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Cholestatic Liver Disease in Children

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Abstract Inherited syndromes of intrahepatic cholestasis and biliary atresia are the most common causes of chronic liver disease and the prime indication for liver transplantation in children. Our understanding of the pathogenesis of these diseases has increased substantially by the discovery of genetic mutations in children with intrahepatic cholestasis and the findings that inflammatory circuits are operative at the time of diagnosis of biliary atresia. Building on this solid foundation, recent studies provide new insight into genotype-phenotype relationships and how mutations produce altered bile composition and cholestasis. New evidence exists that although liver transplantation is curative for patients with end-stage liver disease owing to cholestasis, some patients may develop recurrence of cholestasis because of the emergence of autoantibodies that disrupt canalicular function in the new graft. Progress is also evident in biliary atresia, with recent studies identifying candidate modifier genes and directly implicating lymphocytes and inflammatory signals in the pathogenesis of bile duct injury and obstruction.

Keywords Cirrhosis · Jaundice · Bilirubin · Hemochromatosis · Biliary atresia · Alagille disease · Transplantation

Introduction

Diseases that manifest as cholestasis in children often result from pathologic processes that begin early in postnatal life, when the liver has not reached functional maturity and may be more susceptible to the adverse consequences of endogenous (metabolic, genetic) or environmental insults [1]. Despite the multifactorial nature of the pathology, several diseases are linked to single-gene defects that fundamentally alter physiologic processes and produce clinical syndromes. The discovery of these genes has broadened our understanding of the pathogenesis of disease and improved nosology by incorporating biologic features into disease categories [2, 3]. Now, we are learning how the disruption of molecular pathways regulates mechanisms of disease, about new factors that modify the clinical course, and the implications of discoveries for the development of new therapies, which are the focus of this review.

Before addressing recent advances for specific cholestatic diseases in children, we make note of a study investigating the prevalence of subclinical vitamin deficiency in patients with cholestasis. The demands of postnatal growth and development in the face of fat malabsorption secondary to cholestasis heighten the risk for complications of fat-soluble vitamin deficiency (eg, neurologic deficits, bone disease, and hemorrhage). The study, conducted in children and adults with cholestatic syndromes, determined the plasma concentration of protein induced in vitamin K absence II (PIVKA-II), which measures undercarboxylated prothrombin [4]. Although 29% of the subjects had evidence of coagulopathy as indicated by an increased international normalized ratio (INR), a much higher percentage had increased PIVKA-II (68%) despite ongoing supplementation with vitamin K. If reproducible in a larger patient population and approved for routine clinical use, PIVKA-II may be an important tool to more closely monitor vitamin nutriture in children with chronic cholestasis.

Neonatal Iron Storage Disease

The onset of cholestasis in the first few days of life demands prompt and thorough evaluation. If associated with severe

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synthetic dysfunction and evidence of extrahepatic iron deposition (eg, in the pancreas), cholestasis is caused by neonatal iron storage disease. Survival in affected neonates is poor. Based on a 60% to 80% recurrence of disease in siblings and a proposed role for maternal alloimmunity in pathogenesis of liver injury, investigators explored the potential benefits of intravenous immunoglobulin (IV Ig) during gestation in a pilot study and reported preliminary evidence of improved infant survival [5]. A more definitive open-label trial involving 48 women (and 53 pregnancies) treated with weekly IV Ig beginning at 18 weeks of at-risk gestations reduced the recurrence rate of liver disease in neonates, with an increase in overall survival from 8% in historical controls to 98% in infants with IV Ig therapy [6••]. This study provides greater support for a role of alloimmunity in pathogenesis of neonatal iron storage disease and for the use of maternal IV Ig during at-risk pregnancies to improve the outcome of affected neonates.

