link between the drug and a cellular protein has been formed. HLA proteins are likely to contribute by presenting the complex to T-cells.

Well-established and replicated HLA associations have been described for hypersensitivity reactions affecting various tissues, particularly the skin, and for

Table 7.2 HLA associations with cholestatic DILI

Drug	HLA allele	Odds ratio	Reference
Flucloxacillin	*B*57:01*	80.6 (95% CI 22.8–284.9; $p = 9 \times 10^{-19}$)	[13]
Co-amoxiclav	*A*02:01*	2.3 (95% CI 1.8–2.9; $p = 1.8 \times 10^{-10}$)	[26]
	*DRB1*15:01*	2.8 (95% CI 2.1–3.8; $p = 3.5 \times 10^{-11}$)	
Ticlopidine	*A*33:03*	13.0 (95% CI 4.4–38.6; $p = 1.2 \times 10^{-5}$)	[20]

CI = confidence interval

DILI. Those carrying certain HLA alleles have an increased risk of developing these reactions. For cholestatic DILI, some of the HLA-associated reactions do not show obvious features of a hypersensitivity reaction, so findings of strong HLA associations have been slightly unexpected. The first HLA genotyping studies were candidate gene association studies on co-amoxiclav-related DILI. Two independent candidate gene association studies reported an identical association with the HLA class II *DRB1*15:01* allele [15, 19, 30]. Further studies have found additional HLA class I and II associations for cholestatic DILI (Table 7.2). Effect sizes vary depending on the drug with odds ratios for risk of DILI of between 2 and 80 reported.

The strongest HLA association reported to date for any form of DILI is for the predominantly cholestatic reactions due to the antimicrobial flucloxacillin, which is used in a number of countries to treat gram-positive infections. This association was detected in the first GWAS performed for any form of DILI. A very strong association (odds ratio 80) for the development of flucloxacillin DILI was seen with the class I HLA allele *B*57:01* [13] (Fig. 7.1). This allele had been shown previously to be a strong risk factor for hypersensitivity reactions to the drug abacavir, but these reactions generally do not involve the liver. Importantly, the underlying mechanism involved in the flucloxacillin DILI reactions appears to be different to the mechanism reported for abacavir with flucloxacillin believed to form a covalent complex with protein [28], while abacavir appears to interact directly with the HLA protein, causing inappropriate presentation of cellular peptides to T-cells [21]. No associations with *B*57:01* for any other form of DILI have been described to date.

Early reports of an association between HLA-*DRB1*15:01* and co-amoxiclav DILI [15, 19, 30] have been extended by a relatively large GWAS involving 201 predominantly cholestatic and mixed DILI cases related to this drug from Europe and the USA [26] (Fig. 7.2). This GWAS confirmed the *DRB1*15:01* association but also described a second novel association involving *HLA-A*02:01*. Though the HLA class I *A*02:01* signal is independent of the class II *DRB1*15:01*, those positive for both these alleles have a greater than additive increased risk of DILI, suggesting that the two risk factors interact. Importantly, the odds ratios observed for co-amoxiclav-related DILI are much lower than for *B*57:01* in flucloxacillin DILI. Individually, *DRB1*15:01* and *A*02:01* carriage give odds ratios of approximately 2.5 for DILI development (Table 7.2), while individuals who are positive for both risk alleles show an odds ratio of approximately 9. One further non-HLA risk

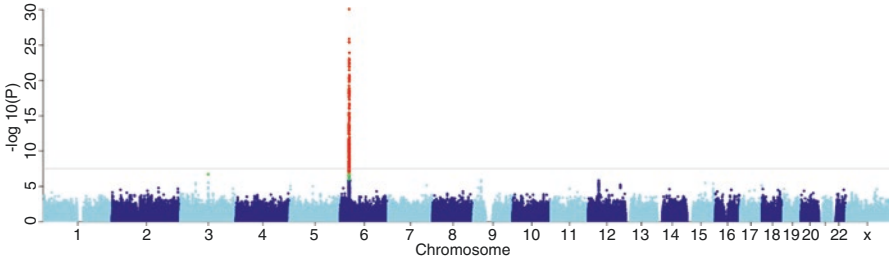

Fig. 7.1 Manhattan plot for a GWAS of flucloxacillin-induced liver injury. Samples were genotyped using the Illumina Human1M-Duo BeadChip. The $-\log_{10}$p values for a number of polymorphisms in the MHC region on chromosome 6 are greater than 7 ($p < 10\text{-}7$) and therefore show genome-wide significance. The study involved 51 cases of DILI and 282 population controls. The polymorphism (rs2395029) showing the most significant difference in frequency between cases and controls is in complete linkage disequilibrium with the HLA class I HLA-B*57:01 allele (Reproduced from [13])

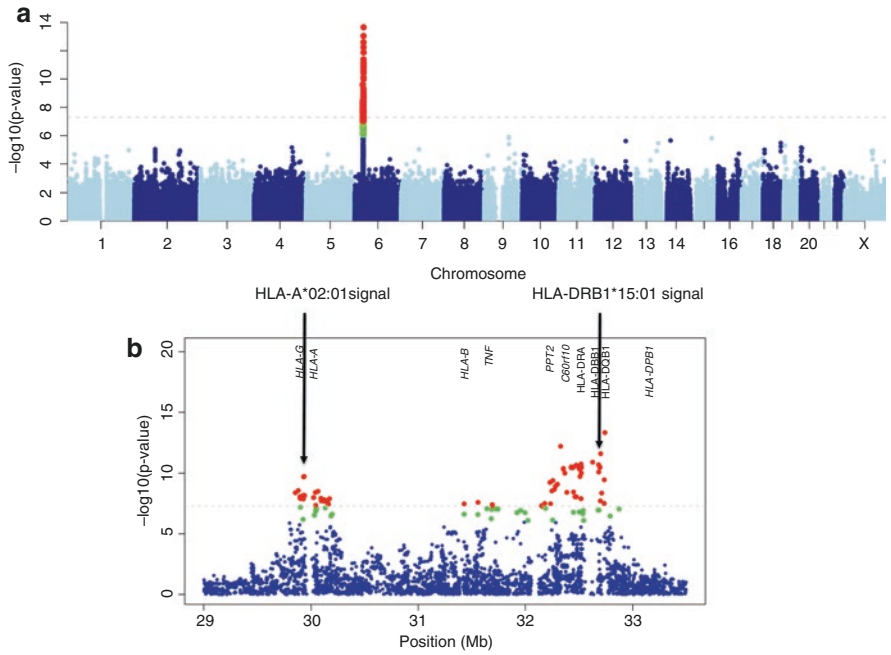

Fig. 7.2 (**a**) Manhattan plot for a GWAS of co-amoxiclav-induced liver injury. Samples were genotyped using the Illumina Human1M-Duo BeadChip. The $-\log_{10}$p values for a number of polymorphisms in the MHC region on chromosome 6 are greater than 7 ($p < 10\text{-}7$) and therefore show genome-wide significance. The study involved 201 cases of DILI and 532 population controls. (**b**) Enlargement of the MHC region from panel (**a**) with specific genes indicated showing the signal from both HLA class I (A*02:01) and class II (DRB1*15:01) (Reproduced from [26])

factor, a non-synonymous polymorphism in the gene *PTPN22,* was also detected when additional analyses involving autoimmune disease-related risk factors were performed. This non-HLA gene codes for a phosphatase enzyme that is believed to contribute to T-cell responses. The variant is also a risk factor for type 1 diabetes and rheumatoid arthritis but not for other forms of cholestatic liver disease such as primary biliary cirrhosis.

A third GWAS involving the DILI cases due to flucloxacillin and co-amoxiclav in the two earlier GWAS studies described above [13, 26], together with approximately 500 additional DILI cases, has also been performed [40]. The study included new cholestatic cases relating to drugs such as azathioprine, sulfamethoxazole/trimethoprim, terbinafine, and tetracyclines, but the numbers for each were relatively small (typically 5–10 cases). No genome-wide signals other than a HLA signal were seen either for the entire study or cholestatic cases alone. When the flucloxacillin and co-amoxiclav cases were excluded from the analysis leaving 77 cholestatic DILI cases due to other drugs, the significant HLA signal was lost suggesting that HLA genotype might not be a universal risk factor for cholestatic DILI.

A separate candidate gene study based in Japan was performed in 22 cases of DILI induced by ticlopidine [20]. The majority of these cases (64%) had suffered cholestatic liver injury. A significant association with the HLA class I allele *A*33:03* was seen (Table 7.1) with the association being strongest in cholestatic cases only (odds ratio 36.5). This study was followed up by further genotyping for the main cytochrome P450 that metabolizes ticlopidine, *CYP2B6* [5]. A significant association with a *CYP2B6* allele that results in high metabolic activity was obtained suggesting that both high levels of a metabolite and the presence of a particular HLA protein might be factors in cholestatic DILI in response to this drug.

While a role for cytochrome P450-mediated metabolism in addition to HLA genotype seems plausible for ticlopidine-related cholestasis, this is less likely for the other well-studied forms of cholestasis due to flucloxacillin and co-amoxiclav. In the case of co-amoxiclav, neither the amoxicillin nor the clavulanic acid components are subject to P450-mediated metabolism, and in the case of flucloxacillin, the extent of this metabolism is very limited. As discussed above, activation of T-cells by flucloxacillin requires presentation of the drug by antigen-presenting cells expressing HLA-*B*57:01*, and this process also appears to require covalent binding of flucloxacillin to protein [28]. Recently, broadly similar findings have been reported for co-amoxiclav-induced T-cell proliferation [23]. This study found that both amoxicillin and clavulanic acid, when presented to T-cells by antigen-presenting cells, induced cell proliferation though the requirement for a specific HLA genotype for antigen presentation was not restricted to one HLA allele, as in the case of flucloxacillin. The role for both amoxicillin and clavulanic acid in inducing T-cell proliferation was slightly unexpected, though rare cases of amoxicillin-induced cholestasis do occur [35]. Strong local T-cell responses in the biliary system would lead to biliary injury.

7.3.3 ABC Transporters

After HLA genes, the ABC transporter superfamily genes are the best studied candidates for a role in drug-induced cholestasis. They are biologically plausible candidates, especially because some ABC transporter family gene products transport both bile acids and drugs, so competition for binding may occur [18]. Additionally, some inherited forms of cholestasis result from specific mutations in the *ABCB4* (MDR3) and *ABCB11* (BSEP) genes [29]. In a study on a small group of patients who had suffered DILI relating to a range of different drugs, an association between cholestatic injury and a polymorphism in exon 13 of *ABCB11* was reported [24]. This polymorphism is very common and has only a small effect on disease risk; therefore, it appears unlikely to be a major risk factor for cholestatic disease. The possibility of a larger association with individual causative drugs deserves further investigation, but has generally not been confirmed in subsequent studies [7, 22, 38], and there is also no evidence from published GWAS for this. ABC transporters other than ABCB11 have also been investigated as risk factors for cholestatic DILI but generally have not reported significant associations apart from some suggestions of an increased frequency for certain genotypes for the ABCC2 transporter which mainly transports glucuronidated drugs [7, 11, 39]. The most recently published GWAS on DILI [40] reported that a secondary analysis of genes relevant to drug metabolism and transport in cholestatic cases only gave a signal for the transporter gene *ABCC1*. This transporter, which is located on the basolateral hepatocyte membrane and is able to transport a range of drugs and their glucuronide conjugates, is present at very low levels normally but its expression is induced during severe liver injury, with regenerating bile ductules at the interface of portal tracts and necrotic areas staining strongly by immunofluorescence [33]. This might be a protective mechanism against further cellular damage. The interesting and biologically plausible association between ABCC1 genotype and cholestatic DILI still needs follow-up.

Post Script: Most recent GWAS has revealed an association between A*33:01 a HLA class I allele and cholestatic or mixed pattern of DILI with odds ratio [OR], 2.7; 95% CI, 1.9-3.8; P=2.4x10-8) [42]. The association with A*33:01 was mediated by large effects for terbinafine-, fenofibrate-, and ticlopidine-related DILI.

Conclusions

To date, the available genetic studies point to inappropriate T-cell responses underlying the majority of cholestatic liver disease. However, the limited data so far available on cholestasis due to drugs other than flucloxacillin, co-amoxiclav, and ticlopidine suggests that not all cholestatic reactions involve HLA associations. Larger genetic studies in relation to relatively common causes of cholestatic liver disease such as azathioprine and sulfamethoxazole/trimethoprim may provide information on additional risk factors. While the observed HLA associations for cholestatic DILI provide interesting insights into the underlying mecha-

nisms, they are generally not helpful in predicting who will develop this toxicity. Even for flucloxacillin DILI where the strongest association has been reported, only 1 in every 500 to 1000 patients carrying *HLA-B*57:01* develop the reaction when exposed to the drug. This means additional risk factors contribute to risk, and these may include factors such as age and gender together with additional non-HLA genes.

References

1. Aithal GP, Watkins PB, Andrade RJ, Larrey D, Molokhia M, Takikawa H, et al. Case definition and phenotype standardization in Drug-Induced Liver Injury (DILI). Clin Pharmacol Ther. 2011;89:806–15.
2. Andrade RJ, Lucena MI, Fernandez ME, et al. Drug induced liver injury: an analysis of 461 incidences submitted to the Spanish registry over a 10-year period. Gastroenterology. 2005;129:512–21.
3. Andrade RJ, Lucena MI, Kaplowitz N, et al. Outcome of acute idiosyncratic drug-induced liver injury: long-term follow-up in a hepatotoxicity registry. Hepatology. 2006;44:1581–8.
4. Arias IM. Cyclosporin, the biology of the bile canaliculus, and cholestasis. Gastroenterology. 1993;104(5):1558–60.
5. Ariyoshi N, Iga Y, Hirata K, Sato Y, Miura G, Ishii I, et al. Enhanced susceptibility of HLA-mediated ticlopidine-induced idiosyncratic hepatotoxicity by CYP2B6 polymorphism in Japanese. Drug Metab Pharmacokinet. 2010;25:298–306.
6. Bharadwaj M, Illing P, Theodossis A, Purcell AW, Rossjohn J, Mccluskey J. Drug hypersensitivity and human leukocyte antigens of the major histocompatibility complex. Annu Rev Pharmacol Toxicol. 2012;52:401–31.
7. Bhatnagar P, Day CP, Aithal G, Pirmohamed M, Bernal W, Daly AK. Genetic variants of hepatic transporters and susceptibility to drug induced liver injury. Toxicology. 2008;253:10–10.
8. Björnsson ES, Bergmann OM, Björnsson HK, Kvaran RB, Olafsson S. Incidence, presentation, and outcomes in patients with drug-induced liver injury in the general population of Iceland. Gastroenterology. 2013;144(7):1419–25. 1425.e1–3; quiz e19–20
9. Bossard R, Stieger B, O'Neill B, Fricker G, Meier PJ. Ethinylestradiol treatment induces multiple canalicular membrane transport alterations in rat liver. J Clin Invest. 1993;91(6):2714–20.
10. Chalasani N, Fontana RJ, Bonkovsky HL, et al. Causes, clinical features, and outcomes from a prospective study of drug-induced liver injury in the United States. Gastroenterology. 2008;135:1924–34.
11. Choi JH, Ahn BM, Yi J, Lee JH, Nam SW, Chon CY, Han KH, et al. MRP2 haplotypes confer differential susceptibility to toxic liver injury. Pharmacogenet Genomics. 2007;17:403–15.
12. Daly AK. Using genome-wide association studies to identify genes important in serious adverse drug reactions. Annu Rev Pharmacol Toxicol. 2012;52:21–35.
13. Daly AK, Donaldson PT, Bhatnagar P, Shen Y, Pe'er I, Floratos A, et al. HLA-B*5701 genotype is a major determinant of drug-induced liver injury due to flucloxacillin. Nat Genet. 2009;41:816–9.
14. Donaghy L, Barry FJ, Hunter JG, Stableforth W, Murray IA, Palmer J, et al. Clinical and laboratory features and natural history of seronegative hepatitis in a nontransplant centre. Eur J Gastroenterol Hepatol. 2013;25(10):1159–64.
15. Donaldson PT, Daly AK, Henderson J, Graham J, Pirmohamed M, Bernal W, et al. Human leucocyte antigen class II genotype in susceptibility and resistance to co-amoxiclav-induced liver injury. J Hepatol. 2010;53(6):1049–53.

16. El Sherrif Y, Potts JR, Howard MR, Barnardo A, Cairns S, Knisely AS, et al. Hepatotoxicity from anabolic androgenic steroids marketed as dietary supplements: contribution from ATP8B1/ABCB11 mutations? Liver Int. 2013;33(8):1266–70.
17. Fontana RJ, Hayashi PH, Barnhart H, Kleiner DE, Reddy KR, Chalasani N, et al. Persistent liver biochemistry abnormalities are more common in older patients and those with cholestatic drug induced liver injury. Am J Gastroenterol. 2015;110(10):1450–9.
18. Geier A, Wagner M, Dietrich CG, Trauner M. Principles of hepatic organic anion transporter regulation during cholestasis, inflammation and liver regeneration. Biochim Biophys Acta. 2007;2007:283–308.
19. Hautekeete ML, Horsmans Y, Van Waeyenberge C, Demanet C, Henrion J, Verbist L, et al. HLA association of amoxicillin-clavulanate-induced hepatitis. Gastroenterology. 1999;117:1181–6.
20. Hirata K, Takagi H, Yamamoto M, Matsumoto T, Nishiya T, Mori K, et al. Ticlopidine-induced hepatotoxicity is associated with specific human leukocyte antigen genomic subtypes in Japanese patients: a preliminary case-control study. Pharmacogenomics J. 2008;8:29–33.
21. Illing PT, Vivian JP, Dudek NL, Kostenko L, Chen Z, Bharadwaj M, et al. Immune self-reactivity triggered by drug-modified HLA-peptide repertoire. Nature. 2012;486:554–8.
22. Kagawa T, Hirose S, Arase Y, Oka A, Anzai K, Tsuruya K, et al. No contribution of the ABCB11 p.444A polymorphism in Japanese patients with drug-induced cholestasis. Drug Metab Dispos. 2015;43:691–7.
23. Kim SH, Saide K, Farrell J, Faulkner I, Tailor A, Ogese M, et al. Characterization of amoxicillin- and clavulanic acid-specific T cells in patients with amoxicillin-clavulanate-induced liver injury. Hepatology. 2015;62:887–99.
24. Lang C, Meier Y, Stieger B, Beuers U, Lang T, Kerb R, et al. Mutations and polymorphisms in the bile salt export pump and the multidrug resistance protein 3 associated with drug-induced liver injury. Pharmacogenet Genomics. 2007;17(1):47–60.
25. Li F, Lu J, Cheng J, Wang L, Matsubara T, Csanaky IL, et al. Human PXR modulates hepatotoxicity associated with rifampicin and isoniazid co–therapy. Nat Med. 2013;19(4):418–20.
26. Lucena MI, Molokhia M, Shen Y, Urban TJ, Aithal GP, Andrade RJ, et al. Susceptibility to amoxicillin-clavulanate-induced liver injury is influenced by multiple HLA class I and II alleles. Gastroenterology. 2011;141:338–47.
27. Mehta NKE. The Hla complex in biology and medicine: a resource book. New Delhi: Jaypee Brothers Medical Publishers Ltd; 2010.
28. Monshi MM, Faulkner L, Gibson A, Jenkins RE, Farrell J, Earnshaw CJ, et al. Human leukocyte antigen (HLA)-B*57:01-restricted activation of drug-specific T cells provides the immunological basis for flucloxacillin-induced liver injury. Hepatology. 2013;57:727–39.
29. Noe J, Kullak-Ublick GA, Jochum W, Stieger B, Kerb R, Haberl M, et al. Impaired expression and function of the bile salt export pump due to three novel ABCB11 mutations in intrahepatic cholestasis. J Hepatol. 2005;43:536–43.
30. O'Donohue J, Oien KA, Donaldson P, Underhill J, Clare M, Macsween RM, et al. Co-amoxiclav jaundice: clinical and histological features and HLA class II association. Gut. 2000;47:717–20.
31. Pauli-Magnus C, Meier PJ, Stieger B. Genetic determinants of drug-induced cholestasis and intrahepatic cholestasis of pregnancy. Semin Liver Dis. 2010;30(2):147–59.
32. Phillips MJ, Oda M, Funatsu K. Evidence for microfilament involvement in norethandrolone-induced intrahepatic cholestasis. Am J Pathol. 1978;93(3):729–44.
33. Ros JE, Libbrecht L, Geuken M, Jansen PL, Roskams TA. High expression of MDR1, MRP1, and MRP3 in the hepatic progenitor cell compartment and hepatocytes in severe human liver disease. J Pathol. 2003;200:553–60.
34. Schachter D. Fluidity and function of hepatocyte plasma membranes. Hepatology. 1984;4(1):140–51.
35. Schwarze C, Schmitz V, Fischer HP, Sauerbruch T, Spengler U. Vanishing bile duct syndrome associated with elevated pancreatic enzymes after short-term administration of amoxicillin. Eur J Gastroenterol Hepatol. 2002;14:1275–7.

36. Stevens JL, Baker TK. The future of drug safety testing: expanding the view and narrowing the focus. Drug Discov Today. 2009;14(3–4):162–7.
37. Stieger B, Fattinger K, Madon J, Kullak-Ublick GA, Meier PJ. Drug- and estrogen-induced cholestasis through inhibition of the hepatocellular bile salt export pump (Bsep) of rat liver. Gastroenterology. 2000;118(2):422–30.
38. Ulzurrun E, Stephens C, Crespo E, Ruiz-Cabello F, Ruiz-Nunez J, Saenz-Lopez P, et al. Role of chemical structures and the 1331 T>C bile salt export pump polymorphism in idiosyncratic drug-induced liver injury. Liver Int. 2013;33:1378–85.
39. Ulzurrun E, Stephens C, Ruiz-Cabello F, Robles-Diaz M, Saenz-Lopez P, Hallal H, et al. Selected ABCB1, ABCB4 and ABCC2 polymorphisms do not enhance the risk of drug-induced hepatotoxicity in a Spanish cohort. PLoS One. 2014;9:e94675.
40. Urban TJ, Shen Y, Stolz A, Chalasani N, Fontana RJ, Rochon J, et al. Limited contribution of common genetic variants to risk for liver injury due to a variety of drugs. Pharmacogenet Genomics. 2012;22:784–95.
41. Watson RG, Olomu A, Clements D, Waring RH, Mitchell S, Elias E. A proposed mechanism for chlorpromazine jaundice – defective hepatic sulphoxidation combined with rapid hydroxylation. J Hepatol. 1988;7(1):72–8.
42. Nicoletti P*, Aithal GP, Bjronsson ES, Andrade RJ, Sawle A, Arrese M, et al. Association of liver injury from specific drugs, or groups of drugs, with polymorphisms in HLA and other genes in a genome-wide association study. Gastroenterology. 2016:pii: S0016-5085(16)35530–5. doi: 10.1053/j.gastro.2016.12.016. [Epub ahead of print]

Primary Biliary Cholangitis: Its Science and Practice

8

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Abstract

Primary biliary cholangitis (PBC) is an autoimmune liver disorder characterized by progressive destruction of intrahepatic bile ducts, leading to cholestasis, cirrhosis, and liver failure. The etiology of PBC is unknown; however a complex interaction between genetic, environmental, and autoimmune factors is believed to play a role. The major hallmark of PBC is the presence of antimitochondrial antibodies (AMA), with sensitivity and specificity for PBC >90–95%. The immunodominant epitopes recognized by AMA are all mapped within the lipoyl domains of the E2 subunits of the pyruvate dehydrogenase complex (PDC-E2). In addition to autoantibodies, PBC is characterized by an enrichment of autoreactive $CD4^+$ and $CD8^+$ T cells, by enhanced natural killer (NK) cell activity and monocyte responses and accumulation of Th17 cells around damaged bile ducts. Controversial findings regarding numbers and function of regulatory T cells have been reported. The development of several animal models has aided the study and understanding of different aspects of PBC pathogenesis; however no "perfect model" has been developed to date. Novel therapeutic avenues targeting bile acids, nuclear receptors, immune cell receptors, and cytokines have been developed with promising results.

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G. Hirschfield et al. (eds.), *Biliary Disease*, DOI 10.1007/978-3-319-50168-0_8

Take-Home Points

- PBC is an autoimmune liver disorder with characteristic loss of tolerance to the E2 subunits of the 2-oxo-acid dehydrogenases.
- There is a strong genetic predisposition, with specific HLA haplotypes and 27 non-HLA risk loci being associated with PBC.
- In addition to genetic factors, environmental factors (vitamin D, heavy metals, smoking, nail polish), infectious factors, and xenobiotics have been reported to be associated with PBC.
- Several animal models that manifest clinical features of PBC have been developed; however to date there is no "perfect model."
- UDCA remains the only drug approved to treat PBC patients; however one third of patients do not respond. Novel therapeutic avenues for PBC patients include bile acid-based therapies (nor-UDCA, FXR agonists, and PPARα agonists), immune-based therapies [anti-IL-12 (ustekinumab), CTLA4-Ig, anti-CD40L, rituximab (anti-CD20)], and cell-based therapies (mesenchymal stromal cells).

8.1 Introduction

8.1.1 History and Definition of PBC

In 1851, Addison and Gull described three patients with light skin lesions, jaundice, and enlarged livers; one of these patients later developed yellowish plaques around her eyes and finger joints. A century later, Ahrens and colleagues coined the term primary biliary cirrhosis (PBC) to describe jaundice, hepatomegaly, and pruritus affecting middle-aged women, in the absence of apparent obstruction of the large bile ducts [1]. However, with increased routine liver biochemistry testing, most cases of PBC are diagnosed in the asymptomatic or even preclinical phase and in the absence of cirrhosis. Recent consensus among medical societies and patient representatives leads to a change in nomenclature of PBC to primary biliary *cholangitis* in order to emphasize the hallmark feature of immune-mediated destruction of the small- and medium-sized intrahepatic bile ducts.

PBC is an autoimmune liver disorder characterized by the progressive destruction of intrahepatic bile ducts, leading to cholestasis, cirrhosis, and liver failure. The etiology of PBC is complex, involving genetic, environmental, and autoimmune factors, the most notable being an almost universal loss of tolerance to the E2 subunits of the 2-oxo-acid dehydrogenases including pyruvate dehydrogenase complex (PDC-E2), branched-chain 2-oxo-acid dehydrogenase complex (BCOADC-E2), 2-oxoglutarate dehydrogenase complex (OGDC-E2), and the E3 binding protein of dihydrolipoamide. Despite significant advances in understanding many aspects of PBC etiopathogenesis, ursodeoxycholic acid (UDCA) remains the only drug approved by the Food and Drug Administration (FDA) to treat PBC. Although treatment with UDCA at a dose of 13–15 mg/kg per day can delay the progression of

Fig. 8.1 The development of science and technology greatly improved the practice of primary biliary cholangitis. The scientific achievements based on basic research and clinical investigation have significantly changed the practice of PBC involving its acknowledgment, pathogenesis, diagnosis, and therapy

histological changes, ameliorate long-term morbidity, and prolong life expectancy in many PBC patients, there remains a group of patients who do not adequately respond to treatment and for whom new therapies are needed (Fig. 8.1).

8.1.2 Clinical Presentation of PBC

Many asymptomatic patients will develop symptomatic liver disease within 5 years of diagnosis; however, a third could remain symptom-free for many years. Although nonspecific, fatigue is the most common symptom of PBC, and more than 40% reported moderate-to-severe symptoms. Related to long-standing cholestasis, pruritus seems to be the most typical complaint and is reported by 20–70% of patients. A reduction in bone density is common in patients with PBC, with features of osteopenia (33%) and, less frequently, osteoporosis (11%). Hypercholesterolemia, typically caused by a rise in HDL cholesterol, is common in patients with PBC but does not increase cardiovascular risk or cause early signs of atherosclerosis. So, the statins may usually not be necessary for those patients.

Because there may exist liver inflammation, some PBC patients will experience right upper quadrant abdominal pain, where the liver is located. Like other liver diseases, cirrhosis is the final stage of PBC, with symptoms of liver dysfunction (ascites, jaundice, spider angiomata, etc.) and portal hypertension (splenomegaly,

esophageal varices, caput medusa, etc.). However, a unique feature of PBC is that about 6% of patients with early-stage disease have varices before the onset of cirrhosis. Further study showed that esophageal varices develop in about a third of patients with stages III–IV disease over a median of 5–6 years; roughly half of these patients will have a bleeding event. The 3-year survival rate after initial variceal bleed is about 50%.

Hepatocellular carcinoma (HCC) and additional autoimmune diseases could also be observed in PBC patients. The most frequently associated autoimmune disease for PBC is Sicca syndrome. It is also known as Sjogren's syndrome (SS) that classically combines dry eyes, dry mouth, and another disease of connective tissue such as rheumatoid arthritis, lupus, scleroderma, or polymyositis. Schirmer's test is highly specific for the diagnosis of SS, and all PBC patients should undergo this easy test at the outpatient clinic. The association between SS and PBC strongly suggested a paradigm for a common immunopathogenesis.

About 10–20% of the PBC cases are associated with thyroid dysfunction (TD). Among thyroid disorders, PBC has been described in association with Hashimoto's thyroiditis, hypothyroidism, euthyroid goiter, and, less commonly, Grave's disease. Until now, no predictor of thyroid disease in PBC or other chronic liver disease has been identified, except possibly end-stage liver disease and treatment of chronic hepatitis C with interferon. Nonetheless, thyroid hormones have an extensive interrelationship with liver function, and prompt diagnosis and management of TD in patients with chronic liver disease can substantially improve the quality of their life.

8.2 Lessons from the Epidemiology and Natural History of PBC

PBC is a rare disease, but can be found in many if not most populations. Several large population-based studies have suggested that the prevalence of antimitochondrial antibodies (AMAs) is higher than the prevalence of diagnosed PBC, suggesting that there is a large undiagnosed cohort of PBC patients or that there is a substantial number of individuals with AMA but not clinically significant PBC. As with other autoimmune diseases, a change in the geoepidemiology of PBC is likely as countries become increasingly developed. In addition, the clinical symptoms and natural history in PBC patients are heterogeneous spanning from asymptomatic and nonprogressive to rapid progression to cirrhosis. These subgroups of PBC patients are increasingly being recognized, and specific criteria for phenotypes are being developed to better understand the underlying mechanisms leading to more severe disease.

8.2.1 The Epidemiology of PBC

The prevalence of PBC varies both on a regional and an international level. This can be explained, in part, by differences in clinical practice and case-finding activity. It is likely, however, that substantive geographical differences exist both in terms of

genetic susceptibility and environmental factors that potentially trigger the disease in genetically susceptible individuals [2].

Triger DR et al. reported in 1984 the first multinational survey of the clinical and epidemiological aspects of PBC involving ten countries in Western Europe with a population of over 24 million. The results showed a female to male sex ratio of 10:1 with the ratio skewed more in later stages of disease (11.4:1 in stages III and IV compared to 6.5:1 in stages I and II). In addition, the prevalence of PBC showed a marked variation from center to center. Further, the annual incidence of PBC was increasing [3]. In 1985, Löfgren et al. estimated the incidence of AMA-positive PBC patients in Sweden during the period 1976–1983 at 140 per million [4]. Subsequent studies reported prevalence rates per 100,000 persons in Australia (1.9), Japan (2.7–5.4), the United Kingdom (20.0–25.1), the USA (40.0), and Finland (18.0). Interestingly, in 2010, Liu et al. reported the prevalence of PBC from southern China at 49.2 cases per 100,00 population [5]. Due to the very high prevalence of hepatitis B in China, which is responsible for the majority of liver disease in this area, the diagnosis of PBC is likely to have been previously underreported. A recent systematic review including 24 studies published between 1972 and 2007 found that the incidence rates ranged from 0.33 to 5.8 per 100,000 persons/year and prevalence rates ranged from 1.91 to 40.2 per 100,000 persons [6].

Whether PBC is increasing in prevalence remains controversial. In 1999, James et al. found an increase in prevalence of PBC in Northern England from 2 to 33.5 cases per 100,000 persons between 1987 and 1994 [7]. Similarly, Myers et al. found an increase in prevalence of PBC in Canada from 10.0 to 22.7 cases per 100,000 persons between 1996 and 2002 [8]. In addition, the systematic review of PBC by Boonstra et al. found that an increasing prevalence of PBC was found in most studies [6].

The true prevalence of PBC however remains unknown due to lack of case-finding studies. These limitations include a largely asymptomatic stage that is likely to escape presentation to a medical provider and the lack of knowledge or awareness of PBC in the general medical community preventing timely diagnosis even when a patient presents for a medical evaluation. Several studies of AMA prevalence in serum collected during routine health checks or blood donation suggest that the prevalence of AMA-positive cases is 430 to 1000 per 100,000 persons [9–13]. Whether those AMA-positive cases have undiagnosed PBC or preclinical PBC or will never develop PBC is not clear. A study of 29 cases with AMA-positive serum but normal liver tests demonstrated that 75% of the cases developed clinical criteria for PBC after a median follow-up of 18 years [14], suggesting that the true prevalence of PBC is much greater than measured by case-finding studies. Further studies with strict case-finding protocols are needed to find the true incidence and prevalence of PBC.

8.2.2 Changing Natural History

Early studies of PBC reported that less than 20% of cases were asymptomatic [15]. However, following the identification of the AMA antigen and the development of a diagnostic test, the corresponding percentage increased to 60% [15, 16]. The

course of PBC disease falls within four phases: (i) a preclinical phase characterized by AMA reactivity but normal liver tests; (ii) an asymptomatic phase, which can last up to 20 years; (iii) a symptomatic phase, in which the patient remains anicteric or mildly jaundiced, lasting for up to 5–10 years; and (iv) a short-lasting preterminal phase characterized by a severe jaundice [17–19]. Without treatment, PBC is progressive even in those without symptoms. Several studies have documented that a quarter or more of asymptomatic patients will develop symptoms and have a decreased survival [20–23].

With the introduction of UDCA treatment, earlier identification, and likely with the identification of less severe cases of PBC, the natural history of the disease appears to have improved. Before the use of UDCA, the 10-year transplant-free survival of PBC was 50–70% in patients who were asymptomatic, and for symptomatic patients, the median transplant-free survival was 5–8 years [24]. Following the introduction of UDCA, the natural history of PBC appears to have changed dramatically. Evidence for this comes from large patient cohorts which demonstrate that survival among patients that respond to UDCA and life expectancy is not different compared to a match control population [25–27]. In addition, the number and rate of liver transplantations done for PBC has decreased significantly over the past decade [28]. However, for the one third of PBC patients who are nonresponders to UDCA, the disease appears to progress more rapidly and is associated with a reduced transplant-free survival [29].

8.2.3 Special Groups of PBC Patients

8.2.3.1 PBC in Young Women

PBC is infrequently diagnosed in patients less than 25 years of age [30], and the youngest PBC patient reported is a 12-year-old girl [31]. PBC patients presenting at an earlier age are more likely to experience significant symptoms, impaired quality of life, less likely to respond to therapy, and potentially to pursue a more aggressive disease course. Such patients should be the focus for novel therapy to improve outcome. Although male patients showed only a weak age-associated UDCA response rate pattern, women showed a clear age-related effect, with age again being an independent predictor of UDCA response on multivariate analysis. Women presenting younger than the age of 45 (the age of equivalent likelihood of response between the groups) were significantly less likely than either men or older women at presentation to respond to UDCA. On the contrary, women presenting older than age 70 had a greater than 90% chance of responding to UDCA [32].

8.2.3.2 PBC in Men

Although typically PBC affects middle-aged women, the disease may also occur in men who make up approximately 10% of most cohorts reported, and some evidence suggests that they have more severe disease. Rubel and colleagues first reported that compared to women, men with PBC had similar AMA titers, but higher serum alkaline phosphatase activities and lower frequency of piecemeal necrosis on liver

histology [33]. Similar to other chronic liver diseases, men with PBC have also been reported to have a higher risk of hepatocellular carcinoma compared to women [34]. More recently, a large cohort from the United Kingdom that included 221 men found that PBC in men was less responsive to UDCA treatment regardless of age [32, 35].

Several reasons have been proposed to explain these sex-based differences in PBC. Firstly, in men, biochemical abnormalities might be attributed to alcohol. Secondly, men report fewer symptoms and, thus, may be less likely to seek medical attention than female patients [36, 37]. Finally, the rarity of PBC in men may prevent the diagnosis of PBC.

8.2.3.3 PBC Overlap with Other Autoimmune Diseases

Additional autoimmune diseases involving the liver or extrahepatic organs are commonly observed in patients with PBC [38]. Within liver disorders, the term "overlap syndrome" is used to define the coexistence of autoimmune hepatitis (AIH) and another hepatic autoimmune condition, namely, PBC or primary sclerosing cholangitis (PSC) [39]. However, overlapping PBC and PSC have been described in only a few cases. The prevalence of the PBC/AIH overlap syndrome has ranged from 2% to 20% in series published since 1998. The variable diagnostic criteria used and the rarity of the condition are likely to have led to the wide variation observed. Currently, the criteria for diagnosing overlap syndrome remain controversial. The two criteria most often used and validated in the literature come from the Paris study group [40] and the International Autoimmune Hepatitis Group (IAIHG) [41]. By analyzing the efficacy of Paris criteria and the revised and the simplified IAIHG scores in diagnosing PBC/AIH overlap syndrome, Paris criteria appeared to diagnose overlap syndrome better than the IAIHG scores [42].

The European Association for the Study of the Liver (EASL) guidelines recommend combined therapy with UDCA and corticosteroids for patients with PBC/AIH overlap syndrome. An alternative approach is to start with UDCA alone, adding corticosteroids if there is not an adequate biochemical response within 3 months. Steroid-sparing agents should be considered in patients requiring long-term immunosuppression [43].

More than 84% of PBC patients have been reported to exhibit features of at least one extrahepatic autoimmune disease, which may include rheumatologic, endocrine, gastrointestinal, pulmonary, or dermatological conditions. Sometime during the clinical course, evidence of two or more extrahepatic autoimmune diseases can be found in nearly 40% of PBC cases [38, 44]. Thyroid dysfunction is frequently (25%) associated with PBC, often predating its diagnosis [45], and xerostomia and/or keratoconjunctivitis sicca, commonly referred to as the sicca syndrome, is seen in up to 70% of patients [46]. The treatment is symptomatic and consists of the use of artificial saliva preparations with a neutral pH that contain a balance of electrolytes corresponding to normal saliva. The frequency of other extrahepatic autoimmune diseases associating with PBC varies with different type of diseases, including scleroderma (8%) [47], rheumatoid arthritis (1.8–5.6%) [48, 49], and systemic lupus erythematosus (2.7–7.5%) [50]. The prevalence of celiac disease in PBC

patients has been reported to be as high as 11% in Northern Ireland, but in other populations, no associations have been found [51–53]. This may be related to true differences in prevalence or differences in the criteria used for diagnosis, particularly the requirement for small bowel biopsy rather than simply serologic testing [51–53].

8.2.3.4 PBC and Cancers

Although there is a risk of hepatocellular carcinoma (HCC) in patients with PBC, relative to other causes of chronic liver disease such as chronic viral hepatitis, the risk is low. In a study of 212 Greek PBC patients with a median follow-up of 6 years, the cumulative HCC incidence at 5 years was 1% and occurred only in those with stage IV disease [54]. In a separate UK study, compared to a control cohort, the hazard ratio for liver cancer mortality was found to be quite high (8.52; 95% CI 3.18–23.06), but the incidence was only 19 per 10,000 person years [55–57]. In Asians, the rates have also been found to be low with a cumulative incidence of 2.4% over a median follow-up of 58 months in Japan [58] and a 5-year cumulative incidence of 2.6% reported from China [59–60]. Several risk factors for those HCC patients have been investigated, including advanced histological stage (stage IV), sex, age, presence of portal hypertension, history of alcohol intake, smoking, prior infection with hepatitis B virus, and lack of response to UDCA [58–63]. Despite this overall low rate of HCC, the AASLD Practice Guidelines recommend regular screening for HCC with cross-sectional imaging with or without alpha-fetoprotein at 6–12-month intervals in PBC patients with cirrhosis [24].

Several extrahepatic malignancies (EMs) have also been suggested to be associated with PBC, but this remains controversial. The most studied cancer aside from HCC has been breast cancer. Early studies found that the incidence of breast cancer was four times the incidence expected from a comparable normal population [64, 65]. However, further studies could not confirm these findings [54, 61, 66]. A systematic review and meta-analysis on PBC and cancer risk, which considered approximately 16,300 PBC patients, concluded that PBC was not associated with extrahepatic malignancies, including breast cancer [67].

8.2.3.5 PBC and Pregnancy

Although a large proportion of the female patients with PBC are diagnosed in the postmenopausal period, up to a quarter of PBC patients may be of childbearing age [32], and nearly 15% may become pregnant after their liver disease is recognized or be diagnosed with PBC in the peripartum period [68]. Early studies noted that PBC patients often have a history of miscarriages, limited fertility, and hysterectomies before the onset of the disease [69] and that they have higher rates of maternal and fetal complications [70–75]. More recent studies however suggest a much better outcome in pregnancies, likely due to the inclusion of early-stage disease in these later studies [76, 77]. Compared to controls, PBC cases have been found to have more pregnancies [78], and two case series totally over 300 pregnancies found them to be mainly uneventful [68, 79]. Specifically, liver chemistries remained stable in 70% of patients throughout pregnancy; no adverse maternal events were observed

during pregnancy or postpartum; and only 6% developed progressive disease following delivery [79]. Based upon these data, it appears that early-stage, non-cirrhotic PBC patients do not have an increased risk of miscarriage or preterm delivery and that most women with PBC maintain a stable disease during pregnancy, but up to 60% of them will develop postpartum biochemical flares. Although UDCA appears to be safe during pregnancy and breastfeeding, formal evaluation of its safety in this setting has not been established [80].

8.3 Understanding the Science of PBC Pathogenesis

The development of PBC is believed to entail the action of environmental stressors in genetically susceptible individuals, as with most other autoimmune diseases. An important role in PBC pathogenesis has been also attributed to toxic bile acids and the disruption of protective mechanisms, which under normal conditions protect biliary epithelial cells from the harmful effects of bile acids [81–83]. However, the etiology of this disease remains enigmatic despite much recent effort, reflecting the true complexity underlying autoimmunity and the difficulties inherent to understanding and testing how environmental factors interact with the genetic background to result in disease.

The greatest insights into PBC pathogenesis have come from the unique autoimmune attack on the mitochondrial proteins to which the AMA autoantibodies are directed. The immunodominant epitopes recognized by AMA are all mapped within the lipoyl domains of PDC-E2 and the other related target antigens [84]. High-resolution structural analysis and modeling studies of the PDC-E2 lipoyl domains from both prokaryotes and eukaryotes demonstrate that lipoic acid is covalently attached to the ε group of lysine (K) via an amide bond and is prominently displayed on the outer surface of PDC-E2 [85]. The change in conformation and the existence of multiple conformations of the lipoyl domain during reductive acylation are important in catalyzing the acyl transfer, but render PDC-E2 susceptible to aberrant chemical modifications. Indeed, many small molecule lipoyl mimics conjugated to PDC-E2 demonstrate specific reactivity to AMA often at levels higher than the native PDC-E2 [86–88]. Further, tolerance to lipoic acid and recapitulation of a PBC-like cholangitis can be induced by immunization of animals with xenobiotics [87–93].

In addition to AMAs, autoreactive CD8+ and CD4+ T cells to PDC-E2 are present in peripheral blood and enriched in the liver of patients with primary biliary cholangitis [94]. Reduced regulatory T cells [95], raised levels of serum polyclonal IgM [96], and hyperresponsiveness to CpG (cytosine-phosphate-guanine dinucleotide motif) [96] enhanced natural killer cell [97, 98], and monocyte responses [99] are all features found in primary biliary cholangitis.

A central conundrum to PBC pathogenesis has been how tolerance to AMAs is once lost to a ubiquitous autoantigen does such a highly specific injury of biliary epithelial cells ensue. Indeed, the answer appears to be linked to the unique processes of biliary epithelial cell apoptosis [100–105]. Unlike other cell types, the E2 component of the pyruvate dehydrogenase complex remains intact in bile duct cells after

apoptosis, thus probably retaining its immunogenicity [100]. This enzyme is also found within apoptotic blebs and is accessible to AMAs and local antigen-presenting cells [101]. In vitro studies have shown an intense and specific immune response when macrophages from patients with primary biliary cholangitis are combined with apoptotic blebs of biliary epithelial cells and AMAs [105]. However, recurrence of primary biliary cholangitis after liver transplantation suggests that this occurrence is not an intrinsic defect of bile duct cells of affected individuals but is a feature of biliary epithelia in general, not seen in other epithelial cells.

8.3.1 Genetic Factors

The heritability of PBC has been clearly established in a series of 16 twin pairs, 8 of which were monozygotic and demonstrated a 63% concordance rates compared to 0% in the dizygotic twin pairs [106]. The prevalence of PBC in first-degree relatives of PBC patients has been also reported to be 4–9%

Although specific human leukocyte antigen (HLA) haplotypes have been associated with PBC, the strength of the association is less than that of other classic autoimmune diseases and is primarily limited to class II alleles. In addition, the associated haplotypes vary significantly across populations [107]. In populations of European origin, PBC has consistently been associated with the DRB1*08:01-DQA1*04:01-DQB1*04:02 risk haplotype [108] and the protective haplotype DRB1*11:01-DQA*05:01-DQB1*03:01 [109]. In Japan, DRB1*08:03-DQB1*06:01 and DRB1*04:05-DQB1*04:01 haplotypes appear to confer susceptibility to PBC, while conversely, DRB1*13:02-DQB1*06:04 and DRB1*11:01-DQB1*03:01 haplotypes are protective [110]. In China, the DRB1*08:03-DQB1*06:01 was also found to be a risk allele along with DRB1*07:01-DQB1*02:02, while the DQB1*03:01 allele and DRB1*12:02-DQB1*03:01 haplotype were protective [111]. Recent genome-wide association studies (GWAS) from North America, Italy, the United Kingdom, and Japan have confirmed that the HLA remains the most strongly associated PBC susceptibility locus with the peak of association lying between DQB1 and DQB2 [112–115].

In addition to the HLA locus, GWAS have identified 27 non-HLA risk loci for PBC including 2q32 (STAT1, STAT4), 2q33 (CTLA-4), 3q25 (IL12A, SCHIP1), 7q32 (IRF5, TNPO3), 11q23 (CXCR5), 12p13 (TNFRSF1A, LTBR), 16p13.13 (SOCS1, CLEC16A), 17q12 (IKZF3), and 19q13.3 (SPIB) [114, 116, 117].

Epigenetic modulation has also been suggested to play a role in PBC with significantly lower levels of DNA methylation of the CD40L promoter in CD4+ T cells, which is inversely correlated with levels of serum IgM in PBC patients [118]. Other DNA modifications include X chromosome inactivation [119], partial and variable methylation of CpG in CLIC2 and PIN4 [120], and changes in microRNA expression [121].

8.3.2 Environmental Factors

Although genetic factors play an important role in the development of complex autoimmune diseases, it is apparent that environmental exposures are equally important. Environmental exposures, including infectious and noninfectious agents, have been linked to PBC with most suggesting a mechanism of molecular mimicry leading to loss of tolerance to PDC-E2 [122]. Multiple studies have reported that PBC patients have more frequent urinary tract infections (UTI) compared with controls and that the infections primarily precede the development of PBC [123, 124]. Several peptides and proteins derived from bacteria frequently causing UTIs including *E. coli*, *Novosphingobium aromaticivorans* [125, 126], and *Pseudomonas aeruginosa* [127] cross-react with PDC-E2 antibodies and activate T-cell clones from PBC patients. Other specific infectious agents implicated in PBC pathogenesis include herpes simplex virus [128], mouse mammary tumor virus [129, 130], Epstein-Barr virus [131, 132], and *Saccharomyces cerevisiae* [125, 127, 133].

In addition to infection factors, xenobiotics have been suggested to play a crucial role in PBC pathogenesis due to the central role of the liver in metabolizing chemicals that in turn may modify cellular proteins to form neo-antigens. As noted above, substantial evidence exists to support the hypothesis that xenobiotic-induced and/or oxidative modification of the lipoic acid on mitochondrial autoantigens can lead to loss of tolerance [90, 91]. In clinical practice, AMA has been observed in subjects with acute liver failure from acetaminophen (APAP), presumably due to the oxidation of the lipoic acid into a neo-antigen [134]. Other environmental factors such as vitamin D, heavy metals, smoking, and nail polish have all been reported to be associated with PBC [135].

8.3.3 Innate and Adaptive Immune Responses

Defects in immune regulation that govern components of both innate and adaptive immunity contribute to the induction and abnormal perpetuation of the immune responses in PBC. From the first cloning of PDC-E2 in 1987 [136], a clearer picture of the immune mechanisms has been developed (Fig. 8.2) [137].

8.3.3.1 Humoral Immunity

Although high titers of serum AMA can be detected in up to 95% of patients with PBC, there is no correlation between the level of serum AMA and the severity of PBC leading to query the significance of the humoral immune response to PDC-E2. However, in vitro experiments have demonstrated the unique ability of AMA to induce a large cytokine release upon co-culture of peripheral blood mononuclear cells and biliary epithelial cells [105]. In addition and in contrast to AMA, PBC-specific antinuclear antibodies are also detected in nearly 50% of patients with PBC, and their presence does correlate with a more severe disease. Yet, whether or how those autoantibodies directly participate in immunopathogenesis of PBC remains unclear.

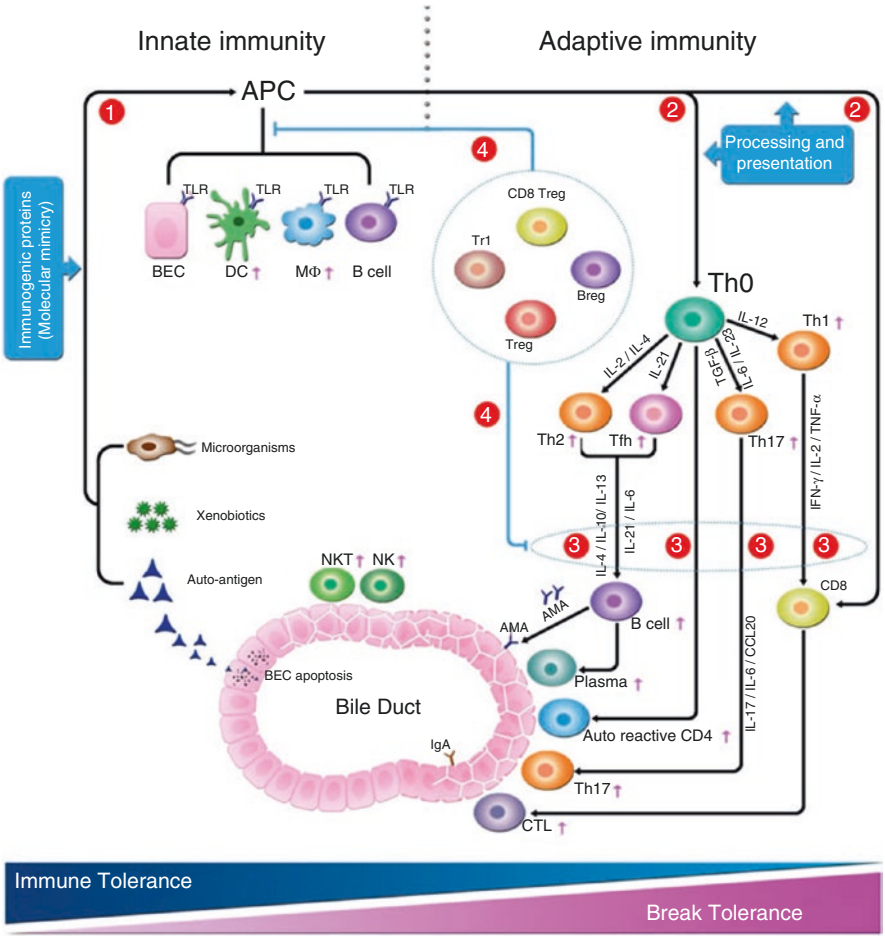

Fig. 8.2 Innate and adaptive immunity in primary biliary cholangitis patients (This figure is cited from Wang et al. [137]). (*1*) Microorganism proteins, xenobiotics, and apoptosis of biliary epithelial cells (BEC) can be recognized and endocytosed by antigen-presenting cells (APCs), which subsequently activate innate immune cells such as Toll-like receptors (TLRs), DCs, macrophages, natural killer (NK) and natural killer T (NKT) cells, and others. (*2*) After being processed by APCs, potentially T-cell immunogenic peptides were generated and presented to uncommitted T helper (Th0) lymphocytes and CD8 T cells. (*3*) Activated Th0 cells then differentiate into Th1, Th2, Tfh, and Th17 cells. Furthermore, Th1 cells secrete cytokines such as interleukin-2 (IL-2) and interferon-γ, which stimulate development of cytotoxic T lymphocytes (CTL). Th2 cells or Tfh cells secrete IL-4, IL-10, IL-13, or IL-21 and may stimulate autoantibody (e.g., AMA) production by B lymphocytes. Finally, CTL (autoreactive CD8þ T), B lymphocytes, Th17, autoreactive CD4 +T, NK, and NKT infiltrate and gather around the small bile duct and participate in the development of autoimmunity. (*4*) Simultaneously, the number and function of immunosuppressive cells (Treg, Breg, Tr1, and CD8 Treg) decrease significantly, which indirectly promotes overactivation of immune responsiveness

Supporting evidence for a role of AMA in PBC include the higher numbers of B cells spontaneously producing disease-specific autoantibodies in blood and liver tissue of PBC patients compared to controls and the correlation of the frequency of PDC-specific B lymphocytes in peripheral blood lymphocyte infiltration in the liver [138, 139]. In addition, the proportion of CD19+CD69+ activated B cells is markedly higher in the liver than in peripheral blood of PBC patients, and the number of AMA-producing cells is five times greater in the liver than in peripheral blood [140]. Further, findings of high levels of autoantigen-specific peripheral plasmablasts indicating recent activation of naive or memory B cells and a continuous and robust activation along with the presence of CXCR7+ $CCR10^{low}$ PDC-E2-specific antibody-secreting cells suggest a mechanistic basis for the migration of circulating antigen-specific plasmablasts to the mucosal epithelial [141].