Intrahepatic Cholestasis

Several genetic disorders present as intrahepatic cholestasis secondary to loss of key functions in organelles or the canalicular membrane. Among them, deficiency of α-1-antitrypsin (A1AT) causes liver disease when patients bear the homozygous Z variant (PiZZ), which features the single amino acid substitution G342K. The mutant PiZZ-A1AT undergoes polymerization and may not be properly cleared by the process known as endoplasmic reticulum (ER)—associated degradation (ERAD). Without adequate clearance, the mutant protein accumulates and becomes toxic to hepatocytes. One important question in the field relates to the biologic basis for the development of clinically significant liver disease in only 8% to 10% over the first 20 years of life and for the variability in clinical course. This question was investigated in a recent article that assessed the potential role of ERManI as a modifier of disease [7]. ERManI is a putative ER mannosidase that plays a role in sorting and targeting misfolded glycoproteins for ERAD. Sequencing of exons of the *ERManI* gene in children with PiZZ uncovered high prevalence of homozygosity of the minor allele 2484G/A in those children with earlier onset of end-stage liver disease [7]. The biologic plausibility for this role was supported by the findings that the minor allele suppressed ERManI translation under ER stress conditions. Thus, polymorphisms in *ERManI* (and other functionally related genes) may contribute to phenotypic differences in children with PiZZ-induced liver disease.

Mutations in the genes *SERPINA1* (for A1AT deficiency), *JAG1* (for Alagille disease), and those encoding the canalicular transport proteins familial intrahepatic cholestasis-1 (FIC1), bile salt export pump (BSEP), and multidrug resistance protein-3 (MDR3) are responsible for the most common recognizable syndromes of intrahepatic cholestasis (Fig. 1). However, a substantial portion of symptomatic children remains with undefined etiology. For these children, potential candidate genes include those encoding nuclear factors that regulate synthesis and trafficking of bile acids to the canaliculus. One example is the forkhead box proteins Foxa1, Foxa2, and Foxa3, which are known to regulate the promoters of the genes encoding A1AT, transthyretin, and several nuclear receptors. A new role for Foxa2 in the pathogenesis of cholestasis was suggested by the functional phenotyping of mice carrying the hepatocyte-specific inactivation of the *Foxa2* gene [8•]. Loss of Foxa2 resulted in intrahepatic cholestasis associated with a decreased expression of genes involved in bile acid transport at the basolateral and canalicular sites. Interestingly, children and adults with cholestatic syndromes also had decreased expression of FOXA2, implying that this transcription factor is important for bile acid homeostasis and may represent an important genetic modifier of liver disease.

FIC1 Deficiency

Mutations in the *ATP8B1* gene decrease the expression and/or disrupt the function of the encoded protein known as FIC1. Patients with FIC1 deficiency present with progressive forms of intrahepatic cholestasis commonly referred to as progressive familial intrahepatic cholestasis type 1 (PFIC-1) and regional variants in the Faeroe Islands and

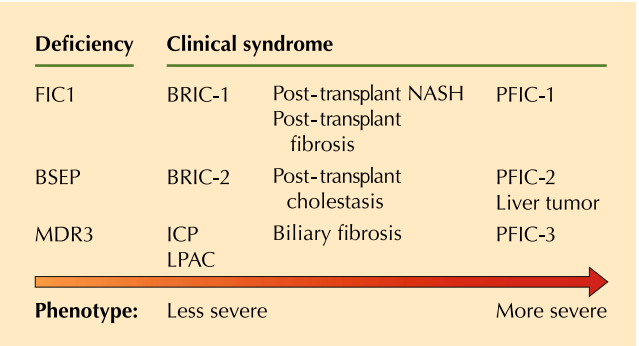

Fig. 1 Clinical phenotypes induced by deficiency of individual canalicular transporters. The phenotypes are grouped based on a perceived level of severity (from less to more severe). BRIC—benign recurrent intrahepatic cholestasis; BSEP—bile salt export pump; FIC—familial intrahepatic cholestasis; ICP—intrahepatic cholestasis of pregnancy; LPAC—low phospholipid–associated cholestasis; MDR—multidrug resistance protein; NASH—nonalcoholic steatohepatitis; PFIC—progressive familial intrahepatic cholestasis