8.3.3.2 Autoreactive T Cells

CD4+ and CD8+ T cells are present in portal tracts and around damaged bile ducts in PBC, strongly supporting their role in the development of biliary damage. Using six HLA-DRB4*01:01-restricted autoreactive CD4+ T cells from four different patients with PBC, Shimoda et al. mapped all six clones to peptide residues 163–176 (GDLLAEIETDKATI) of PDC-E2, which corresponds to the inner lipoyl domain [142]. Further study showed that those PDC-E2-specific autoreactive CD4 T cells are present in peripheral blood and liver with a 100–150-fold increase in the number of PDC-E2-specific CD4+ T cells in the hilar lymph nodes and liver compared to peripheral blood in patients with PBC [143]. Similar results were noted with HLA class I (HLA-A0201) restricted autoreactive CD8+ T cells mapping to residues 159–167 of PDC-E2 in close vicinity to the epitopes recognized by CD4 T cells as well as by AMA [127]. Further, a tenfold higher frequency of PDC-E2 159–167-specific CD8+ T cells was found in the liver compared with blood. Moreover, the frequency of precursor of PDC-E2-specific autoreactive CD8 T cells was significantly higher in early- compared to late-stage disease [144].

8.3.3.3 T Helper Cells and Regulatory T Cells

In addition to the classical CD4+ T helper cells, including Th1 and Th2 that were reported to be abnormal in over a decade ago [145, 146], many new classes of helper T cells have been noted to be abnormal in PBC. Several PBC animal models have impaired T regulatory (Treg) functions including disruption of TGF-β signaling in T cells and targeted deletion of CD25 and Foxp3 [147–149]. In human PBC, FoxP3+ Tregs can be identified in the lymphoid infiltrates localized to portal tracts, and a significantly lower proportion of circulating CD4 + $CD25^{high}$ Tregs are observed in PBC patients and their family members compared to healthy controls [95]. Th17 cells have been noted to accumulate around damaged bile ducts in liver tissue both from a mouse model and PBC patients [150], and follicular T helper cells in PBC have been noted to be increased and have a functional activation including IL-21 production and the ability to promote B-cell maturation and autoantibody production [151].

8.3.3.4 Monocytes

The finding of macrophages among the infiltrate of the chronic nonsuppurative destructive cholangitis classically seen in PBC was noted several decades ago [152]. Subsequently, TLR3 has been noted to be highly expressed on those macrophages and to promote the expression of type I interferons in early-stage PBC [153]. In addition, peripheral blood monocytes in PBC express higher levels of TLR4 and secrete more proinflammatory cytokines in response to infectious stimuli including lipopolysaccharide (LPS) [99, 154]. At the same time, the level of RP105, which is involved in the negative regulation of TLR4 signaling, is decreased in PBC monocytes [154].

8.3.3.5 Dendritic Cells

Localization and activation of dendritic cells have been noticed in the abnormal immune pathogenesis of PBC long time ago. By using S100 protein, dendritic cells were first identified inside the basement membrane between biliary epithelial cells of septal bile ducts in early-stage PBC [155]. Then, high restricted distribution of CD83-positive-activated DC was also found in the liver from patients with primary biliary cholangitis [156].

As antigen-presenting cells, their function in activation of the autoreactive T cells was investigated. In 2001, Akbar et al. firstly demonstrated peripheral blood T cells from PBC and showed PDC-specific proliferation when cultured with PDC-pulsed DCs [157]. In 2002, Kita et al. also found that by pulsing DC with full-length recombinant PDC-E2 protein, PDC-E2-specific cytotoxic T lymphocytes (CTL) could be generated, which indicated that CTL activation could be augmented by immune complexes cross presented by DC [158]. In addition, a phenotype of DC2 with reduced expression of HLA D2 and CD123 in PBC was considered relevant to the breakdown of tolerance to self-antigen [159]. Recently, Langerhans cells (LCs), another subtype of DC, were found existing around or within biliary epithelial layers and closely associated with the periductal cytokine milieu in patients with PBC [160].

8.3.3.6 NK and NKT Cells

With increased numbers in the peripheral blood [161] and higher frequency of cytotoxic activity [97], NK cells have been involved in the pathogenesis of PBC. A higher frequency of CD56dim/CD16pos hepatic NK cells with cytotoxic activity against autologous biliary epithelial cells was present within the liver of PBC patients, which may reflect the breakdown of NK cell immune tolerance [162]. The latest study found that NK cell-mediated innate immune responses are likely critical at the initial stage of PBC, but also facilitate and maintain the chronic cytopathic effect of autoantigen-specific T cells, essential for progression of disease.

Natural killer T (NKT) cells are a regulatory T-cell lineage that has a range of immune activities. Their correlation role with PBC patients was firstly reported by Kita et al. in 2002 [98]. Then, through a series of mice model experiments [125, 163, 164], NKT cells are found to be involved in disease exacerbations. In 2014, Aso-Ishimoto et al. further confirmed that NKT cells were significantly decreased in the liver of patients with early PBC, but increased in advanced PBC, which

suggest that activated NKT cells may contribute to the biliary epithelial cell death resulting in the progression of PBC [165]. At last, by depletion both NK and NKT cells in 2-OA-BSA mouse model, Shimoda et al. found that there is a marked suppression of AMA and cytokine production from autoreactive T cells [95, 98, 127, 138–151, 158, 166–170].

8.3.4 Animal Models

The successfully constructing several murine models with manifest characteristic clinical features of human PBC, the pathogenesis of PBC, were further investigated in earlier stages and at more detailed levels, although the diverse clinical courses and the complexity of the immunological mechanisms of PBC cannot be fully recapitulated by a single animal model [171]. Until now, the PBC models could arbitrary be divided into genetically relative spontaneous models, environmental (xenobiotic and infection) triggered models, and autoimmunity relative models (Table 8.1) [176].

8.3.4.1 Genetically Relative Spontaneous Models

NOD.c3c4 mice is double-congenic mouse strain, which the Idd-resistant alleles from B10 and B6 mice were replaced on chromosomes 3 and 4, in nonobese diabetic (NOD) mice, respectively. In 2004, Koarada et al. [172] first discovered the PBC-like characteristics in the NOD.c3c4 mice, with spontaneous lymphocyte (CD3+, CD4+, and CD8+ T cells and PDCA1+ dendritic cells) infiltrations around the bile duct, the appearance of AMA and ANA (PDC-E2 positivity, 56% for 9–10 weeks; ANA positivity, 80–90% for 20–25 weeks), and biliary destruction [173]. Furthermore, the lack of apoptosis in bile duct epithelium due to decreased expression of Fas antigen is likely responsible for the development of autoimmunity in this model [177].

The Cl^-/HCO_3^- anion exchanger 2 (AE2) plays an important role in acid-base transport and export of biliary bicarbonate, which are involved in intracellular pH regulation. In 2008, AE2-knockdown mice (Ae2a, b −/− mice) were noticed to be another genetic relative PBC mouse model [174]. AMA and increased levels of IgM, IgG, and alkaline phosphatase were observed in Ae2a,b−/− mice, and histologically, 30% of the mice showed infiltration of CD4+ and CD8+ T cells in the portal areas and around the damaged bile duct. However, the disadvantage of this model is that many mice show no changes and are difficult to breed.

8.3.4.2 Environmental Factors Triggered Models

Xenobiotic Trigged Models In 2007, Leung and colleagues immunized groups of guinea pigs with 6-bromohexanoate (6-BH)-conjugated BSA and found that immunized guinea pigs not only developed AMA responses similar to human PBC but also developed autoimmune cholangitis after 18 months [89]. 2-octynoic acid (2-OA) is found in several cosmetic products including nail polish, and their frequent use among women may contribute to the female predominance of PBC. Exposing

Table 8.1 The characteristic of different factor-induced mouse models of PBC

	Genetically relative models		Environmental triggered models			Autoimmunity relative models		
	NOD.ABD	Ae2a,b−/−	2-OA-BSA	*E. coli*	*N. aromaticivorans*	dnTGFβRII	IL-2Ra−/−	Scurfy
Classification	Spontaneous	Spontaneous	Induced	Induced	Induced	Spontaneous	Spontaneous	Spontaneous
Background	NOD.c3c4	?	C57BL/6 or NOD.1101	NOD. B6-Idd10/ Idd18	NOD1101	C57BL/6	C57BL/6	C57BL/6
Gender differences	Female dominant	−	−	−	−	−	−	−
Serum biochemistry	−	Bile stasis	Bile stasis	?	?	Bile stasis	−	−
AMA	50–60%	40–80%	100%	100%	100%	100%	100%	100%
ANA	40–50%	?	?	?	?	100%	80%	?
Ig	IgM+++, IgG+++	IgA+++, IgG++	?	?	?	IgA+++, IgG+	IgA+++, IgG+++	IgM+++, IgA++ IgG +++
Portal lymphocytic infiltration	+++	+~+++	+~++	+	+	+++	+++	++~+++
Bile duct destruction	+	+~+++	+	+	+	+~++	+~++	+~++
Granuloma	+	−	+	−	−	−	−	−
Eosinophilia	+	+	−	−	−	−	−	+
Liver fibrosis	30%	−	-~±	−	−	−	−	−

(continued)

Table 8.1 (continued)

	Genetically relative models		Environmental triggered models			Autoimmunity relative models		
	NOD.ABD	Ae2a,b−/−	2-OA-BSA	*E. coli*	*N. aromaticivorans*	dnTGFβRII	IL-2Ra−/−	Scurfy
Commons	Also develop common biliary dilation and biliary epithelial cell proliferation	Late onset, difficult to breed	Proof of molecular mimics could lead to breach tolerance, late onset, difficult to breed	High light the importance of microbial infections in breach tolerance	High light the importance of microbial infections in breach tolerance	Spontaneously develop inflammatory bowel disease	Severe anemia, inflammatory bowel disease, short life span	Short life span
Ref	[172, 173]	[174]	[92, 93]	[175]	[125]	[147]	[148]	[149]

NOD.1101 [93] or C57BL/6 [92] to 2-octynoic acid-conjugated BSA (2OA-BSA) could induce AMA and PBC-like liver lesion. The 2OA-BSA PBC model was used not only in studying the mechanisms of cytokines [178] and NK/NKT cells [164, 166], but also has been used in preclinical trials, including depleting B cells by two different monoclonal antibodies (CD20 and CD79) [179] and CTLA4-based therapy on cholangitis by using CTLA4-Ig [180].

Infectious Trigged Models In 2014, Wang et al. demonstrated that NOD.B6-Idd10/Idd18 mice infected with *E. coli* developed AMA and severe cholangitis [175]. It has been reported that there are six *E. coli* peptide sequences that mimic the human PDC-E2 autoepitope with 6–8 identical amino acid residues [181], which may account for the *E. coli*-induced anti-PDC-E2 response in the NOD.B6-Idd10/Idd18 mice. In 2008, Mattner et al. reported that mice (common mouse strains C57BL/6, NOD, SJL) infected with *N. aromaticivorans* could also construct a model with PBC-like features [125]. *N. aromaticivorans* exhibits molecular homology with the PDC-E2 epitope and may potentiate the breakdown of self-tolerance in PBC patients [182].

8.3.4.3 Autoimmunity Relative Models

TGF-β receptor II is critical for signal transduction of TGF-β, which regulates the activation of lymphocytes [183]. In 2006, Oertelt S et al. firstly reported that under the direction of the CD4 promoter, a transgenic mouse directly expressing a dominant-negative form of TGF-β receptor type II (dnTGFβRII) mimics several key phenotypic features of human PBC [147]. Using this model, the pathology roles of NKT [44], CD4+, CD8+ T [184, 185], B cells [186], and lots of cytokines (such as IL-12, IL-23, and IL-17) [187–189] were extensively investigated. In addition, a preclinical experiment of B-cell depletion therapeutic was also done in dnTGFβRII mice, which indicated the potential usage of anti-CD20 in early PBC, but, at the same time, raised a cautionary note regarding the use of anti-CD20 in inflammatory bowel disease [186].

The IL-2 and Foxp3 signal pathways both play an important role for differentiation of Treg cells. In 2006, Wakabayashi et al. firstly reported that chronic nonsuppurative destructive cholangitis (CNSDC) features were found in IL-2Rα−/− c57BL/6 mice [148]. In 2009, the scurfy mice, in which the function of Tregs is abolished, were also reported to show similar pathogenesis and clinical feature to PBC patients [149]. However, the short life span makes these two model less attractive.

8.4 Progress in the Diagnosis of PBC

When initially described, PBC was believed to be a rare autoimmune disease with nearly all patients diagnosed at an advanced stage based upon the classic signs and symptoms of biliary cirrhosis with pruritus, jaundice, and xanthelasma. However, advances in diagnostic testing, particularly in autoantibody detection, have significantly changed the ability to confidently diagnose PBC (Fig. 8.3). In fact, no other

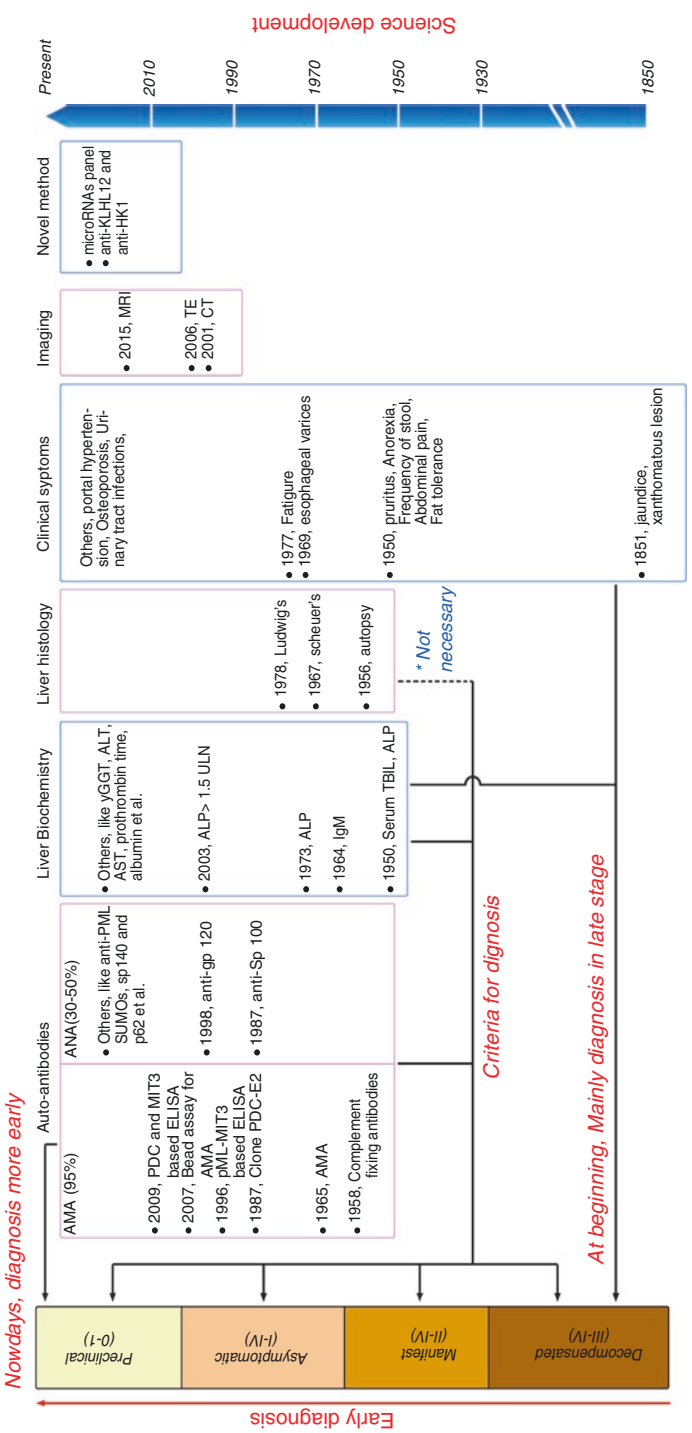

Fig. 8.3 History of the diagnosis of primary biliary cholangitis. At the beginning, PBC were mainly diagnosed in advanced stage depending on classical signs and symptoms (such as pruritus, jaundice, and xanthomatosis). With the development of science (discovering PBC-specific autoantibodies, learning about the characteristic of liver histology, and development of more sensitive and accurate detection methods), the PBC patients were diagnosed in much early stage. Right now, PBC is diagnosed provided two of the following three criteria are satisfied: (1) AMA titer > 1:40, (2) alkaline phosphatase (AP) > 1.5 times the upper limit of normal for > 24 week, and (3) compatible liver histology

autoimmune condition has autoantibodies with such specificity and sensitivity as those found in PBC. Indeed, the diagnosis of PBC requires at least two of three criteria including the presence of AMA, persistent elevation of serum alkaline phosphatase, and liver histology consistent with PBC [24].

8.4.1 Autoantibodies

Since Ian Mackay's first discovery of complement fixing antibodies in PBC [190], more than 60 distinct autoantibodies have been identified in PBC, but only a few are specific to PBC and useful for diagnosis. In addition, some may also assist in the assessment of disease severity, clinical phenotype, and long-term prognosis [191] (Table 8.2).

In 1965, antimitochondrial antibodies (AMA) in PBC sera were first recognized by Walker and his colleagues [207] using indirect immunofluorescence, and subsequently the 74 kD mitochondrial autoantigen, PDC-E2, was cloned and sequenced in 1987 [136, 208]. The epitopes recognized by AMA are often referred to as M2 antigens for historical reasons but more accurately include the lipoylated domains of the E2 and E3 binding protein (E3BP) components of the pyruvate dehydrogenase complex (PDC-E2) and the E2 components of the 2-oxoglutarate dehydrogenase (OADC-E2) and branched-chain 2-oxo-acid dehydrogenase (BCOADC-E2) complexes. Accordingly, the discovery of those autoantigens greatly changed AMA detection, moving from indirect immunofluorescence (IIF) of rat kidney, stomach, liver, or HEp-2 cells to immunoblotting of purified or recombinant mitochondrial antigens and finally to sensitive enzyme-linked immunoassay (ELISA) systems and bead assays using recombinant proteins co-expressing the immunodominant epitopes of PDC-E2, BCOADC-E2, and OACD-E2 [9, 84, 209, 210]. With improvements in the sensitivity of assays for detecting AMAs, up to 95% of PBC patients can now be classified as AMA-positive compared to 80% with older technologies [24].

In addition to AMA, PBC-specific antinuclear autoantibodies (ANA) with membranous/rim-like and multiple nuclear dots (MND) IIF patterns are also detected in approximately 30–50% of PBC patients [211–213]. These antigens include components of the nuclear envelope pore complexes (gp210 and p62), which correspond to a dotted nuclear envelope or rim-like/membranous pattern; lamin A, B, and C and lamin B receptor, which correspond to a smooth nuclear envelope pattern; sp100, PML proteins, sp140, and small ubiquitin-related modifiers (SUMOs), which correspond to a multiple nuclear dot (MND) pattern; and centromere A, B, and C proteins, which correspond to a centromere (CENP) pattern [191]. The detection of these PBC-specific ANA can be used to confirm the diagnosis of PBC in AMA-negative cases, thus increasing the sensitivity of serological tests in diagnosing PBC. Approximately half of AMA-negative PBC patients are positive for at least one of the three PBC-specific ANAs, including anti-gp210, anti-SP100, or antipromyelocytic leukemia (PML) antibodies [9, 214].

Although AMA titers do not change over time and are not associated with disease severity or progression [215], some PBC-specific ANAs have been reported to

Table 8.2 Autoantibodies detected in primary biliary cholangitis

IIF pattern	Autoantibody	Autoantigen properties	Sensitivity	Specificity	Clinical associations	Ref
MIT	AMA	Mitochondrial	90–95%	High	# diagnostic value # prevalence in 0.16%–1% of general population # not associated with disease severity and treatment effect (except for AMA-IgA) # no clinical different between AMA-positive and AMA negative patients	[30, 169]
	Anti-PDC-E2	Outer and inner lipoyl domain	80–90%			
	Anti-PDC-E3BP	Not reported	10%			
	Anti-PDC-E1a	TTP binding and phosphorylation site	5–25%			
	Anti-OGDC-E2	lipoyl domain	20–60%			
	Anti-BCOADC-E2	lipoyl domain	50–80%			
NE	Anti-gp210	Integral glycoprotein of the nuclear pore	20–40%	Very high	# diagnostic value for AMA-negative patients # associated with disease severity and treatment effect	[192, 193]
	Anti-P62	Glycoprotein of the nuclear pore	10–30%	High (also detected in SjS)	# diagnostic value for AMA-negative patients # presence related to the progressive or advanced stage of PBC # indicate marked inflammatory infiltrates in liver biopsy	[194–196]
	Anti-lamin B receptor (LER)	A protein integral to the inner nuclear membrane with a nucleoplasmic	2–6%	High	# diagnostic value for AMA-negative patients # clinical significance is nuclear	[197, 198]

(continued)

Table 8.2 (continued)

IIF pattern	Autoantibody	Autoantigen properties	Sensitivity	Specificity	Clinical associations	Ref
MND	Anti-SP100	Nuclear protein antigen	20–40%	High (also detected in SLE, pSS)	# diagnostic value for AMA-negative patients # associated with disease faster progression	[199–201]
	Anti-PML	A protein fused with the retinoic acid rector-a	15–20%	High	No report	[199, 200, 202]
	Anti-SP140	Promyelocytic leukemia protein nuclear body components	15%	High	# diagnostic value for AMA-negative patients # no association was found in any clinical feature	[203]
	Anti-SUMO-1, 2	Small ubiquitin-related modifiers	2–6%	High	# diagnostic value for AMA-negative patients	[204]
CENP	Anti-centromere A,B,C	Major centromere proteins	10–30%	Not high (also detected in SSc)	# predictive value for progression to portal hypertension for PBC	[205, 206]

associate with a more severe disease course [194, 211]. Antibodies to gp210, which are highly specific for PBC and detected in 20–40% of PBC patients [192, 193, 216], have in particular been associated with disease severity [194, 205, 217–221]. In fact, serum titers of anti-gp210 antibodies change from negative to positive or vice versa depending on disease activity or stage progression. Anti-sp100 and anti-PML antibodies are also highly specific for PBC, with a prevalence of 20–40% and 15–20%, respectively [199, 200, 202, 222], and have also been reported to associate with disease severity and poor prognosis [199, 200, 202].

8.4.2 Liver Biochemistry Changes

Presently, more than half of patients diagnosed with PBC are asymptomatic and only suspected based upon routine liver tests. They generally attract medical attention by findings of elevated serum alkaline phosphatase. In addition, serum alkaline phosphatase is used as a measure of disease activity and response to treatment in PBC. Although there is a linear correlation between serum bile acids and alkaline phosphatase in PBC, there are several limitations to the use of serum alkaline phosphatase that should be considered. Four distinct alkaline phosphatase isoforms are present in humans, namely, intestinal, placental, placenta-like, and liver/bone/kidney, and the liver alkaline phosphatase is found in the serum of patients with cholestatic forms of liver disease including PBC. However, serum alkaline phosphatase activity can be influenced by genetic variants, pregnancy, bone growth, age, and medications [223].

In addition to alkaline phosphatase, elevated levels of serum γ-glutamyl transferase and aminotransferases are usually observed in PBC. Increased serum levels of (conjugated) bilirubin as well as alterations in prothrombin time and serum albumin are usually late phenomena in PBC similar to other cirrhotic states. However, serum bilirubin is a strong and independent predictor of survival with a high impact on all established models of prognosis.

Serum IgM value is often increased in cholestasis, particularly in PBC [224]. The combination of a high serum IgM together with a very high ALP and a normal or only moderately increased serum bilirubin concentration strongly suggests PBC. Increased awareness of the condition and the increasing availability of diagnostic tools, in particular serological testing, have led to a more frequent and earlier diagnosis of PBC.

8.4.3 Liver Histology

The histological changes of PBC are well defined and traditionally based on Ludwig's four classification stages, including stage I, portal inflammation with granulomatous destruction of the bile ducts with or without granulomas; stage II, periportal hepatitis and bile duct proliferation; stage III, fibrous septa or bridging necrosis; and stage IV, cirrhosis [225]. Notably, the liver is not uniformly involved, and features of all four stages of PBC can be found in one biopsy specimen. The role of liver biopsy in

patient management currently remains controversial, and most patients do not undergo liver biopsy as it is not required for diagnosis in most cases and does not alter management. However, there may be some potential prognostic value in obtaining a liver biopsy as the presence of piecemeal necrosis has been associated with a decrease in transplant-free survival [226–230]. In addition, liver biopsy should also be performed if there is concern for an overlap with autoimmune hepatitis.

8.4.4 Novel Diagnosis Methods

In rare cases, the diagnosis of PBC remains challenging when there is an absence of AMA or PBC-specific ANA in a patient with other features suggestive of PBC. In addition, development of less expensive diagnostic tools would be welcomed in clinical practice. In 2014, Tan Y et al. reported a panel of microRNAs (hsa-miR-122-5p, hsa-miR-141-3p, and hsa-miR-26b-5p) that showed a high diagnostic accuracy for PBC but was significantly less accurate compared to AMA [231]. More recently, anti-KLHL12 and anti-HK1 antibodies added to AMA and ANA serological assays significantly improved efficacy in the clinical detection and diagnosis of PBC, especially for AMA-negative subjects [232].

8.5 Current Therapies for PBC Patients

PBC therapies are largely targeted to the liver pathology in order to prevent or reverse bile duct injury, subsequent liver fibrosis and liver-related complications, and death. Secondarily, these effects would be expected to prevent the consequences of chronic cholestasis, including pruritus, fatigue, osteoporosis, etc.

8.5.1 Ursodeoxycholic Acid (UDCA)

Despite major breakthroughs in the understanding of PBC, there remains only one agent approved by the US Food and Drug Administration for the treatment of PBC, ursodeoxycholic acid (UDCA). UDCA dosed at 13–15 mg/kg/day is recommended by the practice guidelines of both the European and the American Associations for the Study of Liver Diseases for PBC patients [233–236].

UDCA may act through multiple mechanisms including the stimulation of hepatocellular and ductular secretions, cytoprotection against bile acid-induced injury, anti-apoptotic effects, and further anti-inflammatory and immunomodulatory effects. Hydrophobic bile acids damage cell membranes and that conjugates of UDCA counteract [237] and modulate the composition of mixed phospholipid-rich micelles in bile [238, 239]. UDCA also stimulates biliary secretion of bile acids [240, 241] by enhancing the expression of transporter proteins [242–244] and targeting the insertion of transporter molecules into the hepatocyte canalicular membrane [245–248] (Figs. 8.4 and 8.5). In addition, accumulation of hydrophobic bile

acids in hepatocytes can lead to apoptosis and subsequent inflammation and liver fibrosis. Anti-apoptotic effects of UDCA in vitro and in vivo in the rat [251, 252] and in human hepatocytes [253] are associated with a reduction in mitochondrial membrane permeability to ions and mitochondrial cytochrome c release [254]. UDCA diminishes Fas ligand-induced apoptosis in mouse hepatocytes [255] and protects rat hepatocytes against bile acid-induced apoptosis by preventing bile acid-induced, c-Jun N-terminal kinase-dependent Fas trafficking to the plasma membrane [256]. Further actions of UDCA include the suppression of IL-2, IL-4, and IFN-y from activated T cells, immunoglobulin production from stimulated B cells [257–259], IL-1 secretion from monocytes/macrophages [260], and suppression of IFN-γ-induced MHC class II expression [261–263] (Fig. 8.6).

The first open-label trial of UDCA at a dose of 12 to 15 mg/kg/d in patients with PBC successfully demonstrated a dramatic improvement in liver biochemistries with few adverse effects [264]. In 1991, the first multicenter, double-blind randomized controlled trial of UDCA further validated the usefulness of UDCA as a therapeutic agent for PBC [265] followed by a series of clinical trials that found a similar favorable effect on improvement of biochemical cholestasis [266–268]. However, when UDCA has been stopped, there is a prompt rebound of serum biochemical

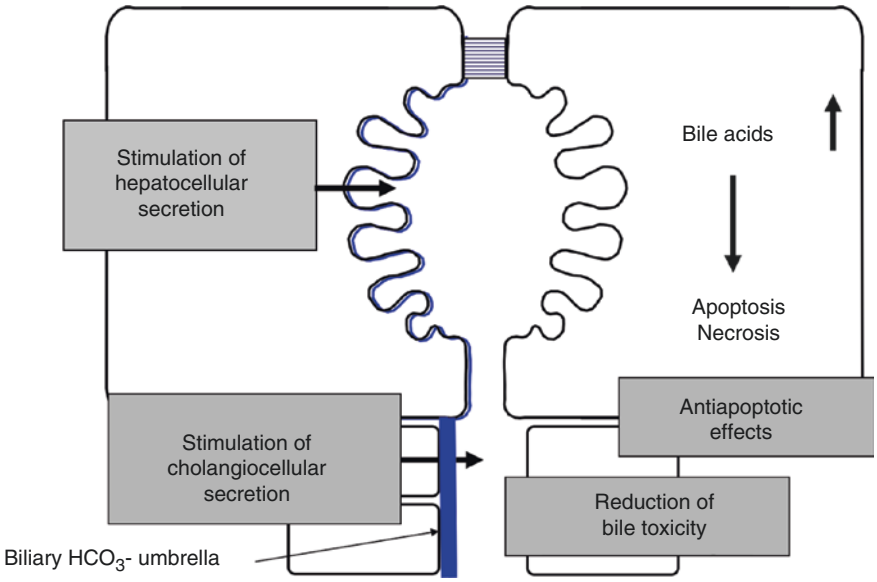

Fig. 8.4 Potential mechanisms and sites of action of ursodeoxycholic acid in cholestatic liver diseases and primary biliary cholangitis. Several mechanisms could contribute to the beneficial effect of ursodeoxycholic acid (UDCA) under various cholestatic conditions: (1) by stimulating impaired hepatobiliary secretion, (2) via inhibition of bile acid-induced hepatocyte (and cholangiocyte?) apoptosis, (3) by decreasing bile cytotoxicity, and (4) via stimulation of cholangiocellular calcium-dependent chloride/bicarbonate anion secretion. The relative contribution of each of these mechanisms to the anti-cholestatic action of UDCA is unknown (This figure is cited from Beuers [249])

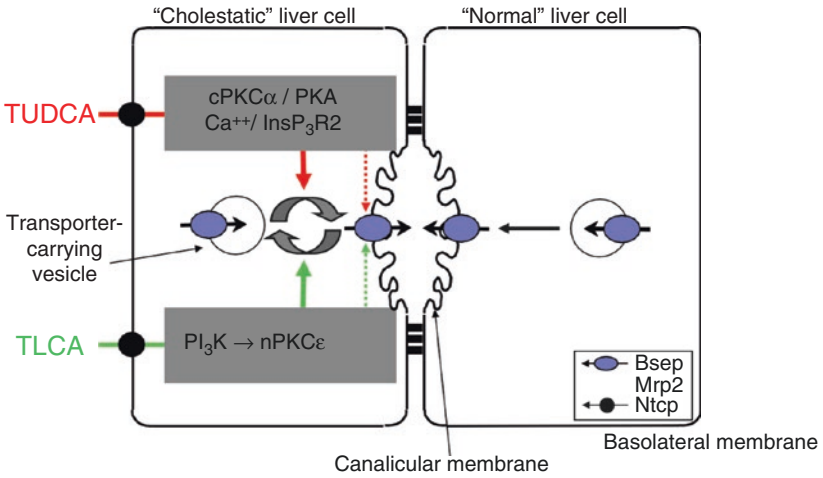

Fig. 8.5 UDCA conjugates act as posttranscriptional secretagogues in experimental cholestasis. Experimental model of TUDCA-induced stimulation of hepatocellular secretion. TUDCA is taken up into the hepatocyte by the Na^+-taurocholate cotransporting polypeptide (Ntcp) and stimulates apical vesicular exocytosis and insertion of key canalicular transporters such as the conjugate export pump Mrp2 and the bile salt export pump, Bsep, via Ca^{2+}- and PKCα-dependent mechanisms or via activation of p38MAPK and Ras-, Raf-, Erk-1/2-dependent mechanisms. TLCA is the most potent cholestatic agent among the major human bile acids. TLCA is a potent signaling molecule which elevates hepatocellular $[Ca^{2+}]_i$ without stimulation of Ca^{2+} influx, selectively translocates nPKCε to canalicular membranes and activates membrane-bound PKC, and induces retrieval of key apical transporters such as the bile acid export pump, Bsep/Abcb11, from canalicular membranes of hepatocytes (This figure is cited from Wimmer et al. [250] and Gustav Paumgartner and Ulrich Beuers [239])

values to pretreatment levels indicating that long-term, perhaps lifelong, treatment is likely to be necessary [266, 267, 269–272].

Because PBC is a slowly progressive and rare disease, establishing benefits of any therapy in terms of histologic or clinical outcomes has been challenging. Nevertheless, histologic improvement with UDCA has been demonstrated with long-term UDCA treatment delaying histological progression [57]. In addition, a Markov model of a randomized, double-blinded, placebo-controlled trial of UDCA found that UDCA therapy was associated with a fivefold lower progression rate from early-stage disease to extensive fibrosis or cirrhosis (7% per year under UDCA vs. 34% per year under placebo), but was not associated with a significant difference in regression rates [273]. Further, analysis of paired liver biopsy specimens from several studies with a time interval of 36 months between biopsies indicated that UDCA had the greatest effects on the subgroup of patients with baseline stages I and II [274].

The effect of UDCA on survival has been more difficult to establish. The first long-term clinical trial involving 145 patients with biopsy-proved PBC randomized to receive UDCA or placebo for 2 years followed by an open-label trial for two additional years showed that UDCA therapy reduces the need for liver transplantation [275]. Several additional clinical trials and meta-analyses have been performed with most, though not all [276–279], concluding that UDCA improves transplant-free

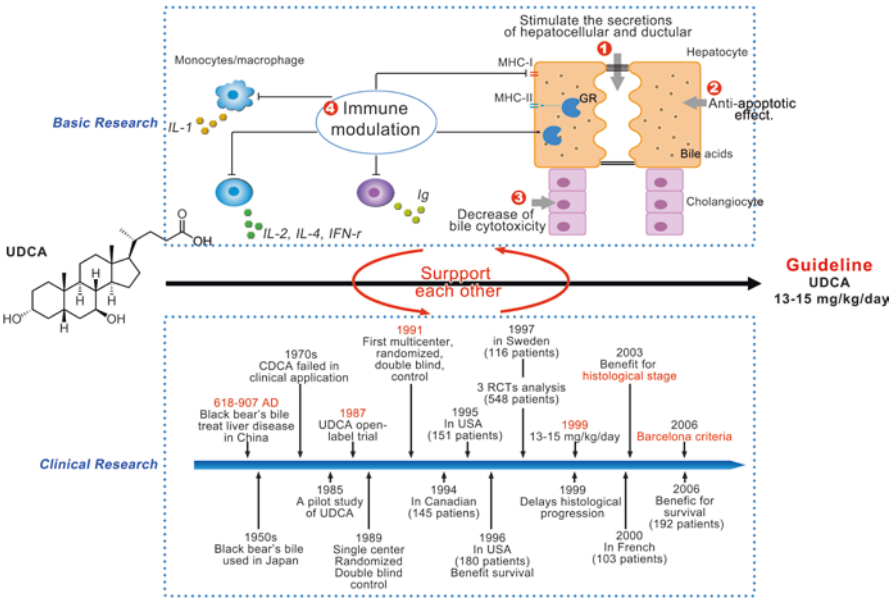

Fig. 8.6 Basic and clinical research made UDCA the only treatment for primary biliary cholangitis. Through clinical research, UDCA could dramatically improve liver biochemistries, significantly delay the progression, and prolong survival free of transplantation for PBC patients. Basic research revealed a series of mechanisms responsible for UDCA's treatment effects: (*1*) stimulate hepatocellular and ductular secretions, (*2*) anti-apoptotic effects, (*3*) decrease of bile cytotoxicity, (*4*) immune modulation. Finally, both the basic and clinical research supporting each other made UDCA, dosage at 13–15 mg/kg/day, to be the only therapy for PBC approved by the US Food and Drug Administration and recommended by the practice guidelines of both the European and the American associations

survival among patients with later-stage disease [280]. In addition, several large cohorts have demonstrated significantly better survival among those treated with UDCA compared to predicted survival using models of historical controls [25–27, 281–284].

Although UDCA appears to be effective in many if not most cases of PBC, there remains a group of patients that continues to progress. The early recognition of PBC patients with a predicted poor long-term outcome is a key issue for patient management and effective design of clinical trials. The biochemical response to UDCA is an independent predictive factor for death and liver transplantation and has been recommended as a study endpoint in clinical trials where clinical endpoints are deemed unfeasible. Thus, it is necessary and important to identify patients with an incomplete biochemical response as soon as possible.

Several criteria have been reported to define an "incomplete" biochemical response to UDCA. Pares et al. from Barcelona defined response to UDCA treatment by an alkaline phosphatase decrease greater than 40% of baseline values or normal levels after 1 year of treatment and successfully predicted the survival for UDCA treatment patients [27]. Several other studies have shown that the biochemical response to UDCA assessed at 1 year or 6 months can predict the long-term outcome

with UDCA responders having a survival similar to that estimated for the matched control population [25, 26, 282–285]. More recent studies of large cohorts have compared these criteria and developed new models, but in general they all conclude that the main predictors are serum levels of alkaline phosphatase and bilirubin [32, 286–288] (Table 8.3). In addition to biochemical response, other predictors of outcome may capture additional information related to outcomes. The AST/platelet ratio index (APRI), which is an indicator of advanced liver fibrosis, has been reported to predict adverse events in patients with PBC independent of UDCA response [289].

8.5.2 Liver Transplantation

Liver transplantation for PBC patients has some of the best survival data compared to any other indication with the 1-, 5-, and 10-year survival rates of 86%, 80%, and 72%, respectively [290]. Until the 1990s, PBC was the most common indication for liver transplantation in the USA accounting for up to 55% of transplanted patients. Currently, PBC accounts for less than 5% of all transplantations in the USA and has steadily decreased, likely reflecting the impact of the widespread use of UDCA.

Following liver transplantation, PBC recurs in 21–37% of patients at 10 years after liver transplantation [291] and in 43% at 15 years with the median time to recurrence of 3–5.5 years [292, 293]. Diagnosis of recurrent PBC (rPBC) is based on the liver histopathology and meeting all diagnostic criteria including PBC as the indication for liver transplantation; histopathology of the graft is suggestive for rPBC, including epithelioid granulomas, mononuclear inflammatory infiltrate, formation of

Table 8.3 Biochemical criteria for the responses to ursodeoxycholic acid (UDCA) in primary biliary cholangitis

Criteria	Issue date	Author	Evaluation time	Biochemical response to UDCA	Ref
Barcelona	2006	Pares A, et al.	After 1 year	Decreased of ALP by > 40%, or ALP normalization	[27]
Paris I	2008	Corpechot C, et al.	After 1 year	ALP ≤3 x ULN, AST≤2 x ULN, and serum bilirubin ≤1 mg/dL	[25]
Rotterdam	2009	Kuiper E.M, et al.	After 1 year	Normalization of abnormal serum bilirubin and /or albumin	[26]
Ehime	2009	Azemoto	After 6 months	Normalization of GGT level or reduction rate of GGT above the ULN> 70%	[285]
Toronto	2010	Kumagi T, et al.	After 2 year	ALP <1.67 × ULN	[282]
Paris II	2011	Corpechot C, et al.	After 1 year	ALP < 1.5 × ULN or AST<1.5 × ULN or Bilirubin ≤1 mg/dL	[283]
Beijing	2013	Zhang L.N, et al.	After 6 months	ALP ≤3 × ULN, decreased bilirubin, increased albumin	[284]

lymphoid aggregates, and bile duct damage; and other causes of graft failure are excluded [294]. Recurrence of PBC has been associated with donor and recipient age [295], cold and warm ischemia times [296], number of HLA mismatches [297, 298], and immunosuppressive regimen including tacrolimus [299] and azathioprine [300]. Recently, male-to-female sex mismatch did not appear to yield a direct negative impact on outcomes following liver transplantation for PBC, but it did appear to aggravate the negative effects of prolonged cold ischemia and blood transfusions [301]. UDCA treatment of liver transplant recipients may improve liver function test results, but it does not impact patient or graft survival [302].

8.5.3 Symptom Therapy

As previously reported, fatigue and pruritus remain the most common symptoms reported by patients with PBC. In addition, patients with fatigue and pruritus at onset are more likely to progress to cirrhosis and are less likely to respond to UDCA [303]. Currently, there are no approved therapies available for these indications, and the data available indicates that off-label use of therapies has limited efficacy for these indications.

8.5.3.1 Fatigue

Fatigue occurs in up to 70% of patients [304, 305]. It is associated with excessive daytime sleepiness and autonomic dysfunction [306, 307]. Severity is measured according to the fatigue impact scale (FIS) [308] or PBC-40 (PBC-40 question profile) [309]. The pathogenesis of fatigue in liver disease is still poorly defined, but it appears to involve both peripheral and central nervous system components [306, 307]. Observational data found some benefit from modafinil in the patients with prominent daytime somnolence [310, 311] with side effects including insomnia, nausea, nervousness, and headaches.

8.5.3.2 Pruritus

Pruritus from cholestasis is mostly generalized, associated with scratching, sometimes violent, and sleep deprivation. Intensity of pruritus is one of the most unbearable symptoms, but not correlated with liver disease severity. Patients with pruritus from liver disease do not have primary pruritic skin lesions, and the lesions are usually secondary to scratching.

Although the exact mediators of cholestatic pruritus remain to be elucidated, two main mechanisms have been proposed: activation of G protein-coupled bile acid receptor 1 (GPBAR1; also known as Takeda G protein-coupled receptor 5, TGR5) and activation of the autotaxin (ATX, also known as ectonucleotide pyrophosphatase/phosphodiesterase 2) [312, 313] (Fig. 8.7). In contrast to other pruritogen candidates, such as bile salts, endogenous opioids, histamine, and serotonin serum, levels of ATX have been reported to correlate with itch intensity. Notably, ATX serum level mirrors treatment response from therapeutic interventions, such as colesevelam, rifampicin, nasobiliary drainage, or MARS® treatment. The beneficial

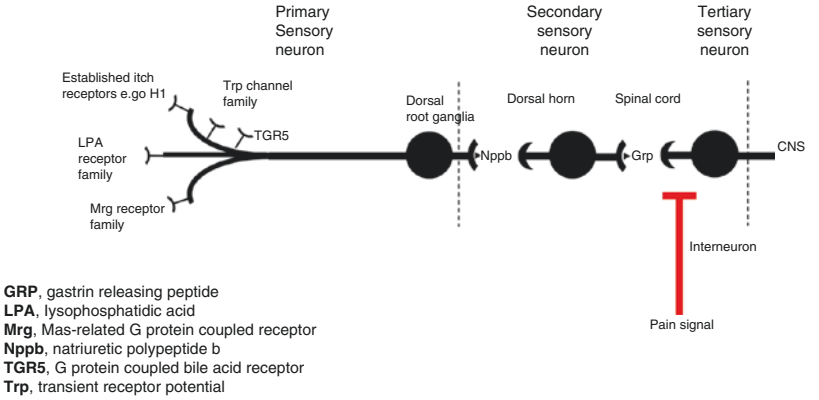

Fig. 8.7 The itch neuron. Schematic representation of receptors and neurotransmitters that may play a role in itch transmission. Pruritogens may bind to their receptors on unmyelinated itch nerve endings in the skin. This may involve established receptors such as histamine receptors, PAR2, IL-31 receptor, and others but also newly discovered receptors, such as those from the transient receptor potential (TRP family, TRPV1, and TRPA1) or from the Mas gene-related (Mrg) family, that are activated by endogenous and exogenous small molecule pruritogens. Itch may also be initiated or potentiated by activation of LPA receptors. Synaptic transmission between the primary and secondary itch neurons may be mediated by Nppb, while transmission between the secondary and tertiary neuron may involve Grp. Finally, at the level of the spinal cord itch transmission is dampened by pain signals. *CNS* central nervous system, *DRG* dorsal root ganglia, *Grp* gastrin-releasing peptide, *LPA* lysophosphatidic acid, *LPC* lysophosphatidylcholine, *Mrg* Mas-related G protein coupled, *Nppb* natriuretic polypeptide b, *Trp* transient receptor potential cation channel, *TGR5* G protein coupled bile acid receptor (This figure is cited from Beuers [314])

antipruritic action of rifampicin may be explained, at least partly, by PXR-dependent transcription inhibition of ATX expression. Targeting the ATX pathway offers considerable hope for novel therapy for PBC and other cholestatic pruritus [315].

Many therapeutic measures have been used, among which cholestyramine and colestipol are the most common. These drugs are nonabsorbable basic polystyrenes that bind anions in the gut lumen, including bile acids and other substances, blocking their absorption [316–318]. Others, like rifampicin [319, 320], naltrexone [321, 322], sertraline [323, 324], propofol, methyltestosterone, ondansetron, gabapentin, S-adenosylmethionine, ultraviolet light exposure, and plasmapheresis, have been tried for relieving itching associated with PBC, but none have been assessed in a formal manner (Fig. 8.8). Moreover, there is no evidence that standard topical therapies for pruritus are effective in patients with PBC [325].

8.5.3.3 Osteoporosis

PBC patients have a 20–44% prevalence of osteoporosis. The prevalence increases with disease progression, and up to 80% of patients with cirrhosis have osteoporosis. The risk factors for osteoporosis in PBC are similar to those in the general population and include old age, female gender, smoking, excessive alcohol consumption, underweight physique (body mass index < 19.0 kg/m^2 in adults), early menopause (< 45 years of age), positive family history of osteoporosis, and corticosteroid therapy [326]. The detailed mechanism of osteoporosis is unclear. For the prevention and treatment of osteoporosis, good nutrition is recommended, as is the suppression

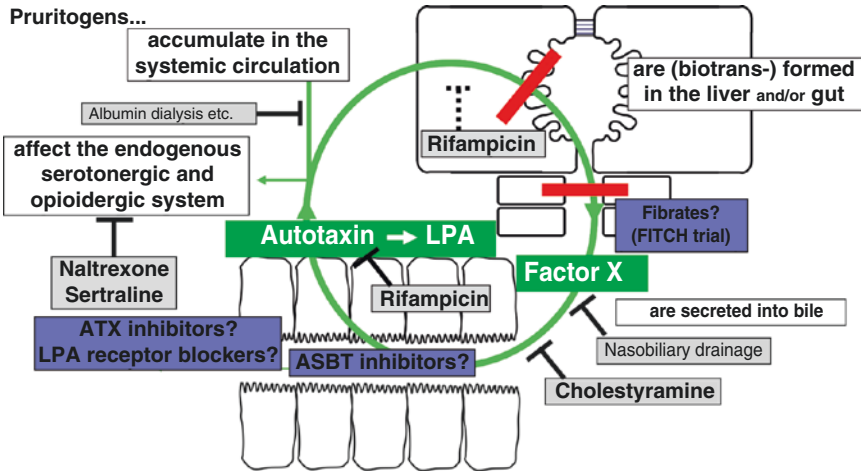

Fig. 8.8 Therapeutic targets in pruritus of cholestasis. Clinical observations indicate that potential pruritogens (1) accumulate in the systemic circulation (as indicated by relief of severe pruritus after treatment with plasmapheresis or albumin dialysis), (2) are secreted into bile (as proven by rapid relief of severe pruritus after nasobiliary drainage or suggested attenuation of pruritus after administration of cholestyramine), (3) are (biotrans-)formed in the liver and/or gut (as indicated by effective treatment with the potent PXR agonist, rifampicin), and (4) affect the endogenous opioidergic and serotoninergic system (as supported by the alleviating effects of naltrexone or sertraline). Recent evidence indicates that LPA formed by ATX represents a long-sought trigger of unmyelinated itch neuron endings. A biliary factor "X" which might stimulate ATX formation remains to be unraveled (This figure is cited from Beuers et al. [314])

of the risk factors for osteoporosis. Supplements of calcium and vitamin D, or the dose required to maintain normal levels, should be provided. Particular care should be taken with patients receiving resins because their administration may reduce the intestinal absorption of vitamin D [327]. Although calcium and vitamin D supplements are recommended, there are no data confirming the efficacy of these supplements in preventing bone loss in PBC patients. Bisphosphonates, such as pamidronate, ibandronate, or zoledronic acid to stabilize bone mineral density in PBC patients, appear to be safe and effective [328].

8.6 Scientifically Based Novel Therapeutic Targets for PBC Patients

8.6.1 Bile Acid-Based Therapies

8.6.1.1 24-norursodeoxycholic Acid (nor-UDCA)

nor-UDCA is a side chain shortened UDCA derivate, which lacks a methylene group resulting in a relative resistance to amidation with taurine or glycine compared with UDCA. *nor*-UDCA is passively absorbed from cholangiocytes and undergoes "cholehepatic shunting" instead of a full enterohepatic cycle. By induction of a HCO_3^--rich hypercholeresis [329–331], *nor*-UDCA could counteract intrinsic bile acid toxicity [332] and reinforce the "biliary HCO_3^- umbrella" [333].

Moreover, *nor*-UDCA has anti-lipotoxic, antiproliferative, antifibrotic, as well as anti-inflammatory effects which may complement stimulation of HCO_3^- secretion with bile acid detoxification and induction of alternative export via overflow systems at the basolateral membrane [334, 335].

8.6.1.2 FXR Agonists

The nuclear receptors farnesoid X receptor (FXR), retinoid X receptor (RXR), peroxisome proliferator-activated receptor α (PPARα), and pregnane X receptor (PXR) are transcriptional modifiers of bile formation and at present are under investigation as promising targets for therapeutic intervention approaches [336]. FXR, present in the liver, intestine, kidneys, and adrenals, plays an important role in the enterohepatic circulation of bile acids. It reduces bile acid synthesis by its action on 7-alpha-hydroxylase, downregulation of bile acid uptake proteins, and increases in expression of bile acid exporter pumps [337].

Obeticholic acid (OCA), an FXR agonist with a 100-fold higher affinity for FXR compared to its natural ligand chenodeoxycholic acid, has been studied in two phase II studies and one phase III study (EudraCT Number: 2011–004728-36). The latter was an international, double-blinded, placebo-controlled, clinical trial in PBC patients with an incomplete biochemical response to UDCA with preliminary results showing a significant improvement in serum alkaline phosphatase and bilirubin. However, a potential limiting adverse effect of OCA is the high incidence of pruritus. In addition to OCA, FXR agonists PX-102, NGM282, and LJN 452 are also in clinical trials for PBC patients.

8.6.1.3 PPARα Agonists

Treatment with PPAR agonists, such as bezafibrate, fenofibrate, and ciprofibrate, increases MDR3 insertion into the canalicular membrane [338], which stimulates phosphatidylcholine secretion and protects cholangiocytes against bile acid toxicity. However, PPAR agonists also repress of CYP7A1 and induce of CYP3A4 enzymes that induce bile salt synthesis and detoxification, respectively [339]. Several small studies of these drugs have been reported and, recently, a meta-analysis comparing treatment effect of UDCA plus bezafibrate versus UDCA found that combination therapy performed better than monotherapy in relation to biochemical parameters, but there was no difference concerning symptoms or survival [340]. Bezafibrate is currently approved in Europe and Japan, while only fenofibrate is available in the USA. Some concerns have been raised regarding the safety of these agents and liver toxicity, but to date none have been reported in PBC.

Others potential agents targeting bile acids are in various phases of development, including INT-777 (a TGR5 agonist), LUM001, A4250, and GSK 2330672, inhibitors of the intestinal apical sodium-bile acid transporter (ASBT) [336].

8.6.2 Immune-Based Therapies

8.6.2.1 Anti-IL12

Based upon results of GWAS implicating the IL-12 signaling pathway in several populations [112, 114] and the efficacy of anti-IL-12 therapy in other autoimmune

conditions, a clinical trial of ustekinumab was initiated. IL-12 acts as a T-cell-stimulating factor involved in the differentiation of naive T cells into Th1 cells. IL-12 is also involved in the activity of natural killer (NK) cells and their cytotoxic activity. In addition, the binding of IL-12 to its receptor is thought to modulate autoimmune responses by evoking IFN-γ production, which may in turn alter IL-23-driven induction of IL-17-producing Th17 lymphocytes. Ustekinumab is a monoclonal antibody directed at p40, a subunit shared by both IL-12 and IL-23, and which has been in clinical trials for the treatment of PBC (NCT01389973) (Fig. 8.9).

8.6.2.2 CTLA-4 Ig

CTLA-4 is an inhibitory receptor expressed on activated and regulatory T lymphocytes, where it functions as a co-inhibitory molecule that interacts with B7.1 (CD80) and B7.2 (CD86) expressed on antigen-presenting cells. Polymorphisms in *CTLA-4* may affect its inhibitory functions and have been associated with PBC [341, 342]. In addition, CTLA-4 Ig (abatacept), a recombinant fusion protein comprised of a fragment of the Fc domain of human IgG1 and the extracellular domain of human CTLA-4 inhibits T-cell activation through binding of CD80/86, was shown to ameliorate liver inflammation in two PBC mouse models [180, 343]. CTLA-4 Ig is currently approved for the treatment of rheumatoid arthritis and juvenile idiopathic

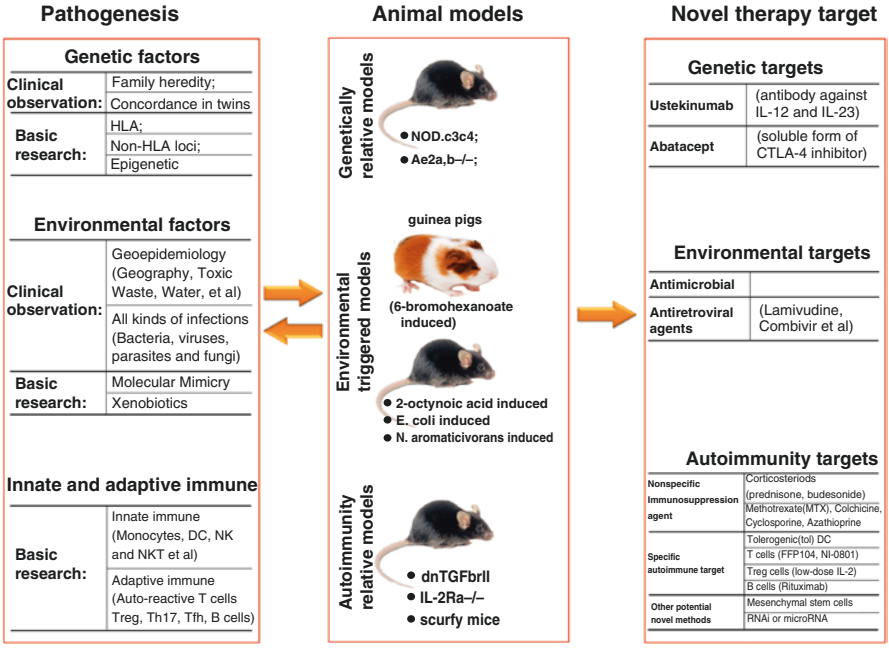

Fig. 8.9 Science provided the novel therapy target for primary biliary cholangitis. Genetic, environmental, and autoimmune factors have been reported to be involved in the pathogenesis of primary biliary cholangitis. Accordingly, genetic, environmental, and autoimmune-based animal models were also constructed for mechanisms and preclinical research. Finally, all those data provided important novel targets for the future treatment of PBC

arthritis and is in clinical trials for PBC patients with an incomplete response to UDCA (NCT02078882).

8.6.2.3 Corticosteroids

Although early studies with corticosteroids in PBC patients led to clinical, biochemical, and histologic improvement in open and controlled trials, the beneficial effects came at the expense of systemic side effects, particularly worsening of osteoporosis, which precluded their long-term use in patients with PBC [344, 345]. More recently, budesonide, a corticosteroid with an extensive first-pass hepatic metabolism and reduced systemic effects, has led to a renewed interest in the use of corticosteroids in PBC. An initial randomized controlled trial of early-stage PBC patients treated with UDCA and budesonide or placebo found a significant histologic improvement at 2 years in those patients receiving budesonide compared to placebo. In addition, there was no significant difference in changes to bone mineral density. A subsequent study of PBC patients with stages I–III disease confirmed the improvement in liver histology in patients treated with budesonide [346]. However, in an open-label trial of budesonide 9 mg daily with UDCA for 1-year in PBC patients who had an incomplete response to UDCA therapy, there was a small but significant improvement in serum levels of total bilirubin and alkaline phosphatase, while at the same time, there was a significant loss of bone mass in the lumbar spine [347]. The differences in these results may be attributed to the altered metabolism of budesonide in late-stage disease which may not only increase the risk of loss of bone density but also increase the risk of portal vein thrombosis [348]. A phase III trial is currently underway for the evaluation of budesonide in UDCA-refractory PBC patients (NCT00746486).

8.6.2.4 Methotrexate

Methotrexate is a nonspecific immunosuppression drug that is useful for treating a number of autoimmune diseases either alone or in combination with other drugs. In a case series from a single center, methotrexate has been reported to be highly effective in PBC patients that do not respond adequately to UDCA [349]. However, in a large, multicenter randomized trial comparing UDCA to UDCA plus methotrexate in PBC patients, after a median treatment period of 7.6 years, there were no differences between treatment groups [350].

8.6.2.5 Anti-CD40L

CD40L is expressed primarily on activated CD4+ T cells, and deregulation of CD40-CD40L has been documented in PBC [351–353]. In the dnTGFβRII mouse model, anti-CD40L decreased activated CD8+T cells and hepatic NKT cells and ameliorated liver inflammation and bile duct destruction [354]. A clinical trial of the anti-CD40 monoclonal antibody FFP104 is currently in phase I/II trials of the treatment of PBC (NCT02193360).

8.6.2.6 Rituximab

Although the pathogenic role of B cells in PBC is still debating, B-cell depletion therapy may have potential value. Rituximab is a mouse-human chimeric

anti-CD20 monoclonal antibody initially developed for the treatment of B-cell lymphomas and later found to be effective for the treatment of rheumatoid arthritis. Initial studies in the dnTGFβRII mouse model found that B-cell depletion with CD20 improved liver inflammation while exacerbating colitis [186]. Notably, depletion of B cells prior to induction of PBC with 2-octinoic acid led to exacerbation of liver inflammation [179]. Two clinical trials have been reported in PBC patients with an incomplete response to UDCA. Six patients with PBC who had suboptimal biochemical response to UDCA treated with rituximab were found to have a reduction in the number of AMA-producing B cells, AMA titers, serum alkaline phosphatase levels (ALP), and plasma levels of immunoglobulins (IgA, IgM, and IgG) 24 weeks after treatment (NCT00364819) [355]. However, there were two patients who experienced upper respiratory infection and reactivation of varicella zoster after the first infusion. Similar results were also found in a study of 14 patients in which autoantibody levels decreased but biochemical improvements were limited [356].

8.6.3 Cell-Based Therapies

Mesenchymal Stem Cells The potential immunomodulatory capacity of mesenchymal stem cells has raised significant clinical interest with reported efficacy in patients with severe autoimmune diseases, including Crohn's disease, multiple sclerosis, refractory systemic lupus erythematosus, and systemic sclerosis [357]. In a polyinosinic-cytidylic acid poly(I:C)-induced mouse model of PBC, Wang et al. reported that mesenchymal stem cell infusion not only induced a biochemical response but also reduced the monocyte infiltration around bile ducts and increased CD4+Foxp3+ regulatory T cells in peripheral blood and lymph nodes [358]. A clinical trial of umbilical cord-derived mesenchymal stem cells administered three times at 4-week intervals to seven PBC patients who had a suboptimal response to UDCA indicated that the treatment in PBC was safe and resulted in a significant decrease in serum alkaline phosphatase and γ-glutamyltransferase levels at the end of the follow-up period as compared with baseline [359]. Similar results using allogeneic bone marrow-derived mesenchymal stem cells in UDCA-resistant PBC patients were also reported [360].

Conclusions

Despite significant progress in our understanding of the immunologic, genetic, and environmental basis of PBC, the clinical practice of managing patients with PBC has changed little over the past two decades since UDCA was first introduced. Surprisingly, therapies targeting specific arms of the immune system have yet to be shown effective and may reflect the difficulties in halting the perpetual activation of innate immunity by a ubiquitous self-antigen. Nevertheless, the science and practice of PBC appear to be at the tipping point at which our understanding of PBC, tools for investigation, and therapeutic agents has converged for a rational and efficient evaluation of new therapies. The next decade is likely to see more change in the management of PBC than has occurred in the past two decades due to the tremendous scientific progress in the field.

Acknowledgments We would like to thank Professor Ulrich Beuers who produced four figures for this chapter.

References

1. Ahrens Jr EH, Payne MA, Kunkel HG, Eisenmenger WJ, Blondheim SH. Primary biliary cirrhosis. Medicine (Baltimore). 1950;29:299–364.
2. Griffiths L, Dyson JK, Jones DE. The new epidemiology of primary biliary cirrhosis. Semin Liver Dis. 2014;34:318–28.
3. Triger DR, Berg PA, Rodes J. Epidemiology of primary biliary cirrhosis. Liver. 1984;4:195–200.
4. Lofgren J, Jarnerot G, Danielsson D, Hemdal I. Incidence and prevalence of primary biliary cirrhosis in a defined population in Sweden. Scand J Gastroenterol. 1985;20:647–50.
5. Liu H, Liu Y, Wang L, Xu D, Lin B, Zhong R, Gong S, et al. Prevalence of primary biliary cirrhosis in adults referring hospital for annual health check-up in Southern China. BMC Gastroenterol. 2010;10:100.
6. Boonstra K, Beuers U, Ponsioen CY. Epidemiology of primary sclerosing cholangitis and primary biliary cirrhosis: a systematic review. J Hepatol. 2012;56:1181–8.
7. OF J, Bhopal R, Howel D, Gray J, Burt AD, Metcalf JV. Primary biliary cirrhosis once rare, now common in the United Kingdom? Hepatology. 1999;30:390–4.
8. Myers RP, Shaheen AA, Fong A, Burak KW, Wan A, Swain MG, Hilsden RJ, et al. Epidemiology and natural history of primary biliary cirrhosis in a Canadian health region: a population-based study. Hepatology. 2009;50:1884–92.
9. Liu H, Norman GL, Shums Z, Worman HJ, Krawitt EL, Bizzaro N, Vergani D, et al. PBC screen: an IgG/IgA dual isotype ELISA detecting multiple mitochondrial and nuclear autoantibodies specific for primary biliary cirrhosis. J Autoimmun. 2010;35:436–42.
10. Turchany JM, Uibo R, Kivik T, Van de Water J, Prindiville T, Coppel RL, Gershwin ME. A study of antimitochondrial antibodies in a random population in Estonia. Am J Gastroenterol. 1997;92:124–6.
11. Mattalia A, Quaranta S, Leung PS, Bauducci M, Van de Water J, Calvo PL, Danielle F, et al. Characterization of antimitochondrial antibodies in health adults. Hepatology. 1998;27:656–61.
12. Lazaridis KN, Juran BD, Boe GM, Slusser JP, de Andrade M, Homburger HA, Ghosh K, et al. Increased prevalence of antimitochondrial antibodies in first-degree relatives of patients with primary biliary cirrhosis. Hepatology. 2007;46:785–92.
13. Shibata M, Onozuka Y, Morizane T, Koizumi H, Kawaguchi N, Miyakawa H, Kako M, et al. Prevalence of antimitochondrial antibody in Japanese corporate workers in Kanagawa prefecture. J Gastroenterol. 2004;39:255–9.
14. Metcalf JV, Mitchison HC, Palmer JM, Jones DE, Bassendine MF, James OF. Natural history of early primary biliary cirrhosis. Lancet. 1996;348:1399–402.
15. Sherlock S. Primary biliary cirrhosis (chronic intrahepatic obstructive jaundice). Gastroenterology. 1959;37:574–86.
16. Tornay Jr AS. Primary biliary cirrhosis: natural history. Am J Gastroenterol. 1980;73:223–6.
17. Selmi C, Bowlus CL, Gershwin ME, Coppel RL. Primary biliary cirrhosis. Lancet. 2011;377:1600–9.
18. Mayo MJ. Natural history of primary biliary cirrhosis. Clin Liver Dis. 2008;12:277–88. viii
19. Pares A, Rodes J. Natural history of primary biliary cirrhosis. Clin Liver Dis. 2003;7:779–94.
20. Beswick DR, Klatskin G, Boyer JL. Asymptomatic primary biliary cirrhosis. A progress report on long-term follow-up and natural history. Gastroenterology. 1985;89:267–71.

21. Balasubramaniam K, Grambsch PM, Wiesner RH, Lindor KD, Dickson ER. Diminished survival in asymptomatic primary biliary cirrhosis. A prospective study. Gastroenterology. 1990;98:1567–71.
22. Mitchison HC, Lucey MR, Kelly PJ, Neuberger JM, Williams R, OF J. Symptom development and prognosis in primary biliary cirrhosis: a study in two centers. Gastroenterology. 1990;99:778–84.
23. Nyberg A, Loof L. Primary biliary cirrhosis: clinical features and outcome, with special reference to asymptomatic disease. Scand J Gastroenterol. 1989;24:57–64.
24. Lindor KD, Gershwin ME, Poupon R, Kaplan M, Bergasa NV, Heathcote EJ, American Association for Study of Liver D. Primary biliary cirrhosis. Hepatology. 2009;50:291–308.
25. Corpechot C, Abenavoli L, Rabahi N, Chretien Y, Andreani T, Johanet C, Chazouilleres O, et al. Biochemical response to ursodeoxycholic acid and long-term prognosis in primary biliary cirrhosis. Hepatology. 2008;48:871–7.
26. Kuiper EM, Hansen BE, de Vries RA, den Ouden-Muller JW, van Ditzhuijsen TJ, Haagsma EB, Houben MH, et al. Improved prognosis of patients with primary biliary cirrhosis that have a biochemical response to ursodeoxycholic acid. Gastroenterology. 2009;136:1281–7.
27. Pares A, Caballeria L, Rodes J. Excellent long-term survival in patients with primary biliary cirrhosis and biochemical response to ursodeoxycholic Acid. Gastroenterology. 2006;130:715–20.
28. Lee J, Belanger A, Doucette JT, Stanca C, Friedman S, Bach N. Transplantation trends in primary biliary cirrhosis. Clin Gastroenterol Hepatol. 2007;5:1313–5.
29. Kuiper EM, Hansen BE, Adang RP, van Nieuwkerk CM, Timmer R, Drenth JP, Spoelstra P, et al. Relatively high risk for hepatocellular carcinoma in patients with primary biliary cirrhosis not responding to ursodeoxycholic acid. Eur J Gastroenterol Hepatol. 2010;22:1495–502.
30. Kaplan MM, Gershwin ME. Primary biliary cirrhosis. N Engl J Med. 2005;353:1261–73.
31. Kitic I, Boskovic A, Stankovic I, Prokic D. Twelve-year-old girl with primary biliary cirrhosis. Case Rep Pediatr. 2012;2012:937150.
32. Carbone M, Mells GF, Pells G, Dawwas MF, Newton JL, Heneghan MA, Neuberger JM, et al. Sex and age are determinants of the clinical phenotype of primary biliary cirrhosis and response to ursodeoxycholic acid. Gastroenterology. 2013;144:560–569 e567. ; quiz e513-564
33. Rubel LR, Rabin L, Seeff LB, Licht H, Cuccherini BA. Does primary biliary cirrhosis in men differ from primary biliary cirrhosis in women? Hepatology. 1984;4:671–7.
34. Nalbandian G, Van de Water J, Gish R, Manns M, Coppel RL, Rudich SM, Prindiville T, et al. Is there a serological difference between men and women with primary biliary cirrhosis? Am J Gastroenterol. 1999;94:2482–6.
35. Lohse AW, Weiler-Normann C. Not all PBC is the same! Gastroenterology. 2013;144:494–7.
36. Owens GM. Gender differences in health care expenditures, resource utilization, and quality of care. J Manag Care Pharm. 2008;14:2–6.
37. Oksuzyan A, Juel K, Vaupel JW, Christensen K. Men: good health and high mortality. Sex differences in health and aging. Aging Clin Exp Res. 2008;20:91–102.
38. Floreani A, Franceschet I, Cazzagon N. Primary biliary cirrhosis: overlaps with other autoimmune disorders. Semin Liver Dis. 2014;34:352–60.
39. Boberg KM, Chapman RW, Hirschfield GM, Lohse AW, Manns MP, Schrumpf E, International Autoimmune Hepatitis G. Overlap syndromes: the International Autoimmune Hepatitis Group (IAIHG) position statement on a controversial issue. J Hepatol. 2011;54:374–85.
40. Chazouilleres O, Wendum D, Serfaty L, Montembault S, Rosmorduc O, Poupon R. Primary biliary cirrhosis-autoimmune hepatitis overlap syndrome: clinical features and response to therapy. Hepatology. 1998;28:296–301.
41. Hennes EM, Zeniya M, Czaja AJ, Pares A, Dalekos GN, Krawitt EL, Bittencourt PL, et al. Simplified criteria for the diagnosis of autoimmune hepatitis. Hepatology. 2008;48:169–76.

42. Kuiper EM, Zondervan PE, van Buuren HR. Paris criteria are effective in diagnosis of primary biliary cirrhosis and autoimmune hepatitis overlap syndrome. Clin Gastroenterol Hepatol. 2010;8:530–4.
43. European Association for the Study of the L. EASL clinical practice guidelines: management of cholestatic liver diseases. J Hepatol. 2009;51:237–67.
44. Culp KS, Fleming CR, Duffy J, Baldus WP, Dickson ER. Autoimmune associations in primary biliary cirrhosis. Mayo Clin Proc. 1982;57:365–70.
45. Crowe JP, Christensen E, Butler J, Wheeler P, Doniach D, Keenan J, Williams R. Primary biliary cirrhosis: the prevalence of hypothyroidism and its relationship to thyroid autoantibodies and sicca syndrome. Gastroenterology. 1980;78:1437–41.
46. Mang FW, Michieletti P, O'Rourke K, Cauch-Dudek K, Diamant N, Bookman A, Heathcote J. Primary biliary cirrhosis, sicca complex, and dysphagia. Dysphagia. 1997;12:167–70.
47. Watt FE, James OF, Jones DE. Patterns of autoimmunity in primary biliary cirrhosis patients and their families: a population-based cohort study. QJM. 2004;97:397–406.
48. Inoue K, Hirohara J, Nakano T, Seki T, Sasaki H, Higuchi K, Ohta Y, et al. Prediction of prognosis of primary biliary cirrhosis in Japan. Liver. 1995;15:70–7.
49. Marasini B, Gagetta M, Rossi V, Ferrari P. Rheumatic disorders and primary biliary cirrhosis: an appraisal of 170 Italian patients. Ann Rheum Dis. 2001;60:1046–9.
50. Matsumoto T, Kobayashi S, Shimizu H, Nakajima M, Watanabe S, Kitami N, Sato N, et al. The liver in collagen diseases: pathologic study of 160 cases with particular reference to hepatic arteritis, primary biliary cirrhosis, autoimmune hepatitis and nodular regenerative hyperplasia of the liver. Liver. 2000;20:366–73.
51. Dickey W, McMillan SA, Callender ME. High prevalence of celiac sprue among patients with primary biliary cirrhosis. J Clin Gastroenterol. 1997;25:328–9.
52. Bardella MT, Quatrini M, Zuin M, Podda M, Cesarini L, Velio P, Bianchi P, et al. Screening patients with celiac disease for primary biliary cirrhosis and vice versa. Am J Gastroenterol. 1997;92:1524–6.
53. Volta U, Rodrigo L, Granito A, Petrolini N, Muratori P, Muratori L, Linares A, et al. Celiac disease in autoimmune cholestatic liver disorders. Am J Gastroenterol. 2002;97:2609–13.
54. Deutsch M, Papatheodoridis GV, Tzakou A, Hadziyannis SJ. Risk of hepatocellular carcinoma and extrahepatic malignancies in primary biliary cirrhosis. Eur J Gastroenterol Hepatol. 2008;20:5–9.
55. Jackson H, Solaymani-Dodaran M, Card TR, Aithal GP, Logan R, West J. Influence of ursodeoxycholic acid on the mortality and malignancy associated with primary biliary cirrhosis: a population-based cohort study. Hepatology. 2007;46:1131–7.
56. Jones DE, Metcalf JV, Collier JD, Bassendine MF, OF J. Hepatocellular carcinoma in primary biliary cirrhosis and its impact on outcomes. Hepatology. 1997;26:1138–42.
57. Angulo P, Batts KP, Therneau TM, Jorgensen RA, Dickson ER, Lindor KD. Long-term ursodeoxycholic acid delays histological progression in primary biliary cirrhosis. Hepatology. 1999;29:644–7.
58. Harada K, Hirohara J, Ueno Y, Nakano T, Kakuda Y, Tsubouchi H, Ichida T, et al. Incidence of and risk factors for hepatocellular carcinoma in primary biliary cirrhosis: national data from Japan. Hepatology. 2013;57:1942–9.
59. Rong G, Wang H, Bowlus CL, Wang C, Lu Y, Zeng Z, Qu J, et al. Incidence and risk factors for hepatocellular carcinoma in primary biliary cirrhosis. Clin Rev Allergy Immunol. 2015;48:132–41.
60. Zhang XX, Wang LF, Jin L, Li YY, Hao SL, Shi YC, Zeng QL, et al. Primary biliary cirrhosis-associated hepatocellular carcinoma in Chinese patients: incidence and risk factors. World J Gastroenterol. 2015;21:3554–63.
61. Floreani A, Baragiotta A, Baldo V, Menegon T, Farinati F, Naccarato R. Hepatic and extrahepatic malignancies in primary biliary cirrhosis. Hepatology. 1999;29:1425–8.
62. Piscaglia F, Sagrini E. Malignancies in primary biliary cirrhosis. Eur J Gastroenterol Hepatol. 2008;20:1–4.

63. Liang Y, Yang Z, Zhong R. Smoking, family history and urinary tract infection are associated with primary biliary cirrhosis: a meta-analysis. Hepatol Res. 2011;41:572–8.
64. Wolke AM, Schaffner F, Kapelman B, Sacks HS. Malignancy in primary biliary cirrhosis. High incidence of breast cancer in affected women. Am J Med. 1984;76:1075–8.
65. Goudie BM, Burt AD, Boyle P, Macfarlane G, Birnie GG, Mills PR, Gillis CR, et al. Breast cancer in women with primary biliary cirrhosis. Br Med J (Clin Res Ed). 1985;291: 1597–8.
66. Floreani A, Spinazze A, Caballeria L, Reig A, Cazzagon N, Franceschet I, Buja A, et al. Extrahepatic malignancies in primary biliary cirrhosis: a comparative study at two European centers. Clin Rev Allergy Immunol. 2015;48:254–62.
67. Liang Y, Yang Z, Zhong R. Primary biliary cirrhosis and cancer risk: a systematic review and meta-analysis. Hepatology. 2012;56:1409–17.
68. Trivedi PJ, Kumagi T, Al-Harthy N, Coltescu C, Ward S, Cheung A, Hirschfield GM. Good maternal and fetal outcomes for pregnant women with primary biliary cirrhosis. Clin Gastroenterol Hepatol. 2014;12:1179–85. e1171
69. Stellon AJ, Williams R. Increased incidence of menstrual abnormalities and hysterectomy preceding primary biliary cirrhosis. Br Med J (Clin Res Ed). 1986;293:297–8.
70. Nir A, Sorokin Y, Abramovici H, Theodor E. Pregnancy and primary biliary cirrhosis. Int J Gynaecol Obstet. 1989;28:279–82.
71. Olsson R, Loof L, Wallerstedt S. Pregnancy in patients with primary biliary cirrhosis--a case for dissuasion? The Swedish Internal Medicine Liver Club. Liver. 1993;13:316–8.
72. Ji H, Haring P, Kirkinen P, Saarikoski S. Glucocorticoid treatment of primary biliary cirrhosis in a pregnant woman. Acta Obstet Gynecol Scand. 1995;74:654–6.
73. Rabinovitz M, Appasamy R, Finkelstein S. Primary biliary cirrhosis diagnosed during pregnancy. Does it have a different outcome? Dig Dis Sci. 1995;40:571–4.
74. Rudi J, Schonig T, Stremmel W. Therapy with ursodeoxycholic acid in primary biliary cirrhosis in pregnancy. Z Gastroenterol. 1996;34:188–91.
75. Goh SK, Gull SE, Alexander GJ. Pregnancy in primary biliary cirrhosis complicated by portal hypertension: report of a case and review of the literature. BJOG. 2001;108:760–2.
76. Floreani A, Paternoster D, Mega A, Farinati F, Plebani M, Baldo V, Grella. Sex hormone profile and endometrial cancer risk in primary biliary cirrhosis: a case-control study. Eur J Obstet Gynecol Reprod Biol. 2002;103:154–7.
77. Poupon R. Ursodeoxycholic acid and bile-acid mimetics as therapeutic agents for cholestatic liver diseases: an overview of their mechanisms of action. Clin Res Hepatol Gastroenterol. 2012;36(Suppl 1):S3–12.
78. Parikh-Patel A, Gold E, Utts J, Gershwin ME. The association between gravidity and primary biliary cirrhosis. Ann Epidemiol. 2002;12:264–72.
79. Floreani A, Infantolino C, Franceschet I, Tene IM, Cazzagon N, Buja A, Baldo V, et al. Pregnancy and primary biliary cirrhosis: a case-control study. Clin Rev Allergy Immunol. 2015;48:236–42.
80. Efe C, Kahramanoglu-Aksoy E, Yilmaz B, Ozseker B, Takci S, Roach EC, Purnak T, et al. Pregnancy in women with primary biliary cirrhosis. Autoimmun Rev. 2014;13:931–5.
81. de Buy Wenniger LJ M, Hohenester S, Maroni L, SJ v V, RP OE, Beuers U. The cholangiocyte glycocalyx stabilizes the 'Biliary HCO3 Umbrella': an integrated line of defense against toxic bile acids. Dig Dis. 2015;33:397–407.
82. Dilger K, Hohenester S, Winkler-Budenhofer U, Bastiaansen BA, Schaap FG, Rust C, Beuers U. Effect of ursodeoxycholic acid on bile acid profiles and intestinal detoxification machinery in primary biliary cirrhosis and health. J Hepatol. 2012;57:133–40.
83. Hohenester S, de Buy Wenniger L M, DM J, RP OE, Beuers U. Biliary bicarbonate secretion constitutes a protective mechanism against bile acid-induced injury in man. Dig Dis. 2011;29:62–5.
84. Moteki S, Leung PS, Coppel RL, Dickson ER, Kaplan MM, Munoz S, Gershwin ME. Use of a designer triple expression hybrid clone for three different lipoyl domain for the detection of antimitochondrial autoantibodies. Hepatology. 1996;24:97–103.

85. Howard MJ, Fuller C, Broadhurst RW, Perham RN, Tang JG, Quinn J, Diamond AG, et al. Three-dimensional structure of the major autoantigen in primary biliary cirrhosis. Gastroenterology. 1998;115:139–46.
86. Amano K, Leung PS, Rieger R, Quan C, Wang X, Marik J, Suen YF, et al. Chemical xenobiotics and mitochondrial autoantigens in primary biliary cirrhosis: identification of antibodies against a common environmental, cosmetic, and food additive, 2-octynoic acid. J Immunol. 2005;174:5874–83.
87. Naiyanetr P, Butler JD, Meng L, Pfeiff J, Kenny TP, Guggenheim KG, Reiger R, et al. Electrophile-modified lipoic derivatives of PDC-E2 elicits anti-mitochondrial antibody reactivity. J Autoimmun. 2011;37:209–16.
88. Rieger R, Leung PS, Jeddeloh MR, Kurth MJ, Nantz MH, Lam KS, Barsky D, et al. Identification of 2-nonynoic acid, a cosmetic component, as a potential trigger of primary biliary cirrhosis. J Autoimmun. 2006;27:7–16.
89. Leung PS, Park O, Tsuneyama K, Kurth MJ, Lam KS, Ansari AA, Coppel RL, et al. Induction of primary biliary cirrhosis in guinea pigs following chemical xenobiotic immunization. J Immunol. 2007;179:2651–7.
90. Leung PS, Quan C, Park O, Van de Water J, Kurth MJ, Nantz MH, Ansari AA, et al. Immunization with a xenobiotic 6-bromohexanoate bovine serum albumin conjugate induces antimitochondrial antibodies. J Immunol. 2003;170:5326–32.
91. Leung PS, Wang J, Naiyanetr P, Kenny TP, Lam KS, Kurth MJ, Gershwin ME. Environment and primary biliary cirrhosis: electrophilic drugs and the induction of AMA. J Autoimmun. 2013;41:79–86.
92. Wakabayashi K, Lian ZX, Leung PS, Moritoki Y, Tsuneyama K, Kurth MJ, Lam KS, et al. Loss of tolerance in C57BL/6 mice to the autoantigen E2 subunit of pyruvate dehydrogenase by a xenobiotic with ensuing biliary ductular disease. Hepatology. 2008;48:531–40.
93. Wakabayashi K, Yoshida K, Leung PS, Moritoki Y, Yang GX, Tsuneyama K, Lian ZX, et al. Induction of autoimmune cholangitis in non-obese diabetic (NOD).1101 mice following a chemical xenobiotic immunization. Clin Exp Immunol. 2009;155:577–86.
94. Shimoda S, Miyakawa H, Nakamura M, Ishibashi H, Kikuchi K, Kita H, Niiro H, et al. CD4 T-cell autoreactivity to the mitochondrial autoantigen PDC-E2 in AMA-negative primary biliary cirrhosis. J Autoimmun. 2008;31:110–5.
95. Lan RY, Cheng C, Lian ZX, Tsuneyama K, Yang GX, Moritoki Y, Chuang YH, et al. Liver-targeted and peripheral blood alterations of regulatory T cells in primary biliary cirrhosis. Hepatology. 2006;43:729–37.
96. Kikuchi K, Lian ZX, Yang GX, Ansari AA, Ikehara S, Kaplan M, Miyakawa H, et al. Bacterial CpG induces hyper-IgM production in CD27(+) memory B cells in primary biliary cirrhosis. Gastroenterology. 2005;128:304–12.
97. Chuang YH, Lian ZX, Tsuneyama K, Chiang BL, Ansari AA, Coppel RL, Gershwin ME. Increased killing activity and decreased cytokine production in NK cells in patients with primary biliary cirrhosis. J Autoimmun. 2006;26:232–40.
98. Kita H, Naidenko OV, Kronenberg M, Ansari AA, Rogers P, He XS, Koning F, et al. Quantitation and phenotypic analysis of natural killer T cells in primary biliary cirrhosis using a human CD1d tetramer. Gastroenterology. 2002;123:1031–43.
99. Mao TK, Lian ZX, Selmi C, Ichiki Y, Ashwood P, Ansari AA, Coppel RL, et al. Altered monocyte responses to defined TLR ligands in patients with primary biliary cirrhosis. Hepatology. 2005;42:802–8.
100. Odin JA, Huebert RC, Casciola-Rosen L, LaRusso NF, Rosen A. Bcl-2-dependent oxidation of pyruvate dehydrogenase-E2, a primary biliary cirrhosis autoantigen, during apoptosis. J Clin Invest. 2001;108:223–32.
101. Lleo A, Selmi C, Invernizzi P, Podda M, Coppel RL, Mackay IR, Gores GJ, et al. Apotopes and the biliary specificity of primary biliary cirrhosis. Hepatology. 2009;49:871–9.

102. Lleo A, Zhang W, McDonald WH, Seeley EH, Leung PS, Coppel RL, Ansari AA, et al. Shotgun proteomics: identification of unique protein profiles of apoptotic bodies from biliary epithelial cells. Hepatology. 2014;60:1314–23.
103. Rong GH, Yang GX, Ando Y, Zhang W, He XS, Leung PS, Coppel RL, et al. Human intrahepatic biliary epithelial cells engulf blebs from their apoptotic peers. Clin Exp Immunol. 2013;172:95–103.
104. Rong G, Zhong R, Lleo A, Leung PS, Bowlus CL, Yang GX, Yang CY, et al. Epithelial cell specificity and apotope recognition by serum autoantibodies in primary biliary cirrhosis. Hepatology. 2011;54:196–203.
105. Lleo A, Bowlus CL, Yang GX, Invernizzi P, Podda M, Van de Water J, Ansari AA, et al. Biliary apotopes and anti-mitochondrial antibodies activate innate immune responses in primary biliary cirrhosis. Hepatology. 2010;52:987–98.
106. Selmi C, Mayo MJ, Bach N, Ishibashi H, Invernizzi P, Gish RG, Gordon SC, et al. Primary biliary cirrhosis in monozygotic and dizygotic twins: genetics, epigenetics, and environment. Gastroenterology. 2004;127:485–92.
107. Invernizzi P. Human leukocyte antigen in primary biliary cirrhosis: an old story now reviving. Hepatology. 2011;54:714–23.
108. Donaldson P, Agarwal K, Craggs A, Craig W, James O, Jones D. HLA and interleukin 1 gene polymorphisms in primary biliary cirrhosis: associations with disease progression and disease susceptibility. Gut. 2001;48:397–402.
109. Invernizzi P, Ransom M, Raychaudhuri S, Kosoy R, Lleo A, Shigeta R, Franke A, et al. Classical HLA-DRB1 and DPB1 alleles account for HLA associations with primary biliary cirrhosis. Genes Immun. 2012;13:461–8.
110. Umemura T, Joshita S, Ichijo T, Yoshizawa K, Katsuyama Y, Tanaka E, Ota M, et al. Human leukocyte antigen class II molecules confer both susceptibility and progression in Japanese patients with primary biliary cirrhosis. Hepatology. 2012;55:506–11.
111. Zhao DT, Liao HY, Zhang X, Liu YM, Zhao Y, Zhang HP, Sun LM, et al. Human leucocyte antigen alleles and haplotypes and their associations with antinuclear antibodies features in Chinese patients with primary biliary cirrhosis. Liver Int. 2014;34:220–6.
112. Hirschfield GM, Liu X, Xu C, Lu Y, Xie G, Lu Y, Gu X, et al. Primary biliary cirrhosis associated with HLA, IL12A, and IL12RB2 variants. N Engl J Med. 2009;360:2544–55.
113. Nakamura M, Nishida N, Kawashima M, Aiba Y, Tanaka A, Yasunami M, Nakamura H, et al. Genome-wide association study identifies TNFSF15 and POU2AF1 as susceptibility loci for primary biliary cirrhosis in the Japanese population. Am J Hum Genet. 2012;91:721–8.
114. Liu X, Invernizzi P, Lu Y, Kosoy R, Lu Y, Bianchi I, Podda M, et al. Genome-wide meta-analyses identify three loci associated with primary biliary cirrhosis. Nat Genet. 2010;42:658–60.
115. Mells GF, Floyd JA, Morley KI, Cordell HJ, Franklin CS, Shin SY, Heneghan MA, et al. Genome-wide association study identifies 12 new susceptibility loci for primary biliary cirrhosis. Nat Genet. 2011;43:329–32.
116. Liu JZ, Almarri MA, Gaffney DJ, Mells GF, Jostins L, Cordell HJ, Ducker SJ, et al. Dense fine-mapping study identifies new susceptibility loci for primary biliary cirrhosis. Nat Genet. 2012;44:1137–41.
117. Mells GF, Kaser A, Karlsen TH. Novel insights into autoimmune liver diseases provided by genome-wide association studies. J Autoimmun. 2013;46:41–54.
118. Lleo A, Liao J, Invernizzi P, Zhao M, Bernuzzi F, Ma L, Lanzi G, et al. Immunoglobulin M levels inversely correlate with CD40 ligand promoter methylation in patients with primary biliary cirrhosis. Hepatology. 2012;55:153–60.
119. Amur S, Parekh A, Mummaneni. Sex differences and genomics in autoimmune diseases. J Autoimmun. 2012;38:J254–65.

120. Mitchell MM, Lleo A, Zammataro L, Mayo MJ, Invernizzi P, Bach N, Shimoda S, et al. Epigenetic investigation of variably X chromosome inactivated genes in monozygotic female twins discordant for primary biliary cirrhosis. Epigenetics. 2011;6:95–102.
121. O'Hara SP, Gradilone SA, Masyuk TV, Tabibian JH, LaRusso NF. MicroRNAs in Cholangiopathies. Curr Pathobiol Rep. 2014;2:133–42.
122. Juran BD, Lazaridis KN. Environmental factors in primary biliary cirrhosis. Semin Liver Dis. 2014;34:265–72.
123. Burroughs AK, Rosenstein IJ, Epstein O, Hamilton-Miller JM, Brumfitt W, Sherlock S. Bacteriuria and primary biliary cirrhosis. Gut. 1984;25:133–7.
124. Varyani FK, West J, Card TR. An increased risk of urinary tract infection precedes development of primary biliary cirrhosis. BMC Gastroenterol. 2011;11:95.
125. Mattner J, Savage PB, Leung P, Oertelt SS, Wang V, Trivedi O, Scanlon ST, et al. Liver autoimmunity triggered by microbial activation of natural killer T cells. Cell Host Microbe. 2008;3:304–15.
126. Selmi C, Balkwill DL, Invernizzi P, Ansari AA, Coppel RL, Podda M, Leung PS, et al. Patients with primary biliary cirrhosis react against a ubiquitous xenobiotic-metabolizing bacterium. Hepatology. 2003;38:1250–7.
127. Kita H, Matsumura S, He XS, Ansari AA, Lian ZX, Van de Water J, Coppel RL, et al. Analysis of TCR antagonism and molecular mimicry of an HLA-A0201-restricted CTL epitope in primary biliary cirrhosis. Hepatology. 2002;36:918–26.
128. Fujiwara K, Yokosuka O. Frequent detection of immunoglobulin M anti-herpes simplex viral antibody in patients with primary biliary cirrhosis. Hepatology. 2012;56:395.
129. Xu L, Shen Z, Guo L, Fodera B, Keogh A, Joplin R, O'Donnell B, et al. Does a betaretrovirus infection trigger primary biliary cirrhosis? Proc Natl Acad Sci U S A. 2003;100:8454–9.
130. Selmi C, Ross SR, Ansari AA, Invernizzi P, Podda M, Coppel RL, Gershwin ME. Lack of immunological or molecular evidence for a role of mouse mammary tumor retrovirus in primary biliary cirrhosis. Gastroenterology. 2004;127:493–501.
131. Morshed SA, Nishioka M, Saito I, Komiyama K, Moro I. Increased expression of Epstein-Barr virus in primary biliary cirrhosis patients. Gastroenterol Jpn. 1992;27:751–8.
132. Uzoegwu PN, Baum H, Williamson J. The occurrence and localization in trypanosomes and other endo-parasites of an antigen cross-reacting with mitochondrial antibodies of primary biliary cirrhosis. Comp Biochem Physiol B. 1987;88:1181–9.
133. Sakly W, Jeddi M, Ghedira I. Anti-*Saccharomyces cerevisiae* antibodies in primary biliary cirrhosis. Dig Dis Sci. 2008;53:1983–7.
134. Leung PS, Rossaro L, Davis PA, Park O, Tanaka A, Kikuchi K, Miyakawa H, et al. Antimitochondrial antibodies in acute liver failure: implications for primary biliary cirrhosis. Hepatology. 2007;46:1436–42.
135. McLean MH, Dieguez Jr D, Miller LM, Young HA. Does the microbiota play a role in the pathogenesis of autoimmune diseases? Gut. 2015;64:332–41.
136. Gershwin ME, Mackay IR, Sturgess A, Coppel RL. Identification and specificity of a cDNA encoding the 70 kd mitochondrial antigen recognized in primary biliary cirrhosis. J Immunol. 1987;138:3525–31.
137. Wang L, Wang FS, Chang C, Gershwin ME. Breach of tolerance: primary biliary cirrhosis. Semin Liver Dis. 2014;34:297–317.
138. Bjorkland A, Loof L, Mendel-Hartvig I, Totterman TH. Primary biliary cirrhosis. High proportions of B cells in blood and liver tissue produce anti-mitochondrial antibodies of several Ig classes. J Immunol. 1994;153:2750–7.
139. Nakamura M, Ishibashi H, Matsui M, Shimoda S, Hayashida K, Koike K, Niho Y. Peripheral B lymphocyte repertoire to mitochondrial antigen in primary biliary cirrhosis--positive correlation between the disease activity and the frequency of circulating B lymphocytes specific for pyruvate dehydrogenase complex. Autoimmunity. 1995;21:253–62.
140. Moritoki Y, Lian ZX, Ohsugi Y, Ueno Y, Gershwin ME. B cells and autoimmune liver diseases. Autoimmun Rev. 2006;5:449–57.

141. Zhang J, Zhang W, Leung PS, Bowlus CL, Dhaliwal S, Coppel RL, Ansari AA, et al. Ongoing activation of autoantigen-specific B cells in primary biliary cirrhosis. Hepatology. 2014;60:1708–16.
142. Shimoda S, Nakamura M, Ishibashi H, Hayashida K, Niho Y. HLA DRB4 0101-restricted immunodominant T cell autoepitope of pyruvate dehydrogenase complex in primary biliary cirrhosis: evidence of molecular mimicry in human autoimmune diseases. J Exp Med. 1995;181:1835–45.
143. Shimoda S, Ishikawa F, Kamihira T, Komori A, Niiro H, Baba E, Harada K, et al. Autoreactive T-cell responses in primary biliary cirrhosis are proinflammatory whereas those of controls are regulatory. Gastroenterology. 2006;131:606–18.
144. Kita H, Matsumura S, He XS, Ansari AA, Lian ZX, Van de Water J, Coppel RL, et al. Quantitative and functional analysis of PDC-E2-specific autoreactive cytotoxic T lymphocytes in primary biliary cirrhosis. J Clin Invest. 2002;109:1231–40.
145. Harada K, Van de Water J, Leung PS, Coppel RL, Ansari A, Nakanuma Y, Gershwin ME. In situ nucleic acid hybridization of cytokines in primary biliary cirrhosis: predominance of the Th1 subset. Hepatology. 1997;25:791–6.
146. Nagano T, Yamamoto K, Matsumoto S, Okamoto R, Tagashira M, Ibuki N, Matsumura S, et al. Cytokine profile in the liver of primary biliary cirrhosis. J Clin Immunol. 1999;19:422–7.
147. Oertelt S, Lian ZX, Cheng CM, Chuang YH, Padgett KA, He XS, Ridgway WM, et al. Anti-mitochondrial antibodies and primary biliary cirrhosis in TGF-beta receptor II dominant-negative mice. J Immunol. 2006;177:1655–60.
148. Wakabayashi K, Lian ZX, Moritoki Y, Lan RY, Tsuneyama K, Chuang YH, Yang GX, et al. IL-2 receptor alpha(−/−) mice and the development of primary biliary cirrhosis. Hepatology. 2006;44:1240–9.
149. Zhang W, Sharma R, Ju ST, He XS, Tao Y, Tsuneyama K, Tian Z, et al. Deficiency in regulatory T cells results in development of antimitochondrial antibodies and autoimmune cholangitis. Hepatology. 2009;49:545–52.
150. Lan RY, Salunga TL, Tsuneyama K, Lian ZX, Yang GX, Hsu W, Moritoki Y, et al. Hepatic IL-17 responses in human and murine primary biliary cirrhosis. J Autoimmun. 2009;32:43–51.
151. Wang L, Sun Y, Zhang Z, Jia Y, Zou Z, Ding J, Li Y, et al. CXCR5+ CD4+ T follicular helper cells participate in the pathogenesis of primary biliary cirrhosis. Hepatology. 2015;61:627–38.
152. Tobe K. Electron microscopy of liver lesions in primary biliary cirrhosis. II. A bile duct with chronic non-suppurative destructive cholangitis. Acta Pathol Jpn. 1982;32:345–57.
153. Takii Y, Nakamura M, Ito M, Yokoyama T, Komori A, Shimizu-Yoshida Y, Nakao R, et al. Enhanced expression of type I interferon and toll-like receptor-3 in primary biliary cirrhosis. Lab Invest. 2005;85:908–20.
154. Honda Y, Yamagiwa S, Matsuda Y, Takamura M, Ichida T, Aoyagi Y. Altered expression of TLR homolog RP105 on monocytes hypersensitive to LPS in patients with primary biliary cirrhosis. J Hepatol. 2007;47:404–11.
155. Demetris AJ, Sever C, Kakizoe S, Oguma S, Starzl TE, Jaffe R. S100 protein positive dendritic cells in primary biliary cirrhosis and other chronic inflammatory liver diseases. Relevance to pathogenesis? Am J Pathol. 1989;134:741–7.
156. Tanimoto K, Akbar SM, Michitaka K, Onji M. Immunohistochemical localization of antigen presenting cells in liver from patients with primary biliary cirrhosis; highly restricted distribution of CD83-positive activated dendritic cells. Pathol Res Pract. 1999;195:157–62.
157. Akbar SM, Yamamoto K, Miyakawa H, Ninomiya T, Abe M, Hiasa Y, Masumoto T, et al. Peripheral blood T-cell responses to pyruvate dehydrogenase complex in primary biliary cirrhosis: role of antigen-presenting dendritic cells. Eur J Clin Invest. 2001;31:639–46.
158. Kita H, Lian ZX, Van de Water J, He XS, Matsumura S, Kaplan M, Luketic V, et al. Identification of HLA-A2-restricted CD8(+) cytotoxic T cell responses in primary biliary cirrhosis: T cell activation is augmented by immune complexes cross-presented by dendritic cells. J Exp Med. 2002;195:113–23.

159. Hiasa Y, Akbar SM, Abe M, Michitaka K, Horiike N, Onji M. Dendritic cell subtypes in autoimmune liver diseases; decreased expression of HLA DR and CD123 on type 2 dendritic cells. Hepatol Res. 2002;22:241–9.
160. Harada K, Shimoda S, Ikeda H, Chiba M, Hsu M, Sato Y, Kobayashi M, et al. Significance of periductal Langerhans cells and biliary epithelial cell-derived macrophage inflammatory protein-3alpha in the pathogenesis of primary biliary cirrhosis. Liver Int. 2011;31:245–53.
161. Panasiuk A, Prokopowicz D, Zak J. Peripheral blood T, B lymphocytes and NK cells in primary biliary cirrhosis. Rocz Akad Med Bialymst. 2001;46:231–9.
162. Hudspeth K, Pontarini E, Tentorio P, Cimino M, Donadon M, Torzilli G, Lugli E, et al. The role of natural killer cells in autoimmune liver disease: a comprehensive review. J Autoimmun. 2013;46:55–65.
163. Chuang YH, Lian ZX, Yang GX, Shu SA, Moritoki Y, Ridgway WM, Ansari AA, et al. Natural killer T cells exacerbate liver injury in a transforming growth factor beta receptor II dominant-negative mouse model of primary biliary cirrhosis. Hepatology. 2008;47:571–80.
164. Wu SJ, Yang YH, Tsuneyama K, Leung PS, Illarionov P, Gershwin ME, Chuang YH. Innate immunity and primary biliary cirrhosis: activated invariant natural killer T cells exacerbate murine autoimmune cholangitis and fibrosis. Hepatology. 2011;53:915–25.
165. Aso-Ishimoto Y, Yamagiwa S, Ichida T, Miyakawa R, Tomiyama C, Sato Y, Watanabe H, et al. Increased activated natural killer T cells in the liver of patients with advanced stage primary biliary cirrhosis. Biomed Res. 2014;35:161–9.
166. Shimoda S, Tsuneyama K, Kikuchi K, Harada K, Nakanuma Y, Nakamura M, Ishibashi H, et al. The role of natural killer (NK) and NK T cells in the loss of tolerance in murine primary biliary cirrhosis. Clin Exp Immunol. 2012;168:279–84.
167. Trivedi PJ, Adams DH. Mucosal immunity in liver autoimmunity: a comprehensive review. J Autoimmun. 2013;46:97–111.
168. Li Y, Wang W, Tang L, He X, Yan X, Zhang X, Zhu Y, et al. Chemokine (C-X-C motif) ligand 13 promotes intrahepatic chemokine (C-X-C motif) receptor 5+ lymphocyte homing and aberrant B-cell immune responses in primary biliary cirrhosis. Hepatology. 2015;61:1998–2007.
169. Tanaka A, Nezu S, Uegaki S, Mikami M, Okuyama S, Kawamura N, Aiso M, et al. The clinical significance of IgA antimitochondrial antibodies in sera and saliva in primary biliary cirrhosis. Ann N Y Acad Sci. 2007;1107:259–70.
170. Takahashi T, Miura T, Nakamura J, Yamada S, Miura T, Yanagi M, Matsuda Y, et al. Plasma cells and the chronic nonsuppurative destructive cholangitis of primary biliary cirrhosis. Hepatology. 2012;55:846–55.
171. Wang J, Yang GX, Tsuneyama K, Gershwin ME, Ridgway WM, Leung PS. Animal models of primary biliary cirrhosis. Semin Liver Dis. 2014;34:285–96.
172. Koarada S, Wu Y, Fertig N, Sass DA, Nalesnik M, Todd JA, Lyons PA, et al. Genetic control of autoimmunity: protection from diabetes, but spontaneous autoimmune biliary disease in a nonobese diabetic congenic strain. J Immunol. 2004;173:2315–23.
173. Irie J, Wu Y, Wicker LS, Rainbow D, Nalesnik MA, Hirsch R, Peterson LB, et al. NOD.c3c4 congenic mice develop autoimmune biliary disease that serologically and pathogenetically models human primary biliary cirrhosis. J Exp Med. 2006;203:1209–19.
174. Salas JT, Banales JM, Sarvide S, Recalde S, Ferrer A, Uriarte I, Oude Elferink RP, et al. Ae2a,b-deficient mice develop antimitochondrial antibodies and other features resembling primary biliary cirrhosis. Gastroenterology. 2008;134:1482–93.
175. Wang JJ, Yang GX, Zhang WC, Lu L, Tsuneyama K, Kronenberg M, Vela JL, et al. *Escherichia coli* infection induces autoimmune cholangitis and anti-mitochondrial antibodies in non-obese diabetic (NOD).B6 (Idd10/Idd18) mice. Clin Exp Immunol. 2014;175:192–201.
176. Katsumi T, Tomita K, Leung PS, Yang GX, Gershwin ME, Ueno Y. Animal models of primary biliary cirrhosis. Clin Rev Allergy Immunol. 2015;48:142–53.

177. Nakagome Y, Ueno Y, Kogure T, Fukushima K, Moritoki Y, Ridgway WM, Eric Gershwin M, et al. Autoimmune cholangitis in NOD.c3c4 mice is associated with cholangiocyte-specific Fas antigen deficiency. J Autoimmun. 2007;29:20–29
178. Kawata K, Tsuda M, Yang GX, Zhang W, Tanaka H, Tsuneyama K, Leung P, et al. Identification of potential cytokine pathways for therapeutic intervention in murine primary biliary cirrhosis. PLoS One. 2013;8:e74225.
179. Dhirapong A, Lleo A, Yang GX, Tsuneyama K, Dunn R, Kehry M, Packard TA, et al. B cell depletion therapy exacerbates murine primary biliary cirrhosis. Hepatology. 2011;53:527–35.
180. Dhirapong A, Yang GX, Nadler S, Zhang W, Tsuneyama K, Leung P, Knechtle S, et al. Therapeutic effect of cytotoxic T lymphocyte antigen 4/immunoglobulin on a murine model of primary biliary cirrhosis. Hepatology. 2013;57:708–15.
181. Bogdanos DP, Baum H, Grasso A, Okamoto M, Butler P, Ma Y, Rigopoulou E, et al. Microbial mimics are major targets of crossreactivity with human pyruvate dehydrogenase in primary biliary cirrhosis. J Hepatol. 2004;40:31–9.
182. Padgett KA, Selmi C, Kenny TP, Leung PS, Balkwill DL, Ansari AA, Coppel RL, et al. Phylogenetic and immunological definition of four lipoylated proteins from Novosphingobium aromaticivorans, implications for primary biliary cirrhosis. J Autoimmun. 2005;24:209–19.
183. AW T. Review of the activation of TGF-beta in immunity. J Leukoc Biol. 2009;85:29–33.
184. Kawata K, Yang GX, Ando Y, Tanaka H, Zhang W, Kobayashi Y, Tsuneyama K, et al. Clonality, activated antigen-specific CD8(+) T cells, and development of autoimmune cholangitis in dnTGFbetaRII mice. Hepatology. 2013;58:1094–104.
185. Leung PS, Yang GX, Dhirapong A, Tsuneyama K, Ridgway WM, Gershwin ME. Animal models of primary biliary cirrhosis: materials and methods. Methods Mol Biol. 2012;900:291–316.
186. Moritoki Y, Lian ZX, Lindor K, Tuscano J, Tsuneyama K, Zhang W, Ueno Y, et al. B-cell depletion with anti-CD20 ameliorates autoimmune cholangitis but exacerbates colitis in transforming growth factor-beta receptor II dominant negative mice. Hepatology. 2009;50:1893–903.
187. Yoshida K, Yang GX, Zhang W, Tsuda M, Tsuneyama K, Moritoki Y, Ansari AA, et al. Deletion of interleukin-12p40 suppresses autoimmune cholangitis in dominant negative transforming growth factor beta receptor type II mice. Hepatology. 2009;50:1494–500.
188. Ando Y, Yang GX, Tsuda M, Kawata K, Zhang W, Nakajima T, Tsuneyama K, et al. The immunobiology of colitis and cholangitis in interleukin-23p19 and interleukin-17A deleted dominant negative form of transforming growth factor beta receptor type II mice. Hepatology. 2012;56:1418–26.
189. Tsuda M, Zhang W, Yang GX, Tsuneyama K, Ando Y, Kawata K, Park O, et al. Deletion of interleukin (IL)-12p35 induces liver fibrosis in dominant-negative TGFbeta receptor type II mice. Hepatology. 2013;57:806–16.
190. Mackay IR. Primary biliary cirrhosis showing a high titer of autoantibody; report of a case. N Engl J Med. 1958;258:185–8.
191. Nakamura M. Clinical significance of autoantibodies in primary biliary cirrhosis. Semin Liver Dis. 2014;34:334–40.
192. Bandin O, Courvalin JC, Poupon R, Dubel L, Homberg JC, Johanet C. Specificity and sensitivity of gp210 autoantibodies detected using an enzyme-linked immunosorbent assay and a synthetic polypeptide in the diagnosis of primary biliary cirrhosis. Hepatology. 1996;23:1020–4.
193. Nakamura M, Shimizu-Yoshida Y, Takii Y, Komori A, Yokoyama T, Ueki T, Daikoku M, et al. Antibody titer to gp210-C terminal peptide as a clinical parameter for monitoring primary biliary cirrhosis. J Hepatol. 2005;42:386–92.
194. Invernizzi P, Podda M, Battezzati PM, Crosignani A, Zuin M, Hitchman E, Maggioni M, et al. Autoantibodies against nuclear pore complexes are associated with more active and severe liver disease in primary biliary cirrhosis. J Hepatol. 2001;34:366–72.