Greenland. Mutations that are less deleterious to FIC1 function manifest as benign recurrent intrahepatic cholestasis type 1 (BRIC-1) (Fig. 1). In addition to hepatic involvement, children with FIC1 deficiency may also have chronic diarrhea, pancreatic insufficiency, and respiratory symptoms. Typically, children develop persistent cholestasis, pruritus, and growth retardation by 1 to 4 years of age, and progress to end-stage liver disease. Liver transplantation is known to restore hepatic function, but a review of the outcome of 11 patients transplanted for FIC1 deficiency in Japan reported microvesicular steatosis in 8 patients as early as 2 months after transplantation, with progression to steatohepatitis in 7 patients by 5 to 6 months; bridging fibrosis was noted in 6 patients, and 2 reached cirrhosis [9•]. All patients with steatosis also had diarrhea, half of which had decreased symptoms after the use of bile salt absorptive resin. Genotype-phenotype relationship showed that steatosis occurred in patients with more severe mutations. Although liver transplantation would not be expected to correct the extrahepatic manifestations, the development of substantial liver disease posttransplant is unexpected. A cause was not established, but the fact that all transplants were living-related raises the possibility that heterozygosity of *ATP8B1* may increase susceptibility of the graft to steatohepatitis.

The multisystem consequences of FIC1 deficiency point to a complex basis of disease. Studies using liver cells showed that FIC1 normally associates with CDC50 proteins during normal ER trafficking, before final anchoring in the canalicular membrane [10]. Interestingly, mutations reported in patients with PFIC-1 that resulted in no detection of the FIC1 protein in canalicular membrane were associated with decreased stability of the mutant protein and/or lack of interaction with CDC50A, whereas mutant proteins from patients with BRIC did not change the cellular localization of the protein [11]. A similar differential effect of mutations was noted on the ability of FIC1 to signal the nuclear receptor farnesoid X receptor (FXR) via the cytoplasmic protein kinase C zeta, suggesting that the consequences of impaired FIC1 are, at least in part, linked to downstream effects of FXR on bile acid homeostasis [12•]. Another study used hepatocytes in a different functional assay and demonstrated that the deficiency of FIC1 disrupts the bile canalicular membrane bilayer structure [13•]. In this study, knocking down *Atp8b1* using RNA interference in rat hepatocytes induced adaptive responses in bile transporters, reduced bile salt excretion, and disrupted the canalicular membrane bilayer with accumulation of phosphatidylserine in the canalicular lumen upon exposure to hydrophobic bile acids. These lines of study are likely to uncover strategies to restore trafficking and proper anchoring of FIC1, or identify ways to activate compensatory circuits to improve bile acid homeostasis.

BSEP Deficiency

The impact of genetic mutations on protein trafficking and anchoring in the canalicular membrane has also been investigated for BSEP, the major transport system for bile salts. Mutations in the *ABCB11* gene, which encodes BSEP, results in low γ-glutamyl transpeptidase (γGTP) cholestasis and manifest as either a mild clinical syndrome of recurrent symptoms known as BRIC-2 or a more severe disease known as PFIC-2, akin to the phenotypes described for FIC1 deficiency, without extrahepatic manifestations (Fig. 1). In experiments designed to examine how mutations change the biology of BSEP, mutant proteins encoded by genetic mutations found in patients with PFIC-2 were expressed in cell lines and were demonstrated to be retained in the ER at variable degrees and processed by the ER-associated degradation machinery [14]. Another study added insight into how different *ABCB11* missense mutations and single nucleotide polymorphisms (SNPs) influence BSEP expression. The investigators found that most mutations/SNPs resulted in aberrant pre-mRNA splicing, retention in the ER, increased degradation, and lowered canalicular expression of the protein [15]. Further dissection of the molecular consequences of disease-causing mutations will enable the development of new therapies based on the genetic makeup of the patient, ultimately aiming at restoring the transport of bile acids.