195. Wesierska-Gadek J, Hohenuer H, Hitchman E, Penner E. Autoantibodies against nucleoporin p62 constitute a novel marker of primary biliary cirrhosis. Gastroenterology. 1996;110:840–7.
196. Davis LI, Blobel G. Nuclear pore complex contains a family of glycoproteins that includes p62: glycosylation through a previously unidentified cellular pathway. Proc Natl Acad Sci U S A. 1987;84:7552–6.
197. Lin F, Noyer CM, Ye Q, Courvalin JC, Worman HJ. Autoantibodies from patients with primary biliary cirrhosis recognize a region within the nucleoplasmic domain of inner nuclear membrane protein LBR. Hepatology. 1996;23:57–61.
198. Courvalin JC, Lassoued K, Worman HJ, Blobel G. Identification and characterization of autoantibodies against the nuclear envelope lamin B receptor from patients with primary biliary cirrhosis. J Exp Med. 1990;172:961–7.
199. Zuchner D, Sternsdorf T, Szostecki C, Heathcote EJ, Cauch-Dudek K, Will H. Prevalence, kinetics, and therapeutic modulation of autoantibodies against Sp100 and promyelocytic leukemia protein in a large cohort of patients with primary biliary cirrhosis. Hepatology. 1997;26:1123–30.
200. Mytilinaiou MG, Meyer W, Scheper T, Rigopoulou EI, Probst C, Koutsoumpas AL, Abeles D, et al. Diagnostic and clinical utility of antibodies against the nuclear body promyelocytic leukaemia and Sp100 antigens in patients with primary biliary cirrhosis. Clin Chim Acta. 2012;413:1211–6.
201. Szostecki C, Krippner H, Penner E, Bautz FA. Autoimmune sera recognize a 100 kD nuclear protein antigen (sp-100). Clin Exp Immunol. 1987;68:108–16.
202. Rigopoulou EI, Davies ET, Pares A, Zachou K, Liaskos C, Bogdanos DP, Rodes J, et al. Prevalence and clinical significance of isotype specific antinuclear antibodies in primary biliary cirrhosis. Gut. 2005;54:528–32.
203. Granito A, Yang WH, Muratori L, Lim MJ, Nakajima A, Ferri S, Pappas G, et al. PML nuclear body component Sp140 is a novel autoantigen in primary biliary cirrhosis. Am J Gastroenterol. 2010;105:125–31.
204. Janka C, Selmi C, Gershwin ME, Will H, Sternsdorf T. Small ubiquitin-related modifiers: a novel and independent class of autoantigens in primary biliary cirrhosis. Hepatology. 2005;41:609–16.
205. Nakamura M, Kondo H, Mori T, Komori A, Matsuyama M, Ito M, Takii Y, et al. Anti-gp210 and anti-centromere antibodies are different risk factors for the progression of primary biliary cirrhosis. Hepatology. 2007;45:118–27.
206. Akbarali Y, Matousek-Ronck J, Hunt L, Staudt L, Reichlin M, Guthridge JM, James JA. Fine specificity mapping of autoantigens targeted by anti-centromere autoantibodies. J Autoimmun. 2006;27:272–80.
207. Walker JG, Doniach D, Roitt IM, Sherlock S. Serological tests in diagnosis of primary biliary cirrhosis. Lancet. 1965;1:827–31.
208. Fussey SP, Guest JR, James OF, Bassendine MF, Yeaman SJ. Identification and analysis of the major M2 autoantigens in primary biliary cirrhosis. Proc Natl Acad Sci U S A. 1988;85:8654–8.
209. Oertelt S, Rieger R, Selmi C, Invernizzi P, Ansari AA, Coppel RL, Podda M, et al. A sensitive bead assay for antimitochondrial antibodies: chipping away at AMA-negative primary biliary cirrhosis. Hepatology. 2007;45:659–65.
210. Dahnrich C, Pares A, Caballeria L, Rosemann A, Schlumberger W, Probst C, Mytilinaiou M, et al. New ELISA for detecting primary biliary cirrhosis-specific antimitochondrial antibodies. Clin Chem. 2009;55:978–85.
211. Czaja AJ. Autoantibodies as prognostic markers in autoimmune liver disease. Dig Dis Sci. 2010;55:2144–61.
212. Hu CJ, Zhang FC, Li YZ, Zhang X. Primary biliary cirrhosis: what do autoantibodies tell us? World J Gastroenterol. 2010;16:3616–29.

213. Liberal R, Grant CR, Sakkas L, Bizzaro N, Bogdanos DP. Diagnostic and clinical significance of anti-centromere antibodies in primary biliary cirrhosis. Clin Res Hepatol Gastroenterol. 2013;37:572–85.
214. Bizzaro N, Covini G, Rosina F, Muratori P, Tonutti E, Villalta D, Pesente F, et al. Overcoming a "probable" diagnosis in antimitochondrial antibody negative primary biliary cirrhosis: study of 100 sera and review of the literature. Clin Rev Allergy Immunol. 2012;42:288–97.
215. Benson GD, Kikuchi K, Miyakawa H, Tanaka A, Watnik MR, Gershwin ME. Serial analysis of antimitochondrial antibody in patients with primary biliary cirrhosis. Clin Dev Immunol. 2004;11:129–33.
216. Courvalin JC, Worman HJ. Nuclear envelope protein autoantibodies in primary biliary cirrhosis. Semin Liver Dis. 1997;17:79–90.
217. Itoh S, Ichida T, Yoshida T, Hayakawa A, Uchida M, Tashiro-Itoh T, Matsuda Y, et al. Autoantibodies against a 210 kDa glycoprotein of the nuclear pore complex as a prognostic marker in patients with primary biliary cirrhosis. J Gastroenterol Hepatol. 1998;13:257–65.
218. Muratori P, Muratori L, Ferrari R, Cassani F, Bianchi G, Lenzi M, Rodrigo L, et al. Characterization and clinical impact of antinuclear antibodies in primary biliary cirrhosis. Am J Gastroenterol. 2003;98:431–7.
219. Miyachi K, Hankins RW, Matsushima H, Kikuchi F, Inomata T, Horigome T, Shibata M, et al. Profile and clinical significance of anti-nuclear envelope antibodies found in patients with primary biliary cirrhosis: a multicenter study. J Autoimmun. 2003;20:247–54.
220. Yang WH, Yu JH, Nakajima A, Neuberg D, Lindor K, Bloch DB. Do antinuclear antibodies in primary biliary cirrhosis patients identify increased risk for liver failure? Clin Gastroenterol Hepatol. 2004;2:1116–22.
221. Wesierska-Gadek J, Penner E, Battezzati PM, Selmi C, Zuin M, Hitchman E, Worman HJ, et al. Correlation of initial autoantibody profile and clinical outcome in primary biliary cirrhosis. Hepatology. 2006;43:1135–44.
222. Szostecki C, Guldner HH, Will H. Autoantibodies against “nuclear dots” in primary biliary cirrhosis. Semin Liver Dis. 1997;17:71–8.
223. Poupon R. Liver alkaline phosphatase: a missing link between choleresis and biliary inflammation. Hepatology. 2015;61:2080–90.
224. Paronetto F, Schaffner F, Popper H. Immunocytochemical and serologic observations in primary biliary cirrhosis. N Engl J Med. 1964;271:1123–8.
225. Ludwig J, Dickson ER, McDonald GS. Staging of chronic nonsuppurative destructive cholangitis (syndrome of primary biliary cirrhosis). Virchows Arch A Pathol Anat Histol. 1978;379:103–12.
226. Corpechot C, Carrat F, Poupon R, Poupon RE. Primary biliary cirrhosis: incidence and predictive factors of cirrhosis development in ursodiol-treated patients. Gastroenterology. 2002;122:652–8.
227. Kaplan MM. Primary biliary cirrhosis. N Engl J Med. 1996;335:1570–80.
228. Taal BG, Schalm SW, ten Kate FW, Hermans J, Geertzen RG, Feltkamp BE. Clinical diagnosis of primary biliary cirrhosis: a classification based on major and minor criteria. Hepatogastroenterology. 1983;30:178–82.
229. Heathcote EJ. Management of primary biliary cirrhosis. The American Association for the Study of Liver Diseases practice guidelines. Hepatology. 2000;31:1005–13.
230. Zein CO, Angulo P, Lindor KD. When is liver biopsy needed in the diagnosis of primary biliary cirrhosis? Clin Gastroenterol Hepatol. 2003;1:89–95.
231. Tan Y, Pan T, Ye Y, Ge G, Chen L, Wen D, Zou S. Serum microRNAs as potential biomarkers of primary biliary cirrhosis. PLoS One. 2014;9:e111424.
232. Norman GL, Yang CY, Ostendorff HP, Shums Z, Lim MJ, Wang J, Awad A, et al. Anti-kelch-like 12 and anti-hexokinase 1: novel autoantibodies in primary biliary cirrhosis. Liver Int. 2015;35:642–51.

233. Hohenester S, Oude-Elferink RP, Beuers U. Primary biliary cirrhosis. Semin Immunopathol. 2009;31:283–307.
234. Hagey LR, Crombie DL, Espinosa E, Carey MC, Igimi H, Hofmann AF. Ursodeoxycholic acid in the Ursidae: biliary bile acids of bears, pandas, and related carnivores. J Lipid Res. 1993;34:1911–7.
235. Hofmann AF, Roda A. Physicochemical properties of bile acids and their relationship to biological properties: an overview of the problem. J Lipid Res. 1984;25:1477–89.
236. Leuschner U, Leuschner M, Sieratzki J, Kurtz W, Hubner K. Gallstone dissolution with ursodeoxycholic acid in patients with chronic active hepatitis and two years follow-up. A pilot study. Dig Dis Sci. 1985;30:642–9.
237. Guldutuna S, Zimmer G, Imhof M, Bhatti S, You T, Leuschner U. Molecular aspects of membrane stabilization by ursodeoxycholate. Gastroenterology. 1993;104:1736–44.
238. Heuman DM, Bajaj RS, Lin Q. Adsorption of mixtures of bile salt taurine conjugates to lecithin-cholesterol membranes: implications for bile salt toxicity and cytoprotection. J Lipid Res. 1996;37:562–73.
239. Paumgartner G, Beuers U. Ursodeoxycholic acid in cholestatic liver disease: mechanisms of action and therapeutic use revisited. Hepatology. 2002;36:525–31.
240. Paumgartner G, Beuers U. Mechanisms of action and therapeutic efficacy of ursodeoxycholic acid in cholestatic liver disease. Clin Liver Dis. 2004;8:67–81. vi
241. Poupon R, Poupon RE. Treatment of primary biliary cirrhosis. Baillieres Best Pract Res Clin Gastroenterol. 2000;14:615–28.
242. Medina JF, Martinez A, Vazquez JJ, Prieto J. Decreased anion exchanger 2 immunoreactivity in the liver of patients with primary biliary cirrhosis. Hepatology. 1997;25:12–7.
243. Fickert P, Zollner G, Fuchsbichler A, Stumptner C, Pojer C, Zenz R, Lammert F, et al. Effects of ursodeoxycholic and cholic acid feeding on hepatocellular transporter expression in mouse liver. Gastroenterology. 2001;121:170–83.
244. Marschall HU, Wagner M, Zollner G, Fickert P, Diczfalusy U, Gumhold J, Silbert D, et al. Complementary stimulation of hepatobiliary transport and detoxification systems by rifampicin and ursodeoxycholic acid in humans. Gastroenterology. 2005;129:476–85.
245. Beuers U, Bilzer M, Chittattu A, Kullak-Ublick GA, Keppler D, Paumgartner G, Dombrowski F. Tauroursodeoxycholic acid inserts the apical conjugate export pump, Mrp2, into canalicular membranes and stimulates organic anion secretion by protein kinase C-dependent mechanisms in cholestatic rat liver. Hepatology. 2001;33:1206–16.
246. Kurz AK, Graf D, Schmitt M, Vom Dahl S, Haussinger D. Tauroursodeoxycholate-induced choleresis involves p38(MAPK) activation and translocation of the bile salt export pump in rats. Gastroenterology. 2001;121:407–19.
247. Dombrowski F, Stieger B, Beuers U. Tauroursodeoxycholic acid inserts the bile salt export pump into canalicular membranes of cholestatic rat liver. Lab Invest. 2006;86:166–74.
248. Kubitz R, Sutfels G, Kuhlkamp T, Kolling R, Haussinger D. Trafficking of the bile salt export pump from the Golgi to the canalicular membrane is regulated by the p38 MAP kinase. Gastroenterology. 2004;126:541–53.
249. Beuers U. Drug insight: mechanisms and sites of action of ursodeoxycholic acid in cholestasis. Nat Clin Pract Gastroenterol Hepatol. 2006;3(6):318–28.
250. Wimmer R, Hohenester S, Pusl T, Denk GU, Rust C, Beuers U. Tauroursodeoxycholic acid exerts anticholestatic effects by a cooperative cPKC{alpha}-/PKA-dependent mechanism in rat liver. Gut. 2008;57:1448–54.
251. Benz C, Angermuller S, Tox U, Kloters-Plachky P, Riedel HD, Sauer P, Stremmel W, et al. Effect of tauroursodeoxycholic acid on bile-acid-induced apoptosis and cytolysis in rat hepatocytes. J Hepatol. 1998;28:99–106.
252. Rodrigues CM, Fan G, Wong PY, Kren BT, Steer CJ. Ursodeoxycholic acid may inhibit deoxycholic acid-induced apoptosis by modulating mitochondrial transmembrane potential and reactive oxygen species production. Mol Med. 1998;4:165–78.

253. Benz C, Angermuller S, Otto G, Sauer P, Stremmel W, Stiehl A. Effect of tauroursodeoxycholic acid on bile acid-induced apoptosis in primary human hepatocytes. Eur J Clin Invest. 2000;30:203–9.
254. Lazaridis KN, Gores GJ, Lindor KD. Ursodeoxycholic acid 'mechanisms of action and clinical use in hepatobiliary disorders'. J Hepatol. 2001;35:134–46.
255. Azzaroli F, Mehal W, Soroka CJ, Wang L, Lee J, Crispe IN, Boyer JL. Ursodeoxycholic acid diminishes Fas-ligand-induced apoptosis in mouse hepatocytes. Hepatology. 2002;36:49–54.
256. Graf D, Kurz AK, Fischer R, Reinehr R, Haussinger D. Taurolithocholic acid-3 sulfate induces CD95 trafficking and apoptosis in a c-Jun N-terminal kinase-dependent manner. Gastroenterology. 2002;122:1411–27.
257. Yoshikawa M, Tsujii T, Matsumura K, Yamao J, Matsumura Y, Kubo R, Fukui H, et al. Immunomodulatory effects of ursodeoxycholic acid on immune responses. Hepatology. 1992;16:358–64.
258. Calmus Y, Guechot J, Podevin P, Bonnefis MT, Giboudeau J, Poupon R. Differential effects of chenodeoxycholic and ursodeoxycholic acids on interleukin 1, interleukin 6 and tumor necrosis factor-alpha production by monocytes. Hepatology. 1992;16:719–23.
259. Lacaille F, Paradis K. The immunosuppressive effect of ursodeoxycholic acid: a comparative in vitro study on human peripheral blood mononuclear cells. Hepatology. 1993;18:165–72.
260. Calmus Y, Weill B, Ozier Y, Chereau C, Houssin D, Poupon R. Immunosuppressive properties of chenodeoxycholic and ursodeoxycholic acids in the mouse. Gastroenterology. 1992;103:617–21.
261. Tanaka H, Makino Y, Miura T, Hirano F, Okamoto K, Komura K, Sato Y, et al. Ligand-independent activation of the glucocorticoid receptor by ursodeoxycholic acid. Repression of IFN-gamma-induced MHC class II gene expression via a glucocorticoid receptor-dependent pathway. J Immunol. 1996;156:1601–8.
262. Miura T, Ouchida R, Yoshikawa N, Okamoto K, Makino Y, Nakamura T, Morimoto C, et al. Functional modulation of the glucocorticoid receptor and suppression of NF-kappaB-dependent transcription by ursodeoxycholic acid. J Biol Chem. 2001;276:47371–8.
263. Weitzel C, Stark D, Kullmann F, Scholmerich J, Holstege A, Falk W. Ursodeoxycholic acid induced activation of the glucocorticoid receptor in primary rat hepatocytes. Eur J Gastroenterol Hepatol. 2005;17:169–77.
264. Poupon R, Chretien Y, Poupon RE, Ballet F, Calmus Y, Darnis F. Is ursodeoxycholic acid an effective treatment for primary biliary cirrhosis? Lancet. 1987;1:834–6.
265. Poupon RE, Balkau B, Eschwege E, Poupon R. A multicenter, controlled trial of ursodiol for the treatment of primary biliary cirrhosis. UDCA-PBC Study Group. N Engl J Med. 1991;324:1548–54.
266. Heathcote EJ, Cauch-Dudek K, Walker V, Bailey RJ, Blendis LM, Ghent CN, Michieletti P, et al. The Canadian Multicenter Double-blind Randomized Controlled Trial of ursodeoxycholic acid in primary biliary cirrhosis. Hepatology. 1994;19:1149–56.
267. Combes B, Carithers Jr RL, Maddrey WC, Lin D, McDonald MF, Wheeler DE, Eigenbrodt EH, et al. A randomized, double-blind, placebo-controlled trial of ursodeoxycholic acid in primary biliary cirrhosis. Hepatology. 1995;22:759–66.
268. Lindor KD, Therneau TM, Jorgensen RA, Malinchoc M, Dickson ER. Effects of ursodeoxycholic acid on survival in patients with primary biliary cirrhosis. Gastroenterology. 1996;110:1515–8.
269. Eriksson LS, Olsson R, Glauman H, Prytz H, Befrits R, Ryden BO, Einarsson K, et al. Ursodeoxycholic acid treatment in patients with primary biliary cirrhosis. A Swedish multicentre, double-blind, randomized controlled study. Scand J Gastroenterol. 1997;32:179–86.
270. Leuschner U, Fischer H, Kurtz W, Guldutuna S, Hubner K, Hellstern A, Gatzen M, et al. Ursodeoxycholic acid in primary biliary cirrhosis: results of a controlled double-blind trial. Gastroenterology. 1989;97:1268–74.

271. Lindor KD, Dickson ER, Baldus WP, Jorgensen RA, Ludwig J, Murtaugh PA, Harrison JM, et al. Ursodeoxycholic acid in the treatment of primary biliary cirrhosis. Gastroenterology. 1994;106:1284–90.
272. Angulo P, Dickson ER, Therneau TM, Jorgensen RA, Smith C, DeSotel CK, Lange SM, et al. Comparison of three doses of ursodeoxycholic acid in the treatment of primary biliary cirrhosis: a randomized trial. J Hepatol. 1999;30:830–5.
273. Corpechot C, Carrat F, Bonnand AM, Poupon RE, Poupon R. The effect of ursodeoxycholic acid therapy on liver fibrosis progression in primary biliary cirrhosis. Hepatology. 2000;32:1196–9.
274. Poupon RE, Lindor KD, Pares A, Chazouilleres O, Poupon R, Heathcote EJ. Combined analysis of the effect of treatment with ursodeoxycholic acid on histologic progression in primary biliary cirrhosis. J Hepatol. 2003;39:12–6.
275. Poupon RE, Poupon R, Balkau B. Ursodiol for the long-term treatment of primary biliary cirrhosis. The UDCA-PBC Study Group. N Engl J Med. 1994;330:1342–7.
276. Goulis J, Leandro G, Burroughs AK. Randomised controlled trials of ursodeoxycholic-acid therapy for primary biliary cirrhosis: a meta-analysis. Lancet. 1999;354:1053–60.
277. Gong Y, Huang Z, Christensen E, Gluud C. Ursodeoxycholic acid for patients with primary biliary cirrhosis: an updated systematic review and meta-analysis of randomized clinical trials using Bayesian approach as sensitivity analyses. Am J Gastroenterol. 2007;102:1799–807.
278. Gong Y, Huang ZB, Christensen E, Gluud C. Ursodeoxycholic acid for primary biliary cirrhosis. Cochrane Database Syst Rev. 2008;12:CD000551.
279. Rudic JS, Poropat G, Krstic MN, Bjelakovic G, Gluud C. Ursodeoxycholic acid for primary biliary cirrhosis. Cochrane Database Syst Rev. 2012;12:CD000551.
280. Poupon RE, Lindor KD, Cauch-Dudek K, Dickson ER, Poupon R, Heathcote EJ. Combined analysis of randomized controlled trials of ursodeoxycholic acid in primary biliary cirrhosis. Gastroenterology. 1997;113:884–90.
281. Poupon RE, Bonnand AM, Chretien Y, Poupon R. Ten-year survival in ursodeoxycholic acid-treated patients with primary biliary cirrhosis. The UDCA-PBC Study Group. Hepatology. 1999;29:1668–71.
282. Kumagi T, Guindi M, Fischer SE, Arenovich T, Abdalian R, Coltescu C, Heathcote EJ, et al. Baseline ductopenia and treatment response predict long-term histological progression in primary biliary cirrhosis. Am J Gastroenterol. 2010;105:2186–94.
283. Corpechot C, Chazouilleres O, Poupon R. Early primary biliary cirrhosis: biochemical response to treatment and prediction of long-term outcome. J Hepatol. 2011;55:1361–7.
284. Zhang LN, Shi TY, Shi XH, Wang L, Yang YJ, Liu B, Gao LX, et al. Early biochemical response to ursodeoxycholic acid and long-term prognosis of primary biliary cirrhosis: results of a 14-year cohort study. Hepatology. 2013;58:264–72.
285. Azemoto N, Abe M, Murata Y, Hiasa Y, Hamada M, Matsuura B, Onji M. Early biochemical response to ursodeoxycholic acid predicts symptom development in patients with asymptomatic primary biliary cirrhosis. J Gastroenterol. 2009;44:630–4.
286. Lammers WJ, Hirschfield GM, Corpechot C, Nevens F, Lindor KD, Janssen HL, Floreani A, et al. Development and validation of a scoring system to predict outcomes of patients with primary biliary cirrhosis receiving ursodeoxycholic acid therapy. Gastroenterology. 2015;149:1804–1812.e4.
287. Lammers WJ, van Buuren HR, Hirschfield GM, Janssen HL, Invernizzi P, Mason AL, Ponsioen CY, et al. Levels of alkaline phosphatase and bilirubin are surrogate end points of outcomes of patients with primary biliary cirrhosis: an international follow-up study. Gastroenterology. 2014;147:1338–1349 e1335. ; quiz e1315
288. Carbone M, Sharp SJ, Flack S, Paximadas D, Spiess K, Adgey C, Griffiths L, et al. The UK-PBC Risk Scores: derivation and validation of a scoring system for long-term prediction of end-stage liver disease in primary biliary cirrhosis. Hepatology. 2016;63:930–50.

289. Trivedi PJ, Bruns T, Cheung A, Li KK, Kittler C, Kumagi T, Shah H, et al. Optimising risk stratification in primary biliary cirrhosis: AST/platelet ratio index predicts outcome independent of ursodeoxycholic acid response. J Hepatol. 2014;60:1249–58.
290. Raczynska J, Habior A, Paczek L, Foroncewicz B, Pawelas A, Mucha K. Primary biliary cirrhosis in the era of liver transplantation. Ann Transplant. 2014;19:488–93.
291. Neuberger J, Portmann B, Macdougall BR, Calne RY, Williams R. Recurrence of primary biliary cirrhosis after liver transplantation. N Engl J Med. 1982;306:1–4.
292. Carbone M, Neuberger J. Liver transplantation in PBC and PSC: indications and disease recurrence. Clin Res Hepatol Gastroenterol. 2011;35:446–54.
293. Schreibman I, Regev A. Recurrent primary biliary cirrhosis after liver transplantation – the disease and its management. MedGenMed. 2006;8:30.
294. Neuberger J. Recurrent primary biliary cirrhosis. Liver Transpl. 2003;9:539–46.
295. Charatcharoenwitthaya P, Pimentel S, Talwalkar JA, Enders FT, Lindor KD, Krom RA, Wiesner RH. Long-term survival and impact of ursodeoxycholic acid treatment for recurrent primary biliary cirrhosis after liver transplantation. Liver Transpl. 2007;13: 1236–45.
296. Liermann Garcia RF, Evangelista Garcia C, McMaster P, Neuberger J. Transplantation for primary biliary cirrhosis: retrospective analysis of 400 patients in a single center. Hepatology. 2001;33:22–7.
297. Morioka D, Egawa H, Kasahara M, Jo T, Sakamoto S, Ogura Y, Haga H, et al. Impact of human leukocyte antigen mismatching on outcomes of living donor liver transplantation for primary biliary cirrhosis. Liver Transpl. 2007;13:80–90.
298. Hashimoto T, Sugawara Y, Makuuchi M. Impact of human leukocyte antigen mismatching on outcomes of living donor liver transplantation for primary biliary cirrhosis. Liver Transpl. 2007;13:938–9.
299. Neuberger J, Gunson B, Hubscher S, Nightingale P. Immunosuppression affects the rate of recurrent primary biliary cirrhosis after liver transplantation. Liver Transpl. 2004;10:488–91.
300. Manousou P, Arvaniti V, Tsochatzis E, Isgro G, Jones K, Shirling G, Dhillon AP, et al. Primary biliary cirrhosis after liver transplantation: influence of immunosuppression and human leukocyte antigen locus disparity. Liver Transpl. 2010;16:64–73.
301. Grat M, Lewandowski Z, Patkowski W, Wronka KM, Grat K, Krasnodebski M, Ligocka J, et al. Relevance of male-to-female sex mismatch in liver transplantation for primary biliary cirrhosis. Ann Transplant. 2015;20:116–23.
302. Guy JE, Qian P, Lowell JA, Peters MG. Recurrent primary biliary cirrhosis: peritransplant factors and ursodeoxycholic acid treatment post-liver transplant. Liver Transpl. 2005;11:1252–7.
303. Quarneti C, Muratori P, Lalanne C, Fabbri A, Menichella R, Granito A, Masi C, et al. Fatigue and pruritus at onset identify a more aggressive subset of primary biliary cirrhosis. Liver Int. 2015;35:636–41.
304. Al-Harthy N, Kumagi T, Coltescu C, Hirschfield GM. The specificity of fatigue in primary biliary cirrhosis: evaluation of a large clinic practice. Hepatology. 2010;52:562–70.
305. Cauch-Dudek K, Abbey S, Stewart DE, Heathcote EJ. Fatigue in primary biliary cirrhosis. Gut. 1998;43:705–10.
306. Newton JL, Hudson M, Tachtatzis P, Sutcliffe K, Pairman J, Burt JA, Jones DE. Population prevalence and symptom associations of autonomic dysfunction in primary biliary cirrhosis. Hepatology. 2007;45:1496–505.
307. Newton JL, Gibson GJ, Tomlinson M, Wilton K, Jones D. Fatigue in primary biliary cirrhosis is associated with excessive daytime somnolence. Hepatology. 2006;44:91–8.
308. Fisk JD, Ritvo PG, Ross L, Haase DA, Marrie TJ, Schlech WF. Measuring the functional impact of fatigue: initial validation of the fatigue impact scale. Clin Infect Dis. 1994;18(Suppl 1):S79–83.

309. Jacoby A, Rannard A, Buck D, Bhala N, Newton JL, James OF, Jones DE. Development, validation, and evaluation of the PBC-40, a disease specific health related quality of life measure for primary biliary cirrhosis. Gut. 2005;54:1622–9.
310. Kaplan MM, Bonis PA. Modafinil for the treatment of fatigue in primary biliary cirrhosis. Ann Intern Med. 2005;143:546–7.
311. Ian Gan S, de Jongh M, Kaplan MM. Modafinil in the treatment of debilitating fatigue in primary biliary cirrhosis: a clinical experience. Dig Dis Sci. 2009;54:2242–6.
312. Alemi F, Kwon E, Poole DP, Lieu T, Lyo V, Cattaruzza F, Cevikbas F, et al. The TGR5 receptor mediates bile acid-induced itch and analgesia. J Clin Invest. 2013;123:1513–30.
313. Dawson PA, Karpen SJ. Bile acids reach out to the spinal cord: new insights to the pathogenesis of itch and analgesia in cholestatic liver disease. Hepatology. 2014;59:1638–41.
314. Beuers U, Kremer AE, Bolier R, Elferink RP. Pruritus in cholestasis: facts and fiction. Hepatology. 2014;60:399–407.
315. Kremer AE, van Dijk R, Leckie P, Schaap FG, Kuiper EM, Mettang T, Reiners KS, et al. Serum autotaxin is increased in pruritus of cholestasis, but not of other origin, and responds to therapeutic interventions. Hepatology. 2012;56:1391–400.
316. Garbutt JT, Kenney TJ. Effect of cholestyramine on bile acid metabolism in normal man. J Clin Invest. 1972;51:2781–9.
317. Javitt NB. Letter: timing of cholestyramine doses in cholestatic liver disease. N Engl J Med. 1974;290:1328–9.
318. Rust C, Sauter GH, Oswald M, Buttner J, Kullak-Ublick GA, Paumgartner G, Beuers U. Effect of cholestyramine on bile acid pattern and synthesis during administration of ursodeoxycholic acid in man. Eur J Clin Invest. 2000;30:135–9.
319. Bachs L, Pares A, Elena M, Piera C, Rodes J. Comparison of rifampicin with phenobarbitone for treatment of pruritus in biliary cirrhosis. Lancet. 1989;1:574–6.
320. Tandon P, Rowe BH, Vandermeer B, Bain VG. The efficacy and safety of bile Acid binding agents, opioid antagonists, or rifampin in the treatment of cholestasis-associated pruritus. Am J Gastroenterol. 2007;102:1528–36.
321. Wolfhagen FH, Sternieri E, Hop WC, Vitale G, Bertolotti M, Van Buuren HR. Oral naltrexone treatment for cholestatic pruritus: a double-blind, placebo-controlled study. Gastroenterology. 1997;113:1264–9.
322. Terg R, Coronel E, Sorda J, Munoz AE, Findor J. Efficacy and safety of oral naltrexone treatment for pruritus of cholestasis, a crossover, double blind, placebo-controlled study. J Hepatol. 2002;37:717–22.
323. Browning J, Combes B, Mayo MJ. Long-term efficacy of sertraline as a treatment for cholestatic pruritus in patients with primary biliary cirrhosis. Am J Gastroenterol. 2003;98:2736–41.
324. Mayo MJ, Handem I, Saldana S, Jacobe H, Getachew Y, Rush AJ. Sertraline as a first-line treatment for cholestatic pruritus. Hepatology. 2007;45:666–74.
325. Pares A. Old and novel therapies for primary biliary cirrhosis. Semin Liver Dis. 2014;34:341–51.
326. Raszeja-Wyszomirska J, Miazgowski T. Osteoporosis in primary biliary cirrhosis of the liver. Prz Gastroenterol. 2014;9:82–7.
327. Pares A, Guanabens N. Osteoporosis in primary biliary cirrhosis: pathogenesis and treatment. Clin Liver Dis. 2008;12:407–24. x
328. Treeprasertsuk S, Silveira MG, Petz JL, Lindor KD. Parenteral bisphosphonates for osteoporosis in patients with primary biliary cirrhosis. Am J Ther. 2011;18:375–81.
329. Hofmann AF, Zakko SF, Lira M, Clerici C, Hagey LR, Lambert KK, Steinbach JH, et al. Novel biotransformation and physiological properties of norursodeoxycholic acid in humans. Hepatology. 2005;42:1391–8.
330. Yoon YB, Hagey LR, Hofmann AF, Gurantz D, Michelotti EL, Steinbach JH. Effect of side-chain shortening on the physiologic properties of bile acids: hepatic transport and effect on biliary secretion of 23-nor-ursodeoxycholate in rodents. Gastroenterology. 1986;90:837–52.

331. Hofmann AF, Hagey LR. Key discoveries in bile acid chemistry and biology and their clinical applications: history of the last eight decades. J Lipid Res. 2014;55:1553–95.
332. Trauner M, Fickert P, Halilbasic E, Moustafa T. Lessons from the toxic bile concept for the pathogenesis and treatment of cholestatic liver diseases. Wien Med Wochenschr. 2008;158:542–8.
333. Hohenester S, Wenniger LM, Paulusma CC, van Vliet SJ, Jefferson DM, Elferink RP, Beuers U. A biliary HCO3- umbrella constitutes a protective mechanism against bile acid-induced injury in human cholangiocytes. Hepatology. 2012;55:173–83.
334. Moustafa T, Fickert P, Magnes C, Guelly C, Thueringer A, Frank S, Kratky D, et al. Alterations in lipid metabolism mediate inflammation, fibrosis, and proliferation in a mouse model of chronic cholestatic liver injury. Gastroenterology. 2012;142:140–51. e112
335. Trauner M, Halilbasic E, Kazemi-Shirazi L, Kienbacher C, Staufer K, Traussnigg S, Hofer H. Therapeutic role of bile acids and nuclear receptor agonists in fibrosing cholangiopathies. Dig Dis. 2014;32:631–6.
336. Beuers U, Trauner M, Jansen P, Poupon R. New paradigms in the treatment of hepatic cholestasis: from UDCA to FXR, PXR and beyond. J Hepatol. 2015;62:S25–37.
337. Pellicciari R, Fiorucci S, Camaioni E, Clerici C, Costantino G, Maloney PR, Morelli A, et al. 6alpha-ethyl-chenodeoxycholic acid (6-ECDCA), a potent and selective FXR agonist endowed with anticholestatic activity. J Med Chem. 2002;45:3569–72.
338. Matsumoto T, Miyazaki H, Nakahashi Y, Hirohara J, Seki T, Inoue K, Okazaki K. Multidrug resistance3 is in situ detected in the liver of patients with primary biliary cirrhosis, and induced in human hepatoma cells by bezafibrate. Hepatol Res. 2004;30:125–36.
339. Honda A, Ikegami T, Nakamuta M, Miyazaki T, Iwamoto J, Hirayama T, Saito Y, et al. Anticholestatic effects of bezafibrate in patients with primary biliary cirrhosis treated with ursodeoxycholic acid. Hepatology. 2013;57:1931–41.
340. Zhang Y, Chen K, Dai W, Xia Y, Wang F, Shen M, Cheng P, et al. Combination therapy of bezafibrate and ursodeoxycholic acid for primary biliary cirrhosis: a meta-analysis. Hepatol Res. 2015;45:48–58.
341. Gough SC, Walker LS, Sansom DM. CTLA4 gene polymorphism and autoimmunity. Immunol Rev. 2005;204:102–15.
342. Oertelt S, Kenny TP, Selmi C, Invernizzi P, Podda M, Gershwin ME. SNP analysis of genes implicated in T cell proliferation in primary biliary cirrhosis. Clin Dev Immunol. 2005;12:259–63.
343. Tanaka H, Yang GX, Tomiyama T, Tsuneyama K, Zhang W, Leung PS, Coppel RL, et al. Immunological potential of cytotoxic T lymphocyte antigen 4 immunoglobulin in murine autoimmune cholangitis. Clin Exp Immunol. 2015;180:371–82.
344. Mitchison HC, Bassendine MF, Malcolm AJ, Watson AJ, Record CO, James OF. A pilot, double-blind, controlled 1-year trial of prednisolone treatment in primary biliary cirrhosis: hepatic improvement but greater bone loss. Hepatology. 1989;10:420–9.
345. Mitchison HC, Palmer JM, Bassendine MF, Watson AJ, Record CO, James OF. A controlled trial of prednisolone treatment in primary biliary cirrhosis. Three-year results. J Hepatol. 1992;15:336–44.
346. Rautiainen H, Karkkainen P, Karvonen AL, Nurmi H, Pikkarainen P, Nuutinen H, Farkkila M. Budesonide combined with UDCA to improve liver histology in primary biliary cirrhosis: a three-year randomized trial. Hepatology. 2005;41:747–52.
347. Angulo P, Jorgensen RA, Keach JC, Dickson ER, Smith C, Lindor KD. Oral budesonide in the treatment of patients with primary biliary cirrhosis with a suboptimal response to ursodeoxycholic acid. Hepatology. 2000;31:318–23.
348. Hempfling W, Grunhage F, Dilger K, Reichel C, Beuers U, Sauerbruch T. Pharmacokinetics and pharmacodynamic action of budesonide in early- and late-stage primary biliary cirrhosis. Hepatology. 2003;38:196–202.
349. Leung J, Bonis PA, Kaplan MM. Colchicine or methotrexate, with ursodiol, are effective after 20 years in a subset of patients with primary biliary cirrhosis. Clin Gastroenterol Hepatol. 2011;9:776–80.

350. Combes B, Emerson SS, Flye NL, Munoz SJ, Luketic VA, Mayo MJ, McCashland TM, et al. Methotrexate (MTX) plus ursodeoxycholic acid (UDCA) in the treatment of primary biliary cirrhosis. Hepatology. 2005;42:1184–93.
351. Afford SC, Ahmed-Choudhury J, Randhawa S, Russell C, Youster J, Crosby HA, Eliopoulos A, et al. CD40 activation-induced, Fas-dependent apoptosis and NF-kappaB/AP-1 signaling in human intrahepatic biliary epithelial cells. FASEB J. 2001;15:2345–54.
352. Oertelt S, Invernizzi P, Selmi C, Podda M, Gershwin ME. Soluble CD40L in plasma of patients with primary biliary cirrhosis. Ann N Y Acad Sci. 2005;1051:205–10.
353. Higuchi M, Horiuchi T, Kojima T, Nishizaka H, Ishibashi H, Hayashi K, Niho Y, et al. Analysis of CD40 ligand gene mutations in patients with primary biliary cirrhosis. Scand J Clin Lab Invest. 1998;58:429–32.
354. Tanaka H, Yang GX, Iwakoshi N, Knechtle SJ, Kawata K, Tsuneyama K, Leung P, et al. Anti-CD40 ligand monoclonal antibody delays the progression of murine autoimmune cholangitis. Clin Exp Immunol. 2013;174:364–71.
355. Tsuda M, Moritoki Y, Lian ZX, Zhang W, Yoshida K, Wakabayashi K, Yang GX, et al. Biochemical and immunologic effects of rituximab in patients with primary biliary cirrhosis and an incomplete response to ursodeoxycholic acid. Hepatology. 2012;55:512–21.
356. Myers RP, Swain MG, Lee SS, Shaheen AA, Burak KW. B-cell depletion with rituximab in patients with primary biliary cirrhosis refractory to ursodeoxycholic acid. Am J Gastroenterol. 2013;108:933–41.
357. Figueroa FE, Carrion F, Villanueva S, Khoury M. Mesenchymal stem cell treatment for autoimmune diseases: a critical review. Biol Res. 2012;45:269–77.
358. Wang D, Zhang H, Liang J, Gu Z, Ma X, Huang J, Lin J, et al. Effect of allogeneic bone marrow-derived mesenchymal stem cells transplantation in a polyI:C-induced primary biliary cirrhosis mouse model. Clin Exp Med. 2011;11:25–32.
359. Wang L, Li J, Liu H, Li Y, Fu J, Sun Y, Xu R, et al. Pilot study of umbilical cord-derived mesenchymal stem cell transfusion in patients with primary biliary cirrhosis. J Gastroenterol Hepatol. 2013;28(Suppl 1):85–92.
360. Wang L, Han Q, Chen H, Wang K, Shan GL, Kong F, Yang YJ, et al. Allogeneic bone marrow mesenchymal stem cell transplantation in patients with UDCA-resistant primary biliary cirrhosis. Stem Cells Dev. 2014;23:2482–9.

Primary Sclerosing Cholangitis (PSC): Current Concepts in Biology and Strategies for New Therapy

9

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Abstract

Primary sclerosing cholangitis (PSC) is characterised morphologically by fibro-inflammatory stricture formation and typified phenotypically by strong clinical association with inflammatory bowel disease. Although rare, PSC harbours a significant and disproportionate clinical need, in which ~50% of patients develop advancing disease necessitating transplantation. These unacceptably poor outcomes highlight a critical therapeutic shortfall in hepatology, whereby no existing intervention has been shown to improve transplant-free survival. The clinical course in PSC can be unpredictable; and advances in clinical practice highlight the breadth of disease heterogeneity, that exists, whilst equally providing a more individualised assessment of patient risk. These developments are paralleled by notable pathological discovery, wherein recognition of enteric dysbiosis, essential lymphocyte recruitment pathways, and mucosal immunogenicity have driven a resurgence in clinical trial activity for appropriately stratified patient populations.

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G. Hirschfield et al. (eds.), *Biliary Disease*, DOI 10.1007/978-3-319-50168-0_9

Take-Home Points

- The vast majority of PSC patients (>70%) develop inflammatory bowel disease (IBD) at some point in their lifetime.
- Present understanding of pathobiology is incomplete, but disease development is likely to involve alterations to the host microbiome, perturbed tolerogenic immune responses, and conserved leucocyte homing pathways between afflicted disease sites.
- Clinical course is impacted by ductal as well as IBD phenotypes; however the 'natural' history is highly variable between patients, with current biochemical predictors of outcome being imprecise. Emerging prognostic tools include non-invasive surrogates of liver fibrosis that are awaiting prospective, large-scale validation.
- No medical therapy has been proven to consistently slow disease progression, and liver transplantation remains the only life-extending intervention.
- Mechanistic insights spanning genetic risks, microbiome manipulation and biological pathways to liver injury and fibrosis have led to a renewed interest in therapies.

9.1 Introduction

The term 'sclerosing cholangitis' encompasses, a spectrum of disorders morphologically represented by multilevel stricturing throughout the biliary tree and often patchy bile duct inflammation and concentric periductal fibrosis histologically [1]. A variety of aetiologies can give rise to these histopathological changes as the endpoint of a final common pathway of chronic biliary injury. The prefix 'primary' refers to the commonest form of sclerosing cholangitis (PSC), which although idiopathic in aetiology bears a strident clinical association with inflammatory bowel disease (IBD) (Fig. 9.1). Presently, PSC represents the greatest unmet need in modern hepatology, with up to 50% of patients developing progressive liver disease requiring transplantation within 10–20 years after diagnosis, in addition to a significantly increased lifetime risk of hepatobiliary and colorectal malignancy (up to 15%) [3].

The unacceptably high morbidity and mortality for patients with PSC are representative of the critical absence of definitive medical therapy and reflection of an as yet indeterminate explanation for disease pathogenesis. Various mechanistic aspects tracking the development of typical bile duct lesions are under concentrated study, stimulating an ongoing debate as to whether the principal injury is caused by immune-mediated mechanisms or biochemical aspects related to bile physiology [4]. However, any pathogenic model also needs to take into consideration that hepatobiliary injury in PSC runs an independent clinical course to that of the associated bowel disease affliction.

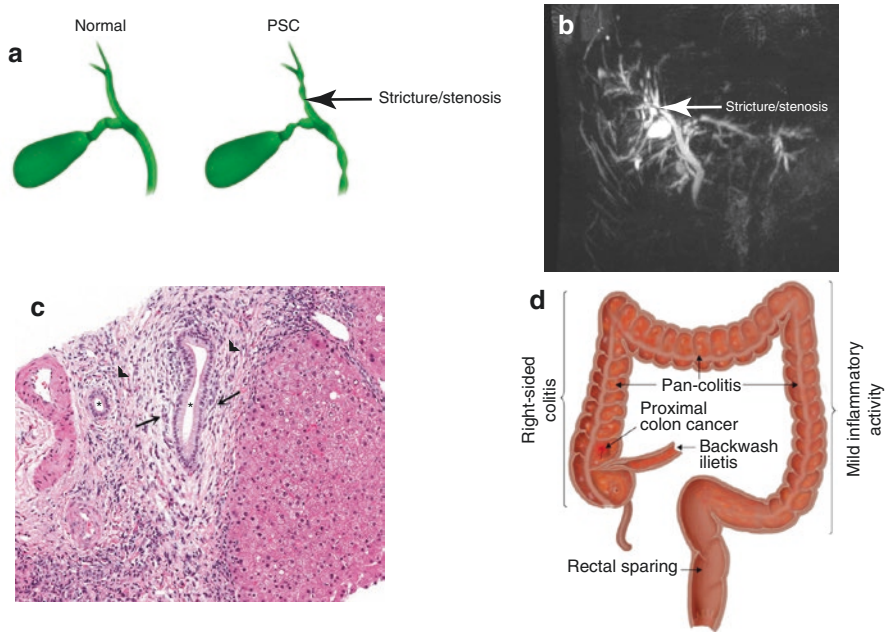

Fig. 9.1 Phenotypic characteristics. Sclerosing cholangitis represents the development of multifocal stricturing throughout the biliary tree (**a**). Such changes can be subtle, and the gold standard radiological method of detection is cholangiography, conventionally via magnetic resonance imaging (**b**). Histological characteristics vary and include lymphocytic infiltration (*arrows*) and the classic 'onion skin' periductal fibrotic lesion (*arrowheads*) which surround the bile ducts (*asterisks*) (**c**). Although periductal lesions are widely described in the literature, such features are present in fewer than 20% of biopsies in patients with primary sclerosing cholangitis (PSC). The latter is a syndrome with incompletely understood aetiopathogenesis, albeit an overwhelming coexistence with inflammatory bowel disease (IBD); most often a distinct form of colitis (**d**); typified by pan-colonic inflammation (right sided predominant in ~25%), backwash ileitis (~50%), rectal sparing (50–65%) and a significantly increased risk of colonic malignancy. Liver histology image (Credited to Dr. Maura O'Neil [2])

9.2 PSC as an Immune-Mediated Disease

The majority of the liver's blood supply (>70%) derives from the intestine (via portal circulation), and therefore the liver is continually exposed to a wide range of nutrients, xenobiotics and potentially antigenic components derived from the gut. Exemplifying this close relationship, expression of innate immune pathogen recognition receptors (PRR) is conserved between sites, in order to respond to the constant exposure of microbial-derived products [5]. Characterising the integrity of the gut barrier and hepatic immune responses to gut-derived factors is therefore potentially relevant to the development of new therapies to treat immune-mediated liver disease, including PSC [6].

9.2.1 The Gut-Liver Block and Biliary Inflammation

The intestinal mucosa is host to a large number of commensal microbes, which in humans are dominated by the bacterial phyla *Firmicutes* and *Bacteroides* and less so *Actinobacteria* and *Proteobacteria*. Commensal flora resident in the gastrointestinal tract have co-evolved with man and, through a symbiotic relationship, perform a broad range of essential physiological functions ranging from the promotion of intestinal defences against outgrowth of pathogenic species to a hitherto unknown level of host-related co-metabolism [7]. The intestinal epithelial monolayer underneath provides a structural barrier that prevents invasion of bacteria beyond the gut and also expresses a variety of PRR, such as toll-like receptors (TLR) and nucleotide-binding oligomerisation domain-containing proteins (NOD)-1 and NOD2, which are involved in the recognition of cellular injury and damage [8]. The ability of the intestinal epithelial cells to secrete cytokines and chemokines in response to enteric microbial fluctuations, pathogens or injury allows them to actively shape local immune responses and modulate sub-epithelial dendritic cell (DC) and lymphocyte positioning and activation. A degree of mutualism between commensal flora and the human host is illustrated by the fact that the activation of epithelial TLR2 or TLR9 increases gut barrier function, whereas mice deficient in the downstream TLR-signalling molecule MyD88 are susceptible to experimentally induced IBD [9]. However, it remains tentative how exactly the mucosal epithelium distinguishes between commensal bacteria and pathogenic strains. Disturbances in immune or epithelial homeostasis can lead to gut inflammation, and in certain circumstances, commensal flora may act as pathogens. Evidence to support impaired tolerance to commensal flora in the pathogenesis of IBD is provided by the interleukin (*Il*)-*10*$^{-/-}$ murine model, wherein intestinal inflammation is abrogated under germ-free conditions and in *Il-2*-deficient animals which develop colitis following enteric exposure to *Escherichia coli* but not *Bacteroides vulgates* [10, 11].

Ordinarily, gut commensals and pathogens are confined to the gut by the mucosal epithelium and mesenteric lymph nodes (MLN). It is conceivable, however, that in the presence of intestinal inflammation and a disturbed epithelial barrier, bacteria can enter the portal circulation and the liver where further levels of regulation exist to prevent uncontrolled systemic immune activation. Under normal circumstances, liver-resident antigen-presenting cells (APC) display attenuated responses to endotoxin exposure, and the liver functions as a second 'firewall' that clears commensals from the circulation if intestinal defences are overwhelmed [12]. Intestinal $CX_3CR_1^+$ macrophages are a critical component of the intestinal barrier and express TLR to sense microorganisms and activate innate lymphoid cells (ILC) to secrete IL-22, thereby directly promoting epithelial integrity and repair [13]. Deletion of CX_3CR_1 in mice not only results in increased bacterial translocation to MLN and susceptibility to colitis, but in a diet-induced model of liver disease to hepatitis, demonstrating how defects in gut integrity can drive hepatic inflammation [14]. Compositional changes to the gut microbiota as a consequence of defective inflammasome pathway signalling have also been shown to induce liver inflammation driven by hepatic TNFα activation as a consequence of TLR4 and TLR9 agonists in the portal

circulation [15]. Such observations indicate how defective pathogen sensing as a consequence of a divergent enteric microbiome, or perhaps through genetic variations affecting the threshold for PRR signalling [16], might change the gut microbiota leading to hepatic inflammation in association with IBD. This concept is supported by data from mouse models in which changes in intestinal bacterial populations or infusion of bacterial antigens into the portal circulation lead to peri-cholangitis [17].

Analysis of colonic mucosal biopsies in British patients with PSC and IBD suggests a restriction in the biodiversity of adherent microbiota, highlighted by a near absence of *Bacteroides* and by significant increases in *Escherichia*, *Lachnospiraceae* and *Megasphaera*, when compared to individuals with IBD alone and healthy controls [5, 18]. Restricted biodiversity was also reported when analysing stool samples in a Norwegian PSC cohort, albeit with a marked increase in the anaerobic genus *Veillonella* [19]. These subtle, yet important, differences are likely to reflect the impact of dietary and environmental influences on enteric flora composition and possibly disparate genetic risk factors that alter microbial handling between patient populations [20, 21].

Seropositivity for perinuclear anti-neutrophil cytoplasmic antibodies (pANCA) is frequently observed in patients with PSC, and although not disease specific, these antibodies are highly reactive against the microbial cell division protein FtsZ present in virtually every bacteria of the intestinal microflora [22]. Some autoantibodies detected in PSC also bind to biliary epithelium, which, as a contiguous component of the mucosal epithelium, can be activated through enhanced TLR4 and TLR9 expression resulting in the secretion of pro-inflammatory cytokines and chemokines that drive hepatobiliary inflammation [23].

9.2.2 Loss of Immune Tolerance

Based on pathological observations, hepatic innate immune responses have traditionally been considered a primary inciting event in PSC, with disease development initiated via bacteria or pathogen-associated molecular patterns (PAMPs) that enter the portal circulation via a permeable intestinal mucosa [24]. Progressive injury occurs in small, medium and large bile ducts with resultant inflammation and concentric periductal fibrosis. In early disease, these changes are confined to portal tracts, with a mixed inflammatory cell infiltrate composed of natural killer (NK)-cells, NKT-cells, B-cells, macrophages and predominantly activated effector or memory T-cells [25]. The ratio of $CD4^+$ to $CD8^+$ T-cells in PSC patients shows considerable inconsistencies in different studies although $CD4^+$ T-cells are more commonly observed in the portal tracts, whereas $CD8^+$ T-cells populate portal areas and the parenchyma [26]. DCs which prime intrahepatic T-cell responses are inherently tolerogenic in normal liver and express low levels of the co-stimulatory molecules required for full T-cell activation [27]. Furthermore, liver-derived DCs preferentially secrete the immunoregulatory cytokine IL-10 and are also capable of inducing peripheral regulatory T-cells (T_{reg}), a cell population critical for

suppression of immune responses. However, patients with PSC often exhibit reduced T-cell expression of the IL-2 receptor, particularly in association with *IL-2Rα* genetic polymorphisms and defective T_{reg} responses [28]. These observations are of clinical interest given that *Il2Rα*$^{-/-}$ mice develop spontaneous T-cell-mediated cholangitis and colitis in the context of defective regulatory T_{reg} activity [29].

A subpopulation of $CD4^+$ and $CD8^+$ T-cells have been identified, which lack the co-stimulatory molecule CD28. These CD28-negative T-cells are $IL2R\alpha^{lo}$ and enriched in PSC relative to normal liver, where they express phenotypic surface markers in keeping with an activated memory phenotype ($CD45RA^-$ $CCR7^-$) in addition to containing high quantities of cytotoxic molecules granzyme B and perforin. Liver-infiltrating $CD28^-$ T-cells are equipped with chemokine homing receptors that facilitate recruitment and positioning in proximity to bile ducts, where they induce activation of biliary epithelial cells (BEC) [26]. CD28-negative T-cells appear to be chronically activated immunopathogenic lymphocytes that are less susceptible to regulation by conventional T_{reg}, making them potentially important drivers of hepatobiliary inflammation.

Defective numbers of functional intrahepatic and peripheral blood T_{reg} are implicated across the spectrum of autoimmunity, often within the expanse of heightened effector T_h17 responses to pathogen stimulation [30]. IL-17-producing cells, including mucosal-associated invariant T-cells (MAIT-cells), T_c17-cells and T_h17-cells, are abundant in the intestinal lamina propria as well as the liver. In the gut, they are maintained by commensal bacteria which induce innate lymphoid cells (ILC) to secrete IL-22, a critical factor for IL-17A expression in T-cells [31].

9.2.3 Overlapping Lymphocyte Recruitment Pathways Between Gut and Liver (Table 9.1)

Intra-organ recruitment and localisation of leucocytes is a highly coordinated process regulated through the selective expression of integrins and chemokine receptors (expressed by lymphocytes), which allow interactions with tissue-specific adhesion molecules and chemokine ligands expressed by endothelial structures – the first port of entry into solid organs within the human body. In the gut, the endothelial phenotype is highlighted by expression of the chemokine CCL25 and the adhesion molecule mucosal addressin cell adhesion molecule-1 (MAdCAM)-1. This pairing facilitates recruitment of lymphocytes imprinted with gut tropism; specifically those which express the chemokine receptor CCR9 and integrin $\alpha4\beta7$. Under homeostatic conditions, MAdCAM-1 and CCL25 are critically involved in the recruitment of lymphocytes to the lamina propria; and although their expression increases significantly during inflammation [32], effector cells may also engage other adhesion molecules including vascular adhesion protein (VAP)-1, intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1, which become induced in response to pro-inflammatory signals. In addition, human lamina propria lymphocytes express chemokine receptors CCR2, CCR5, CCR6 and

Table 9.1 Molecular determinants of leucocyte recruitment to the liver and gut

Site	Constitutively expressed	Increased in response to inflammation
Small bowel	α4β7 – MAdCAM-1 αEβ7 – E-cadherin (intraepithelial compartment) CCR6 – CCL20 CCR9 – CCL25 CCR10 – CCL28 CXCR1 – CXCL5/6/8 CXCR2 – CXCL1/2/5/6 CXCR6 – CXCL16 CX3CR1 – CX3CL1	E-selectin P-selectin – PSGL-1 α4β7 – MAdCAM-1 VAP-1 α4β1 – VCAM-1 αΛβ2 – ICAM-1 CCR2 – CCL2/7/8 CCR5 – CCL3/4/5/8 CCR9 – CCL25 CXCR3 – CXCL9/10/11 CX3CR1 – CX3CL1
Colon	α4β7 – MAdCAM-1 L-selectin[a] – PNAd / MAdCAM-1 CCR5 – CCL3/4/5/8 CCR6 – CCL20 CCR10 – CCL28 CX3CR1 – CX3CL1	α4β7 – MAdCAM-1 L-selectin – PNAd/MAdCAM-1 α4β1 – VCAM-1 αLβ2 – ICAM-1 VAP-1 E-selectin P-selectin – PSGL-1 CCR2 – CCL2/7/8 CCR3 – CCL11 (colitis) CCR9 – CCL25 (colitis) CXCR1 – CXCL5/6/8 CXCR2 – CXCL1/2/5/6 CXCR3 – CXCL9/10/11
Portal vessels	CCR5 – CCL3/4/5	E-selectin P-selectin – PSGL-1 CX3CR1 – CX3CL1
Sinusoids	VAP-1 CLEVER-1 ICAM-1 (low levels) - αLβ2 CXCR6 – CXCL16 CXCR4 – CXCL12 CXCR3 – CXCL9/10/11 (low)	CD44 VAP-1 α4β7 – MAdCAM-1 αLβ2 – ICAM-1 α4β1 – VCAM-1 CCR9 – CCL25 CXCR3 – CXCL9/10/11 CX3CR1 – CX3CL1 CXCR4 – CXCL12
Biliary epithelium	CCR10 – CCL28	αLβ2 – ICAM-1 α4β1 – VCAM-1 CCL20 – CCR6 CXCR1 – CXCL8 CXCR4 – CXCL12 CXCR6 – CXCL16 CX3CR1 – CX3CL1

[a]L-selectin predominantly involved in recruitment of naïve T-cells

CXCR3, which might play a more pertinent role than CCR9 during later stages of intestinal inflammation [33].

Lymphocytes can enter the liver via several routes, including post-capillary portal venules and hepatic sinusoids. Effector T-cells infiltrating the inflamed human liver commonly express high levels of CXCR3, which allow them to respond to the chemokine ligands CXCL9 and CXCL10, both of which are induced by interferon (IFN)-γ and tumour necrosis factor (TNF)α and detectable on sinusoidal endothelium [34]. CXCR3 appears to be a common signal for recruitment of T_h1, T_h17 and T_{reg}, although subsequent positioning within the liver may involve other chemokines including CCR4 and CCR10, which localise T_{reg} in portal tracts. The chemokine receptor CXCR6 may have a role in recruitment of effector T-cells and the patrolling of sinusoids by NKT-cells [34].

Animal models of immune-mediated hepatobiliary injury have demonstrated inflammation-dependent roles for ICAM-1, VCAM-1 and several pro-inflammatory chemokines, although no liver specific adhesion molecules have been identified. The liver endothelial phenotype is also characterised by expression of adhesion molecules more commonly associated with lymphatic structures such as VAP-1 and common lymphatic endothelial and vascular endothelial receptor-1 (CLEVER-1) [6]. Moreover, in PSC, MAdCAM-1 and CCL25 are also detectable on liver endothelium, and collectively facilitate the recruitment of $\alpha4\beta7^+CCR9^+$ mucosal lymphocytes directly from the gut [35, 36]. Most of the liver-infiltrating $\alpha4\beta7^+CCR9^+$ cells in PSC are $CD45RA^-$ $CCR7^-$ $CD11a^{hi}$ and secrete interferon (IFN)-γ upon stimulation in vitro, in keeping with a long-lived memory phenotype. This suggests that upon entry to the liver, $CCR9^+$ T-cells can become reactivated following exposure to a common antigenic trigger shared with the intestine [35].

The exact factors leading to aberrant expression of MAdCAM-1 in the PSC liver are incompletely understood. Recent work has demonstrated that catabolism of biological amines by VAP-1, an adhesion molecule upregulated in the inflamed liver which also behaves as a selective amine oxidase, is able to induce expression of functional MAdCAM-1 on the surface of hepatic sinusoidal endothelial cells (HSEC) [37]. Increased levels of enterobacterial-derived amines, perhaps due to enhanced absorption via the inflamed gut, may thus act as a substrate for VAP-1, thereby increasing MAdCAM-1 expression in the inflamed mucosa and hepatic endothelium. Such mechanisms could promote the unrestrained recruitment of mucosal effector cells and result in tissue damage that is characteristic of both IBD and PSC.

The capacity to imprint gut tropism on naïve lymphocytes is dependent on the ability of APC to convert retinol to all-trans retinoic acid (ATRA), which complexes with intracellular retinoid receptors to activate transcription of gut-homing receptors (CCR9 and α4β7). This ability can be conferred *in vitro* by the isolated addition of exogenous ATRA suggesting that retinoids in bile or stored in stellate cells, one of the main storage cells for RA, might be able to provide ATRA within the liver, allowing liver APCs to induce a gut-homing phenotype. However, isolated human hepatic DC and stellate cells are unable to induce high-level α4β7 or CCR9 expression on T-cells suggesting that the ability to imprint naïve lymphocytes with gut tropism is restricted to intestinal DCs [38]. Nevertheless, a study in mice by Neumann *et al*. illustrated that

priming of naïve T-cells by HSEC is able to induce expression of α4β7 on $CD4^+$ T-cells in a retinoic acid dependent manner [39]. Of interest, there is emerging evidence from spontaneous models of ileitis, which show that the onset of portal and lobular liver inflammation may actually develop before that observed in the gut [40].

Gut-derived lymphocytes also utilise other chemokine receptors utilise to localise to biliary epithelium, following which they can destroy bile ducts. Moreover, the biliary and intestinal epithelia are in direct mucosal continuity with one another, thus it is perhaps unsurprising that these two structures share certain chemokine expression patterns. For instance, some of the $\alpha4\beta7^+$ T-cells also express $\alpha E\beta7^+$ which binds to E-cadherin expressed on the adherens junction of gut epithelial cells or bile ducts [36]. Similarly, the inflamed biliary epithelium expresses CCL20, and effector IL-17-secreting T-cells that infiltrate the human liver express high levels of its cognate receptor CCR6, facilitating their recruitment and positioning around bile ducts [41].

9.3 Bile Toxicity

As previously discussed, several lines of evidence implicate mucosal dysbiosis and restricted biodiversity of the intestinal microbiome as being a pivotal event in the pathogenesis of IBD. Following secretion into the gut lumen, enzymatic reactions catalysed by intestinal commensals are also responsible for bile acid transformation before their recirculation via the enterohepatic circulation. Intriguingly, disease distribution in PSC follows the machinery of the enterohepatic circulation, with affections occurring throughout the entire biliary system, as well as a colonic IBD phenotype with right-sided predominance and frequent accompanying terminal ileitis. It has been hypothesised that IBD-associated dysbiosis disturbs this balance, leading to aberrant bile acid metabolism [42]. Altered bile acid transformation in the gut lumen, possibly by a divergent enteric microbiome, could conceivably erase the anti-inflammatory effects of protective bile acid types on epithelial cells, and participatie in the inflammatory loop of IBD. Moreover, thinking derived from the dissection of Mendelian cholestasis syndromes (i.e. progressive familial intrahepatic cholestasis type I–III, cystic fibrosis cholangiopathy) has put a strong bias on the perception of cholestatic tissue injury towards mechanisms involving aberrations of bile acid homeostasis and bile acid transport [43].

In a number of animal models of PSC, genetic modification of bile composition has also been shown to induce sclerosing cholangitis and biliary fibrosis. Mice with targeted disruption of the *Mdr2* (*Abcb4*) gene encoding a canalicular phospholipid flippase spontaneously develop cholangitis and typical 'onionskin type' periductal fibrosis mirroring some of the key features of human PSC [17]. Although $Mdr2^{-/-}$ mice do not develop IBD, dextran sulphate sodium (DSS)-provoked colitis in heterozygous $Mdr2^{+/-}$ mice as a 'two-hit' model is able to induce portal inflammation in animals that are otherwise free of hepatobiliary disease [44]. It is unclear whether commensal organisms provide a degree of protection against development of sclerosing cholangitis induced by toxic bile acids; for in an axenic variant of the $Mdr2^{-/-}$ model, mice were seen to develop increased intestinal permeability, bacterial translocation and cholangiocyte senescence associated with exacerbated biliary injury [45]. Further evidence to

support the role of disturbed bile composition in the development of sclerosing cholangitis and biliary type of liver fibrosis is derived from studies wherein mice were fed either 3,5-diethoxycarbonyl-1,4-dihydrocollidine or lithocholic acid, both of which result in histological features resembling human PSC [46].

Genome-wide studies have not thus far identified any significant associations with the human ortholog *MDR3* (*ABCB4*) and PSC. However, genetic variation of key components of the bile homeostasis machinery may still play a role in pathogenesis by altering bile composition and by proxy the aggressiveness of bile, thereby influencing downstream response to immune-mediated bile duct injury. Indeed, the key observation from clinical trials of ursodeoxycholic acid (UDCA) in PSC is that of an altered disease phenotype (i.e. improvement in hepatic biochemistry) however not yet translating into a survival benefit for the patients. For an in depth overview of bile acid secretion and its regulatory aspects in health and disease, we refer the reader to previous chapters (please see Chap. 4 – Beuers et al.).

9.4 Biliary Versus Parenchymal Fibrosis

Accumulation of myofibroblasts is a key feature of fibrosis and mostly derive from activation of resident mesenchymal hepatic stellate cells (HSC). However, as biliary epithelium is the principal site of injury in chronic cholangiopathies such as PSC wherein fibrosis originates in the peri-ductular region, the portal localisation of portal fibroblasts (versus the peri-sinusoidal location of HSC) would make them attractive candidates as mediators of biliary fibrosis. Evidence regarding the relative contributions of various myofibroblast sources (and function) in fibrogenic liver disease has been limited to studies of isolated cell populations, that was up until the advent of *in vivo* cellular fate-tracing studies. In murine models of fibrosis, HSC have been found to exert a dominant role across all aetiologies, including toxin-induced, steatotic and biliary injury [47]. Indeed, HSC constituted the majority (>80%) of collagen-producing cells even in cholestatic liver disease, with only low-level fibrotic gene expression by the less abundant portal fibroblast-like population. It has therefore been proposed that HSC behave as universal responders in hepatic wound repair, irrespective of the underlying cause of injury. A higher proportion of portal myofibroblast populations are nevertheless observed in biliary as opposed to parenchymal injury, and whilst portal fibroblasts may not contribute significantly to hepatic fibrosis, their localisation adjacent to bile ducts is likely indicative of more specialised functions. Detailed and specific mechanistic insights pertaining to development of liver fibrosis are beyond the scope of this chapter, for which we refer the reader elsewhere [48].

9.5 PSC as a Genetic Disease

Heritable aspects of PSC are evinced through registry studies [49], wherein disease prevalence in first-degree relatives of affected patients appears approximately ten times greater than that observed across unrelated comparator populations. Clinical

associations between PSC and IBD are well described, and the risk of developing PSC and/or ulcerative colitis (UC) is also significantly increased in families of afflicted individuals compared to controls [49]. Importantly, despite evidence of familial aggregation, PSC does not display classical Mendelian inheritance, rather exhibits a complex inheritance pattern suggesting a vast array of gene-gene and gene-environment interactions contribute to disease manifestations at various disease stages. As such, some of the currently proposed genes may influence disease risk by determining how a given individual responds to a particular environmental antigen. Others may act in concert and express the consequence of variation in a stepwise manner and be responsible for diverse clinical phenotypes depending on coexistence of other distinct genetic and environmental co-variables [50]. Putative environmental factors are ill-defined, although clinical observations indicate that patients with PSC are frequently non-smokers; and there is emerging evidence to suggest disease development and progression is enhanced in patients with reduced coffee consumption – of note given that caffeine is a partial antagonist of VAP-1 enzyme activity [51, 52].

Inspired by the success of the genetics in Mendelian cholestasis syndromes, the heritable aspects of PSC have fostered a wealth of genetic research [50, 53–55]. As reviewed elsewhere [50, 56], most striking was the discovery of an overwhelming overlap between PSC and risk loci inherent to many other autoimmune conditions, including coeliac disease, type-I diabetes mellitus and immune-mediated spondyloarthropathy. Moreover, a number of discovered genetic associations implicate the fundamental role of breaks in immune tolerance and mucosal immunogenicity in the pathogenesis of biliary disease development in PSC. However, the combined output from genome-wide association studies (GWAS) provides explanation for less than 10% of overall disease liability [56]. Accordingly, clinical merits of genomic studies will only be fully realised when genetic and epigenetic data can link to the gut microbiome and environmental influences populating the complex interplay of disease pathogenesis (Fig. 9.2), akin to that which has been described for autoimmune diseases with known antigenic triggers such as coeliac disease [58].

9.5.1 Human Leucocyte Antigen (HLA) Associations

The highly polymorphic major histocompatibility complex (MHC) has been implicated in the aetiopathogenesis of human autoimmunity for decades [59], with strong albeit distinct HLA signals recently confirmed for autoimmune liver disease through GWAS. Comprehension of how HLA impacts cholestatic disease mechanistically is somewhat limited, although the fact that an association has been identified in the first instance suggests a defect in the direction and precision of T-cell-related and antigen-specific immune responses. Variation within the MHC region represents the most significant genetic risk factor for PSC [60], with proposed single-nucleotide polymorphisms in near-perfect linkage disequilibrium with alleles at both *HLA-B* (HLA class I region) and *DRB1* (HLA class II region). Despite the striking coexistence with colitis, the majority of HLA associations in PSC are distinct from those identified in IBD [56, 61]. A key

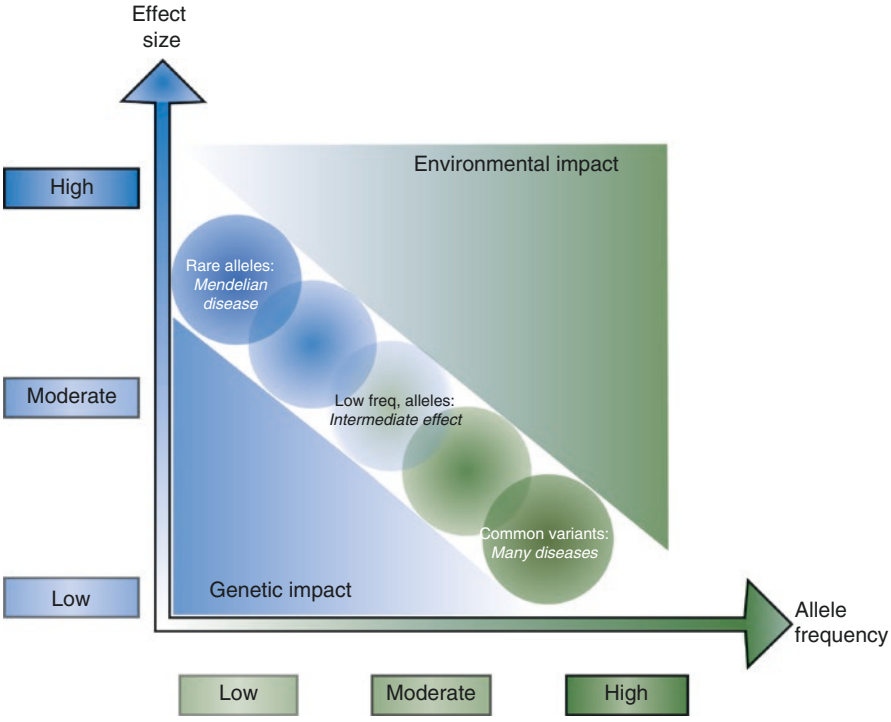

Fig. 9.2 Genetic risk in complex genetic disease. Genome-wide association studies (GWAS) in complex diseases have identified many risk loci harbouring several plausible candidate genes. However, the fundamental nature of such a 'case-based' approach means that variants common to the general population are identified readily but actually exert a relatively small effect on disease development, participating in a complex interplay with environmental triggers, which, in PSC, have yet to be identified. Consequently, only a small proportion of the predicted heritability has been uncovered by currently available findings, and it remains plausible that a smaller number of undetected loci comprise rarer causal variants exerting relatively high risk towards disease pathogenesis (Figure adapted from Gershon et al. [57])

opportunity for post-GWAS research is the delineation of the antigenic repertoire of PSC-associated HLA types and potential specific triggers of T-cell activation in PSC.

9.5.2 Mucosal Immune Activation and Autoimmunity

Genetic links to mucosal immunity are particularly evident in PSC [6]. The importance of IL-2/IL-2Rα polymorphisms, suggested through associations at the 4q27 and 10p15 loci, respectively [56], is supported by the fact that mice lacking IL-2Rα develop autoantibodies and a T-cell-mediated cholangitis together with colitis. Moreover, liver-derived lymphocytes from patients with PSC show reduced expression of the IL-2 receptor and an impaired proliferative response to stimulation *in vitro* [62]. IL-2 can contribute to termination of inflammatory immune responses,

by promoting the development, survival and function of T_{reg}; and a loss of IL2Rα signalling function in PSC is supported by the observation that patients who harbour variant polymorphisms exhibit reduced circulating T_{reg} populations [28]. A prominent role for TNFα in the immunopathogenesis of PSC has also been suggested through induction of immunopathogenic T-cell phenotypes as well as indirectly through the hepatic endothelial induction of mucosal chemokines and adhesion molecules that are normally 'gut restricted' in an NFκB-dependent manner [37]. Moreover, PSC genetic risk-associations include the 1p36 locus that encompasses the TNF-superfamily receptor TNFRSF14 – a protein expressed on $CD4^+$ and $CD8^+$ T-cells, B-cells, monocytes, neutrophils, dendritic cells and mucosal epithelium, which behaves as a molecular switch modulating lymphocyte activation [63].

An immunosuppressive role for histone deacetylase (HDAC)-7 – a gene implicated in the negative selection of T-cells in the thymus and development of tolerogenic immune responses – is supported by a genetic association at 12q13 in PSC GWAS, in which the most associated polymorphism was located within an intron encoding serine-threonine protein kinase (PRK)-D2 (19q13). When T-cell receptors of thymocytes are engaged, PRKD2 phosphorylates HDAC7 resulting in loss of its gene regulatory functions. This gives rise to apoptosis and negative selection of immature T-cells. Notably, this negative selection takes place owing to a loss of HDAC7-mediated repression of the leucocyte transcription factor *Nur77* [56]. *Nur77* expression parallels that of *IL-10* and is heavily influenced by salt-inducible kinase *(SIK)-2* polymorphisms, the latter of which is also proposed as a genetic risk locus in PSC.

Further impression of impaired mucosal tolerance is suggested through a genetic association at 18q21, which contains transcription factor (TCF)-4; congenital deficiency of which results in partial blockade of early B- and T-cell development and also attenuated development of immunoregulatory plasmacytoid dendritic cells (pDC) in murine models [64]. Caspase-recruitment domain (CARD)-9 is an important downstream mediator of signalling from mucosal PRR, and genetic associations suggest a link between defective intestinal mucosal microbial handling and the development of PSC. *Card9*$^{-/-}$ mice appear more susceptible to experimentally induced colitis, typified by defective IFNγ and T_h17 responses, as well as reduced transcription of the mucosal chemokine *CCL20*, signifying the critical importance of CARD9 in the maintenance of epithelial immunostasis [65]. CCL20 has recently been identified as a GWAS risk locus in PSC [66] and as a chemokine facilitates the recruitment of T_h17 cells to epithelial structures. Moreover, CCL20 expression is under control of a positive feedback loop, wherein release of cytokines by T_h17 cells results in overexpression of CCL20 by BEC and intestinal epithelial cells in a paracrine manner [31, 41].

Another one of the strongest non-HLA associations in PSC is macrophage-stimulating (*MST*)*-1*, which is also associated with UC and Crohn's disease (CD). MST-1 is expressed by BEC and involved in regulating innate immune responses to bacterial ligands, as well as modulating lymphocyte trafficking in lymphoid tissues through integrin- and selectin-mediated adhesion [67]. Glutathione peroxidase (GPX)-1 is an antioxidant enzyme located close to MST-1, and polymorphisms in *GPX* may also confer an increased disease susceptibility to PSC. Moreover,

Gpx1/2−/− mice develop a chronic ileocolitis with an increased frequency of colonic malignancy [68]; particularly noteworthy given the increased premalignant nature of colitis in PSC patients.

Variants in *FUT2*, an enzyme encoding galactoside 2-alpha-l-fucosyltransferase-2, have also been suggested to confer increased susceptibility to PSC (as well as Crohn's disease), although fall short of reaching significance at a genome-wide level [69]. Fucosyltransferase variants alter the recognition and binding of various pathogens to carbohydrate receptors on the mucosal surface and are associated with changes in the commensal phyla in affected PSC patients characterised by elevated *Firmicutes* and reduced *Proteobacteria*. This is akin to changes found in *FUT2* polymorphisms associated with Crohn's colitis and again links defective immune responses to the gut microbiota in PSC. Moreover, variants in *FUT2* have been described as a risk factor for development of dominant biliary stenosis in PSC – a phenotype associated with adverse clinical outcomes [70]. Colorectal malignancy may result as a direct consequence of altered fucosylation of the epithelial adhesion molecule E-cadherin [71], and a recent study in mice also illustrates how congenital *E-cadherin* deletion results in spontaneous periportal inflammation, periductal fibrosis and an enhanced susceptibility towards hepatobiliary cancer – akin to clinical PSC [72].

Collectively, genetic findings suggest that cholangitis in PSC may be a direct result of defective pathogen sensing, disrupted barrier functions, and askew in effector versus regulatory immune responses as part of a bigger picture where T-cell responses towards an hitherto unidentified specific antigen (or autoantigen) play a major role (Fig. 9.3).

9.6 Clinical Presentation

In the largest, most comprehensive population-based study to date ($n = 590$), PSC was validated as being male predominant (~60%) with a median age at diagnosis of ~40 years [74]. However, PSC can develop at any age, with younger patients frequently manifesting a more hepatitic biochemical profile (Table 9.2) [75]. Associations with colonic inflammatory bowel disease (IBD) are well recognised, and >70% of PSC patients have a history of colitis at diagnosis [76]. Historic descriptors have likened the pattern of intestinal inflammation to that of ulcerative colitis (UC), although the IBD in PSC is a unique clinical phenotype with regard to distribution, inflammatory activity and oncogenic potential (Fig. 9.1) [77]. Relapsing-remitting episodes of acute cholangitis are a frequent complaint, and data from representative cohorts suggest symptomatic presentations carry poorer transplant-free and malignancy-free survival [78].

9.6.1 Natural History

Presently, there is no known medical treatment which is consistently demonstrated to attenuate disease progression in patients with PSC [79]. Consequently, clinical

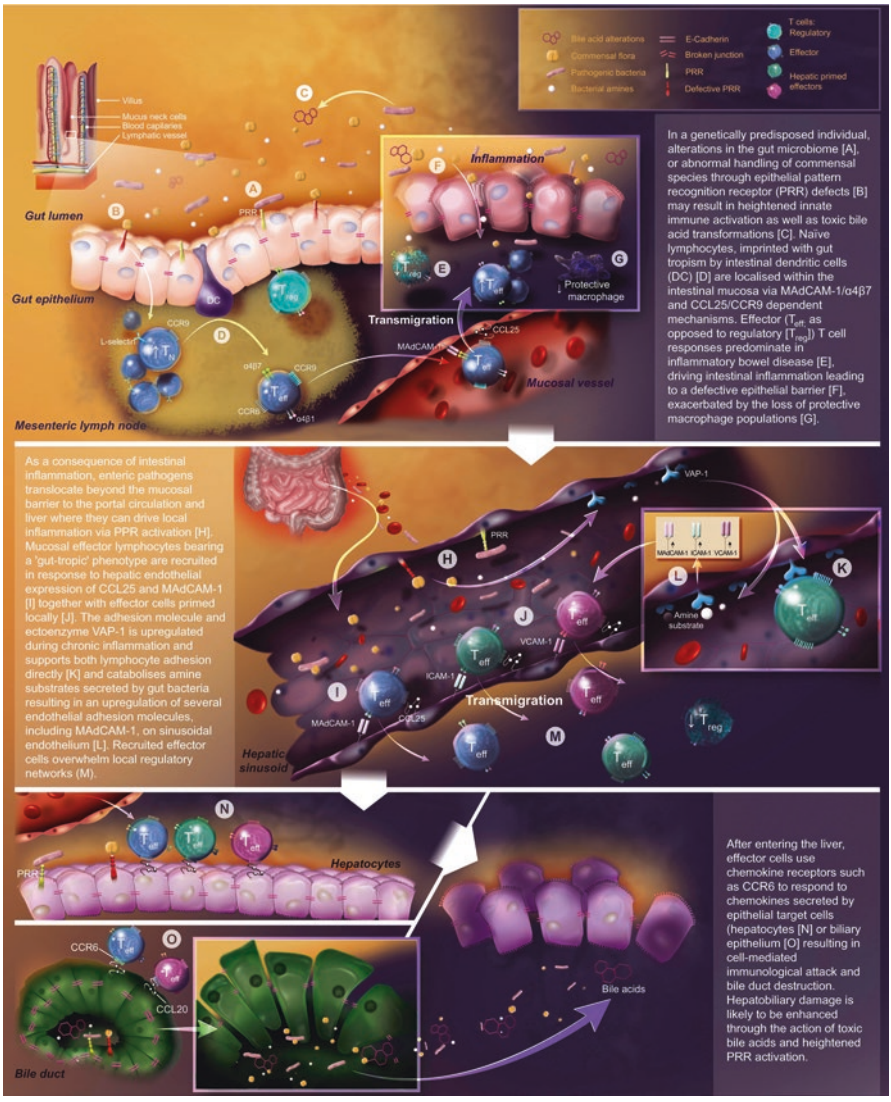

Fig. 9.3 (Figure obtained with permission from the article by Trivedi and Adams [73])

outcome is largely dictated by the development of cirrhosis, portal hypertension and variable predisposition to the development of colonic and/or hepatobiliary malignancy. Up to 50% of symptomatic patients develop progressive liver disease requiring transplantation, although modelling the natural history has proven challenging as a result of changes in diagnostic paradigms (magnetic resonance cholangiography now the 'gold standard'), discrepant transplantation indications and phenotypic differences in inflammatory bowel disease globally. The absence of a defined serological marker makes case recognition difficult, and unbiased cholangiography

Table 9.2 Clinical features of primary sclerosing cholangitis

Manifestation	Description/comments
Presenting age	Median: between 30–40 years; can present at any age
Gender distribution	> 60% men
Symptoms	Pruritus and fatigue represent archetypal symptoms of cholestasis Symptomatic presentations at time of PSC diagnosis vary: 36–56% > 20% develop symptoms during follow-up Relapsing-remitting episodes of cholangitis can occur at any stage
Liver biochemistry	Predominantly elevated serum ALP; ~40% experience spontaneous normalisation Bilirubin also elevated during acute flares of cholangitis and in late-stage disease Persistently elevated bilirubin (~3 times ULN) in the presence of IBD should raise concern over cholangiocarcinoma
Immunoserology	Elevated pANCA (non-specific) in >80% Elevated ANA (non-specific) in 70–80% Antibody titres do not correlate with disease severity or indicate overlap with AIH when present in isolation Elevated serum IgG4 concentration in ~10%; clinical significance uncertain
Coexisting IBD	~70–80% develop coexisting IBD at some point; mostly (>90%) a form of colitis Coexistence with Crohn's disease reported in a minority: more often women (50%) and a greater prevalence of small duct disease
Imaging	Cholangiography (usually MRC) the gold standard for diagnosis: Multifocal stricturing/beading and prestenotic dilatations 10–15% have biochemical, histological and clinical features of small duct disease (normal cholangiogram)
Histology	Biopsy not mandatory for diagnosis (except in small duct disease) Common hepatic histological changes include interface activity, copper-binding protein and ductopenia Concentric periductal fibrosis ('duct lesion') present in <20% of cases

assessments in patients with long-standing IBD found clinically manifest PSC in 2.7% of IBD patients compared with a genuine frequency of PSC-like lesions in up to 7.4% of the IBD population [80].

Despite the relatively low population frequency, chronic cholestatic liver diseases such as PSC remain a significant cause of morbidity and mortality for patients, accounting for a significant fraction of first liver transplantations in the Western world (up to 20–25% in Scandinavian areas) [81, 82]. As such, PSC portends a standardised mortality ratio (SMR) greater than fourfold that of a matched control population [83], although epidemiological registries illustrate significant discrepancy between event-free survival times across transplant centres versus true population-based cohorts (median 13.2 vs. 21.3 years [74]). Such study cohorts highlight the significant challenges in prognostic modelling, particularly in the unmasking of patients with early clinical disease, in addition to the inherent selection bias that stems from tertiary centre restricted reporting.

Table 9.3 Hepatobiliary malignancies in PSC

Manifestation	Risk	Approach to surveillance
Colorectal carcinoma	Increased risk in patients with coexisting colitis relative to those with colitis and no PSC Cumulative risk increases with duration or colitis (10% and 30% at 5 and 10 yrs.)	Annual surveillance colonoscopy in all with PSC and colitis
Cholangiocarcinoma	Lifetime risk 10–15% > 30% manifest in the same yr. as PSC diagnosis; annual incidence thereafter 0.5–1.0% per year	No consensus to surveillance Cholangiographically indistinguishable from PSC alone Low yield from single-pass brush cytology Karyotyping with in situ hybridisation may facilitate differentiation in specialist centres (but not yet validated)
Gallbladder carcinoma	Prevalence of mass lesions up to 14% > 50% of all gallbladder polyps are an adenocarcinoma in PSC	Annual ultrasound surveillance Early cholecystectomy if polyp increasing in size, or >0.8 cm when first identified
Pancreatic carcinoma	Unclear whether risk increased in PSC Risk found to be increased ~14-fold in one study	Regular surveillance not advocated at present
Hepatocellular carcinoma	Risk increased in all patients with liver cirrhosis Risk outweighed by that of primary biliary malignancies	6 monthly ultrasound in patients with cirrhosis

9.6.2 Hepatobiliary Malignancy

Distinct from the risk of progressive liver disease, patients with PSC harbour an increased predisposition towards hepatobiliary malignancy (Table 9.3), far beyond that posed by hepatocellular carcinoma (HCC) in the context of established liver cirrhosis. Cholangiocarcinoma (CCA) develops in up to 10–15% of patients. One-third of CCA are diagnosed within the first year of PSC diagnosis (annual incidence thereafter 0.5–1.5%); therefore, a heightened index of suspicion is advised for acute/subacute symptomatic PSC presentations, particularly men with persistently elevated bilirubin and coexisting colitis [84]. Abrupt changes in biochemical or clinical presentation of any PSC patient should also heighten suspicion of CCA. Biliary malignancy is cholangiographically indistinguishable from benign disease, although emerging endoscopic and molecular techniques may help discern malignant versus benign appearances (see Chap. 12: Gores et al.) [85]. Many centres in Europe and the USA apply biliary brush cytology with or without concomitant DNA analysis (by fluorescence in situ hybridisation [FISH], digital image analysis [DIA] or flow cytometry); however, the appropriate action upon

pathological findings varies and lacks scientific justification [79]. Presently, however, no evidence-based screening protocols are available, and no current methodology facilitates effective identification of at-risk individuals prior to cancer development.

Gallbladder mass lesions are also more common in PSC with an estimated prevalence of 3–14%, with over 50% being established as adenocarcinomas at time of diagnosis [86]. Therefore, annual ultrasound surveillance is advocated in all patients to detect gallbladder polyps [87, 88], which if present warrant consideration for cholecystectomy.

9.7 Risk Stratification

Disease progression in PSC is highly variable, with the predictive utility of most currently proposed strategies reliant on representing how far advanced disease already is, rather than what it will become. Moreover, when interrogating the thesis of risk stratification in any liver disease, one must remain mindful of study methodology and applicability from which interpretations are framed, so as to correctly inform patients of their individual level of risk and to do so with an appropriate level of confidence [78].

With these caveats in mind, selection of current and emergent tools utilized in clinical practice are shown in Fig. 9.4. In particular, the small duct variant of PSC (10–15% of the disease spectrum); in which identical clinical, biochemical and histological features manifest in the context of a normal cholangiogram, is less often symptomatic than classical PSC (30% vs. 53%), and exhibits a relatively benign clinical course (median transplant-free survival: 29 years vs. 17 years) [74, 89]. As survival patterns mirror those of an age- and sex-matched population, the need for investigative therapy is perhaps less perceptible in those with small duct disease, but serial non-invasive cholangiographic follow-up is advocated, given that 25% of patients develop progressive biliary changes every ~7 years.

By contrast, the presence of ‘dominant strictures (DS)’ in PSC, which reportedly manifest in 12–60% of patients presenting to specialist endoscopy units, has been repeatedly proven to predict significantly poorer clinical outcomes. The reduced overall survival is partly attributable to CCA being indistinguishable from non-malignant biliary stenosis cholangiographically. In one prospective study, the actuarial transplant-free survival in patients with DS was only 25% over 20 years, relative to 73% in the remainder of the studied cohort [90].

The presence of colitis may also impact on the risk of developing CCA, and transplant-free survival, as was evident in two population-based series [74, 91], with further support lent by well-characterised single-centre cohorts [92, 93]. Over 90% who develop CCA report prolonged duration of colitis prior to PSC diagnosis, compared to patients without malignancy (17 years vs. 9 years according to one group; $p = 0.009$) [94]. The negative clinical impact of colitis on development of biliary malignancy irrespective of cholangiographic phenotype

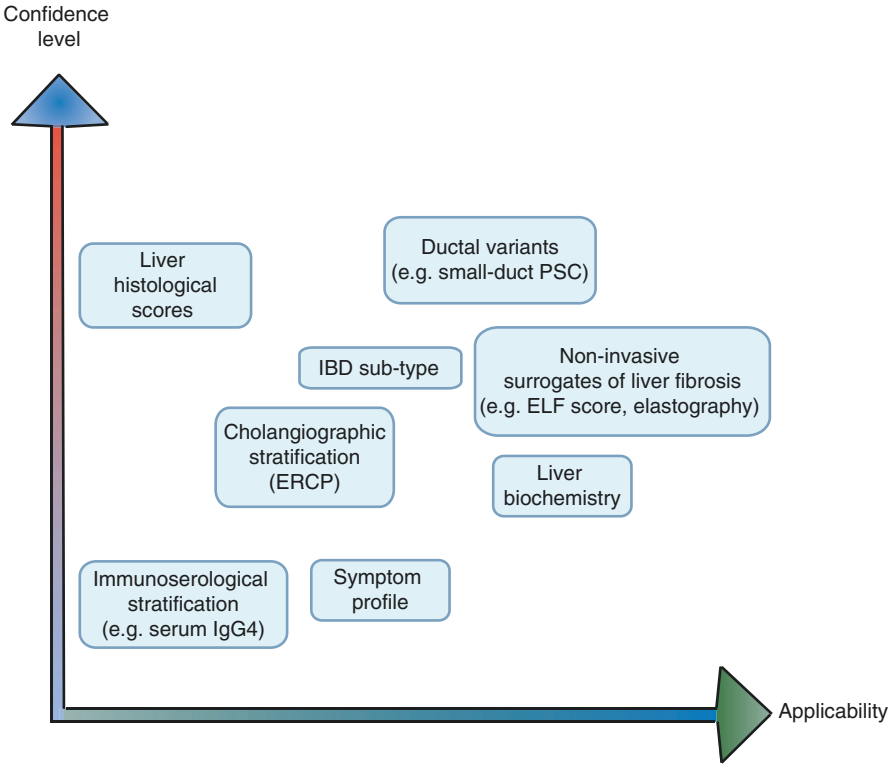

Fig. 9.4 Clinical risk stratification. Several predictive methods and prognostic scoring systems have been proposed in primary sclerosing cholangitis (PSC). Unfortunately, very few yield a high confidence level as well as high clinical applicability. For instance, liver histology is accepted by many as representing a robust surrogate of disease progression regardless of aetiology however is not validated in terms of predictive utility neither acceptable in routine clinical practice in terms of routine applicability. By contrast, serum ALP has been investigated by several groups, but despite ready availability of biochemical testing, this has repeatedly failed validation at prespecified cut points and has surrogacy questioned when determined as a continuous variable. The strongest, most consistent predictors of outcome appear to be phenotypic characteristics, specifically small duct disease (good outcome) and coexistence of colitis (adverse outcome), whereas immunoserological parameters (e.g. IgG4) command further investigation given the existing divergence in findings. Non-invasive surrogates of liver fibrosis are also promising, but it is as yet unclear whether these modalities are able to capture early, progressive injury or just presence of late-stage advanced fibrosis

has since been confirmed in a Dutch cohort of 161 patients [95]. The difficulty, however, in applying colitis as a discriminator rests in poor correlation between activity of hepatobiliary disease and that of colonic inflammation; wherein involvement of afflicted sites is frequently chronologically displaced, with no current study addressing the prognostic impact of IBD as a time-dependent covariate. Importantly, as of yet, pre-emptive colectomy in patients with PSC and IBD should not be advocated.

Biochemical stratification in PSC has been attempted, with early efforts focussing on the predictive utility of serum bilirubin either in isolation or as part of detailed computational algorithms such as the PSC Mayo score. Although persistently elevated bilirubin for >3 months raises concern for the development of hepatobiliary malignancy, levels have a propensity to fluctuate with flares of cholangitis activity and potentially influenced by biliary intervention. Moreover, the series from which Mayo score was derived antedates modern management of variceal bleeding and is further criticised by the inability to forecast adverse events in prior PSC clinical trials. More recent efforts centre on the predictive utility of serum alkaline phosphatase (ALP) assessed at various time points following original diagnosis [96, 97]. However, unlike in other cholestatic liver diseases, serum ALP lacks validation as a continuous variable in terms of predicting outcome, and has not been used to compare survival patterns in PSC with that of a matched control population. Therefore, serum ALP cannot be recommended as a 'stand-alone' risk stratifier in PSC.

The 'gold standard' for assessing disease progression in many liver diseases is liver histology, although biopsy is not part of the diagnostic algorithm in PSC. Nevertheless, histological fibrosis stage (according to the Nakanuma PBC staging system) has been shown to correlate with transplant-free survival [98]. Given the intrusive nature of liver biopsy, several non-invasive modalities have emerged such as transient elastography and the enhanced liver fibrosis (ELF®) test [99, 100]. The latter in particular has been externally validated as predictive of clinical outcome across a multicentre patient registry, wherein PSC patients exhibited significantly elevated scores than counterparts with IBD alone and matched controls, but also disparate transplant-free survival curves according to tertile distribution or through a dichotomous (Youden index-derived) cut point. These internationally substantiated findings represent a major step in risk stratification, and more serum-based fibrosis markers of outcome predication in PSC continue to emerge.

9.8 Therapeutic Shortfalls in Existing Pharmacotherapy

In the absence of definitive medical therapy, the mainstay of managing PSC rests in the appropriate treatment of symptoms and infective complications, surveillance of hepatobiliary and colonic malignancy, and in the early identification of those at risk of progressive liver fibrosis who require timely listing for liver transplantation (Tables 9.4 and 9.5).

9.8.1 Ursodeoxycholic Acid (UDCA)

Numerous studies have tried to address the efficacy of UDCA in PSC, a drug with pleiotropic anti-inflammatory, anti-apoptotic and choleretic effects. Moreover, UDCA is hypothesised to enhance the biliary 'bicarbonate umbrella', thus affording biliary epithelial cells with a level of protection against the deleterious effects of more toxic bile acids [101]. UDCA is often associated with a reduction in serum

Table 9.4 Approaches to patient management [3]

Manifestation	Stratagem
Symptoms	Pruritus guidelines: Exclude/treat dominant stricture Pharmacotherapy (e.g. cholestyramine > rifampicin > opioid antagonists > SSRIs) Referral to specialist centre if failure to settle Fatigue more difficult: Treat pruritus first Exclude coexisting hypothyroidism, hypovitaminosis D, diabetes mellitus
Biliary sepsis	Biliary intervention for dominant strictures Empirical antibiotic prophylaxis prior to any planned biliary intervention Timely antibiotic treatment for acute flares of cholangitis Rotating antibiotics reasonable for those with recurrent episodes in the absence of lesions amenable to biliary intervention and who fall short of transplant criteria
Dominant strictures	Biliary intervention according to centre-specific expertise; unclear whether dilatation or stenting superior Empirical antibiotic prophylaxis prior to any planned biliary intervention
Osteodystrophy	Render vitamin D replete Screening bone densitometry to assess fracture risk in all with cirrhosis and elevated serum bilirubin
Cancer surveillance	See Table 9.3
Timing for liver transplant	See Table 9.5

Table 9.5 Liver transplantation in PSC

Absolute indications
Cirrhosis with decompensation or within MELD-sodium score (or equivalent) meeting transplantation criteria
Recurrent acute cholangitis
Biliary obstruction not amenable to percutaneous or endoscopic therapy
Hepatocellular carcinoma
Debated indications
Intractable pruritus
Hilar cholangiocarcinoma
Compensated cirrhosis with sarcopenia
Proven biliary dysplasia

Abbreviation: *MELD* model for end-stage liver disease

ALP in patients with PSC, an effect that is lost upon drug cessation [102]. However, results from clinical trials of UDCA in PSC (15–20 mg/kg/day) demonstrate inconsistent benefit on transplant-free or malignancy-free survival, whereas high dosages (28–30 mg/kg/day) appear to confer harm to patients in terms of cholangiocarcinoma development as well as death and transplantation relative to placebo [103,

104]. These findings have led to cautious and discrepant recommendations as to the clinical utility of UDCA [88, 105, 106]. Nevertheless, from the pathophysiological point of view, data lends support to a role of bile acid homeostasis in key aspects of PSC pathophysiology.

The previously conceived chemopreventative effects of UDCA in terms of colorectal carcinoma originate from reports wherein a significantly reduced prevalence of colonic dysplasia was seen in patients receiving therapy [107]. However, more contemporary clinical trials in PSC report no difference in the frequency of colorectal dysplasia, cancer or dysplasia/cancer-free survival over a 5-year period [108]. Moreover, the Mayo clinic study found a greater association of colonic dysplasia in patients with PSC/IBD taking UDCA compared to those receiving placebo (hazard ratio: 4.44) when receiving high dosages (28–30 mg/kg/day) [109]. Consequently, liver disease guidelines from Europe and the USA do not presently recommend routine administration of UDCA in PSC and advise complete avoidance of high dosages.

9.8.2 Immunosuppression

A host of immunomodulatory agents have been evaluated in PSC, including cyclosporine, tacrolimus, corticosteroids, azathioprine, sirolimus and anti-TNFα therapy. Although they may induce an improvement in biochemical indices, these therapies have proven largely disappointing and fail to slow disease progression or improve prognosis with the anecdotal exception of carefully selected patients having overlapping histological features with autoimmune hepatitis (AIH) [110–112]. Clinical response under such situations may call into question the presence of autoimmune cholangiopathy, particularly in young patients wherein a form of sclerosing cholangitis associated with florid autoimmune features has been described [75]. Under such circumstances, parenchymal liver damage may respond well to immunosuppression but biliary disease progression still occurs in ~50% of patients. The lack of clinical efficacy of immunosuppressive treatments remains a paradox in PSC in light of the wealth of data on a likely involvement of immune-driven mechanisms.

In individuals with suspected PSC and markedly elevated serum immunoglobulin subclass 4 (IgG4) levels and an IgG4-positive plasma cell infiltrate evident histologically, treatment with corticosteroids may unmask an underlying IgG4-associated cholangiopathy that is responsive to immunosuppression (see Chap. 14; Webster et al.). Between 9 and 15% of patients with true PSC also display moderate/high serum levels of immunoglobulin subclass 4 (IgG4), which although distinct from true IgG4-associated cholangiopathy is believed to represent a unique immunogenetic background [113]. Several studies support clinical distinctions in PSC based on serum IgG4 values, with those having higher than normal values exhibiting significantly greater ALP titres and higher PSC Mayo risk scores. Shorter median time to transplantation has also been illustrated by some groups although this observation has failed replication in recent reports spanning several international centres [114].

9.8.3 Antibiotics

Patients suffering from recurrent episodes of bacterial cholangiosepsis may obtain relief from a pragmatic approach to care in terms of rotating, prophylactic antibiotics [88, 105, 106]. To seek out therapeutic efficacy of antimicrobial therapy, longer term has also been attempted, deriving from the interrelationship among bile acid metabolism, systemic and mucosal inflammation and the gut microbiota [115]. Metronidazole together with UDCA was shown to significantly improve serum ALP in PSC compared with UDCA alone; however, an impact on liver histology or cholangiography was not found to be statistically significant in randomised controlled trials. Studies of vancomycin in the paediatric, and later adult PSC populations, similarly demonstrate improvement in biochemical parameters as well as symptom profile after several months of therapy; however, the number of individual clinical trial patients remains small (<20). Moreover, it is unclear as to the appropriateness of liver biochemistry as a surrogate endpoint in clinical trials of antibiotic therapy, particularly given that readouts may reflect the impact of antibiotics on intestinal as opposed to liver-derived serum ALP. Nevertheless, the data provide proof-of-concept support to an involvement of the gut microbiota in PSC pathophysiology in need of refinement.

9.8.4 Biliary Intervention

Endoscopic treatment remains a mainstay for the treatment of clinically significant biliary strictures in PSC [88, 105, 106]. Several investigators report biochemical and clinical improvements following endoscopic therapy of DS, and relieving biliary obstruction may reverse secondary liver fibrosis [116]. However, a consensus agreement grounded in high-quality evidence, specifically which endoscopic strategy to use (dilation only or dilation and stenting) and when, does not yet exist, and whether such intervention improves, clinical outcome in PSC is the subject of a large multicentre clinical trial (NCT01398917).

9.9 Emergent Therapeutic Strategies (Table 9.6)

9.9.1 Targeting Leucocyte Homing

There exists a large gap between the available immunogenetic information to date and permeation into clinical practice, a providence that PSC shares with many other complex diseases studied on a genome-wide scale. Nevertheless, the advances in understanding genetics of chronic cholestasis speak broadly to the ultimate goal of all such studies: to guide treatment that is biologically driven and mechanistically linked. Accordingly, strategies targeting the activation or recruitment of IL-17-secreting T-cells are particularly attractive, particularly given their pending incorporation into IBD treatment pathways [117, 118]; in addition to chemokine receptor

Table 9.6 Putative therapeutic strategies

Pathway	Intervention and rationale	Successfully applied precedents	Caveats
A. *Immune tolerance* vs. *activation*			
IL-12 / IL-23	IL-12 drives differentiation of activated, naïve T-cells to IFN-producing T_h1 cells, contributing to loss of tolerance in experimental models of autoimmunity Murine models of cholangiopathy also exhibit a milder hepatobiliary phenotype in the absence of functional IL-12 IL-23 (which shares a common p40 subunit with IL-12) is essential for differentiation of IL- 17 release from $CD4^+$ and $CD8^+$ T-cells, and implicated in the breakdown of immune self-tolerance	Anti-IL-12/23 (ustekinumab) – psoriasis, CD Anti-IL-17A (secukinumab/ ixekizumab) – psoriasis Anti-IL17RA (brodalumab) – uveitis, CD, AS	Unclear how best to evaluate the efficacy of immunomodulatory therapies Efficacy endpoints of Ustekinumab (e.g. ELF score) were not reached in PBC clinical trials
B. *Leucocyte recruitment*			
α4β7 – MAdCAM-1 inhibition	An axis critically involved in the recruitment of mucosal lymphocytes to the intestine during homeostasis and inflammation. Increased activity of this system is also evident in the PSC liver	Anti-α4β7 (vedolizumab) – UC, CD Clinical trial in PSC imminent	Liver-infiltrating regulatory T-cells in PSC may also be $\alpha4\beta7^+$ Targeting of α4 exacerbated liver injury in a murine model of hepatitis
VAP−1 antagonism/VAP-1 enzyme inhibition	Pleiotropic roles in mediating liver injury through recruitment of (predominantly) effector leucocytes, in addition to mediating expression of downstream endothelial adhesion molecules Absence of VAP-1 illustrated to attenuate liver inflammation and fibrosis in murine models	BTT-1023 – psoriasis, RA Currently under clinical trial in PSC	VAP-1 ligand unknown Potential role in recruitment of regulatory phenotypes to the liver Targeted deletion in murine models impairs microbial handling
CCR6 – CCL20	Responsible for the recruitment and positioning of T-cells (predominantly T_h17 cells) around inflamed biliary epithelium	Antibody in preclinical development	No data suggesting efficacy in vivo

CCR9 – CCL25 inhibition	An axis critically involved in the recruitment of mucosal lymphocytes to the intestine during homeostasis (small bowel) and inflammation (large bowel). Increased activity of this system is also evident in the PSC liver (predominantly effector memory T-cells) In early pharmacological studies, neutralising antibodies inhibited CCR9-mediated Ca^{2+} mobilisation of T-cells and was an equipotent inhibitor of CCL25-directed chemotaxis	Anti-CCR9 (Traficet ^{EN}) – CD	Limited efficacy demonstrated in IBD (marginal benefit over placebo that was restricted to colonic disease)
C. *Bile acid metabolism*			
NorUDCA	Increases the flow of bicarbonate-rich choleresis Anti-inflammatory, anti-fibrotic and anti-proliferative effects demonstrated in multiple murine cholangiopathy models	Currently under clinical trial in PSC	Efficacy evaluation restricted to serum ALP changes (may not be truly representative of outcome surrogacy in PSC)
FXR/FGF19 agonists	FXR agonists exhibit pluripotent effects including reduced bile acid synthesis, increased bile acid export and bile formation, as well as anti-inflammatory and increased hepatic regeneration	Obeticholic acid – PBC	FXR expression diminished in the proximal colon in PSC; high drug dosages might be required to demonstrate efficacy Concerns over potential proliferative effects (potentially neoplastic). Exacerbates pruritus in PBC
TGR5 agonists	Genetic associations in UC (and to a lesser extent PSC) at the 2q35 locus TGR5 ligation results in enhanced epithelial barrier function as well as synergistic effects on bile acid homeostasis with FXR	Antibody in clinical development	See FXR/FGF19
ASBT inhibitors	Blocks the ileal reabsorption of bile acids that are potentially harmful to the biliary epithelium	ASBT inhibitors ameliorate cholangitis in murine models, and potentially attenuate itch intensity	May increase colonic cancer risk through increased exposure of colon to secondary bile acids

(continued)

Table 9.6 (continued)

Pathway	Intervention and rationale	Successfully applied precedents	Caveats
D. *Liver fibrosis*			
LOXL2-antagonists	Catalyses the cross-linking of extracellular collagens and stabilises the fibrotic matrix Believed to be critical in determining reversibility of fibrosis in liver disease	Currently under clinical trial in PSC	Premature termination of clinical trials across other fibrotic (pulmonary) and neoplastic (pancreatic) due to lack of efficacy
VAP−1 antagonism/VAP-1 enzyme inhibition	VAP-1 activity controls/supercedes that of LOXL2 Targeted deletion/enzyme inhibition abrogates liver fibrosis in murine models	Currently under clinical trial in PSC	See above
αvβ6 integrin antagonists	Expression on hepatic progenitors of ductular reactive cells in liver fibrosis Targeting αvβ6 ameliorate biliary fibrosis in vivo	In clinical development	Relative contribution of ductular reactive cells and portal fibroblasts vs. hepatic stellate cells in mediating human liver fibrosis uncertain
E. *Microbiome manipulation*			
Antibiotics	Loss of tolerance to enteric commensals as well as development of an intrinsically divergent microbiome postulated as critical inciting events in PSC	Preliminary data using antibiotics (vancomycin) show reduction in serum ALP in adults and children with PSC (small *n* numbers)	Enteric microbiome exhibits heterogeneity from one patient population (country) to the next. Impacted by diet, climate and IBD phenotype Unclear whether impact on serum ALP reflects lower levels secreted from the intestine as oppose to liver
Probiotics		Clinical trials in IBD ongoing Success with VSL#3 in Successful applications in a host of intestinal inflammatory disorders (e.g. *Clostridium difficile* infection)	
Faecal microbiota transplantation			

Abbreviations: *AS* ankylosing spondylitis, *CD* Crohn's disease, *FXR* farnesoid X receptor, *LOXL* lysyl oxidase-like, *MAdCAM* mucosal addressin cell adhesion molecule, *PPAR* peroxisome proliferator, *PBC* primary biliary cirrhosis, *RA* rheumatoid arthritis, *TGR* transmembrane G-protein coupled receptor, *UC* ulcerative colitis

antagonists targeting CCR6 that would facilitate the rational targeting of lymphocyte trafficking at sites of epithelial inflammation.

The monoclonal antibody vedolizumab, targeting α4β7-MAdCAM-1 interactions, has demonstrated efficacy in the induction and maintenance of ulcerative colitis and Crohn's disease also [119]. Given the existence of overlapping lymphocyte recruitment pathways, such intervention may be permissive to clinical trials in patients with PSC. Furthermore, the clinical utility of vedolizumab in the management of IBD exacerbations in patients with PSC post-transplant holds great promise [120]. In a similar vein, therapeutic initiatives targeting CCR9 may provide a distinct therapeutic advantage over pan-α4β7-inhibition, given that pre-clinical studies show imprinting of α4β7 by HSEC, which can skew T-cells toward a regulatory phenotype [39].

Neutralising antibodies against VAP-1 are also candidates for future therapy, given the constitutively selective expression of VAP-1 within the human liver and upregulation during chronic inflammation where it facilitates recruitment of $CD4^+$, $CD8^+$ and monocyte populations [121]. The discovery that VAP-1 amine oxidase activity is able to modulate expression of downstream endothelial adhesion molecules opens up further therapeutic avenues permissive to enzyme inhibition, which could indirectly regulate trafficking of mucosal as well as liver-tropic lymphocytes to PSC liver.

9.9.2 Anti-fibrotic Therapies

In addition to its endothelial expression, VAP-1 is also released by HSC and activated myofibroblasts in chronic liver disease, having been shown to promote wound-healing responses and enhance expression of pro-fibrotic genes such as lysyl oxidase homologue (*LOXL*)–2 [122]. The latter is responsible for catalysing cross-linking of extracellular collagens, thereby providing a degree of stability to the extracellular matrix. To this effect, clinical trials are underway of a monoclonal antibody targeting LOXL2 in PSC (NCT01672853) alongside similar efforts in other fibrotic liver, lung and skin diseases. The characteristic histopathological lesion of sclerosing cholangitis, PSC in particular, is potentially pathognomonic for particular fibrotic pathways. However, the molecular details leading to development of the 'onion-skinning fibrotic scar' are thus far largely unknown; as mechanistic insights continue to evolve, more specific approaches to targeting these pathways may emerge. Notably, in the event of positive trial outcomes, anti-fibrotic treatments are still likely to see clinical implementation only in combination regimens with other strategies, since none of them is likely to target the driving events of PSC development.

The 'ductular reaction' defined as the proliferation of small duct-like structures in response to liver injury is a near universal finding in chronic biliary disease, representing heterogenic cellular proliferation of cells in pre-existing ductules, activated progenitors, reactive BEC and intermediate hepatocytes [123]. From a clinical perspective, progression of liver fibrosis is strongly correlated with the development

of a ductular reaction [124]. Precursors of reactive BEC, as well as a subset of intermediate 'hepatocyte-like' cells within fibrotic septa, are strongly immunoreactive for the integrin αvβ6, which upon genetic or therapeutic inactivation potently suppresses murine ductular reaction *in vivo*, consequently inhibiting biliary fibrosis progression and hepatobiliary cancer development [125]. The development of a human therapeutic antibody targeting αvβ6 may therefore provide a novel opportunity to hinder progression of biliary fibrosis and epithelial oncogenesis in patients with PSC [126].

9.9.3 Bile Acid/Nuclear Receptors (Read in Conjunction with Chap. 4: Beuers et al. Application of NorUDCA)

24-Nor-ursodeoxycholic acid (NorUDCA) is the C23-homologue of UDCA, largely being secreted in an unchanged or glucuronidated form. Because of predominant cholehepatic (as opposed to enterohepatic) shunting, norUDCA is highly enriched within the disease relevant compartment and increases the flow of a bicarbonate-rich choleresis. Application of NorUDCA in multiple murine models of PSC demonstrate anti-inflammatory, anti-fibrotic and anti-proliferative effects, increasing the hydrophilicity of biliary bile acids whilst stimulating a bicarbonate-rich choleresis [127]. These encouraging results can hopefully be extrapolated to human PSC, and clinical trials are currently underway (NCT01755507). Other approaches for amending the enterohepatic or cholehepatic circulation of bile acids exist in terms of blockade at the level of the ileal reabsorption (ASBT inhibition) [128]. To what extent such inhibition may impact on the severity of IBD and colonic transformation of bile acids in PSC warrants further assessment.

Targeting nuclear bile acid hormone receptors is another avenue of exploration in the treatment of chronic cholestatic liver disease. In particular, obeticholic acid – the most potent ligand for farnesoid X receptor available in humans – has been shown to improve biochemical surrogates of disease activity in patients with PBC [129], fuelling therapeutic exploration in patients with PSC. Of note, FXR expression is inversely correlated with the severity of inflammation in colitis, with diminished levels particularly evident in the proximal colon of patients with PSC [130]. In this regard, higher dosages of potential FXR ligands may be required to demonstrate the desired effects in PSC patients. Furthermore, concerns have been raised as to potential proliferative effects through FGF19-mediated FGFR4 signalling in a disease with a high neoplastic propensity. Targeting of other nuclear receptors (e.g. PXR by means of budesonide or rifampicin) similarly needs further experimental backing before rational and safe applications can be elaborated [131].

The membrane-bound G-protein-coupled bile acid receptor (TGR5) acts synergistically with FXR, although ligand-mediated activation is also implicated in the modulation of intestinal inflammation, motility and intestinal barrier function. These pleiotropic effects are highlighted in the *Tgr5* knockout mouse which

displays a decreased total bile acid pool size yet increased hydrophobic bile acid composition, as well as aggravated liver injury following challenge with bacterial endotoxins (e.g. lipopolysaccharide) [132]. Of further interest is the fact that genetic associations in UC (and to a lesser extent PSC) at 2q35 encompass several genes including TGR5, and in the *Mdr2*$^{-/-}$ mouse a dual agonist targeting FXR and TGR5 was able to attenuate biliary inflammation and fibrosis histologically [133]. As for FGF19-related interventions, there may be concerns regarding proliferative side effects in PSC, given the inherent risk of CCA [134].

Conclusions

Over the last two decades, mechanistic insights spanning genetic risks and bile acid metabolism have been paralleled by discovery of overlapping lymphocyte recruitment pathways across the afflicted sites in PSC/IBD. These findings are leading to a resurgence in PSC-related clinical trial activity, through pathways targeting bile acid homeostasis and tissue-specific lymphocyte homing. These models need to be tested and confirmed but, if correct, suggest novel approaches to therapy by preventing the exposure to toxic bile acid subtypes, attenuating the recruitment of immunopathogenic lymphocyte populations and lessening the burden of liver fibrosis. The first round of trials, already ongoing at time of writing, target all these aspects and are likely to provide proof-of-concept insights as to which avenue is likely to prove more effective, tentatively guiding future, more refined therapeutic approaches. As of yet, the disease course in PSC remains unpredictable in timing yet intrinsically progressive in nature, with a high risk of cholangiocellular neoplasia, reflecting the critical absence of definitive medical therapy for this disease.

References

1. Abdalian R, Heathcote EJ. Sclerosing cholangitis: a focus on secondary causes. Hepatology. 2006;44:1063–74. doi:10.1002/hep.21405.
2. O'Neil M, Damjanov I, Taylor RM. Liver pathology for clinicians. Cham: Springer International Publishing; 2015.
3. Hirschfield GM, Karlsen TH, Lindor KD, Adams DH. Primary sclerosing cholangitis. Lancet. 2013;382:1587–99. doi:10.1016/S0140-6736(13)60096-3.
4. Karlsen TH, Boberg KM. Update on primary sclerosing cholangitis. J Hepatol. 2013;59:571–82. doi:10.1016/j.jhep.2013.03.015.
5. Marchesi JR, Adams DH, Fava F, Hermes GDA, Hirschfield GM, Hold G, et al. The gut microbiota and host health: a new clinical frontier. Gut. 2015; gutjnl – 2015–309990. doi:10.1136/gutjnl-2015-309990.
6. Trivedi PJ, Adams DH. Mucosal immunity in liver autoimmunity: a comprehensive review. J Autoimmun. 2013;46:97–111. doi:10.1016/j.jaut.2013.06.013.
7. Flint HJ, Scott KP, Louis P, Duncan SH. The role of the gut microbiota in nutrition and health. Nat Rev Gastroenterol Hepatol. 2012;9:577–89. doi:10.1038/nrgastro.2012.156.
8. Swamy M, Jamora C, Havran W, Hayday A. Epithelial decision makers: in search of the "epimmunome.". Nat Immunol. 2010;11:656–65. doi:10.1038/ni.1905.
9. Frantz AL, Rogier EW, Weber CR, Shen L, Cohen DA, Fenton LA, et al. Targeted deletion of MyD88 in intestinal epithelial cells results in compromised antibacterial immunity associated

with downregulation of polymeric immunoglobulin receptor, mucin-2, and antibacterial peptides. Mucosal Immunol. 2012;5:501–12. doi:10.1038/mi.2012.23.
10. Sellon RK, Tonkonogy S, Schultz M, Dieleman LA, Grenther W, Balish E, et al. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. Infect Immun. 1998;66:5224–31.
11. Waidmann M, Bechtold O, Frick J-S, Lehr H-A, Schubert S, Dobrindt U, et al. Bacteroides vulgatus protects against *Escherichia coli*-induced colitis in gnotobiotic interleukin-2-deficient mice. Gastroenterology. 2003;125:162–77.
12. Balmer ML, Slack E, de Gottardi A, Lawson MAE, Hapfelmeier S, Miele L, et al. The liver may act as a firewall mediating mutualism between the host and its gut commensal microbiota. Sci Transl Med. 2014;6:237ra66. doi:10.1126/scitranslmed.3008618.
13. Moriwaki K, Balaji S, McQuade T, Malhotra N, Kang J, Chan FK-M. The necroptosis adaptor RIPK3 promotes injury-induced cytokine expression and tissue repair. Immunity. 2014;41:567–78. doi:10.1016/j.immuni.2014.09.016.
14. Schneider KM, Bieghs V, Heymann F, Hu W, Dreymueller D, Liao L, et al. CX3CR1 is a gatekeeper for intestinal barrier integrity in mice: limiting steatohepatitis by maintaining intestinal homeostasis. Hepatology. 2015;62(5):1405–16. doi:10.1002/hep.27982.
15. Henao-Mejia J, Elinav E, Jin C, Hao L, Mehal WZ, Strowig T, et al. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. Nature. 2012;482:179–85. doi:10.1038/nature10809.
16. Janse M, Lamberts LE, Franke L, Raychaudhuri S, Ellinghaus E, Muri Boberg K, et al. Three ulcerative colitis susceptibility loci are associated with primary sclerosing cholangitis and indicate a role for IL2, REL, and CARD9. Hepatology. 2011;53:1977–85. doi:10.1002/hep.24307.
17. Fickert P, Pollheimer MJ, Beuers U, Lackner C, Hirschfield G, Housset C, et al. Characterization of animal models for Primary Sclerosing Cholangitis (PSC). J Hepatol. 2014; doi:10.1016/j.jhep.2014.02.006.
18. Quraishi MN, Sergeant M, Kay G, Iqbal T, Chan J, Constantinidou C, et al. The gut adherent microbiota of PSC-IBD is distinct to that of IBD. Gut. 2016.
19. Kummen M, Holm K, Anmarkrud JA, Nygård S, Vesterhus M, Høivik ML, et al. The gut microbial profile in patients with primary sclerosing cholangitis is distinct from patients with ulcerative colitis without biliary disease and healthy controls. Gut. 2016;gutjnl – 2015–310500. doi:10.1136/gutjnl-2015-310500.
20. Kevans D, Tyler AD, Holm K, Jørgensen KK, Vatn MH, Karlsen TH, et al. Characterization of intestinal microbiota in ulcerative colitis patients with and without primary sclerosing cholangitis. J Crohns Colitis. 2016;10:330–7. doi:10.1093/ecco-jcc/jjv204.
21. Torres J, Bao X, Goel A, Colombel J-F, Pekow J, Jabri B, et al. The features of mucosa-associated microbiota in primary sclerosing cholangitis. Aliment Pharmacol Ther. 2016;43:790–801. doi:10.1111/apt.13552.
22. Terjung B, Söhne J, Lechtenberg B, Gottwein J, Muennich M, Herzog V, et al. p-ANCAs in autoimmune liver disorders recognise human beta-tubulin isotype 5 and cross-react with microbial protein FtsZ. Gut. 2010;59:808–16. doi:10.1136/gut.2008.157818.
23. Karrar A, Broomé U, Södergren T, Jaksch M, Bergquist A, Björnstedt M, et al. Biliary epithelial cell antibodies link adaptive and innate immune responses in primary sclerosing cholangitis. Gastroenterology. 2007;132:1504–14. doi:10.1053/j.gastro.2007.01.039.
24. O'Mahony CA, Vierling JM. Etiopathogenesis of primary sclerosing cholangitis. Semin Liver Dis. 2006;26:3–21. doi:10.1055/s-2006-933559.
25. Borchers AT, Shimoda S, Bowlus C, Keen CL, Gershwin ME. Lymphocyte recruitment and homing to the liver in primary biliary cirrhosis and primary sclerosing cholangitis. Semin Immunopathol. 2009;31:309–22. doi:10.1007/s00281-009-0167-2.
26. Liaskou E, Jeffery LE, Trivedi PJ, Reynolds GM, Suresh S, Bruns T, et al. Loss of CD28 expression by liver-infiltrating T cells contributes to pathogenesis of primary sclerosing cholangitis. Gastroenterology. 2014;147:221–32.e7. doi:10.1053/j.gastro.2014.04.003.
27. Lau AH, de Creus A, Lu L, Thomson AW. Liver tolerance mediated by antigen presenting cells: fact or fiction? Gut. 2003;52:1075–8. doi:10.1136/gut.52.8.1075.

28. Sebode M, Peiseler M, Franke B, Schwinge D, Schoknecht T, Wortmann F, et al. Reduced FOXP3(+) regulatory T cells in patients with primary sclerosing cholangitis are associated with IL2RA gene polymorphisms. J Hepatol. 2014;60:1010–6. doi:10.1016/j.jhep.2013.12.027.
29. Wakabayashi K, Lian Z-X, Moritoki Y, Lan RY, Tsuneyama K, Chuang Y-H, et al. IL-2 receptor alpha(−/−) mice and the development of primary biliary cirrhosis. Hepatology. 2006;44:1240–9. doi:10.1002/hep.21385.
30. Katt J, Schwinge D, Schoknecht T, Quaas A, Sobottka I, Burandt E, et al. Increased Th17 response to pathogen stimulation in patients with primary sclerosing cholangitis. Hepatology. 2013;58(3):1084–93. doi:10.1002/hep.26447.
31. Sano T, Huang W, Hall JA, Yang Y, Chen A, Gavzy SJ, et al. An IL-23R/IL-22 circuit regulates epithelial serum amyloid A to promote local effector Th17 responses. Cell. 2015; doi:10.1016/j.cell.2015.08.061.
32. Trivedi PJ, Bruns T, Ward S, Mai M, Schmidt C, Hirschfield GM, et al. Intestinal CCL25 expression is increased in colitis and correlates with inflammatory activity. J Autoimmun. 2016;68:98–104. doi:10.1016/j.jaut.2016.01.001.
33. Eksteen B, Liaskou E, Adams DH. Lymphocyte homing and its role in the pathogenesis of IBD. Inflamm Bowel Dis. 2008;14:1298–312. doi:10.1002/ibd.20453.
34. Oo YH, Adams DH. The role of chemokines in the recruitment of lymphocytes to the liver. J Autoimmun. 2010;34:45–54. doi:10.1016/j.jaut.2009.07.011.
35. Eksteen B, Grant AJ, Miles A, Curbishley SM, Lalor PF, Hübscher SG, et al. Hepatic endothelial CCL25 mediates the recruitment of CCR9+ gut-homing lymphocytes to the liver in primary sclerosing cholangitis. J Exp Med. 2004;200:1511–7. doi:10.1084/jem.20041035.
36. Grant AJ, Lalor PF, Hübscher SG, Briskin M, Adams DH. MAdCAM-1 expressed in chronic inflammatory liver disease supports mucosal lymphocyte adhesion to hepatic endothelium (MAdCAM-1 in chronic inflammatory liver disease). Hepatology. 2001;33:1065–72. doi:10.1053/jhep.2001.24231.
37. Liaskou E, Karikoski M, Reynolds GM, Lalor PF, Weston CJ, Pullen N, et al. Regulation of mucosal addressin cell adhesion molecule 1 expression in human and mice by vascular adhesion protein 1 amine oxidase activity. Hepatology. 2011;53:661–72. doi:10.1002/hep.24085.
38. Eksteen B, Mora JR, Haughton EL, Henderson NC, Lee-Turner L, Villablanca EJ, et al. Gut homing receptors on CD8 T cells are retinoic acid dependent and not maintained by liver dendritic or stellate cells. Gastroenterology. 2009;137:320–9. doi:10.1053/j.gastro.2009.02.046.
39. Neumann K, Kruse N, Szilagyi B, Erben U, Rudolph C, Flach A, et al. Connecting liver and gut: murine liver sinusoidal endothelium induces gut tropism of CD4+ T cells via retinoic acid. Hepatology. 2012;55:1976–84. doi:10.1002/hep.24816.
40. Omenetti S, Brogi M, Goodman WA, Croniger CM, Eid S, Huang AY, et al. Dysregulated intrahepatic CD4(+) T-cell activation drives liver inflammation in ileitis-prone SAMP1/YitFc mice. Cell Mol Gastroenterol Hepatol. 2015;1:406–19. doi:10.1016/j.jcmgh.2015.05.007.
41. Oo YH, Banz V, Kavanagh D, Liaskou E, Withers DR, Humphreys E, et al. CXCR3-dependent recruitment and CCR6-mediated positioning of Th-17 cells in the inflamed liver. J Hepatol. 2012;57:1044–51. doi:10.1016/j.jhep.2012.07.008.
42. Duboc H, Rajca S, Rainteau D, Benarous D, Maubert M-A, Quervain E, et al. Connecting dysbiosis, bile-acid dysmetabolism and gut inflammation in inflammatory bowel diseases. Gut. 2013;62:531–9. doi:10.1136/gutjnl-2012-302578.
43. Karlsen TH, Hov JR. Genetics of cholestatic liver disease in 2010. Curr Opin Gastroenterol. 2010;26:251–8. doi:10.1097/MOG.0b013e328336807d.
44. Jahnel J, Fickert P, Langner C, Högenauer C, Silbert D, Gumhold J, et al. Impact of experimental colitis on hepatobiliary transporter expression and bile duct injury in mice. Liver Int. 2009;29:1316–25. doi:10.1111/j.1478-3231.2009.02044.x.
45. Tabibian JH, O'Hara SP, Trussoni CE, Tietz PS, Splinter PL, Mounajjed T, et al. Absence of the intestinal microbiota exacerbates hepatobiliary disease in a murine model of primary sclerosing cholangitis. Hepatology. 2015. doi:10.1002/hep.27927.

46. Pollheimer MJ, Fickert P. Animal models in primary biliary cirrhosis and primary sclerosing cholangitis. Clin Rev Allergy Immunol. 2015;48(2–3):207–17. doi:10.1007/s12016-014-8442-y.
47. Mederacke I, Hsu CC, Troeger JS, Huebener P, Mu X, Dapito DH, et al. Fate-tracing reveals hepatic stellate cells as dominant contributors to liver fibrosis independent of its etiology. Nat Commun. 2013;4:2823. doi:10.1038/ncomms3823.
48. Trautwein C, Friedman SL, Schuppan D, Pinzani M. Hepatic fibrosis: concept to treatment. J Hepatol. 2015;62:S15–24. doi:10.1016/j.jhep.2015.02.039.
49. Bergquist A, Montgomery SM, Bahmanyar S, Olsson R, Danielsson A, Lindgren S, et al. Increased risk of primary sclerosing cholangitis and ulcerative colitis in first-degree relatives of patients with primary sclerosing cholangitis. Clin Gastroenterol Hepatol. 2008;6:939–43. doi:10.1016/j.cgh.2008.03.016.
50. Folseraas T, Liaskou E, Anderson CA, Karlsen TH. Genetics in PSC: what do the "risk genes" teach us? Clin Rev Allergy Immunol. 2014;48:154–64. doi:10.1007/s12016-014-8417-z.
51. Lammert C, Juran BD, Schlicht E, Xie X, Atkinson EJ, de Andrade M, et al. Reduced coffee consumption among individuals with primary sclerosing cholangitis but not primary biliary cirrhosis. Clin Gastroenterol Hepatol. 2014; doi:10.1016/j.cgh.2013.12.036.
52. Che B, Wang L, Zhang Z, Zhang Y, Deng Y. Distribution and accumulation of caffeine in rat tissues and its inhibition on semicarbazide-sensitive amine oxidase. Neurotoxicology. 2012;33:1248–53. doi:10.1016/j.neuro.2012.07.004.
53. Henriksen EKK, Melum E, Karlsen TH. Update on primary sclerosing cholangitis genetics. Curr Opin Gastroenterol. 2014;30:310–9. doi:10.1097/MOG.0000000000000052.
54. Karlsen TH, Lammert F, Thompson RJ. Genetics of liver disease: from pathophysiology to clinical practice. J Hepatol. 2015;62:S6–14. doi:10.1016/j.jhep.2015.02.025.
55. Hirschfield GM, Chapman RW, Karlsen TH, Lammert F, Lazaridis KN, Mason AL. The genetics of complex cholestatic disorders. Gastroenterology. 2013;144:1357–74. doi:10.1053/j.gastro.2013.03.053.
56. Liu JZ, Hov JR, Folseraas T, Ellinghaus E, Rushbrook SM, Doncheva NT, et al. Dense genotyping of immune-related disease regions identifies nine new risk loci for primary sclerosing cholangitis. Nat Genet. 2013; doi:10.1038/ng.2616.
57. Gershon ES, Grennan KS. Genetic and genomic analyses as a basis for new diagnostic nosologies. Dialogues Clin Neurosci. 2015;17:69–78.
58. Sollid LM, Jabri B. Triggers and drivers of autoimmunity: lessons from coeliac disease. Nat Rev Immunol. 2013;13:294–302. doi:10.1038/nri3407.
59. Thorsby E. A short history of HLA. Tissue Antigens. 2009;74:101–16. doi:10.1111/j.1399-0039.2009.01291.x.
60. Karlsen TH, Franke A, Melum E, Kaser A, Hov JR, Balschun T, et al. Genome-wide association analysis in primary sclerosing cholangitis. Gastroenterology. 2010;138:1102–11. doi:10.1053/j.gastro.2009.11.046.
61. Goyette P, Boucher G, Mallon D, Ellinghaus E, Jostins L, Huang H, et al. High-density mapping of the MHC identifies a shared role for HLA-DRB1*01:03 in inflammatory bowel diseases and heterozygous advantage in ulcerative colitis. Nat Genet. 2015;47:172–9. doi:10.1038/ng.3176.
62. Bo X, Broome U, Remberger M, Sumitran-Holgersson S. Tumour necrosis factor alpha impairs function of liver derived T lymphocytes and natural killer cells in patients with primary sclerosing cholangitis. Gut. 2001;49:131–41.
63. Steinberg MW, Shui J-W, Ware CF, Kronenberg M. Regulating the mucosal immune system: the contrasting roles of LIGHT, HVEM, and their various partners. Semin Immunopathol. 2009;31:207–21. doi:10.1007/s00281-009-0157-4.
64. Reizis B. Regulation of plasmacytoid dendritic cell development. Curr Opin Immunol. 2010;22:206–11. doi:10.1016/j.coi.2010.01.005.
65. Sokol H, Conway KL, Zhang M, Choi M, MORIN B, CAO Z, et al. Card9 mediates intestinal epithelial cell restitution, T-helper 17 responses, and control of bacterial infection in mice. Gastroenterology. 2013;145:591–601.e3. doi:10.1053/j.gastro.2013.05.047.
66. Ellinghaus D, Jostins L, Spain SL, Cortes A, Bethune J, Han B, et al. Analysis of five chronic inflammatory diseases identifies 27 new associations and highlights disease-specific patterns at shared loci. Nat Genet. 2016; doi:10.1038/ng.3528.

67. Häuser F, Deyle C, Berard D, Neukirch C, Glowacki C, Bickmann JK, et al. Macrophage-stimulating protein polymorphism rs3197999 is associated with a gain of function: implications for inflammatory bowel disease. Genes Immun. 2012;13:321–7. doi:10.1038/gene.2011.88.
68. Lee D-H, Esworthy RS, Chu C, Pfeifer GP, Chu F-F. Mutation accumulation in the intestine and colon of mice deficient in two intracellular glutathione peroxidases. Cancer Res. 2006;66:9845–51. doi:10.1158/0008-5472.CAN-06-0732.
69. Folseraas T, Melum E, Rausch P, Juran BD, Ellinghaus E, Shiryaev A, et al. Extended analysis of a genome-wide association study in primary sclerosing cholangitis detects multiple novel risk loci. J Hepatol. 2012;57:366–75. doi:10.1016/j.jhep.2012.03.031.
70. Rupp C, Friedrich K, Folseraas T, Wannhoff A, Bode KA, Weiss K-H, et al. Fut2 genotype is a risk factor for dominant stenosis and biliary candida infections in primary sclerosing cholangitis. Aliment Pharmacol Ther. 2014;39:873–82. doi:10.1111/apt.12663.
71. Gaj P, Maryan N, Hennig EE, Ledwon JK, Paziewska A, Majewska A, et al. Pooled sample-based GWAS: a cost-effective alternative for identifying colorectal and prostate cancer risk variants in the Polish population. PLoS One. 2012;7 doi:10.1371/journal.pone.0035307.
72. Nakagawa H, Hikiba Y, Hirata Y, Font-Burgada J, Sakamoto K, Hayakawa Y, et al. Loss of liver E-cadherin induces sclerosing cholangitis and promotes carcinogenesis. Proc Natl Acad Sci U S A. 2014;111:1090–5. doi:10.1073/pnas.1322731111.
73. Trivedi PJ, Adams DH. Gut – liver immunity. J Hepatol. 2015; doi:10.1016/j.jhep.2015.12.002.
74. Boonstra K, Weersma RK, van Erpecum KJ, Rauws EA, Spanier BWM, Poen AC, et al. Population-based epidemiology, malignancy risk, and outcome of primary sclerosing cholangitis. Hepatology. 2013;58:2045–55. doi:10.1002/hep.26565.
75. Gregorio GV, Portmann B, Karani J, Harrison P, Donaldson PT, Vergani D, et al. Autoimmune hepatitis/sclerosing cholangitis overlap syndrome in childhood: a 16-year prospective study. Hepatology. 2001;33:544–53. doi:10.1053/jhep.2001.22131.
76. Trivedi PJ, Chapman RW. PSC, AIH and overlap syndrome in inflammatory bowel disease. Clin Res Hepatol Gastroenterol. 2012;36:420–36. doi:10.1016/j.clinre.2011.10.007.
77. Boonstra K, van Erpecum KJ, van Nieuwkerk KMJ, Drenth JPH, Poen AC, Witteman BJM, et al. Primary sclerosing cholangitis is associated with a distinct phenotype of inflammatory bowel disease. Inflamm Bowel Dis. 2012;18:2270–6. doi:10.1002/ibd.22938.
78. Trivedi PJ, Corpechot C, Pares A, Hirschfield GM. Risk stratification in autoimmune cholestatic liver diseases: opportunities for clinicians and trialists. Hepatology. 2015; doi:10.1002/hep.28128.
79. Karlsen TH, Vesterhus M, Boberg KM. Review article: controversies in the management of primary biliary cirrhosis and primary sclerosing cholangitis. Aliment Pharmacol Ther. 2014;39:282–301. doi:10.1111/apt.12581.
80. Lunder A, Hov J, Borthne A, Gleditsch J, Johannsen G. et al. Prevalence of sclerosing cholangitis detected by magnetic resonance cholangiography in patients with long-term inflammatory bowel disease. Gastroenterology 2016;151(4):660–9.
81. Charman S, Copley L, Tovikkai C, Meulen J van der. UK liver transplant audit (NHS Blood and Transplant). 2012.
82. Fosby B, Melum E, Bjøro K, Bennet W, Rasmussen A, Andersen IM, et al. Liver transplantation in the Nordic countries - an intention to treat and post-transplant analysis from The Nordic Liver Transplant Registry 1982–2013. Scand J Gastroenterol. 2015;50:797–808. doi:10.3109/00365521.2015.1036359.
83. de Valle MB, Björnsson E, Lindkvist B. Mortality and cancer risk related to primary sclerosing cholangitis in a Swedish population-based cohort. Liver Int. 2012;32:441–8. doi:10.1111/j.1478-3231.2011.02614.x.
84. Tischendorf JJW, Hecker H, Krüger M, Manns MP, Meier PN. Characterization, outcome, and prognosis in 273 patients with primary sclerosing cholangitis: a single center study. Am J Gastroenterol. 2007;102:107–14. doi:10.1111/j.1572-0241.2006.00872.x.
85. Rizvi S, Eaton JE, Gores GJ. Primary sclerosing cholangitis as a pre-malignant biliary tract disease: surveillance and management. Clin Gastroenterol Hepatol. 2015; doi:10.1016/j.cgh.2015.05.035.

86. Razumilava N, Gores GJ, Lindor KD. Cancer surveillance in patients with primary sclerosing cholangitis. Hepatology. 2011;54:1842–52. doi:10.1002/hep.24570.
87. Karlsen TH, Schrumpf E, Boberg KM. Gallbladder polyps in primary sclerosing cholangitis: not so benign. Curr Opin Gastroenterol. 2008;24:395–9. doi:10.1097/MOG.0b013e3282f5727a.
88. EASL. Clinical practice guidelines: management of cholestatic liver diseases. J Hepatol. 2009;51:237–67. doi:10.1016/j.jhep.2009.04.009.
89. Angulo P, Maor-Kendler Y, Lindor KD. Small-duct primary sclerosing cholangitis: a long-term follow-up study. Hepatology. 2002;35:1494–500. doi:10.1053/jhep.2002.33202.
90. Rudolph G, Gotthardt D, Klöters-Plachky P, Kulaksiz H, Rost D, Stiehl A. Influence of dominant bile duct stenoses and biliary infections on outcome in primary sclerosing cholangitis. J Hepatol. 2009;51:149–55. doi:10.1016/j.jhep.2009.01.023.
91. Ngu JH, Gearry RB, Wright AJ, Stedman CAM. Inflammatory bowel disease is associated with poor outcomes of patients with primary sclerosing cholangitis. Clin Gastroenterol Hepatol. 2011;9:1092–7. doi:10.1016/j.cgh.2011.08.027.
92. Burak K, Angulo P, Pasha TM, Egan K, Petz J, Lindor KD. Incidence and risk factors for cholangiocarcinoma in primary sclerosing cholangitis. Am J Gastroenterol. 2004;99:523–6. doi:10.1111/j.1572-0241.2004.04067.x.
93. Rudolph G, Gotthardt D, Kloeters-Plachky P, Rost D, Kulaksiz H, Stiehl A. In PSC with dominant bile duct stenosis, IBD is associated with an increase of carcinomas and reduced survival. J Hepatol. 2010;53:313–7. doi:10.1016/j.jhep.2010.02.030.
94. Boberg KM, Bergquist A, Mitchell S, Pares A, Rosina F, Broomé U, et al. Cholangiocarcinoma in primary sclerosing cholangitis: risk factors and clinical presentation. Scand J Gastroenterol. 2002;37:1205–11.
95. Janse M, Lamberts LE, Verdonk RC, Weersma RK. IBD is associated with an increase in carcinoma in PSC irrespective of the presence of dominant bile duct stenosis. J Hepatol. 2012;57:473–4. doi:10.1016/j.jhep.2012.02.034.
96. Al Mamari S1, Djordjevic J, Halliday JS, Chapman RW. Improvement of serum alkaline phosphatase to <1.5 upper limit of normal predicts better outcome and reduced risk of cholangiocarcinoma in primary sclerosing cholangitis. J Hepatol. 2013;58(2):329–34.
97. Lindström L, Hultcrantz R, Boberg KM, Friis-Liby I, Bergquist A. Association between reduced levels of alkaline phosphatase and survival times of patients with primary sclerosing cholangitis. Clin Gastroenterol Hepatol. 2013;11(7):841–6.
98. de Vries EMG, Verheij J, Hubscher SG, Leeflang MMR, Boonstra K, Beuers U, et al. Applicability and prognostic value of histologic scoring systems in primary sclerosing cholangitis. J Hepatol. 2015; doi:10.1016/j.jhep.2015.06.008.
99. Corpechot C, Gaouar F, El Naggar A, Kemgang A, Wendum D, Poupon R, et al. Baseline values and changes in liver stiffness measured by transient elastography are associated with severity of fibrosis and outcomes of patients with primary sclerosing cholangitis. Gastroenterology. 2014;146:970–9. doi:10.1053/j.gastro.2013.12.030.
100. Vesterhus M, Hov JR, Holm A, Schrumpf E, Nygård S, Godang K, et al. Enhanced liver fibrosis score predicts transplant-free survival in primary sclerosing cholangitis. Hepatology. 2015;62:188–97. doi:10.1002/hep.27825.
101. Beuers U. Drug insight: mechanisms and sites of action of ursodeoxycholic acid in cholestasis. Nat Clin Pract Gastroenterol Hepatol. 2006;3:318–28. doi:10.1038/ncpgasthep0521.
102. Wunsch E, Trottier J, Milkiewicz M, Raszeja-Wyszomirska J, Hirschfield GM, Barbier O, et al. Prospective evaluation of ursodeoxycholic acid withdrawal in patients with primary sclerosing cholangitis. Hepatology. 2014;60:931–40. doi:10.1002/hep.27074.
103. Lindor KD, Kowdley KV, Luketic VAC, Harrison ME, McCashland T, Befeler AS, et al. High-dose ursodeoxycholic acid for the treatment of primary sclerosing cholangitis. Hepatology. 2009;50:808–14. doi:10.1002/hep.23082.
104. Imam MH, Sinakos E, Gossard AA, Kowdley KV, Luketic VA, Edwyn Harrison M, et al. High-dose ursodeoxycholic acid increases risk of adverse outcomes in patients with early stage primary sclerosing cholangitis. Aliment Pharmacol Ther. 2011;34:1185–92. doi:10.1111/j.1365-2036.2011.04863.x.

105. Chapman R, Fevery J, Kalloo A, Nagorney DM, Boberg KM, Shneider B, et al. Diagnosis and management of primary sclerosing cholangitis. Hepatology. 2010;51:660–78. doi:10.1002/hep.23294.
106. Lindor KD, Kowdley KV, Harrison ME. ACG clinical guideline: primary sclerosing cholangitis. Am J Gastroenterol. 2015;110:646–59. doi:10.1038/ajg.2015.112.
107. Pardi DS, Loftus EV, Kremers WK, Keach J, Lindor KD. Ursodeoxycholic acid as a chemopreventive agent in patients with ulcerative colitis and primary sclerosing cholangitis. Gastroenterology. 2003;124:889–93. doi:10.1053/gast.2003.50156.
108. Lindström L, Boberg KM, Wikman O, Friis-Liby I, Hultcrantz R, Prytz H, et al. High dose ursodeoxycholic acid in primary sclerosing cholangitis does not prevent colorectal neoplasia. Aliment Pharmacol Ther. 2012;35:451–7. doi:10.1111/j.1365-2036.2011.04966.x.
109. Eaton JE, Silveira MG, Pardi DS, Sinakos E, Kowdley KV, Luketic VAC, et al. High-dose ursodeoxycholic acid is associated with the development of colorectal neoplasia in patients with ulcerative colitis and primary sclerosing cholangitis. Am J Gastroenterol. 2011;106:1638–45. doi:10.1038/ajg.2011.156.
110. Lüth S, Kanzler S, Frenzel C, Kasper HU, Dienes HP, Schramm C, et al. Characteristics and long-term prognosis of the autoimmune hepatitis/primary sclerosing cholangitis overlap syndrome. J Clin Gastroenterol. 2009;43:75–80. doi:10.1097/MCG.0b013e318157c614.
111. Abdo AA, Bain VG, Kichian K, Lee SS. Evolution of autoimmune hepatitis to primary sclerosing cholangitis: a sequential syndrome. Hepatology. 2002;36:1393–9. doi:10.1002/hep.1840360615.
112. Floreani A, Rizzotto ER, Ferrara F, Carderi I, Caroli D, Blasone L, et al. Clinical course and outcome of autoimmune hepatitis/primary sclerosing cholangitis overlap syndrome. Am J Gastroenterol. 2005;100:1516–22. doi:10.1111/j.1572-0241.2005.41841.x.
113. Berntsen NL, Klingenberg O, Juran BD, Benito de Valle M, Lindkvist B, Lazaridis KN, et al. Association between HLA haplotypes and increased serum levels of IgG4 in patients with primary sclerosing cholangitis. Gastroenterology. 2015;148:924–7.e2. doi:10.1053/j.gastro.2015.01.041.
114. Benito de Valle M, Müller T, Björnsson E, Otten M, Volkmann M, Guckelberger O, et al. The impact of elevated serum IgG4 levels in patients with primary sclerosing cholangitis. Dig Liver Dis. 2014;46:903–8. doi:10.1016/j.dld.2014.06.010.
115. Tremaroli V, Bäckhed F. Functional interactions between the gut microbiota and host metabolism. Nature. 2012;489:242–9. doi:10.1038/nature11552.
116. Hammel P, Couvelard A, O'Toole D, Ratouis A, Sauvanet A, Fléjou JF, et al. Regression of liver fibrosis after biliary drainage in patients with chronic pancreatitis and stenosis of the common bile duct. N Engl J Med. 2001;344:418–23. doi:10.1056/NEJM200102083440604.
117. Teng MWL, Bowman EP, McElwee JJ, Smyth MJ, Casanova J-L, Cooper AM, et al. IL-12 and IL-23 cytokines: from discovery to targeted therapies for immune-mediated inflammatory diseases. Nat Med. 2015;21:719–29. doi:10.1038/nm.3895.
118. Danese S, Vuitton L, Peyrin-Biroulet L. Biologic agents for IBD: practical insights. Nat Rev Gastroenterol Hepatol. 2015;12:537–45. doi:10.1038/nrgastro.2015.135.
119. Feagan BG, Rutgeerts P, Sands BE, Hanauer S, Colombel J-F, Sandborn WJ, et al. Vedolizumab as induction and maintenance therapy for ulcerative colitis. N Engl J Med. 2013;369:699–710. doi:10.1056/NEJMoa1215734.
120. Meszaros M, Pageaux G-P, Altwegg R. Management of ulcerative colitis using vedolizumab after liver transplantation for primary sclerosing cholangitis. J Crohns Colitis. 2016;10:236. doi:10.1093/ecco-jcc/jjv182.
121. Weston CJ, Adams DH. Hepatic consequences of vascular adhesion protein-1 expression. J Neural Transm. 2011;118:1055–64. doi:10.1007/s00702-011-0647-0.
122. Schuppan D, Kim YO. Evolving therapies for liver fibrosis. J Clin Invest. 2013;123:1887–901. doi:10.1172/JCI66028.
123. Carpino G, Cardinale V, Renzi A, Hov JR, Berloco PB, Rossi M, et al. Activation of biliary tree stem cells within peribiliary glands in primary sclerosing cholangitis. J Hepatol. 2015;63:1220–8. doi:10.1016/j.jhep.2015.06.018.

124. Williams MJ, Clouston AD, Forbes SJ. Links between hepatic fibrosis, ductular reaction, and progenitor cell expansion. Gastroenterology. 2014;146:349–56. doi:10.1053/j.gastro.2013.11.034.
125. Peng Z-W, Ikenaga N, Liu SB, Sverdlov DY, Vaid KA, Dixit R, et al. Integrin αvβ6 critically regulates hepatic progenitor cell function and promotes ductular reaction, fibrosis and tumorigenesis. Hepatology. 2015; doi:10.1002/hep.28274.
126. Eberlein C, Kendrew J, McDaid K, Alfred A, Kang JS, Jacobs VN, et al. A human monoclonal antibody 264RAD targeting αvβ6 integrin reduces tumour growth and metastasis, and modulates key biomarkers in vivo. Oncogene. 2013;32:4406–16. doi:10.1038/onc.2012.460.
127. Beuers U, Trauner M, Jansen P, Poupon R. New paradigms in the treatment of hepatic cholestasis: from UDCA to FXR, PXR and beyond. J Hepatol. 2015;62:S25–37. doi:10.1016/j.jhep.2015.02.023.
128. Baghdasaryan A, Fuchs CD, Österreicher CH, Lemberger UJ, Halilbasic E, Påhlman I, et al. Inhibition of intestinal bile acid absorption improves cholestatic liver and bile duct injury in a mouse model of sclerosing cholangitis. J Hepatol. 2016;64:674–81. doi:10.1016/j.jhep.2015.10.024.
129. Hirschfield GM, Mason A, Luketic V, Lindor K, Gordon SC, Mayo M, et al. Efficacy of obeticholic acid in patients with primary biliary cirrhosis and inadequate response to ursodeoxycholic acid. Gastroenterology. 2015;148:751–61.e8. doi:10.1053/j.gastro.2014.12.005.
130. Torres J, Bao X, Iuga AC, Chen A, Harpaz N, Ullman T, et al. Farnesoid X receptor expression is decreased in colonic mucosa of patients with primary sclerosing cholangitis and colitis-associated neoplasia. Inflamm Bowel Dis. 2013;19:275–82. doi:10.1097/MIB.0b013e318286ff2e.
131. Karlsen TH, Lie BA, Frey Frøslie K, Thorsby E, Broomé U, Schrumpf E, et al. Polymorphisms in the steroid and xenobiotic receptor gene influence survival in primary sclerosing cholangitis. Gastroenterology. 2006;131:781–7. doi:10.1053/j.gastro.2006.05.057.
132. Wang Y-D, Chen W-D, Yu D, Forman BM, Huang W. The G-protein-coupled bile acid receptor, Gpbar1 (TGR5), negatively regulates hepatic inflammatory response through antagonizing nuclear factor κ light-chain enhancer of activated B cells (NF-κB) in mice. Hepatology. 2011;54:1421–32. doi:10.1002/hep.24525.
133. Baghdasaryan A, Claudel T, Gumhold J, Silbert D, Adorini L, Roda A, et al. Dual farnesoid X receptor/TGR5 agonist INT-767 reduces liver injury in the Mdr2-/- (Abcb4-/-) mouse cholangiopathy model by promoting biliary HCO^{-3} output. Hepatology. 2011;54:1303–12. doi:10.1002/hep.24537.
134. Reich M, Deutschmann K, Sommerfeld A, Klindt C, Kluge S, Kubitz R, et al. TGR5 is essential for bile acid-dependent cholangiocyte proliferation in vivo and in vitro. Gut. 2016;65:487–501. doi:10.1136/gutjnl-2015-309458.

Cholangiocarcinoma: Disease Pathogenesis and New Treatment Paradigms

10

Gregory J. Gores and Boris Blechacz

Abstract

Cholangiocarcinoma (CCA) is a malignancy arising in the biliary tree. Its incidence rates have increased, and it has surpassed gallbladder carcinoma as the most common biliary tract malignancy. Most patients are diagnosed with advanced stage disease and are not amenable to potentially curative surgical therapies. The prognosis is dismal with a median survival of <24 months. Novel possible epidemiologic risk factors (i.e., diabetes, cirrhosis, metabolic syndrome) have been identified. Based upon clinical behavior and distinct molecular patterns, intrahepatic, perihilar, and distal CCAs are characterized as distinct malignancies that need to be treated as such in their management and clinical trials. While cholestasis and inflammation are key factors for all three CCA subtypes, their genetic profile differs. Because knowledge of the genetic landscape of CCA has resulted in multiple new therapeutic strategies and clinical trials, it will become increasingly possible to develop precision medicine-based therapies for CCA patients.

Take-Home Points

- CCA has surpassed gallbladder carcinoma as the most common biliary malignancy.
- Inflammation and cholestasis are key factors in cholangiocarcinogenesis.
- Intra- and extrahepatic CCAs are clinically and genetically distinct.

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G. Hirschfield et al. (eds.), *Biliary Disease*, DOI 10.1007/978-3-319-50168-0_10

- Several clinical trials are ongoing evaluating novel targeted agents for CCA.
- Precision medicine-based approaches are necessary to improve outcomes in CCA.

10.1 Introduction

Cholangiocarcinoma (CCA) is the most common biliary tract malignancy and the second most common primary hepatic malignancy [10]. It is classified into intrahepatic (iCCA), perihilar (pCCA), and distal (dCCA) subtypes. The latter two subtypes were previously grouped as extrahepatic CCA but are now considered distinct entities based upon differences in their tumor biology and management. pCCA is the most common subtype. The prognosis of CCA is considered dismal. However, our understanding of its molecular tumor biology has increased, and advances in its surgical and nonsurgical management have resulted in improved outcomes and potentially curative treatments for selected patients.

10.2 Etiology

The majority of patients develop CCA in the absence of identifiable risk factors (Table 10.1) [9]. PSC patients have a 5–20% lifetime risk to develop CCA. However, only 10% of CCAs are attributed to PSC. Usually, CCA is diagnosed after a median of 4 years following the PSC diagnosis [3]. Inflammatory bowel disease (IBD) is not an independent risk factor for CCA in PSC [7]. Caroli's disease and types I and IV biliary cysts increase the risk for cholangiocarcinogenesis by 30-fold [26]. Importantly, excision of cysts reduces but does not eliminate the risk [17]. Hepatolithiasis has high incidence rates in Southeast Asia and is associated with a 6- to 50-fold increased risk for iCCA [26]. Cirrhosis has been identified as a possible independent risk factor for iCCA [25]. Data on HBV and HCV as risk factors for CCA show prevalence-based variability and require further validation [26]. Importantly, obesity, diabetes, and the metabolic syndrome have recently been suggested as risk factors for CCA, but data are inconsistent [26, 29]. In the context of globally increasing incidence rates of obesity, metabolic syndrome, and iCCA, clarification of their associations will be important.

10.3 Pathology and Cells of Origin

Based upon their macroscopic growth pattern, CCA may be classified as mass-forming, periductal-infiltrating, or intraductal-papillary. iCCA is predominantly mass-forming, while pCCA is typically periductal-infiltrating. Histopathologically,

Table 10.1 Genetic aberrations in cholangiocarcinoma

Type of genetic aberration	Gene	iCCA	eCCA
Copy number alterations	MYC		
	MDM2		
	EGFR		
	KRAS		
	CCND1/CCND3		
	AKT3		
	FGFR1/3		
Deletions	CDKN2A		
	UTY		
	KDM5D		
Fusion genes	FGFR2	6–45%	0%
	ATP1B	2.5%	0%
Mutations	KRAS	3–18%	12–21%
	PIK3CA/PIK3CA/ PIK3C2G/PIK3C2A/ PTEN	22%	
	IDH1/IDH2	6–22%	0%
	ERBB2		
	TP53	6–7%	7–14% dCCA > pCCA
	ARID1A/ARID2	4–14%	5–7%
	PBMR1	8–13%	0%
	BAP1	12–22%	0–3%
	SMAD4	0%	21%
	TGFBR2	3%	
	FGFR2	8–13%	0%
	CDKN2A	3%	
	ARAF	11%	
	EGFR	4%	1%
Epigenetic regulators (total 29%)	IDH1/IDH2		
	BAP1		
	TET1/2/3		
	MLL2/3		

Genetic aberrations identified by next-generation sequencing. Differences in incidence rates of these genetic changes are indicated where the information was available

90–95% of CCAs are adenocarcinomas of moderate to poor differentiation, with characteristic mucin expression and highly desmoplastic stroma [9].

CCA is thought to originate from transformed cholangiocytes. This concept received recently support by a cholangiocyte-lineage tracing model in which p53-knockout mice were treated with thioacetamide resulting in labeled-cholangiocyte-positive, NOTCH1-overexpressing CCA tumors [12]. Interestingly, certain primary

liver cancers such as cholangiocellular carcinoma, cholangiocarcinoma-like hepatocellular carcinoma (HCC), and mixed CCA-HCC are characterized by features of both tumor types possibly suggestive of common cells of origin [1, 18, 31]. Indeed, there is also evidence that CCA might originate from stem cells, hepatic progenitor cells, or transdifferentiated hepatocytes. Hepatic progenitor cells have been described in the intrahepatic biliary ductules, the canals of Hering, and peribiliary glands (PBGs) (Fig. 10.1a, b) ([5, 6] #153). These cells have the potential to differentiate in hepatocytes or cholangiocytes, and their differentiation into cholangiocytes underlies regulation by EGFR-induced NOTCH1 [16]. Cells with stem cell features have been described in extramural PBGs of large intrahepatic bile ducts and extrahepatic ducts. In PSC, PBG areas are increased, and they contain inflammatory infiltrates and increased numbers of cells with stem cell features, and cell proliferation is markedly increased [6]. Biliary dysplastic lesions in PSC are found in intrahepatic large ducts, extrahepatic ducts, and PBGs, and the number of cells with stem cell features is significantly increased in these dysplastic lesions [6].

The concept of transdifferentiation is supported by a recent study in which H-Ras and SV40 large T-antigen-transformed hepatic stem cells, hepatoblasts, and hepatocytes gave rise to primary liver cancers with features of HCC and CCA. However, CCA formation was predominantly observed with transformed hepatoblasts [13]. Further, a recent study reported cholangiocarcinogenesis after intrahepatic NOTCH1 and AKT overexpression in a mouse model [11].

In summary, the controversy about the cellular origin remains. However, important insights in the carcinogenesis were gained from studies evaluating the cellular origin of CCA. Interestingly, a common finding of these studies was the upregulation of EGFR and NOTCH1 signaling independent of the cellular origin. These findings provide the rationale for an EGFR- or NOTCH1-targeted chemoprevention strategy in patients at risk for cholangiocarcinogenesis. Indeed, Mayo Clinic recently completed a clinical phase 1 trial evaluating erlotinib in PSC patients with biliary epithelial trisomy 7 (clinicaltrials.gov, NCT00955149).

10.4 Pathogenesis

Inflammation and cholestasis are key factors in cholangiocarcinogenesis. Proinflammatory cytokines (i.e., *IL*-6) activate inducible nitric oxide synthase (iNOS) resulting in excess nitric oxide that mediates oxidative DNA damage, inhibition of DNA repair enzymes, and expression of cyclooxygenase-2 (COX-2). Further, iNOS has been shown to upregulate NOTCH1 in cholangiocytes [14]. Proinflammatory pathways downregulate hepatobiliary transporters, thereby contributing to cholestasis [19]. Bile acids and oxysterols activate EGFR and enhance COX-2 expression [33]. COX-2 dysregulates CCA growth and apoptosis resistance and positively regulates pro-oncogenic signaling pathways such as HGF, IL-6, and EGFR.

Next-generation sequencing identified somatic mutations in oncogenes (i.e., KRAS), tumor suppressor genes (i.e., TP53, SMAD4), and chromatin-modifying genes (i.e., ARID1A, BAP1, PBMR1) in CCA [5, 8, 15]. These studies have also

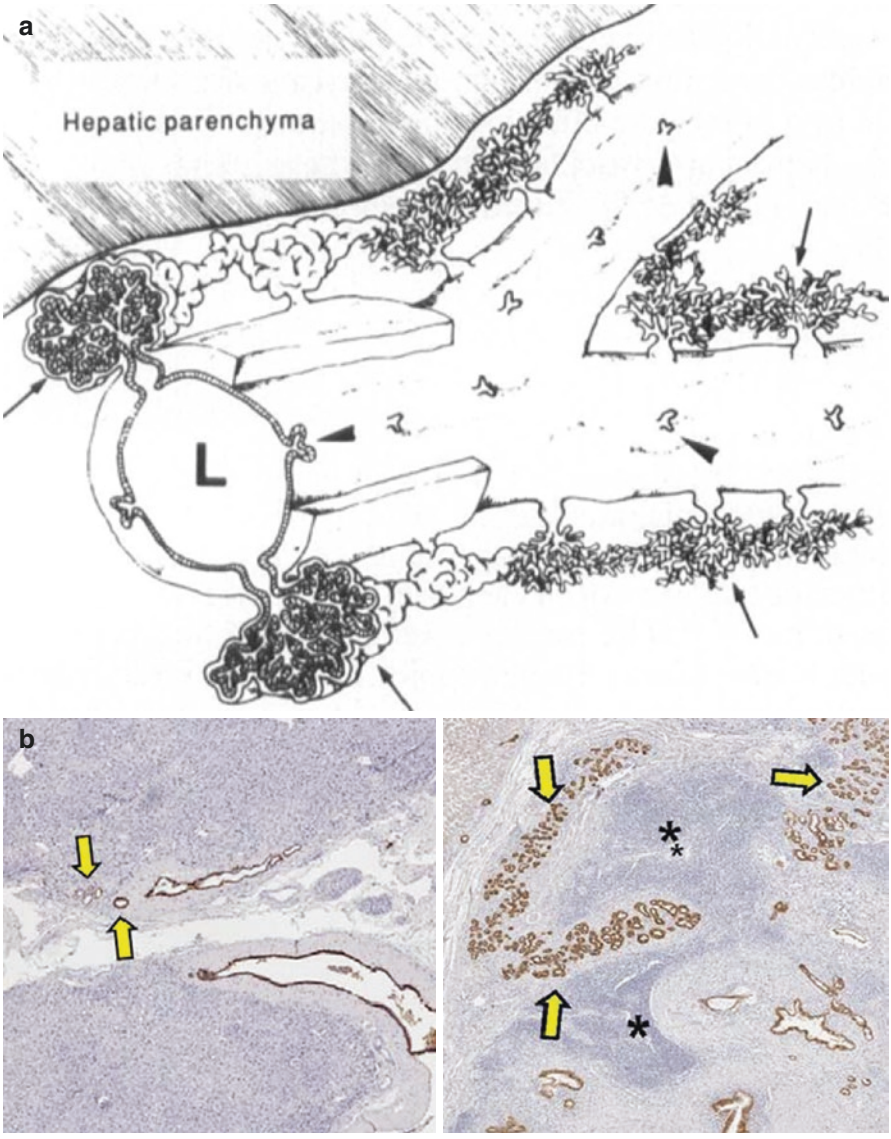

Fig. 10.1 Peribiliary glands. (**a**) Schematic of peribiliary glands (PBGs) including intramural (*arrow heads*) and extramural (*arrows*); *L* bile duct lumen (Nakanuma et al. 1994). (**b**) Immunohistochemistry for CK7 of normal hepatic parenchyma (*left*) and hepatic parenchyma in primary sclerosing cholangitis (PSC). *Arrows* indicate areas with PBGs (Carpino et al. [6])

shown the distinct mutational landscape of different etiologies and anatomic locations [2, 5, 8, 15]. KRAS mutations are more common in pCCA (22–53%) than iCCA (9–17%), while IDH1/2 mutations are more characteristic of iCCA [2, 8]. Mutant IDH1/2 (isocitrate dehydrogenase) inhibits hepatocyte but not biliary

differentiation and causes expansion of hepatic progenitor cells, resulting in iCCA formation in genetic mouse models [24]. Liver fluke-associated CCA is more commonly associated with mutations of TP53 and SMAD4, while BAP1 and IDH1/2 mutations are more frequent in non-liver fluke-associated CCA [5, 8]. Mutations of genes coding for components of oncogenic pathways such as PI3KCA and MET have been described in iCCA and pCCA [28]. Recently, gene rearrangements resulting in oncogenic fibroblast growth factor 2 (FGFR2) fusion proteins were identified in up to 45% of iCCA patients [6, 32]. Also non-genomic upregulation of EGFR, HER2, and MET was found, especially in patients with poor outcomes [2]. Whole-genome expression profiling confirmed activation of pathway driving proliferation (i.e., EGF, RAS, AKT, MET), angiogenesis (i.e., VEGFR, PDGFR), and inflammation (i.e., *IL*-6) [4].

Receptor tyrosine kinases such as IL-6 receptor, c-MET, and the EGFR family members ERBB2 and ERBB1 are key signaling pathways in cholangiocarcinogenesis. CCA cells and cancer-associated fibroblasts (CAFs) express and secrete cytokines and other mitogenic growth factors (i.e., *IL*-6, hepatocyte growth factor [HGF]) with subsequent auto- and paracrine stimulation of their cognate receptors. Receptor overexpression (i.e., *IL*-6R, c-MET, and EGFR), inactivation of negative feedback mechanisms, and transactivation between receptors (i.e., c-MET/EGFR, COX-2/IL-6) further contribute to constitutive pathway activation. Aberrant activation of these receptor tyrosine kinases causes constitutive activation of downstream signaling cascades (i.e., JAK/STAT3, PI3K/Akt, ERK1/2, and p38MAPK) resulting in dysregulation of cell senescence, cell cycle regulation and proliferation, and apoptosis.

10.5 Future Treatment Directions

Traditionally considered chemotherapy resistant, the phase III randomized controlled ABC-02 trial reported a 6-month survival benefit in CCA patients treated with gemcitabine/cisplatin combination therapy versus gemcitabine monotherapy [27]. A recent phase III randomized controlled trial showed higher objective response rates (31% vs. 14%, $p = 0.004$) and longer progression-free survival (5.9 months vs. 3.0 months, $p = 0.049$) through the addition of the EGFR inhibitor erlotinib to gemcitabine/cisplatin [20]. The data indicate the potential of molecular targeting to improve outcomes in CCA.

Limitations of current clinical trials include small sample size, lack of randomization, and combined analysis of CCA and gallbladder carcinoma. The latter point is critical as the recent studies further demonstrated the significant genetic differences between gallbladder carcinoma and CCA [22]. Hence, the subgroup analyses in combined CCA/gallbladder carcinoma studies are imperative. Very few studies evaluated the therapeutic efficacy of targeted agents combined with retrospective analysis of transcriptomic data.

Preclinical and early clinical trials support targeting EGFR in combination with other molecular targets (i.e., HER2, VEGFR) and/or chemotherapeutics

[20]. Interestingly, mutations in KRAS are associated with resistance to RTK inhibition and poor survival [2, 5]. Currently, the K-Ras inhibitor panitumumab is evaluated in CCA in a phase 2 trial in combination with chemotherapy (clinicaltrials.gov, NCT00779454); its combination with another targeted agent might be a promising therapeutic strategy to overcome RTK-inhibitor resistance. Mutations of the chromatin-remodeling gene BAP1 were identified in up to 11% of non-liver fluke-associated iCCAs, thereby indicating the therapeutic potential of targeting chromatin remodeling in CCA [23]. Given the important role of IL-6 in cholangiocarcinogenesis, tumor progression, and chemotherapy resistance, IL-6-targeted therapies (e.g., tocilizumab) are another promising strategy [32]. The JAK/STAT3 pathway is downstream of multiple receptor and non-receptor tyrosine kinases, and its targeting is, therefore, another highly promising approach based on preclinical studies [4], especially as JAK inhibitors are already in use in patients for other indications. The Bcr-Abl and Src inhibitor dasatinib and other direct IDH1/2 inhibitors are currently evaluated in a clinical phase 1 and 2 trial in iCCA patients with IDH1/2 mutations (clinicaltrials.gov, NCT00779454, NCT02073994). The identification of fusion FGFR2 in iCCA provides another druggable target currently evaluated in clinical phase I and II trials in CCA patients (clinicaltrials.gov, NCT02508467, NCT02150967) [32]. Given the highly desmoplastic character of CCA, an innovative approach in CCA includes targeting the microenvironment. Cancer-associated fibroblasts (CAFs) are myofibroblasts shown to be apoptosis resistant in CCA and are thought to promote tumor progression, invasion, and metastases. Recently, it was shown that these cells can be sensitized to apoptosis by the antiapoptotic Bcl-2 family protein inhibitor navitoclax, a drug shown to have a relatively benign safety profile in phase 1 trials [30]. In an orthotopic CCA rat model, navitoclax reduced tumor burden and metastases and prolonged survival [21].

The outcomes of these studies will be highly interesting. However, CCAs are genetically highly heterogeneous, and, therefore, precision medicine will be required to improve outcomes. Transcriptomic analysis revealed prognostic classifiers that were independent of anatomic location [2, 15], and next-generation gene profiling demonstrated distinct subclasses predicting survival and recurrence, thereby allowing patient stratification based upon their prognostic and oncogenic pathway analysis [4]. Expansion of OLT or resection criteria could be based upon such classifiers in the future. Randomized controlled trials are needed that prospectively assign patients based upon their transcriptomic and genetic profile, but for outcome comparison, better staging systems will be needed.

Conclusions

CCA is the second most common primary hepatic malignancy and has surpassed gallbladder carcinoma as the most common biliary malignancy. Clinically as well as on the molecular level, intrahepatic and extrahepatic cholangiocarcinomas are distinct entities and should be treated as such. Epidemiologic studies suggest that beyond the commonly known risk factors (i.e., PSC, liver fluke

infections), conditions such as cirrhosis, chronic HBV and HCV infection, and the metabolic syndrome might increase the risk for cholangiocarcinogenesis. The cellular origin of CCA remains controversial with data supporting cholangiocytes as well as hepatic progenitor cells as cells of origin. Multiple genetic aberrations of oncogenes, tumor suppressor genes, and chromatin-modifying genes have been identified in CCA and are influenced by anatomic location and etiology. iCCA is characterized by IDH1/2 mutations and pCCA by K-Ras mutations. In the molecular pathogenesis, in particular, cholestasis and inflammation are key mediators of cholangiocarcinogenesis via dysregulation of signaling pathways mediating apoptosis resistance, cell proliferation, and EMT. The identification of key genetic and molecular signaling abnormalities has resulted in the development of multiple novel therapeutic strategies, of which several are currently under investigation in clinical trials. However, the molecular characterization of CCA has also identified a significant interindividual tumor heterogeneity, making precision medicine-based therapeutic approaches necessary in order to improve outcomes of this dismal disease.

References

1. Allen RA, Lisa JR. Combined liver cell and bile duct carcinoma. Am J Pathol. 1949;25:647–55.
2. Andersen JB, Spee B, Blechacz BR, Avital I, Komuta M, Barbour A, Conner EA, Gillen MC, Roskams T, Roberts LR, et al. Genomic and genetic characterization of cholangiocarcinoma identifies therapeutic targets for tyrosine kinase inhibitors. Gastroenterology. 2012;142:1021–31. e1015
3. Burak K, Angulo P, Pasha TM, Egan K, Petz J, Lindor KD. Incidence and risk factors for cholangiocarcinoma in primary sclerosing cholangitis. Am J Gastroenterol. 2004;99:523–6.
4. Cardinale V, Bragazzi MC, Carpino G, Torrice A, Fraveto A, Gentile R, Pasqualino V, Melandro F, Aliberti C, Bastianelli C, et al. Cholangiocarcinoma: increasing burden of classifications. Hepatobiliary Surg Nutr. 2013;2:272–80.
5. Cardinale V, Wang Y, Carpino G, Mendel G, Alpini G, Gaudio E, Reid LM, Alvaro D. The biliary tree--a reservoir of multipotent stem cells. Nat Rev Gastroenterol Hepatol. 2012;9:231–40.
6. Carpino G, Cardinale V, Renzi A, Hov JR, Berloco PB, Rossi M, Karlsen TH, Alvaro D, Gaudio E. Activation of biliary tree stem cells within peribiliary glands in primary sclerosing cholangitis. J Hepatol. 2015;63:1220–8.
7. Chalasani N, Baluyut A, Ismail A, Zaman A, Sood G, Ghalib R, McCashland TM, Reddy KR, Zervos X, Anbari MA, Hoen H. Cholangiocarcinoma in patients with primary sclerosing cholangitis: a multicenter case-control study. Hepatology. 2000;31:7–11.
8. Chan-On W, Nairismagi ML, Ong CK, Lim WK, Dima S, Pairojkul C, Lim KH, McPherson JR, Cutcutache I, Heng HL, et al. Exome sequencing identifies distinct mutational patterns in liver fluke-related and non-infection-related bile duct cancers. Nat Genet. 2013;45:1474–8.
9. Endo I, Gonen M, Yopp AC, Dalal KM, Zhou Q, Klimstra D, D'Angelica M, DeMatteo RP, Fong Y, Schwartz L, et al. Intrahepatic cholangiocarcinoma: rising frequency, improved survival, and determinants of outcome after resection. Ann Surg. 2008;248:84–96.
10. Everhart JE, Ruhl CE. Burden of digestive diseases in the United States Part III: Liver, biliary tract, and pancreas. Gastroenterology. 2009;136:1134–44.

11. Fan B, Malato Y, Calvisi DF, Naqvi S, Razumilava N, Ribback S, Gores GJ, Dombrowski F, Evert M, Chen X, Willenbring H. Cholangiocarcinomas can originate from hepatocytes in mice. J Clin Invest. 2012;122:2911–5.
12. Guest RV, Boulter L, Kendall TJ, Minnis-Lyons SE, Walker R, Wigmore SJ, Sansom OJ, Forbes SJ. Cell lineage tracing reveals a biliary origin of intrahepatic cholangiocarcinoma. Cancer Res. 2014;74:1005–10.
13. Holczbauer A, Factor VM, Andersen JB, Marquardt JU, Kleiner DE, Raggi C, Kitade M, Seo D, Akita H, Durkin ME, Thorgeirsson SS. Modeling pathogenesis of primary liver cancer in lineage-specific mouse cell types. Gastroenterology. 2013;145:221–31.
14. Ishimura N, Bronk SF, Gores GJ. Inducible nitric oxide synthase up-regulates Notch-1 in mouse cholangiocytes: implications for carcinogenesis. Gastroenterology. 2005;128:1354–68.
15. Jiao Y, Pawlik TM, Anders RA, Selaru FM, Streppel MM, Lucas DJ, Niknafs N, Guthrie VB, Maitra A, Argani P, et al. Exome sequencing identifies frequent inactivating mutations in BAP1, ARID1A and PBRM1 in intrahepatic cholangiocarcinomas. Nat Genet. 2013;45:1470–3.
16. Kitade M, Factor VM, Andersen JB, Tomokuni A, Kaji K, Akita H, Holczbauer A, Seo D, Marquardt JU, Conner EA, et al. Specific fate decisions in adult hepatic progenitor cells driven by MET and EGFR signaling. Genes Dev. 2013;27:1706–17.
17. Kobayashi S, Asano T, Yamasaki M, Kenmochi T, Nakagohri T, Ochiai T. Risk of bile duct carcinogenesis after excision of extrahepatic bile ducts in pancreaticobiliary maljunction. Surgery. 1999;126:939–44.
18. Komuta M, Spee B, Vander Borght S, De Vos R, Verslype C, Aerts R, Yano H, Suzuki T, Matsuda M, Fujii H, et al. Clinicopathological study on cholangiolocellular carcinoma suggesting hepatic progenitor cell origin. Hepatology. 2008;47:1544–56.
19. Kosters A, Karpen SJ. The role of inflammation in cholestasis: clinical and basic aspects. Semin Liver Dis. 2010;30:186–94.
20. Lee J, Park SH, Chang HM, Kim JS, Choi HJ, Lee MA, Jang JS, Jeung HC, Kang JH, Lee HW, et al. Gemcitabine and oxaliplatin with or without erlotinib in advanced biliary-tract cancer: a multicentre, open-label, randomised, phase 3 study. Lancet Oncology. 2012;13:181–8.
21. Mertens JC, Fingas CD, Christensen JD, Smoot RL, Bronk SF, Werneburg NW, Gustafson MP, Dietz AB, Roberts LR, Sirica AE, Gores GJ. Therapeutic effects of deleting cancer-associated fibroblasts in cholangiocarcinoma. Cancer Res. 2013;73:897–907.
22. Nakamura H, Arai Y, Totoki Y, Shirota T, Elzawahry A, Kato M, Hama N, Hosoda F, Urushidate T, Ohashi S, et al. Genomic spectra of biliary tract cancer. Nat Genet. 2015;47:1003–10.
23. Rizvi S, Borad MJ, Patel T, Gores GJ. Cholangiocarcinoma: molecular pathways and therapeutic opportunities. Semin Liver Dis. 2014;34:456–64.
24. Saha SK, Parachoniak CA, Ghanta KS, Fitamant J, Ross KN, Najem MS, Gurumurthy S, Akbay EA, Sia D, Cornella H, et al. Mutant IDH inhibits HNF-4alpha to block hepatocyte differentiation and promote biliary cancer. Nature. 2014;513:110–4.
25. Shaib YH, El-Serag HB, Davila JA, Morgan R, McGlynn KA. Risk factors of intrahepatic cholangiocarcinoma in the United States: a case-control study. Gastroenterology. 2005;128:620–6.
26. Tyson GL, El-Serag HB. Risk factors for cholangiocarcinoma. Hepatology. 2011;54:173–84.
27. Valle J, Wasan H, Palmer DH, Cunningham D, Anthoney A, Maraveyas A, Madhusudan S, Iveson T, Hughes S, Pereira SP, et al. Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. N Engl J Med. 2010;362:1273–81.
28. Voss JS, Holtegaard LM, Kerr SE, Fritcher EG, Roberts LR, Gores GJ, Zhang J, Highsmith WE, Halling KC, Kipp BR. Molecular profiling of cholangiocarcinoma shows potential for targeted therapy treatment decisions. Hum Pathol. 2013;44:1216–22.
29. Welzel TM, Graubard BI, Zeuzem S, El-Serag HB, Davila JA, McGlynn KA. Metabolic syndrome increases the risk of primary liver cancer in the United States: a study in the SEER-Medicare database. Hepatology. 2011;54:463–71.

30. Wilson WH, O'Connor OA, Czuczman MS, LaCasce AS, Gerecitano JF, Leonard JP, Tulpule A, Dunleavy K, Xiong H, Chiu YL, et al. Navitoclax, a targeted high-affinity inhibitor of BCL-2, in lymphoid malignancies: a phase 1 dose-escalation study of safety, pharmacokinetics, pharmacodynamics, and antitumour activity. Lancet Oncol. 2010;11:1149–59.
31. Woo HG, Lee JH, Yoon JH, Kim CY, Lee HS, Jang JJ, Yi NJ, Suh KS, Lee KU, Park ES, et al. Identification of a cholangiocarcinoma-like gene expression trait in hepatocellular carcinoma. Cancer Res. 2010;70:3034–41.
32. Yamada D, Rizvi S, Razumilava N, Bronk SF, Davila JI, Champion MD, Borad MJ, Bezerra JA, Chen X, Gores GJ. IL-33 facilitates oncogene-induced cholangiocarcinoma in mice by an interleukin-6-sensitive mechanism. Hepatology. 2015;61:1627–42.
33. Yoon JH, Higuchi H, Werneburg NW, Kaufmann SH, Gores GJ. Bile acids induce cyclooxygenase-2 expression via the epidermal growth factor receptor in a human cholangiocarcinoma cell line. Gastroenterology. 2002;122:985–93.

Gallstone Disease: Scientific Understanding and Future Treatment

11

Frank Lammert

Abstract

Gallstones are a widespread disease caused by interaction of exogenous and genetic risk factors. The most frequent stones are cholesterol gallbladder stones, which are a consequence of increased hepatic secretion into bile. Multiple genetic factors confer gallstone risk, with the largest effect contributed by a common variant of the hepatic cholesterol transporter ABCG8. A distinct subgroup of patients develops gallstones due to low phosphatidylcholine concentrations in bile, which is caused by ABCB4 transporter defects. Later in the twenty first century, general lifestyle interventions and precise molecular prevention might overcome the current invasive therapy of gallstone disease with cholecystectomy.

Take-Home Points

- Cholesterol gallbladder stones develop because of cholesterol supersaturation of bile.
- Genetic and environmental factors account for its development. Women develop significantly more gallstones than men; the prevalence of stones also increases with age.
- ABCG8 has been identified as the most frequent genetic risk factor for gallstone disease in humans.

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G. Hirschfield et al. (eds.), *Biliary Disease*, DOI 10.1007/978-3-319-50168-0_11

- A significant association with ABCB4 has been identified in recent GWAS in Icelandic patients; ABCB4 variants have not only associated with intrahepatic cholestasis of pregnancy but also with liver cirrhosis and cancer.
- Development of genotype-based therapies is needed as at present surgery is the main treatment with only 60% success rate and several complications.

11.1 Introduction

Gallstones are common. In Europe, approximately 20% of the population have gallstones. Current treatment of symptomatic gallbladder stones is surgical removal of the gallbladder (cholecystectomy), since medical therapy (stone dissolution by oral administration of bile acids) is limited by a high recurrence rate as long as the gallbladder is in situ [1]. Of note, cholecystectomy rates vary with lower annual rates in Scandinavia (65 per 100,000 inhabitants) and higher rates in Germany and the United States (200 per 100,000 inhabitants); in countries with the highest prevalence of gallstones, such as Chile, the cholecystectomy rate exceeds 220 per 100,000 inhabitants [2]. This variation appears not to merely be due to differences in prevalence but could also reflect differences in indicating surgery in patients with mild or non-specific symptoms.

Based on chemical composition, gallstones are classified into cholesterol as well as black or brown bilirubin (pigment) stones. Cholesterol and black pigment stones develop in the gallbladder; brown pigment stones precipitate in obstructed and infected bile ducts. Of note, many gallbladder stones are cholesterol stones with a central pigment nidus [3], while bile duct stones often contain a cholesterol-rich nucleus [4]. The clinical course of gallbladder and bile duct stones differs. Primary gallbladder stones can cause acute cholecystitis but can also migrate into and occlude the common bile duct (secondary stones). Additional common and potentially life-threatening complications of bile duct stones include acute cholangitis and biliary pancreatitis. The outcomes of different treatment decisions in clinical practice have yet to be evaluated in future studies.

11.2 Pathobiology

The primary mechanism leading to cholesterol gallbladder stones is cholesterol supersaturation of bile, which is caused by environmental and genetic factors. Hydrophobic cholesterol molecules are secreted into bile via the ATP-dependent hemitransporters ABCG5 and ABCG8 in the hepatocanalicular membrane [5]. In hepatic bile, cholesterol is solubilized in vesicles and mixed micelles composed of bile acids and phosphatidylcholine [6]. These biliary lipids enter bile via the bile salt export pump ABCB11 and the phosphatidylcholine floppase ABCB4 [5]. If bile contains more cholesterol than can be solubilized by mixed micelles at equilibrium, it is supersaturated with cholesterol, multilamellar vesicles (“liquid crystals”) occur

and the aggregation of these cholesterol-rich vesicles precedes the formation of solid cholesterol crystals.

Cholesterol supersaturation causes secondary gallbladder hypomotility, because large amounts of cholesterol are absorbed by epithelial cells lining the gallbladder. Excess cholesterol is deposited in the gallbladder wall, where it stiffens the sarcolemmal membrane of the smooth muscle cells and disrupts signal transduction [2].

The rare black pigment stones are often a consequence of chronic hemolysis, resulting in increased bilirubin concentrations in bile [7]. Furthermore, bile salt loss (ileal resection, Crohn's disease, liver cirrhosis) increases the colonic absorption, enterohepatic circulation and biliary concentrations of bilirubin. In bile conjugated bilirubin can be hydrolysed by bacterial β-glucuronidases.

11.3 Twin Studies

Twin studies allowed the systematic analysis of exogenous and genetic risk factors for gallstones. A large study in Swedish twins combined the Swedish Twin Registry with the inpatient-discharge and causes-of-death registries for symptomatic gallstone disease and gallstone surgery-related diagnoses in 43,141 twin pairs born between 1900 and 1958 [8]. This study investigated not only the factors that determine gallstones but their clinical sequelae (biliary colic and complications). The concordance rate (i.e. the quantitative statistical measurement for the presence of a genetic trait in both members of a pair of twins) was higher in monozygotic compared with dizygotic twins (12% versus 6%). However, the rather low concordance between monozygotic twins indicates that environmental factors are also important for gallstone disease. Final risk estimates were derived by structural equation modelling. This analysis of variance and correlations models genotypic and environmental effects as the contribution of unmeasured (latent) variables to the potentially multivariate phenotypic differences. As Fig. 11.1 illustrates, genetic factors accounted for 25% (95% confidence interval (CI) 9–40%), shared environmental effects (e.g. diet in childhood) for 13% (CI 1–25%) and individual environmental effects for 62% (CI 56–68%) of the phenotypic variation among twins (Fig. 11.1).

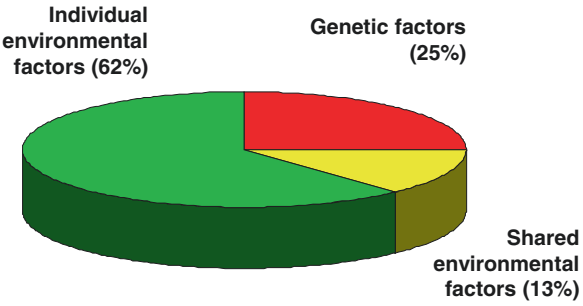

Fig. 11.1 Pie chart illustrating the contribution of genetic, shared and individual environmental factors to gallstone disease in twins. Structural equation modelling was used to estimate the quantitative effects of the factors on the phenotypic variation (Data from Katsika et al. [8])

Table 11.1 Selected exogenous risk factors for gallstone disease in cohort studies

Risk factor	N of cases	Relative risk	95% CI	Reference
Physical inactivity	6958	1.33	1.2–1.5	[13]
High carbohydrate intake	5771	1.35	1.2–1.6	[14]
High saturated fat intake	2350	1.24	1.0–1.5	[15]
Obesity	55,670	1.63*	1.5–1.8	[16]
Diabetes mellitus	223,651	1.56	1.3–1.9	[17]

Abbreviations: *BMI* body mass index, *CI* confidence interval
*per 5 kg/m^2 BMI increase

11.4 Exogenous Risk Factors

Common risk factors for gallbladder stones include physical inactivity and overnutrition, i.e. high calorie intake, in particular high carbohydrate intake, high glycaemic load, and low fibre intake [9]. These contribute to obesity (particularly central adiposity) as well as insulin resistance and diabetes, hence gallstones have been considered a "fellow traveler" of the metabolic syndrome [10]. Hyperinsulinaemia is associated with increased hepatic cholesterol uptake and secretion as well as hyposecretion of bile acids [11]. Low-fibre diet prolongs intestinal transit and thereby increases the synthesis of the lithogenic hydrophobic bile acid deoxycholic acid by intestinal bacteria that belong to the genus *Clostridium* [9]. Large prospective studies have estimated the risks associated with exogenous risk factors (Table 11.1). Since effects of these risk factors accumulate over time, it follows – together with age-dependent alterations of cholesterol homeostasis, bile secretion and gallbladder motility – that the prevalence of stones increases with age. For example, in Germany, the maximum prevalence rate of symptomatic gallstones of 57% was observed in women aged 70–79 years, estimated based on previous cholecystectomy and abdominal ultrasound [12].

11.5 Genetic Risk Factors

Associations between multiple lithogenic (that is, promoting the formation of stones in an organic body) gene variants and gallstone formation have been observed, indicating that gallstone disease is a polygenic complex disorder. Quantitative trait locus analysis in inbred strains of mice fed a lithogenic diet containing a supraphysiological concentration of cholesterol has identified at least 25 lithogenic genes to date, including the hepatobiliary cholesterol transporter *Abcg5/g8* [2]. The disease susceptibility loci have been mapped by identifying the genomic regions where the distribution of marker genotypes correlates with differences in stone phenotypes in experimental crosses of inbred mouse strains.

A well-known genetic risk factor in humans is sex, with women developing significantly more gallstones than men. This difference is, at least in part, due to

the increased incidence of gallstones in pregnancy (5%). Estrogens induce hepatic cholesterol synthesis and secretion, and progesterone causes gallbladder hypomotility [2]. Genome-wide association studies (GWAS) in humans identified a single variant of the hepatobiliary cholesterol transporter *ABCG8* (p.D19H) as the most frequent genetic risk factor for gallstone disease in humans [18]. About 5% of the European populations carry the p.D19H mutation of the *ABCG8* gene. Subsequent studies showed that, for example, obese women over the age of 60 years who are homozygous carriers of the p.D19H risk allele have a 13% absolute 10-year risk of symptomatic gallstone disease compared to 2–4% in non-carriers in the same risk stratum [19]. The genetic association was replicated in multiple international cohorts (Table 11.2); hence this genetic factor represents a common gallstone risk factor worldwide [20]. Functional in vitro studies [21] and measurements of phytosterol and cholesterol precursor concentrations demonstrate that individuals predisposed to gallstone disease display increased biliary output of cholesterol in the setting of relatively low intestinal cholesterol absorption, indicating enhanced whole-body sterol clearance (Fig. 11.2). The comparison of serum sterols showed lower levels of phytosterols, which represent surrogate markers for intestinal cholesterol absorption and higher levels of cholesterol precursors reflecting cholesterol synthesis in gallstone disease patients than in controls [22]. An ethnic gradient in the ratios of phytosterols to cholesterol precursors is also apparent (Germans > Hispanics > Amerindians). Together with the common Gilbert promoter variant of the UDP glucuronosyl transferase gene (*UGT1A1*), which appears to be an additional risk factor predominantly in men, both genes confer about 15% of the population attributable gallstone risk [23]. The *UGT1A1* variant might promote the formation of the bilirubin nidus of cholesterol gallstones.

A large meta-analysis of GWAS [24] identified additional susceptibility loci for gallstone disease, all of which confer low additional risk (Table 11.3). They might affect the rate-limiting enzyme of bile salt synthesis (cholesterol 7α-hydroxylase) and a sulfo-conjugation enzyme for bile salts as well as a glucokinase regulator known to be associated with diabetes and glycaemic traits. Of note, the recent GWAS in a Latin Chilean population comprising 529 cases (489 women) and 566 controls confirmed the previously known *ABCG5/G8* association but also revealed a highly significant signal inside the *GPR30* gene, which encodes the a G

Table 11.2 Studies investigating the role of *ABCG8* p.D19H in gallstone disease

	Year	Population	N	Odds ratio	Risk allele frequency (%)
Buch et al.	2007	Germany	1,832	2.2, 7.1*	5.0
		Chile	167	1.9	7.0
Grünhage et al.	2007	Romania	178	3.0	8.5
Kuo et al.	2008	China	74	3.5	1.4
Katsika et al.	2010	Sweden	341	2.5	6.8
Siddapuram et al.	2010	India	226	2.3	8.2
Stender et al.	2010	Denmark	3,124	1.9**, 3.3*	6.4

OR values are given for the carriership of the 19H risk allele, except for *carriers of the heterozygous genotype DH, and **homozygous carriers of the HH genotype

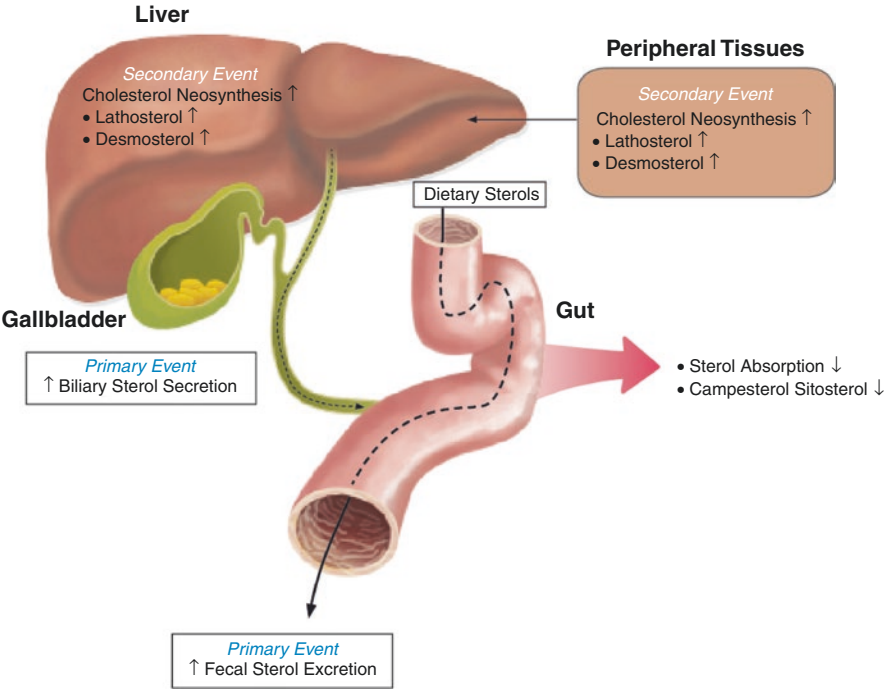

Fig. 11.2 Schematic illustration of the integrated hypothesis on sterol transport and synthesis in individuals at risk for cholesterol gallstones. Based on phytosterols and cholesterol precursors as surrogate markers, these individuals are characterized by an increased clearance/excretion of cholesterol (and phytosterols). This primary event would be followed by a compensatory increase in cholesterol synthesis in liver and peripheral tissues. The critical phenotype, especially increased excretion of sterols into intestine and bile, is present in at-risk individuals already before gallstones develop (From Krawczyk et al. [22])

protein-coupled transmembrane estrogen receptor expressed in liver and gallbladder, in women [25]. The roles of *ELMO1*, *TM6SF4* and *TRAF3* in gallstone pathobiology have yet to be explored further.

In addition, rare mutations in *ABCB4*, *ABCB11*, *CFTR* (cystic fibrosis transmembrane conductance regulator, also known as *ABCC7*) or the *CYP7A1* gene cause gallstone formation by leading to altered bile composition. A specific subgroup of patients with gallstones have low phospholipid associated-cholelithiasis (LPAC) syndrome. LPAC syndrome is defined by early-onset cholelithiasis (< 40 years), concurrent gallbladder, bile duct and/or intrahepatic cholesterol gallstones and recurrence of biliary symptoms after cholecystectomy [26]. LPAC syndrome is caused by mutations of the *ABCB4* gene and is part of the phenotypic spectrum of ABCB4 deficiency, which also comprises severe cholestatic diseases in children [5]. Interestingly a recent GWAS for gallstone disease in 8,258 Icelandic patients, for whom discharge diagnoses were available from the Landspítali University Hospital

Table 11.3 Genetic polymorphisms associated with gallstone disease in genome-wide association studies

Gene locus	Variants	Cases (N)	Population	Odds ratio	95% CI	*P*-value	Reference
ABCB4	p.G622E p.L445GfsX22	4,958	Iceland	2.74 3.10		7.2×10^{-10} 1.8×10^{-9}	[27]
ABCG8	p.D19H	8,720	Meta-analysis	1.78	1.70–1.86	2.0×10^{-75}	[24]
CYP7A1	rs6471717	8,720	Meta-analysis	1.11	1.08–1.14	3.2×10^{-6}	[24]
ELMO1		529	Chile			5.9×10^{-6}	[25]
GCKR	p.P446L	8,720	Meta-analysis	1.12	1.09–1.15	7.7×10^{-8}	[24]
GPR30		489[F]	Chile			5.8×10^{-6}	[25]
SULT21	rs296391	8,720	Meta-analysis	1.17	1.13–1.22	1.1×10^{-7}	[24]
TM4SF4	rs9843304	8,720	Meta-analysis	1.11	1.08–1.14	3.0×10^{-6}	[25]
TRAF3		529	Chile			5.3×10^{-6}	[25]

Abbreviation: *CI* confidence intervals, *F* females

in Reykjavík, revealed a significant association with *ABCB4*. The two *ABCB4* mutations that were significantly associated with gallstones at the genome-wide level are the missense single nucleotide polymorphism p.G622E and the frameshift insertion p.L445GfsX22, which conferred odds ratios for gallstones of 2.74 and 3.10, respectively [27]. Interestingly in the Iceland population, *ABCB4* variants were not only associated with intrahepatic cholestasis of pregnancy but with liver cirrhosis and cancer [27].

11.6 Current Clinical Management

11.6.1 Gallbladder Stones

Treatment algorithms for gallstone disease have been recently reviewed and summarized in Clinical Practice Guidelines of the European Association for the Study of the Liver (EASL). The following sections are based on and adapted from these recommendations [28]. Overall, approximately 20% of patients with gallbladder stones develop symptoms, with multiple and larger stones (> 10 mm) as well as female sex representing independent risk factors [29]. The characteristic symptoms of gallbladder stones are episodic attacks of severe pain (known as "biliary colic") in the right upper abdominal quadrant or epigastrium for at least 15–30 minutes with radiation to the right back or shoulder and a positive reaction to analgesics. Abdominal ultrasound is the imaging of choice for these patients, since its accuracy for detecting gallbladder stones exceeds 95%. Laparoscopic cholecystectomy (with four ports) is the standard method of cholecystectomy for symptomatic gallbladder

stones; mini-laparotomy-cholecystectomy is an alternative to laparoscopic cholecystectomy [28]. The yearly incidence of complications in symptomatic patients is 1–2%. Hence, characteristic biliary colic represents a warning sign for complications and in fact the majority of patients presenting with complications report previous biliary colic [30].

There have been no randomized controlled trials that assess cholecystectomy in asymptomatic patients with gallbladder stones. Overall, 1–2% of these patients develop symptoms related to gallstones every year; the annual incidence of complications, such as acute cholecystitis, acute cholangitis or biliary pancreatitis, is 0.1–0.2% [28]. Cholecystectomy in asymptomatic patients does not increase their life expectancy, because the risks of surgery outweigh the probability of complications. In European countries with low gallbladder carcinoma prevalence, the slight but low risk of gallbladder cancer in asymptomatic cholecystolithiasis does not justify cholecystectomy either [2].

Acute cholecystitis is the most common complication of gallstone disease. Early laparoscopic cholecystectomy within 24 h of hospital admission is the preferred treatment strategy, as recently demonstrated by the randomized controlled ACDC trial [31]. Early laparoscopic cholecystectomy decreases morbidity from 34% to 12%, shortens the length of hospital stay from 10.0 to 5.4 days but does not increase surgical complication rates [31].

11.6.2 Bile Duct Stones

Common bile duct stones are suspected in patients with jaundice, acute cholangitis or acute pancreatitis. Abdominal ultrasound is the first imaging method when bile duct stones are suspected but overall sensitivity is low. Stones in the gallbladder, a dilated common bile duct, acute cholangitis and hyperbilirubinemia are strong predictors for bile duct stones. Patients with an intermediate probability of bile duct stones have to undergo further evaluation with endoscopic ultrasound (or magnetic resonance cholangiopancreatography). According to a recent Cochrane review, the sensitivity of endoscopic ultrasound is 95% with a specificity of 97%, whereas the sensitivity of magnetic resonance cholangiopancreatography is 93% with a specificity of 96% [32].

Endoscopic retrograde cholangiography (ERC) with sphincterotomy and stone extraction is the recommended treatment of bile duct stones. In case of failed standard stone extraction, extracorporeal shock wave, electrohydraulic or laser lithotripsy are performed; in the case of failed endoscopic therapy, cholecystectomy combined with bile duct exploration or intraoperative ERC are performed.

In a randomized trial to evaluate the timing of laparoscopic cholecystectomy after endoscopic sphincterotomy, laparoscopic cholecystectomy within 72 h after endoscopic bile duct clearance leads to significantly less recurrent biliary events (0% versus 28%) as compared to delayed laparoscopic cholecystectomy (after 6–8 weeks); there were no differences in conversion rate, surgical complications or length of hospital stay [33].

The treatment of acute cholangitis has to include immediate broad spectrum antibiotics and endoscopic biliary decompression. For acute biliary pancreatitis with suspected coexistent acute cholangitis, antibiotics should be initiated and ERC with sphincterotomy and stone extraction should be performed, with timing depending on the severity of cholangitis but preferably within 24 h. An ERC is probably indicated in patients with biliary pancreatitis and obstructed bile duct but probably not in patients with biliary pancreatitis in the absence of cholangitis or obstructed bile duct [28]. Importantly, in patients with suspected biliary pancreatitis without cholangitis, endoscopic ultrasound (or magnetic resonance cholangiopancreatography) may prevent ERC and its risks if no stones are detected.

In patients with mild acute biliary pancreatitis, early laparoscopic cholecystectomy is preferable to laparoscopic cholecystectomy performed on the routine waiting list to avoid recurrent gallstone-related complications. The large randomized controlled PONCHO trial has confirmed that performing laparoscopic cholecystectomy during the same hospital admission reduces the rate of recurrent pancreatitis, cholecystitis, choledocholithiasis needing ERC or gallstone colic from 17% to 5% [34]. In contrast, it is reasonable to postpone cholecystectomy in patients with severe biliary pancreatitis with peripancreatic collections until these collections are dissolved or in case of persistent collections, until at least 6 weeks after pancreatitis onset [35].

11.7 Future Clinical Management and Prevention

Surgery currently represents the mainstay of treatment of symptomatic gallstones, and it eliminates biliary colic as the most leading symptom. However, patients with uncomplicated symptomatic cholecystolithiasis may report persistent pain after cholecystectomy. In a prospective cohort multicentre study in the Netherlands, postoperative absence of abdominal pain was reported by 60.5% of patients only. A high preoperative Gastrointestinal Quality of Life Index (GIQLI) revealed that, episodic pain and duration of pain of 1 year or less were associated with postoperative absence of pain [36]. However, these factors were not associated with patient-reported improvement of abdominal symptoms or patient-reported positive cholecystectomy results, highlighting the variation of internal standards and patient expectations. With a 60% success rate, surgery can be considered only a mediocre treatment in patients with uncomplicated gallstones. Therefore, the prevention of gallstone formation or development of symptoms in gallstone carriers would be more beneficial than to stick to an invasive treatment for these patient groups with a success rate that is too low and significant complication rate [2].

Healthy lifestyle and balanced nutrition, regular physical activity and maintenance of an ideal body weight might prevent cholesterol gallbladder stones and symptomatic gallstones, but randomized controlled trials are lacking. However, a randomized intervention to increase physical activity (from 15.7 to 18.6 in the first and 10.2 to 12.1 MET-hours per week in the third trimester) did not decrease the incidence of gallbladder sludge or stones during pregnancy [37].

Specific pharmacological prevention is probably indicated in the subgroup of patients with ABCB4 deficiency. Currently it is recommended to initiate long-term treatment with ursodeoxycholic acid early in young adults with confirmed diagnosis of LPAC syndrome to prevent the occurrence or recurrence of the syndrome and its complications [38]. In fact, genetic testing can be helpful to establish the diagnosis of ABCB4 deficiency. Ursodeoxycholic acid has been demonstrated to stimulate hepatocellular and cholangiocellular secretion, reduce the cytotoxicity of the endogenous hydrophobic bile acids, increase the pool of hydrophilic bile acids, and decrease the lithogenicity of bile [39]. Ursodeoxycholic acid is also an evidence-based stone-preventive measure in situations that are associated with rapid weight loss (e.g. very low-calorie diet, bariatric surgery) [40].

Recently the following classification of ABCB4 variants has been suggested: (i) nonsense variations, missense variations that primarily affect the maturation (ii), activity (iii) or stability (iv) of the protein, or (v) mutations without detectable effect [41]. This classification provides a basis for the development of genotype-based therapies. In fact, functional rescue of trafficking-impaired ABCB4 mutants might be feasible with chemical chaperones such as 4-phenoxybutyrate [42]. In the case of LPAC syndrome-associated hypercholesterolemia, statins should be preferred. In a recent meta-analysis involving a total of 622,868 participants from six studies, current statin use was associated with a lower general risk of cholecystectomy as compared with non-use [43], but controlled studies are required to confirm these findings before statins are proposed for the primary prevention of gallstones in the general population [28].

Conclusions

Gallstone disease is a complex disorder with both genetic (i.e. *ABCG8, ABCB4)* and environmental (over nutrition, physical inactivity) factors accounting for its development. Currently surgery is the main treatment for symptomatic gallstones. However, with a 60% success rate, surgery can be considered only an ordinary treatment. Genotype-based therapies that aim to prevent gallstone formation or the development of symptoms are foreseeable.

References

1. Paumgartner G, Sauter GH. Extracorporeal shock wave lithotripsy of gallstones: 20th anniversary of the first treatment. Eur J Gastroenterol Hepatol. 2005;17:525–7.
2. Lammert F, Gurusamy K, Ko CW, Miquel JF, Mendez-Sanchez N, Portincasa P, Van Erpecum KJ, Van Laarhoven CJ, Wang DQ. Gallstones. Nat Rev Dis Primers. 2016;2:16024.
3. Ostrow JD. Unconjugated bilirubin and cholesterol gallstone formation. Hepatology. 1990;12:219S–26S.
4. Sauerbruch T, Stellaard F, Soehendra N, Paumgartner G. Cholesterol content of bile-duct stones. Dtsch Med Wochenschr. 1983;108:1099–102.
5. Hirschfield GM, Chapman RW, Karlsen TH, Lammert F, Lazaridis KN, Mason AL. The genetics of complex cholestatic disorders. Gastroenterology. 2013;144:1357–74.

6. Wang DQ, Cohen DE, Carey MC. Biliary lipids and cholesterol gallstone disease. J Lipid Res. 2009;50:S406–11.
7. Vitek L, Carey MC. New pathophysiological concepts underlying pathogenesis of pigment gallstones. Clin Res Hepatol Gastroenterol. 2012;36:122–9.
8. Katsika D, Grjibovski A, Einarsson C, Lammert F, Lichtenstein P, Marschall HU. Genetic and environmental influences on symptomatic gallstone disease: a Swedish study of 43,141 twin pairs. Hepatology. 2005;41:1138–43.
9. Lammert F, Sauerbruch T. Mechanisms of disease: the genetic epidemiology of gallbladder stones. Nat Clin Pract Gastroenterol Hepatol. 2005;2:423–33.
10. Grundy SM. Cholesterol gallstones: a fellow traveler with metabolic syndrome? Am J Clin Nutr. 2004;80:1–2.
11. Biddinger SB, Haas JT, Yu BB, Bezy O, Jing E, Zhang W, Unterman TG, Carey MC, Kahn CR. Hepatic insulin resistance directly promotes formation of cholesterol gallstones. Nat Med. 2008;14:778–82.
12. Völzke H, Baumeister SE, Alte D, Hoffmann W, Schwahn C, Simon P, John U, Lerch MM. Independent risk factors for gallstone formation in a region with high cholelithiasis prevalence. Digestion. 2005;71:97–105.
13. Aune D, Leitzmann M, Vatten LJ. Physical activity and the risk of gallbladder disease: a systematic review and meta-analysis of cohort studies. J Phys Act Health. 2016;13:788–95. [epub ahead of print]
14. Tsai CJ, Leitzmann MF, Willett WC, Giovannucci EL. Glycemic load, glycemic index, and carbohydrate intake in relation to risk of cholecystectomy in women. Gastroenterology. 2005;129:105–12.
15. Tsai CJ, Leitzmann MF, Willett WC, Giovannucci EL. Long-chain saturated fatty acids consumption and risk of gallstone disease among men. Ann Surg. 2008;247:95–103.
16. Aune D, Norat T, Vatten LJ. Body mass index, abdominal fatness and the risk of gallbladder disease. Eur J Epidemiol. 2015;30:1009–19.
17. Aune D, Vatten J. Diabetes mellitus and the risk of gallbladder disease: a systematic review and meta-analysis of prospective studies. J Diabetes Complicat. 2016;30:368–73.
18. Buch S, Schafmayer C, Völzke H, Becker C, Franke A, von Eller-Eberstein H, Kluck C, Bässmann I, Brosch M, Lammert F, Miquel JF, Nervi F, Wittig M, Rosskopf D, Timm B, Höll C, Seeger M, ElSharawy A, Lu T, Egberts J, Fändrich F, Fölsch UR, Krawczak M, Schreiber S, Nürnberg P, Tepel J, Hampe J. A genome-wide association scan identifies the hepatic cholesterol transporter ABCG8 as a susceptibility factor for human gallstone disease. Nat Genet. 2007;39:995–9.
19. Stender S, Frikke-Schmidt R, Nordestgaard BG, Tybjaerg-Hansen A. Sterol transporter adenosine triphosphate-binding cassette transporter G8, gallstones, and biliary cancer in 62,000 individuals from the general population. Hepatology. 2011;53:640–8.
20. Krawczyk M, Wang DQ, Portincasa P, Lammert F. Dissecting the genetic heterogeneity of gallbladder stone formation. Semin Liver Dis. 2011;31:157–72.
21. Von Kampen O, Buch S, Nothnagel M, Azocar L, Molina H, Brosch M, Erhart W, Von Schönfels W, Egberts J, Seeger M, Arlt A, Balschun T, Franke A, Lerch MM, Mayerle J, Kratzer W, Boehm BO, Huse K, Schniewind B, Tiemann K, Jiang ZY, Han TQ, Mittal B, Srivastava A, Fenger M, Jørgensen T, Schirin-Sokhan R, Tönjes A, Wittenburg H, Stumvoll M, Kalthoff H, Lammert F, Tepel J, Puschel K, Becker T, Schreiber S, Platzer M, Völzke H, Krawczak M, Miquel JF, Schafmayer C, Hampe J. Genetic and functional identification of the likely causative variant for cholesterol gallstone disease at the ABCG5/8 lithogenic locus. Hepatology. 2013;57:2407–17.
22. Krawczyk M, Lütjohann D, Schirin-Sokhan R, Villarroel L, Nervi F, Pimentel F, Lammert F, Miquel JF. Phytosterol and cholesterol precursor levels indicate increased cholesterol excretion and biosynthesis in gallstone disease. Hepatology. 2012;55:1507–17.
23. Buch S, Schafmayer C, Völzke H, Seeger M, Miquel JF, Sookoian SC, Egberts JH, Arlt A, Pirola CJ, Lerch MM, John U, Franke A, von Kampen O, Brosch M, Nothnagel M, Kratzer W,

Boehm BO, Bröring DC, Schreiber S, Krawczak M, Hampe J. Loci from a genome-wide analysis of bilirubin levels are associated with gallstone risk and composition. Gastroenterology. 2010;139:1942–51.
24. Joshi AD Andersson C, Buch S, Stender S, Noordam R, Weng LC, Weeke PE, Auer PL, Boehm B, Chen C, Choi H, Curhan G, Denny JC, De Vivo I, Eicher JD, Ellinghaus D, Folsom AR, Fuchs C, Gala M, Haessler J, Hofman A, Hu F, Hunter DJ, Janssen HL, Kang JH, Kooperberg C, Kraft P, Kratzer W, Lieb W, Lutsey PL, Darwish Murad S, Nordestgaard BG, Pasquale LR, Reiner AP, Ridker PM, Rimm E, Rose LM, Shaffer CM, Schafmayer C, Tamimi RM, Uitterlinden AG, Völker U, Völzke H, Wakabayashi Y, Wiggs JL, Zhu J, Roden DM, Stricker BH, Tang W, Teumer A, Hampe J, Tybjærg-Hansen A, Chasman DI, Chan AT, Johnson AD. Four susceptibility loci for gallstone disease identified in a meta-analysis of genome-wide association studies. Gastroenterology. 2016; 151(2):351–363.
25. Bustos BI, Pérez-Palma E, Buch S, Azócar L, Moraga C, Toilat M, Hampe J, Nürnberg P, Gutiérrez RA, De Ferrari GV, Miquel JF. Genome-wide association study for gallstone disease in Latin Chilean population. Abstract 910F, presented at the 65th annual meeting of the American Society of Human Genetics. 7 Oct 2015, Baltimore.
26. Poupon R, Rosmorduc O, Boëlle PY, Chrétien Y, Corpechot C, Chazouillères O, Housset C, Barbu V. Genotype-phenotype relationships in the low-phospholipid-associated cholelithiasis syndrome: a study of 156 consecutive patients. Hepatology. 2013;58:1105–10.
27. Gudbjartsson DF, Helgason H, Gudjonsson SA, Zink F, Oddson A, Gylfason A, Besenbacher S, Magnusson G, Halldorsson BV, Hjartarson E, Sigurdsson GT, Stacey SN, Frigge ML, Holm H, Saemundsdottir J, Helgadottir HT, Johannsdottir H, Sigfusson G, Thorgeirsson G, Sverrisson JT, Gretarsdottir S, Walters GB, Rafnar T, Thjodleifsson B, Bjornsson ES, Olafsson S, Thorarinsdottir H, Steingrimsdottir T, Gudmundsdottir TS, Theodors A, Jonasson JG, Sigurdsson A, Bjornsdottir G, Jonsson JJ, Thorarensen O, Ludvigsson P, Gudbjartsson H, Eyjolfsson GI, Sigurdardottir O, Olafsson I, Arnar DO, Magnusson OT, Kong A, Masson G, Thorsteinsdottir U, Helgason A, Sulem P, Stefansson K. Large-scale whole-genome sequencing of the Icelandic population. Nat Genet. 2015;47:435–44.
28. Lammert F, Acalovschi M, Ercolani G, van Erpecum KJ, Gurusamy KS, van Laarhoven CJ, Portincasa P; European Association for the Study of the Liver. EASL Clinical Practice Guidelines on the prevention, diagnosis and treatment of gallstones. J Hepatol. 2016;65(1):146–81.
29. Shabanzadeh DM, Sørensen LT, Jørgensen T. A prediction rule for risk stratification of incidentally discovered gallstones: results from a large cohort study. Gastroenterology. 2016;150:156–67.
30. Besselink MG, Venneman NG, Go PM, Broeders IA, Siersema PD, Gooszen HG, et al. Is complicated gallstone disease preceded by biliary colic? J Gastrointest Surg. 2009;13:312–7.
31. Gutt CN, Encke J, Köninger J, Harnoss JC, Weigand K, Kipfmüller K, et al. Acute cholecystitis: early versus delayed cholecystectomy, a multicenter randomized trial (ACDC study, NCT00447304). Ann Surg. 2013;258:385–93.
32. Giljaca V, Gurusamy KS, Takwoingi Y, Higgie D, Poropat G, Štimac D, Davidson BR. Endoscopic ultrasound versus magnetic resonance cholangiopancreatography for common bile duct stones. Cochrane Database Syst Rev. 2015;2:CD011549.
33. Reinders JS, Goud A, Timmer R, Kruyt PM, Witteman BJ, Smakman N, Breumelhof R, Donkervoort SC, Jansen JM, Heisterkamp J, Grubben M, van Ramshorst B, Boerma D. Early laparoscopic cholecystectomy improves outcomes after endoscopic sphincterotomy for choledochocystolithiasis. Gastroenterology. 2010;138:2315–20.
34. Da Costa DW, Bouwense SA, Schepers NJ, Besselink MG, van Santvoort HC, van Brunschot S, Bakker OJ6, Bollen TL, Dejong CH, van Goor H, Boermeester MA, Bruno MJ, van Eijck CH, Timmer R, Weusten B, Consten EC, Brink MA, Spanier BW, Bilgen EJ, Nieuwenhuijs VB, Hofker HS, Rosman C, Voorburg AM, Bosscha K, van Duijvendijk P, Gerritsen JJ, Heisterkamp J, de Hingh IH, Witteman BJ, Kruyt PM, Scheepers JJ, Molenaar IQ, Schaapherder AF, Manusama ER, van der Waaij LA, van Unen J, Dijkgraaf MG, van Ramshorst B, Gooszen HG, Boerma D. Dutch Pancreatitis Study Group. Same-admission versus interval cholecystec-

tomy for mild gallstone pancreatitis (PONCHO): a multicentre randomised controlled trial. Lancet. 2015;386:1261–8.
35. Nealon WH, Bawduniak J, Walser EM. Appropriate timing of cholecystectomy in patients who present with moderate to severe gallstone-associated acute pancreatitis with peripancreatic fluid collections. Ann Surg. 2004;239:741–9.
36. Lamberts MP, Den Oudsten BL, Gerritsen JJ, Roukema JA, Westert GP, Drenth JP, van Laarhoven CJ. Prospective multicentre cohort study of patient-reported outcomes after cholecystectomy for uncomplicated symptomatic cholecystolithiasis. Br J Surg. 2015;102:1402–9.
37. Ko CW, Napolitano PG, Lee SP, Schulte SD, Ciol MA, Beresford SA. Physical activity, maternal metabolic measures, and the incidence of gallbladder sludge or stones during pregnancy: a randomized trial. Am J Perinatol. 2014;31:39–48.
38. Rosmorduc O, Poupon R. Low phospholipid associated cholelithiasis: association with mutation in the MDR3/ABCB4 gene. Orphanet J Rare Dis. 2007;2:29.
39. Paumgartner U, Beuers U. Ursodeoxycholic acid in cholestatic liver disease: mechanisms of action and therapeutic use revisited. Hepatology. 2002;36:525–31.
40. Stokes CS, Gluud LL, Casper M, Lammert F. Ursodeoxycholic acid and diets higher in fat prevent gallbladder stones during weight loss: a meta-analysis of randomized controlled trials. Clin Gastroenterol Hepatol. 2014;12:1090–100.
41. Delaunay JL, Durand-Schneider AM, Dossier C, Falguières T, Gautherot J, Anne DS, Aït-Slimane T, Housset C, Jacquemin E, Maurice M. A functional classification of ABCB4 variations causing progressive familial intrahepatic cholestasis type 3. Hepatology. 2015;63:1620–31.
42. Gordo-Gilart R, Andueza S, Hierro L, Jara P, Alvarez L. Functional rescue of trafficking-impaired ABCB4 mutants by chemical chaperones. PLoS One. 2016;11:e0150098. epub ahed of print
43. Kan HP, Guo WB, Tan YF, Zhou J, Liu CD, Huang YQ. Statin use and risk of gallstone disease: a meta-analysis. Hepatol Res. 2014; epub.

IgG4-Related Sclerosing Cholangitis

12

Emma L. Culver and George Webster

Abstract

Immunoglobulin G4-related sclerosing cholangitis (IgG4-SC) is the biliary manifestation of a multi-organ chronic fibroinflammatory condition, IgG4-related disease. Clinically it may mimic other biliary disease, including primary sclerosing cholangitis and cholangiocarcinoma. IgG4-SC may lead to progressive fibrosclerotic disease and cirrhosis. There is no specific test to make the diagnosis, which is based on clinical, laboratory, radiological and pathological features. Steroids and other immunosuppressive therapy may lead to clinical and radiological improvement when given in the early phase of disease, but the clear evidence base for treatment regimens is limited. Over the last decade, progress has been made in understanding the immunopathogenesis of IgG4-SC, including the role of HLA class II susceptibility molecules, circulating memory B cells and plasmablasts, T helper 2 and regulatory cells, chemokine-mediated trafficking and the innate immune system.

Take-Home Points

- Immunoglobulin G4-related sclerosing cholangitis (IgG4-SC) is the biliary manifestation of a multisystem fibroinflammatory condition, IgG4-related disease (IgG4-RD).
- IgG4-SC can present with biliary strictures and/or masses, which makes it difficult to differentiate from primary sclerosing cholangitis (PSC), or malignancies such as cholangiocarcinoma (CCA) and pancreatic cancer.

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G. Hirschfield et al. (eds.), *Biliary Disease*, DOI 10.1007/978-3-319-50168-0_12

- Diagnosis of IgG4-SC is based on a combination of clinical, biochemical, radiological and histological findings. A gold standard diagnostic test for IgG4-SC is lacking, warranting the identification of more specific disease markers to aid clinicians, such as the ratio of IgG1/IgG4 to distinguish IgG4-SC from PSC high IgG4, PCR-based clonal assays and plasmablast markers in peripheral blood.
- Treatment with corticosteroids and other disease-modifying immunosuppressive and biological agents is not standardised or supported by prospective randomised controlled trials, and this will require international collaboration.
- Insights into the genetic and immunological aspects of the disease have increased over the last decade, with emphasis on HLA class II susceptibility molecules, circulating memory B cells and plasmablasts, T helper 2 and regulatory cells, chemokine-mediated trafficking and the role of the innate immune system in disease.

12.1 Introduction

Over the last decade, significant clinical and scientific attention has surrounded the concept and definition of IgG4-related disease (IgG4-RD). This fibroinflammatory multiple-system disease may particularly affect the pancreas (autoimmune pancreatitis (AIP) type 1, or IgG4-related pancreatitis) and the biliary tree. The focus of this chapter is the biliary manifestation of IgG4-RD, termed IgG4-related sclerosing cholangitis (IgG4-SC). The epidemiology, clinical features, diagnostic classification, disease mimics and treatment strategies will be outlined. Our present understanding of the aetiopathogenesis of IgG4-SC, and how that might be applied to future therapies, will be particularly explored.

12.2 Epidemiology

The epidemiology of IgG4-SC appears to mirror that of IgG4-related pancreatitis/AIP type 1 and is incompletely defined. This may relate in parts to the only recent recognition of the condition, the absence of a single or simple diagnostic test, and the lack of consensus on criteria by which to make a definitive diagnosis. IgG4-SC has a male preponderance and usually presents in the fifth and sixth decades of life. IgG4-SC appears to be the commonest extra-pancreatic manifestation in patients with of AIP type 1, occurring in 20–88% of cases.

12.3 Clinical Presentation

The clinical presentation may be varied but most usually is that of obstructive jaundice, weight loss and abdominal pain. Jaundice is the most common presenting symptom affecting 70–80% of patients. There are no specific symptoms that allow reliable

differentiation from other causes of biliary obstruction. The diagnosis may be reached during the investigation of non-specific general symptoms in the setting of abnormal liver function tests. IgG4-SC may be diagnosed asymptomatically and incidentally in a patient presenting with symptoms related to other organ affected by IgG4-related disease.

12.4 Diagnostic Features

The diagnosis of IgG4-SC depends on a wide range of clinical, radiological, pathological and laboratory parameters. No single test in isolation allows a diagnosis to be made, although IgG4-SC diagnostic criteria have been created from a combination of findings [1].

12.4.1 Imaging

Imaging may allow the initial clinical suspicion of IgG4-SC to be raised. Abdominal ultrasound may demonstrate biliary dilatation or pancreatic enlargement (a hint towards AIP), but cross-sectional imaging is vital. CT scanning may demonstrate biliary stricturing, with thickened bile duct walls and an associated liver/hilar mass ('inflammatory pseudotumour'). Importantly it may define 'other organ involvement', including pancreatic, renal, retroperitoneal disease and generalised lymphadenopathy. Magnetic resonance imaging and cholangiopancreatogram (MRI/MRCP) may provide similar information, and also cholangiography and pancreatic duct definition. The findings of proximal biliary disease in conjunction with imaging features suggestive of AIP (e.g. diffuse pancreatic swelling, with a thin, diffusely narrowed pancreatic duct) should particularly raise the question of IgG4-SC. Endoscopic retrograde cholangiopancreatogram (ERCP) is indicated in many patients presenting with symptomatic biliary structuring, as a means to decompress the biliary tree, usually by means of stent insertion. At the same time, cholangiography may advance the diagnosis (or at least raise the clinical suspicion) of IgG4-SC.

IgG4-SC may be manifested by involvement of any part of the biliary tree, and a classification based on four sites of involvement has been described [2] (Fig. 12.1). Type 1 is defined by a low bile duct stricture, which is most frequently associated with AIP and corresponds with compression of the intra-pancreatic bile duct by inflammation/fibrosis within the head of the pancreas. Type 2 is defined by both a

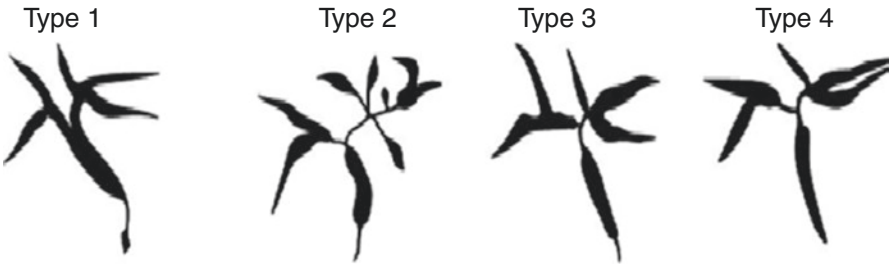

Fig. 12.1 Classification of IgG4-SC by cholangiographic features [2]

diffuse intrahepatic cholangiopathy and a lower CBD stricture. Type 3 includes a hilar and lower CBD stricture, and type 4 is defined by hilar stricturing alone. The type determines the differential diagnosis for IgG4-SC. Type 1: pancreatic cancer, distal cholangiocarcinoma, chronic pancreatitis. Type 2: primary sclerosing cholangitis (PSC) and those for type 1. Type 3: hilar cholangiocarcinoma and those for type 1. Type 4: hilar cholangiocarcinoma and is the most difficult to differentiate.

Particular cholangiographic features have been reported as favouring a diagnosis of IgG4-SC, including long strictures, multifocal strictures and mild upstream dilatation only [3–5]. However, in a study in which experts were required to differentiate IgG4-SC, PSC and CCA based on cholangiogram alone, this modality provided 88% specificity but only 45% sensitivity [6]. Cholangioscopy allows direct visualisation of intrabiliary mucosa and stricture assessment. As well as facilitating targeted biopsies, cholangioscopic features in IgG4-SC may be characteristic [7].

12.4.2 Serum IgG4

Raised levels of serum IgG4 have historically been used in diagnostic criteria of AIP, with >95% specificity reported in early studies [8]. More recent studies in AIP have suggested a much lower sensitivity (30–70%) and specificity (62–83%). Serum IgG4 level has been used to attempt to distinguish IgG4-SC from primary sclerosing cholangitis (PSC) and cancer (both pancreatic and cholangiocarcinoma). One study attempted to establish the optimal cut-off of IgG4 levels in order to differentiate between the subgroups of IgG4-SC, identifying a cut-off of 182 mg/dL and greater than 207 mg/dL for distinguishing types 3 and type 4 IgG4-SC from CCA with a specificity of 97% and 100%, respectively [9]. Another study described the utility of a serum IgG1/IgG4 ratio of 0.24 in differentiating IgG4-SC from PSC patients with an elevated serum IgG4 level between 140 mg/dL and 2.8 mg/dL [10]. However, serum IgG4 levels alone are not a sufficiently sensitive or specific tool to be able to reliably distinguish IgG4-SC from PSC or HPB malignancy.

12.4.3 Tissue Sampling

Tissue acquisition and a formal pathological diagnosis are increasingly viewed as a central focus of establishing the diagnosis of IgG4-RD, and the same is true of IgG4-SC. This is particularly important given the extent to which clinical and radiological features of IgG4-SC may mimic those of other benign and malignant conditions, including PSC and CCA, and the divergent treatment approaches to each. Cytological samples, whether from brush cytology of biliary strictures or endoscopic ultrasound (EUS)-guided fine-needle aspiration (FNA) may be important in excluding malignancy (albeit with sensitivity for biliary cytology of only 20–50% for malignancy) but rarely allow a definitive diagnosis of IgG4-SC to be made. Intrabiliary biopsies may be obtained at the time of ERCP through either fluoroscopy-directed biopsies or more recently via the use of cholangioscopy, allowing visually directed biopsies [11]. Ampullary biopsies may advance the diagnosis of IgG4-SC, in the setting of an IgG4-positive lymphoplasmacytic infiltrate, but other diagnostic features are rarely present [12].

A similar pathological lesion is seen in all tissues affected in IgG4-RD and is central to the recently agreed international consensus on the diagnosis of IgG4-RD [13]. Tissue IgG4 and IgG immunostaining should be performed on biopsy and resection specimens, and a mean IgG4 count (in three high-power fields (HPF)) and ratio of IgG4 to total IgG should be calculated. For IgG4-SC, >10 IgG4+cells/HPF in a biopsy specimen, or >50 IgG4+cells/HPF in a resection specimen, and an IgG4/IgG ratio >40%, in the context of two of three classical features, are considered diagnostic in the appropriate clinical context. However, tissue sampling can be patchy and not all features may be seen in a single specimen [14].

12.5 Differential Diagnosis

Differentiating IgG4-SC from other benign and malignant causes of biliary strictur-ing is of fundamental importance. PSC appears to be particularly associated with raised levels of serum IgG4, occurring in 9%–18% of patients, compared with <1% of other biliary conditions [15]. Although there is considerable disease overlap between IgG4-SC and PSC [16], IgG4+ PSC appears to be distinct, with different HLA associations and carries a higher risk of disease progression than IgG4-ve PSC [17]. In this instance, a ratio of IgG1/IgG4 may be useful in distinguishing IgG4+ PSC from IgG4-SC [10]. CCA may occur in up to 30% of patients with PSC and also be a de novo cause of biliary stricturing and a hilar mass. An inflammatory 'pseudo-tumour' may be seen in IgG4-SC, with imaging features that may mimic malignancy. In a retrospective study of patients who had undergone liver surgery for presumed CCA, 8% were found to have histology on resection consistent with IgG4-SC [18].

12.6 Treatment

The mainstay of medical treatment in IgG4-SC is the use of systemic corticoste-roids, despite the absence of randomised placebo controlled trials. Current approaches to medical therapy are largely extrapolated from the treatment of other manifestations of IgG4-RD, and AIP in particular. Although spontaneous remission can occur in AIP, steroids have been shown to induce remission quicker, more consistently and have a lower relapse rate. Significant spontaneous cholangiographic improvement of type 1 IgG4-SC may be seen (with stricture improvement probably correlating with reduced pancreatic inflammation around the low bile duct), but in types 2–4 IgG4-SC an improvement without treatment is unusual.

International consensus regarding the role of oral corticosteroids in initiation therapy in IgG4-RD has recently been reached [19], and a starting dose of prednisolone 30-40 mg daily for 4 weeks, before reducing by 5 mg every subsequent 14 days, is widely employed. Patients are reviewed regularly, given steroid-induced side effects and the increased risk of cholangitis/sepsis with use of steroids and biliary obstruction. Improvement in bile duct stricturing and normalisation of liver enzymes following the introduction of steroid therapy have been shown [1]. In the setting of biliary obstruction and hilar or dominant extrahepatic strictures, biliary stenting at ERCP is usually indicated, even if a response to steroids is expected. A clinical and

cholangiographic improvement should be seen within 4–6 weeks of starting treatment and be seen on repeat imaging (MRCP, ERCP or CT). Non-response to steroid therapy may be representative of a less inflammatory 'burnt-out' disease, and a more fibrotic phenotype, or an alternative diagnosis.

12.7 Relapse

Patients with IgG4-SC are at high risk of relapse, either after stopping steroid treatment or during the initial taper. In a study of patients with IgG4-SC and AIP, 57% (13 of 23) of patients relapsed [20]. In a further study of 53 patients with IgG4-SC, a similar relapse rate was reported in patients that underwent surgery ($n = 18$) or treatment with steroids ($n = 30$) (44% vs 54%, $p = 0.1$). Factors predictive of relapse included the presence of proximal strictures (IgG4-SC type 2–4) and increased IgG4 levels [1]. Relapses that occur after steroid withdrawal/reduction may be treated with a further course of steroids followed by additional immunomodulatory drugs, e.g. azathioprine (target dose 2 mg/kg/day). However the benefits of the addition of immunomodulators over low-dose steroids alone in reducing time to further relapses are uncertain [21]. Predictors of resistance to immunomodulator therapy include evidence of other organ involvement other than IgG4-SC and retroperitoneal fibrosis ($p < 0.03$).

Certain patients may be intolerant of immunomodulatory drugs and become dependent on high-dose steroids in order to maintain remission. Rituximab, a monoclonal antibody depletion therapy directed against the CD20 antigen on B cells, has been described in case series and a prospective open-label study of patients with AIP and IgG4-RD [21, 22]. In the Mayo study, complete radiographic remission was achieved in 80% of AIP patients, at a median of 4.5 months, although all these patients required additional maintenance therapy with rituximab. Rituximab may find a role in the treatment of patients with Ig4-SC who have failed previous steroid therapy or immunomodulatory therapy or perhaps in a more 'top-down' approach for sparing of long-term steroid-related side effects.

12.8 Clinical Course

Long-term outcome in patients with IgG4-SC is lacking. However, in a UK study of 116 patients with IgG4-RD, with 34 months of follow-up, cirrhosis in the setting of IgG4-SC occurred in 5% of patients [23]. An increased risk of CCA in patients with IgG4-SC is not proven, although there have been case reports suggesting an association. An all-cause increased risk of malignancy in IgG-SC and AIP patients, including HPB cancers, has been shown [23].

12.9 Pathogenesis of Disease

Although the pathogenic mechanisms underlying IgG4-SC are poorly understood, insights into the genetic and immunological aspects of the disease have increased over the last decade. Most of these studies have focused on patients with AIP and/or

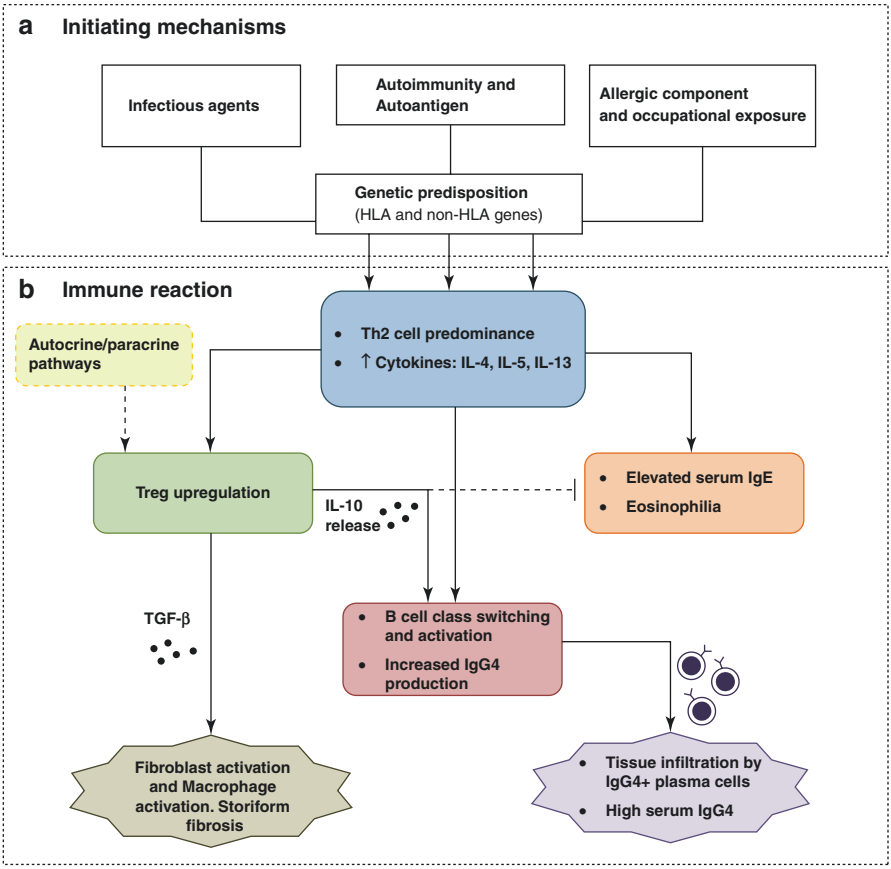

Fig. 12.2 Disease pathways considered in the pathogenesis of IgG4-RD (Proposed mechanisms involved in disease initiation include infectious agents (such as *Helicobacter pylori* via molecular mimicry), local autoantigens (including environmental antigens) and allergic components in a genetically predisposed individual. This is thought to activate a Th2 and T regulatory immune reaction characterised by increased production of IL4, IL5, IL10, IL13 and TGF-β at affected sites. Activated B cells may class switch to IgG4-producing plasmablasts in the circulation and IgG4 plasma cells in the tissue, whilst T regulatory responses drive tissue fibrosis)

IgG4-related sialoadenitis, and thus the findings are extrapolated to IgG4-SC. Disease pathways believed to be involved in the disease are shown in Fig. 12.2.

12.10 Genetic Studies

Studies have identified human leukocyte antigen (HLA) molecules and other immune-regulatory genes as determinants of disease susceptibility to AIP, disease relapse after steroid therapy and extra-pancreatic disease [24–26].

12.10.1 HLA Associations

HLA molecules are highly polymorphic and have a central role in the T-cell response. HLA-DR and CD4+ lymphocytes are expressed on the pancreatic duct and acinar cells in AIP patients suggesting a plausible role for the HLA class II pathway in disease. The first HLA association study reported higher frequencies of the HLA-DRB1*0405-DQB1*0401 haplotype in Japanese AIP patients compared to healthy individuals and in patients with chronic calcifying pancreatitis [24]. Such haplotypes have similarly been reported in autoimmune diseases such as rheumatoid arthritis, autoimmune hepatitis and type I diabetes mellitus. A non-aspartic amino acid at DQB1*57 was significantly associated with relapse in Korean AIP patients [25], although these findings were not confirmed in a Japanese cohort [26]. Different class II HLA associations have been described in a systemic IgG4-RD UK cohort (personal comm.). The differences in class II HLA associations are likely due to ethnic variation, which has been reported in other diseases such as multiple sclerosis, but will require further validation.

12.10.2 Single Nucleotide Polymorphisms in Immune-Regulatory Genes

Single nucleotide polymorphisms involved in disease susceptibility or recurrence have been reported to be present within gene-encoding proteins including cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), tumour necrosis factor-alpha (TNFα) and Fc receptor-like 3 (FcR-3) [27–29] (Table 12.1).

CTLA-4 49A haplotype, a critical attenuator of T-cell activation and an essential component of the regulatory system involved in several autoimmune diseases, was reported at higher frequency in AIP patients compared with healthy individuals in China [29]. SNPs of CTLA-4 have been linked to AIP disease susceptibility in China, disease resistance in Japan and an increased risk of relapse. TNF-a promoter 863A haplotype was suggested to be important in extra-pancreatic disease in Chinese patients. Polymorphisms of the FCRL3 molecule, expressed on B cells and

Table 12.1 Genetic susceptibility factors in patients with AIP and IgG4-SC

Proteins	SNP	Association
CTLA-4	49A haplotype	Higher frequencies in Chinese patients
	−318C/+49A/CT60G	AIP susceptibility in Chinese patients
	+6230 3′-untranslated region	AIP susceptibility in Japanese patients
	+6230G/G	AIP susceptibility in Japanese patients
	+6230A	AIP resistance in Japanese patients
	49A/A and +6230A/A genotypes	AIP relapse in Japanese patients
TNF-α	863A haplotype	Extra-pancreatic involvement in Chinese patients
FcRL-3	−110A/A genotype	AIP susceptibility in Japanese patients

known to augment autoantibody production in individuals with disease-susceptible genotypes, have been shown to contribute to susceptibility to autoimmune diseases. FCRL3 –110A/A has been associated with disease susceptibility in Japanese AIP patients - the number of susceptible alleles correlating with serum IgG4 concentrations [28].

12.11 Antigen Targets

There has been a sustained quest to find an antigen initiating and/or driving AIP/IgG4-SC, and although no specific antigen has yet been identified, the presence of oligoclonal plasmablasts and B cells in peripheral blood and tissues supports an antigen-mediated response [30–32].

12.11.1 Autoantigens

Based on the hypothesis that AIP (and IgG4-SC) is an autoimmune disorder, antibodies against a range of autoantigens have been proposed, including antinuclear antigens, lactoferrin (LF), carbonic anhydrase (CA)-II and IV, pancreatic secretory inhibitor, trypsinogens, anti-amylase α-2A and anti-heat-shock protein 10 [33–35] (Table 12.2). However, none has been consistently found in the disease, and those tested are of IgG1 and not IgG4 subclass. Autoantibodies against CA-II and LF are frequently detected in the serum of patients with AIP (54% and 73%, respectively) but they are non-specific. A proteomics study identified a 13.1 kDa protein as a candidate autoantigen, but the sequence has never been clarified.

12.11.2 Microbial Antigens

Gastric *Helicobacter pylori* infection was proposed to trigger AIP in genetically predisposed individuals through a process of antibody cross-reactivity (molecular mimicry). This theory was based on finding sequence homology between human CA-II and α-CA of *H. pylori*, the homologous segment containing the binding motif of the susceptibility HLA-DRB1*0405 molecule, in a Japanese AIP cohort [36], and sequence homology between the plasminogen-binding protein (PBP) of *H. pylori* and ubiquitin-protein ligase E3 component n-recognin 2 expressed in pancreatic acinar cells, in an Italian AIP cohort [37]. Antibodies against *H. pylori* PBP were detected in 94% of AIP patients but were not disease specific. A recent study has suggested no increased risk of *H. pylori* infection, peptic ulceration or immunological memory to *H. pylori* PBP in patients with IgG4-SC and AIP from a UK cohort (personal comm.). Other studies could not detect *H. pylori* DNA in tissue or pancreatic juice from AIP patients, suggesting no direct infection.

Table 12.2 Autoantibodies in AIP

Autoantibody/ autoantigen	Number of AIP patients	Origin of patients	Frequency in AIP (%)	Frequency in disease and healthy controls (%)
Anticarbonic anhydrase-II	17	Japanese	59	–
	54	Japanese	28	10.5 alcoholic pancreatitis, 64 Sjögren's, 1.9 HCs
	48	European	12.5	0 HCs
Antilactoferrin	17	Japanese	76	–
	48	European	20.8	0
Plasminogen-binding protein	35	European	94	10 pancreatic cancer, 0 alcoholic pancreatitis, 0 IPMN
Anticarbonic anhydrase-IV	–	Japanese	27	0 HCs, 45 Sjögren's
Heat-shock protein 10	–	Japanese	92	81 type I DM, 8 alcoholic pancreatitis, 1.4 HCs
Amylase-2α	15	Japanese	100	88 type 1 DM, 6 type II DM, 0 alcoholic pancreatitis, 0 pancreatic cancer
Antitrypsinogen	19	German	79	10 non-AIP chronic cholangitis and HCs
Anti-pancreatic secretory trypsin inhibitor	26	Japanese	30.8	0

12.11.3 IgG4 Autoantibodies

Convincing evidence that IgG4 itself is driving the pathology of IgG4-RD is lacking. Importantly, IgG4-type autoantibodies have not been detected in IgG4-SC, although IgG4 from AIP patient's serum can bind to normal human tissue [38]. IgG4 autoantibodies have however been shown to play an important role in immune-mediated disorders unrelated to AIP/IgG4-SC. These include IgG4 antibodies against desmoglein 1 in pemphigus vulgaris and foliaceus, MuSK protein in myasthenia gravis, the M-type phospholipase A2 receptor in patients with idiopathic membranous glomerulonephritis and IgG4-containing immune complexes that damage renal glomeruli in subsets of childhood membranous glomerulonephritis.

12.11.4 Environmental and Occupational Antigens

The concept of chronic exposure to one or multiple antigens has recently been explored in IgG4-SC. One study has provided evidence for a polyclonal IgG4 response to multiple non-infectious environmental antigens in IgG4-SC and to a lesser extent in PSC patients with high serum IgG4 levels [39]. Antigen-specific

responses correlated with the serum IgG4 level, suggesting that elevated IgG4 could be the result of a polyclonal expansion of many IgG4 B cells irrespective of their specificity, and thus that pre-existing subsets of IgG4 B cells expand and differentiate in the course of developing IgG4-RD. Observational data highlighted a history of chronic occupational and environmental exposures in Dutch and UK IgG4-SC cohorts, supported by the production of IgG4 antibodies in the context of chronic antigenic stimulation [40]. Furthermore, next-generation sequencing demonstrated oligoclonal expansion of plasmablasts in the blood of IgG4-RD patients in a US cohort (Mattoo et al. 2014) and oligoclonal IgG4+ B cells in whole blood and tissue of AIP/IgG4-SC patients in Dutch and UK cohorts (Maillette de Buy Wenniger et al. 2013; Hubers et al. 2015), with different dominant clones present at disease relapse.

Whilst the oligoclonality of the IgG4 response makes it unlikely that these B cells initiate IgG4-RD, it does not preclude it to be an important contributing factor to maintenance of disease progression once established. Factors other than antigen can drive proliferation and expansion of IgG4-switched cells (such as IL21), enhance class switch to the IgG4 subclass (such as IL4, IL13 and IL10) and drive class switch recombination, somatic hypermutation and affinity maturation (such as the upregulation of activation-induced cytidine deaminase, B lymphocyte-induced maturation protein 1 and Xbox protein 1), all of which have been shown to be present in IgG4-RD patients [41]. This however does not exclude the possibility that a single antigen (self or exogenous) serves as the initial trigger of disease.

12.12 Immune-Mediated Pathways

An immune-mediated phenomenon in IgG4-SC is supported by the presence of serological abnormalities (elevated IgG and IgG4, non-specific autoantibodies), infiltration of the affected tissues with lymphocytes and plasma cells (mostly CD4+ T cells, CD20+ B cells and IgG4-positive CD138+ plasma cells) and a dramatic response to corticosteroids.

12.12.1 T Regulatory Cells

Interestingly, regulatory immune reactions are activated in AIP/IgG4-SC rather than suppressed as in many other autoimmune disorders. An infiltration of inducible-memory T regulatory cells (Tregs) in the affected tissue and blood of patients with IgG4-SC and AIP are associated with upregulation of regulatory cytokines IL-10 and transforming growth factor-β (TGF-β), which have been suggested to play important roles in IgG4 class switching and fibroplasia, respectively [42]. Numbers of infiltrating Tregs in bile duct tissue correlate with IgG4-positive plasma cells in IgG4-SC, whilst numbers of circulating Tregs in peripheral blood correlate with serum IgG4 levels in AIP.

12.12.2 T Helper 2 Cells

A T helper 2 (Th2)-dominant immune response is seen in peripheral blood and tissues in IgG4-SC and AIP, with an increased production of Th2 cytokines such as IL4, IL5, IL10 and IL13. However, recent studies suggest these circulating Th2 cells may be restricted to atopic patients [43]. It is postulated that these Th2 responses mediate humoral IgG4 responses, eosinophilia and elevated serum IgE levels, supported by in vivo evidence of IL4 and IL13-induced switch of naïve B cells to both IgG4 and IgE. Furthermore, IL10 can potentiate IL-4-mediated class switch to IgG4, whilst suppressing production of IgE.

12.12.3 Memory B Cells and Plasmablasts

Important differences in the frequency and phenotype of IgG1 and IgG4 B cells in health and IgG4-RD have been identified. In particular, differences in their potential to react to complement activation and immune complexes may help explain the differential regulation of the IgG4 antibody response. The frequency of IgG4 memory B cells in the blood correlates with serum IgG4 levels in IgG4-RD, providing support that these cell types are responsible for the elevated serum IgG4 level [44]. Expansions of plasmablasts in the blood have recently been reported in active disease and decrease in response to the B cell depletion agent, rituximab [30]. Given the high levels of surface MHC class II (HLA-DR) on plasmablasts, one could speculate that these cells serve as vital antigen-presenting cells to CD4+ T cells in patients with IgG4-RD, but it remains possible that these plasmablasts are secondarily induced bystanders in this disease.

12.12.4 Mast Cells and IGE

An elevated serum IgE and the presence of IgE-positive mast cells in involved tissues of patients with IgG4-RD points to the role of an IgE-mediated response. The strong links between IgG4 and IgE are well established. Importantly IL4, IL13 and IL10 cytokines are secreted not only by Th2 and Treg cells but also by mast cells, of which IgE is a key stimulator. In a subset of individuals with IgG4-RD with a history of allergy/atopy and elevated IgE levels, it is plausible that elevated serum IgG4, resulting from polyclonal expansion of many IgG4 B cells irrespective of antigen specificity, may be linked to IgE B-cell expansion. A specific allergen-driving disease has not been identified.

12.12.5 Chemokine Signalling

Involvement of chemokine signalling pathways, which is believed to involve the pathogenesis of immune-mediated hepatobiliary disorders such as primary

sclerosing cholangitis and primary biliary cirrhosis (cholangitis), has also been implicated in IgG4-SC/AIP. A role for CCL1-CCR8 interaction in lymphocytic recruitment in IgG4-SC/AIP is supported by high levels of expression of CCL1 in the pancreatic duct epithelium, peribiliary glands and vascular endothelial cells of patients, with infiltration of the CCL1-expressing sites by CCR8-positive lymphocytes, the infiltrate consisting predominantly of Tregs and Th2 cells [45]. Chemokine ligands CCL23 and CCL25, which are important in homing to the gut-liver axis and as biomarkers for other autoimmune diseases, have also been implicated by gene expression analysis in the peripheral blood of IgG4-SC/AIP patients (Culver et al. 2014). Furthermore, chemokine receptor CXCR5 expressed on T follicular helper cells has also been increased in IgG4-related lesions.

12.12.6 Innate Immunity

Macrophages, eosinophils and basophils are often detected in affected tissue although their role in disease is not understood. Toll-like receptor and Nod-like receptor stimulation have been implicated in IgG4-RD, since IL10 and high levels of IgG4 are produced in response to stimulation in a B-cell activating factor-dependent manner [46]. The relevance of these findings remains unclear.

12.13 IgG4 Biology

IgG4 is the least prevalent of the four IgG subclasses, representing 3–6% of total IgG in the serum of adults. It can however account for up to 80% of total IgG after chronic exposure to certain antigens. IgG4 is a unique antibody in both structure and function [47]. Amino acid differences in the CH2 domain of IgG4 lead to negligible binding to the C1q protein complex and reduced binding to the Fc-gamma receptors of effector cells. This means that IgG4 is unable to activate the classical complement pathway and has a limited role in immune activation. In the core hinge region, an amino acid switch from proline in IgG1 to serine in IgG4 allows for IgG4 to undergo a process of Fab-arm exchange (exchange of half antibodies) in vivo [48]. This generates functionally monovalent antibodies which cannot cross-link antigens or form large immune complexes. IgG4 can bind the Fc portion of other IgG molecules at its constant domain, which may contribute to its anti-inflammatory function [49]. It can also interfere with complement-activating and immune-precipitating activities of IgG1. Physiological IgG4 responses can be induced by repeat or high antigen exposure and is associated with tolerance induction such as in immunotherapy. IgG4 production is driven predominantly by Th2 cytokines in particular IL4 and IL13, which induce IgG4 and IgE and IL10, IL21 and IL12 that shifts this balance towards IgG4, known as the ‘modified Th2 response’.

12.14 Bench to Bedside: Therapeutic Targets

Corticosteroids interfere with the production of cytokines critical for T-cell proliferation, and the binding of interleukins to B cells suppressing proliferation and antibody production. Serum IgG4, IgG4+ memory B cells and plasmablasts have been reported to fall with corticosteroid therapy, which may be explained by suppressed proliferation of expanded IgG4-switched B cells or suppressed differentiation into IgG4 plasmablasts. The steroid effect highlights the rapid turnover of the IgG4-producing plasma cells, as opposed to production by long-lived plasma cells in the bone marrow. This is also supported in patients treated with the B-cell depletion agent rituximab, which has been shown to reduce disease activity and plasmablast number [30, 50].

Another possible therapeutic target is the prostaglandin D2 (PGD2) receptor, CRTh2 (chemoattractant receptor – homologous molecule expressed on Th2 cells), known to be important in allergic inflammation. It is expressed on Th2 cells and innate cells such as eosinophils and responds to mast cell-derived factors. The frequency of CRTh2 CD4+ T cells is increased in patients with IgG4-siloadenitis, with a correlation of these cells with the serum IgE and peripheral blood eosinophilia [51]. An upregulation of PGD2 and CRTh2 is similarly reported in gene expression analysis of peripheral blood in AIP/IgG4-SC patients [52]. Blockade of CRTh2 can reduce allergic inflammation in rodent models of antigen-induced airway inflammation, allergic rhinitis, atopic dermatitis and hyperresponsiveness in asthma, and therefore this may be applied in those IgG4-RD patients with a history of allergy, elevated IgE and eosinophilia.

Conclusions

Over a decade after the first reports of elevated serum IgG4 concentrations in AIP, our understanding of this disease and its triggers is gradually progressing. Reliable diagnosis remains a significant challenge, as there is currently no validated non-invasive test with good diagnostic accuracy. The recently described peripheral blood plasmablast levels and PCR-based assay based on clonal analysis requires careful validation and scrutiny. Whilst we now understand that this is not a benign disease, the ability to reliably target those patients who require longer duration and early escalation of therapy is in its early stages. The pathophysiology underlying the recently described association with malignancy also requires further attention. Treatment options are not supported by prospective randomised controlled trials, and none are in progress, and this will require international collaboration. The role and action of rituximab and other disease-modifying biological agents needs to be understood.

The initial trigger that sets off aberrant IgG4 production is not known, although evidence points to proliferation of polyclonal non-specific IgG4+ B cells and plasmablasts in the disease. The supportive role of IgE and mast cells driving this disease in subsets of patients with allergy and/or atopy requires further clarification. The role of T-cell subsets in initiation and maintenance of the

inflammatory state has received recent attention, and our ongoing work looks specifically at the role of T follicular helper cells in this capacity. Ultimately, finding the trigger for inflammation and the driver for fibrosis-induced tissue destruction, as well as developing novel therapeutic steroid-sparing approaches to prevent long-term sequelae, must be the aim.

References

1. Ghazale A, Chari ST, Zhang L, Smyrk TC, Takahashi N, Levy MJ, et al. Immunoglobulin G4-associated cholangitis: clinical profile and response to therapy. Gastroenterology [Internet]. 2008 [cited 2015 Oct 4];134(3):706–15. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18222442.
2. Nakazawa T, Ohara H, Sano H, Ando T, Joh T. Schematic classification of sclerosing cholangitis with autoimmune pancreatitis by cholangiography. Pancreas [Internet]. 2006 [cited 2015 Oct 4];32(2):229. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16552350.
3. Nakazawa T, Ohara H, Sano H, Aoki S, Kobayashi S, Okamoto T, et al. Cholangiography can discriminate sclerosing cholangitis with autoimmune pancreatitis from primary sclerosing cholangitis. Gastrointest Endosc [Internet]. 2004 [cited 2015 Oct 4];60(6):937–44. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15605009.
4. Oh H-C, Kim M-H, Lee KT, Lee JK, Moon S-H, Song TJ, et al. Clinical clues to suspicion of IgG4-associated sclerosing cholangitis disguised as primary sclerosing cholangitis or hilar cholangiocarcinoma. J Gastroenterol Hepatol [Internet]. 2010 [cited 2015 Oct 4];25(12):1831–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21091993.
5. Tabata T, Kamisawa T, Hara S, Kuruma S, Chiba K, Kuwata G, et al. Differentiating immunoglobulin g4-related sclerosing cholangitis from hilar cholangiocarcinoma. Gut Liver [Internet]. 2013 [cited 2015 Oct 4];7(2):234–8. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3607779&tool=pmcentrez&rendertype=abstract.
6. Kalaitzakis E, Levy M, Kamisawa T, Johnson GJ, Baron TH, Topazian MD, et al. Endoscopic retrograde cholangiography does not reliably distinguish IgG4-associated cholangitis from primary sclerosing cholangitis or cholangiocarcinoma. Clin Gastroenterol Hepatol [Internet]. 2011 [cited 2015 Oct 4];9(9):800–3. e2. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3246637&tool=pmcentrez&rendertype=abstract.
7. Okano N, Igarashi Y, Kishimoto Y, Ito K, Sasai D. Case of immunoglobulin G4-related cholangitis accompanying autoimmune pancreatitis: diagnosis by peroral cholangioscopy and treatment by endoscopic biliary stenting. Dig Endosc [Internet]. 2012 [cited 2015 Oct 4];24:62–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22533755.
8. Hamano H, Kawa S, Horiuchi A, Unno H, Furuya N, Akamatsu T, et al. High serum IgG4 concentrations in patients with sclerosing pancreatitis. N Engl J Med [Internet]. 2001 [cited 2015 Aug 26];344(10):732–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11236777.
9. Ohara H, Nakazawa T, Kawa S, Kamisawa T, Shimosegawa T, Uchida K, et al. Establishment of a serum IgG4 cut-off value for the differential diagnosis of IgG4-related sclerosing cholangitis: a Japanese cohort. J Gastroenterol Hepatol [Internet]. 2013 [cited 2015 Oct 4];28(7):1247–51. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23621484.
10. Boonstra K, Culver EL, de Buy Wenniger LM, van Heerde MJ, van Erpecum KJ, Poen AC, et al. Serum immunoglobulin G4 and immunoglobulin G1 for distinguishing immunoglobulin G4-associated cholangitis from primary sclerosing cholangitis. Hepatology [Internet]. 2014 [cited 2015 Oct 4];59(5):1954–63. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4489327&tool=pmcentrez&rendertype=abstract.
11. Kalaitzakis E, Sturgess R, Kaltsidis H, Oppong K, Lekharaju V, Bergenzaun P, et al. Diagnostic utility of single-user peroral cholangioscopy in sclerosing cholangitis. Scand. J Gastroenterol

[Internet]. 2015 [cited 2015 Oct 4];49(10):1237–44. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25007715.
12. Moon S-H, Kim M-H, Park DH, Song TJ, Eum J, Lee SS, et al. IgG4 immunostaining of duodenal papillary biopsy specimens may be useful for supporting a diagnosis of autoimmune pancreatitis. Gastrointest Endosc [Internet]. Elsevier Inc.; 2010 [cited 2013 Mar 9];71(6):960–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20304394.
13. Deshpande V, Zen Y, Chan JK, Yi EE, Sato Y, Yoshino T, et al. Consensus statement on the pathology of IgG4-related disease. Mod Pathol [Internet]. 2012 [cited 2013 Mar 9];25(9):1181–92. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22596100.
14. Culver EL, Bateman AC. IgG4-related disease: can non-classical histopathological features or the examination of clinically uninvolved tissues be helpful in the diagnosis?. J Clin Pathol [Internet]. 2012 [cited 2013 Mar 9];65(11):963–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22685258.
15. Mendes FD, Jorgensen R, Keach J, Katzmann JA, Smyrk T, Donlinger J, et al. Elevated serum IgG4 concentration in patients with primary sclerosing cholangitis. Am J Gastroenterol [Internet]. 2006 [cited 2015 Oct 4];101(9):2070–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16879434.
16. Webster GJM, Pereira SP, Chapman RW. Autoimmune pancreatitis/IgG4-associated cholangitis and primary sclerosing cholangitis – overlapping or separate diseases?. J Hepatol [Internet]. European Association for the Study of the Liver. 2009 [cited 2013 Mar 9];51(2):398–402. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19505739.
17. Berntsen NL, Klingenberg O, Juran BD, Benito De Valle M, Lindkvist B, Lazaridis KN, et al. Association between HLA haplotypes and increased serum levels of IgG4 in patients with primary sclerosing cholangitis. Gastroenterology [Internet]. 2015 [cited 2015 Oct 4];148(5):924–7. e2. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25655558.
18. Erdogan D, Kloek JJ, ten Kate FJW, Rauws EAJ, Busch ORC, Gouma DJ, et al. Immunoglobulin G4-related sclerosing cholangitis in patients resected for presumed malignant bile duct strictures. Br J Surg [Internet]. 2008 [cited 2015 Oct 4];95(6):727–34. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18418862.
19. Khosroshahi A, Wallace ZS, Crowe JL, Akamizu T, Azumi A, Carruthers MN, et al. International consensus guidance statement on the management and treatment of IgG4-Related disease. Arthritis Rheumatol (Hoboken, NJ) [Internet]. 2015 [cited 2015 Jul 5];67(7):1688–99. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25809420.
20. Sandanayake NS, Church NI, Chapman MH, Johnson GJ, Dhar DK, Amin Z, et al. Presentation and management of post-treatment relapse in autoimmune pancreatitis/immunoglobulin G4-associated cholangitis. Clin Gastroenterol Hepatol [Internet]. 2009 [cited 2015 Oct 4];7(10):1089–96. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19345283.
21. Hart PA, Topazian MD, Witzig TE, Clain JE, Gleeson FC, Klebig RR, et al. Treatment of relapsing autoimmune pancreatitis with immunomodulators and rituximab: the Mayo Clinic experience. Gut [Internet]. 2013 [cited 2015 Oct 4];62(11):1607–15. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22936672.
22. Carruthers MN, Topazian MD, Khosroshahi A, Witzig TE, Wallace ZS, Hart PA, et al. Rituximab for IgG4-related disease: a prospective, open-label trial. Ann Rheum Dis [Internet]. 2015 [cited 2015 Sep 8];74(6):1171–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25667206.
23. Huggett MT, Culver EL, Kumar M, Hurst JM, Rodriguez-Justo M, Chapman MH, et al. Type 1 autoimmune pancreatitis and IgG4-related sclerosing cholangitis is associated with extrapancreatic organ failure, malignancy, and mortality in a prospective UK cohort. Am J Gastroenterol [Internet]. 2014 [cited 2015 Oct 4];109(10):1675–83. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4552254&tool=pmcentrez&rendertype=abstract.
24. Kawa S, Ota M, Yoshizawa K, Horiuchi A, Hamano H, Ochi Y, et al. HLA DRB10405-DQB10401 haplotype is associated with autoimmune pancreatitis in the Japanese population. Gastroenterology [Internet]. 2002 [cited 2015 Jun 21];122(5):1264–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11984513.

25. Park DH, Kim M-H, Oh HB, Kwon O-J, Choi Y-J, Lee S-S, et al. Substitution of aspartic acid at position 57 of the DQbeta1 affects relapse of autoimmune pancreatitis. Gastroenterology [Internet]. 2008 [cited 2015 Jun 21];134(2):440–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18155707.
26. Hirano K, Asaoka Y, Tada M, Isayama H, Sasahira N, Tsujino T, et al. No significant relation between relapse of autoimmune pancreatitis and substitution of aspartic acid at position 57 of DQbeta1. J Gastroenterol [Internet]. 2009 [cited 2015 Jun 21];44(7):799–800. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19468670.
27. Umemura T, Ota M, Hamano H, Katsuyama Y, Muraki T, Arakura N, et al. Association of autoimmune pancreatitis with cytotoxic T-lymphocyte antigen 4 gene polymorphisms in Japanese patients. Am J Gastroenterol [Internet]. 2008 [cited 2015 Jun 21];103(3):588–94. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18341485.
28. Umemura T, Ota M, Hamano H, Katsuyama Y, Kiyosawa K, Kawa S. Genetic association of Fc receptor-like 3 polymorphisms with autoimmune pancreatitis in Japanese patients. Gut [Internet]. 2006 [cited 2015 Jun 21];55(9):1367–8. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1860039&tool=pmcentrez&rendertype=abstract.
29. Chang M-C, Chang Y-T, Tien Y-W, Liang P-C, Jan I-S, Wei S-C, et al. T-cell regulatory gene CTLA-4 polymorphism/haplotype association with autoimmune pancreatitis. Clin Chem [Internet]. 2007 [cited 2015 Jun 21];53(9):1700–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17712006
30. Mattoo H, Mahajan VS, Della-Torre E, Sekigami Y, Carruthers M, Wallace ZS, et al. De novo oligoclonal expansions of circulating plasmablasts in active and relapsing IgG4-related disease. J Allergy Clin Immunol [Internet]. 2014 [cited 2015 Jun 21];134(3):679–87. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24815737.
31. Maillette de Buy Wenniger LJ, Doorenspleet ME, Klarenbeek PL, Verheij J, Baas F, Elferink RPO, et al. Immunoglobulin G4+ clones identified by next-generation sequencing dominate the B cell receptor repertoire in immunoglobulin G4 associated cholangitis. Hepatology [Internet]. 2013 [cited 2015 Jun 21];57(6):2390–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23300096.
32. Hubers LM, Doorenspleet ME, Culver EL, Maillette de Buy Wenniger LJ, Klarenbeek PL, Van de Graaf SF, et al. O087 : IgG4+ B-cell receptor clones in peripheral blood distinguish IgG4-associated cholangitis/autoimmune pancreatitis from primary sclerosing cholangitis. J Hepatol [Internet]. Elsevier; 2015 [cited 2015 Sep 15];62:S233–4. Available from: http://www.journal-of-hepatology.eu/article/S0168827815301008/fulltext.
33. Asada M, Nishio A, Uchida K, Kido M, Ueno S, Uza N, et al. Identification of a novel autoantibody against pancreatic secretory trypsin inhibitor in patients with autoimmune pancreatitis. Pancreas [Internet]. 2006 [cited 2015 May 13];33(1):20–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16804408.
34. Aparisi L, Farre A, Gomez-Cambronero L, Martinez J, De Las Heras G, Corts J, et al. Antibodies to carbonic anhydrase and IgG4 levels in idiopathic chronic pancreatitis: relevance for diagnosis of autoimmune pancreatitis. Gut [Internet]. 2005 [cited 2015 Jun 21];54(5):703–9. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1774474&tool=pmcentrez&rendertype=abstract.
35. Nishimori I, Miyaji E, Morimoto K, Nagao K, Kamada M, Onishi S. Serum antibodies to carbonic anhydrase IV in patients with autoimmune pancreatitis. Gut [Internet]. 2005 [cited 2015 May 13];54(2):274–81. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1774835&tool=pmcentrez&rendertype=abstract.
36. Guarneri F, Guarneri C, Benvenga S. Helicobacter pylori and autoimmune pancreatitis: role of carbonic anhydrase via molecular mimicry? J Cell Mol Med [Internet]. 2015;9(3):741–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16202223
37. Frulloni L, Lunardi C, Simone R, Dolcino M, Scattolini C, Falconi M, et al. Identification of a novel antibody associated with autoimmune pancreatitis. N Engl J Med [Internet]. 2009 [cited 2015 May 13];361(22):2135–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19940298.

38. Aoki S, Nakazawa T, Ohara H, Sano H, Nakao H, Joh T, et al. Immunohistochemical study of autoimmune pancreatitis using anti-IgG4 antibody and patients' sera. Histopathology [Internet]. 2005 [cited 2015 Jun 21];47(2):147–58. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16045775.
39. Culver EL, Vermeulen E, Makuch M, van Leeuwen A, Sadler R, Cargill T, et al. Increased IgG4 responses to multiple food and animal antigens indicate a polyclonal expansion and differentiation of pre-existing B cells in IgG4-related disease. Ann Rheum Dis [Internet]. 2015 [cited 2015 Sep 18];74(5):944–7. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4392210&tool=pmcentrez&rendertype=abstract.
40. De Buy Wenniger LJM, Culver EL, Beuers U. Exposure to occupational antigens might predispose to IgG4-related disease. Hepatology [Internet]. 2014 [cited 2015 May 13];60(4):1453–4. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4258085&tool=pmcentrez&rendertype=abstract.
41. Tsuboi H, Matsuo N, Iizuka M, Tsuzuki S, Kondo Y, Tanaka A, et al. Analysis of IgG4 class switch-related molecules in IgG4-related disease. Arthritis Res Ther [Internet]. 2012 [cited 2015 Jun 21];14(4):R171. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3580565&tool=pmcentrez&rendertype=abstract.
42. Zen Y, Fujii T, Harada K, Kawano M, Yamada K, Takahira M, et al. Th2 and regulatory immune reactions are increased in immunoglobin G4-related sclerosing pancreatitis and cholangitis. Hepatology [Internet]. 2007 [cited 2015 Apr 20];45(6):1538–46. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17518371.
43. Mattoo H, Della-Torre E, Mahajan VS, Stone JH, Pillai S. Circulating Th2 memory cells in IgG4-related disease are restricted to a defined subset of subjects with atopy. Allergy [Internet]. 2014 [cited 2015 Jun 21];69(3):399–402. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3944385&tool=pmcentrez&rendertype=abstract.
44. Lighaam LC, Vermeulen E, Bleker T den, Meijlink KJ, Aalberse RC, Barnes E, et al. Phenotypic differences between IgG4+ and IgG1+ B cells point to distinct regulation of the IgG4 response. J Allergy Clin Immunol [Internet]. 2014 [cited 2015 Jun 21];133(1):267–70. e1–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24074895.
45. Zen Y, Liberal R, Nakanuma Y, Heaton N, Portmann B. Possible involvement of CCL1-CCR8 interaction in lymphocytic recruitment in IgG4-related sclerosing cholangitis. J Hepatol [Internet]. 2013 [cited 2015 Jun 21];59(5):1059–64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23811304.
46. Akitake R, Watanabe T, Zaima C, Uza N, Ida H, Tada S, et al. Possible involvement of T helper type 2 responses to Toll-like receptor ligands in IgG4-related sclerosing disease. Gut [Internet]. 2010 [cited 2015 Jun 21];59(4):542–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20332525.
47. Aalberse RC, Stapel SO, Schuurman J, Rispens T. Immunoglobulin G4: an odd antibody. Clin Exp Allergy [Internet]. 2009 [cited 2015 May 20];39(4):469–77. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19222496.
48. Rispens T, Ooijevaar-de Heer P, Bende O, Aalberse RC. Mechanism of immunoglobulin G4 Fab-arm exchange. J Am Chem Soc [Internet]. 2011 [cited 2015 Jun 21];133(26):10302–11. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21627172.
49. Rispens T, Ooievaar-De Heer P, Vermeulen E, Schuurman J, van der Neut Kolfschoten M, Aalberse RC. Human IgG4 binds to IgG4 and conformationally altered IgG1 via Fc-Fc interactions. J Immunol [Internet]. 2009 [cited 2015 Jun 21];182(7):4275–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19299726.
50. Khosroshahi A, Bloch DB, Deshpande V, Stone, JH. Rituximab therapy leads to rapid decline of serum IgG4 levels and prompt clinical improvement in IgG4-related systemic disease. Arthritis Rheum [Internet]. 2010 [cited 2015 Jun 21];62(6):1755–62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20191576.

51. Saito Y, Kagami S, Kawashima S, Takahashi K, Ikeda K, Hirose K, et al. Roles of CRTH2+ CD4+ T cells in immunoglobulin G4-related lacrimal gland enlargement. Int Arch Allergy Immunol [Internet]. 2012 [cited 2015 Jun 21];158 Suppl :42–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22627365.
52. Culver EL, Marchi E, Manganis C, Smit W, Makuch M, Ramamurthy N, et al. P355 gene expression analysis identifies immune signaling and complement pathways in igg4-related disease. J Hepatol [Internet]. 2014 Apr [cited 2015 Oct 4];60(1):S185–6. Available from: https://www.infona.pl//resource/bwmeta1.element.elsevier-879551ba-75c2-3046-8d9d-99ee73c4e914.

Conclusions and Future Opportunities

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David Adams and Evaggelia Liaskou

Abstract

Biliary diseases, also known as cholangiopathies, are a group of diseases affecting the intra- and extra- hepatic bile ducts with severe morbidity and mortality. The aetiology of the majority of biliary diseases remains unknown so far. Understanding the immunobiological functions played by cholangiocytes in health and disease, developing new animal models that mirror closely human biliary disease, studying the composition of the gut microbiome and understanding the genetics of biliary diseases are some of the aspects to be covered in this book. These undergoing efforts will hopefully advance our understanding of how these disorders initiate and develop and will guide future more refined therapeutic approaches.

Biliary diseases represent an important group of inborn and acquired diseases of the intra- and extra- hepatic bile ducts with severe morbidity and mortality. The spectrum of cholangiopathies is heterogeneous with respect to underlying mechanisms, clinical course and presentation; however, they all share a common target: the cholangiocyte. An increasing number of evidence has highlighted the ability of cholangiocytes to undergo changes in phenotype, proliferation and secretory activity in response to liver damage. A growing body of data have also supported the involvement of cholangiocytes in inflammation and innate immunity, which is not surprising considering the capabilities of cholangiocytes to mount a reparative response to many forms of liver damage. The imbalance of signals and pathways that orchestrate the immunobiological competence of cholangiocytes has been an emerging new pathogenetic mechanism in specific biliary disease settings. A thorough knowledge of the complex immunobiological functions played by cholangiocytes, both in normal and diseased conditions, is a field that needs further investigation.

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G. Hirschfield et al. (eds.), *Biliary Disease*, DOI 10.1007/978-3-319-50168-0_13

The etiology of the majority of biliary diseases remains unknown so far and the use of rodent models has been a valuable tool to understand the mechanisms of their pathogenesis. Within the last 10 years a significant progress has been made in developing new animal models that exhibit several features of different biliary diseases. Still, a "perfect model" that mirrors human biliary disease in full is lacking. Various aspects of different models to study particular pathogenetic steps is possibly the way forward.

The study of the composition of the gut microbiome has attracted huge interest the last few years, with the field of gut microbiome now advancing rapidly. There is now justified hope that the gut microbiome will be part of individualised treatment in several diseases. The importance of diet in shaping the gut microbiome opens the door for more efficient disease preventive lifestyle advice on the population level. In biliary diseases, little is known about disease-associated and healthy diets; the gut microbiome profile could be therefore speculated to provide guidance, thus future work on this field is interesting.

Remarkable medical achievements for biliary diseases include the Kasai portoenterostomy surgical procedure, which along with liver transplantation (when necessary) have increased survival into adulthood with a good quality of life in cases of biliary atresia. In PBC novel therapeutic avenues include bile acid based therapies, immune-based therapies and cell-based therapies. In PSC therapeutic initiative affecting bile acid homeostasis and tissue specific lymphocyte homing are under trial efforts and will likely provide proof-of-concept insights as to which avenues may prove more effective, thus guiding future more refined therapeutic approaches.

The next few years will be an exciting new era in the field of biliary diseases. Research results in cellular and immunological aspects of the pathogenetic pathways will advance further our understanding of how these disorders initiate and develop. Understanding of the composition of the gut microbiome and involvement of lifestyle in biliary diseases is an exciting unexplored field with great therapeutic potential. Ultimately, the results from the undergoing trial efforts will hopefully provide useful therapeutic avenues for patients with biliary diseases.