The recessive mode of inheritance for BSEP deficiency implies that mutations affect both alleles. This biological setting was supported by a comprehensive DNA sequence analysis of 109 families with BSEP deficiency, which identified 82 different biallelic mutations [16••]. It is notable that seven families carried only a single heterozygous mutation, but it remains unclear how a single allele mutation produces a clinical phenotype. Interestingly, 93% of the mutations produced abnormal or absent BSEP expression on liver biopsies; immunostaining identified a variable pattern of BSEP expression in patients carrying the most common E297G or D482G mutations, thus limiting the use of immunohistochemistry to reliably pinpoint BSEP deficiency. This study also showed how the biologic consequences of BSEP deficiency might be far more reaching than previously recognized, with the development of hepatocellular carcinoma or cholangiocarcinoma in 15% of the patients [16••]. Earlier reports had described liver tumors in patients with *ABCB11* mutations, but the strength of the association was unknown [17, 18]. In the cohort of 109 families, a higher incidence of malignancy (35%) was linked to patients carrying biallelic protein-truncating mutations (vs 10% with less severe genotypes) [16••]. These findings emphasize the need to maintain close surveillance for the development of malignancy in subjects with chronic cholestasis due to BSEP deficiency.

The evolution of liver disease in children with BSEP can be rapid, reach end-stage cirrhosis, and require transplantation for long-term survival. Transplantation is curative, but a new report described the recurrence of cholestatic liver disease following transplantation because of the development of autoantibodies against BSEP [19••]. In this report, the child had three homozygous nonsynonymous nucleotide changes that produced incomplete expression of the protein or improper clearance in the ER-associated degradation pathways. Re-transplantation was required because of progressive cholestasis. After observing recurrence of cholestasis in the second functional graft, the investigators uncovered the presence of autoantibodies in the patient's serum, which recognized the first extracellular loop of the BSEP protein. This new mechanism of recurrence of disease was reported by a different group of investigators in three patients with recurrence of low γGTP cholestasis and giant cell transformation (with no evidence of cellular rejection) following liver transplantation for BSEP deficiency [20••]. These patients were also shown to have antibodies that recognized BSEP. Together, the findings provide a rationale for the development of trials that assess whether a change in the type of immunosuppression to decrease the production of antibodies (eg, anti-CD20 antibodies) may be more beneficial to patients than an intensification of standard drugs to modulate T-cell function (eg, calcineurin inhibitors) in transplanted patients.

MDR-3 Deficiency

Mutations in *ABCB4* disrupt the function of the encoded MDR3, a phospholipid translocase that normally flips phosphatidylcholine from the inner to the outer layer of the canalicular membrane. One key mechanism for the lack of phospholipid transport induced by MDR3 mutants is the lack of final localization of MDR3 in the canalicular membrane. This mechanism was illustrated in one study by tracking the signal produced by the I541F MDR3 mutant in liver and kidney cell lines, which was detected in the cytoplasm because of trapping within the ER and cis-Golgi [21]. Interestingly, the trafficking of the mutant protein toward the canalicular membrane was rescued by low temperature, opening perspectives for the development of new therapies.

MDR3 deficiency leads to chronic cholestasis akin to patients with FIC1 and BSEP deficiencies; however, in contrast, patients with MDR3 deficiency have high serum levels of γGTP [22, 23]. The spectrum of diseases caused by mutations in the *ABCB4* gene includes PFIC-3, low phospholipid—associated cholelithiasis, adult biliary cirrhosis, and intrahepatic cholestasis of pregnancy [24]. The onset of cholestasis is typically in early life, but adult phenotypes have been described. A recent article reporting the gene sequence analysis of 32 adults with anicteric cholestasis of unknown etiology identified heterozygous mutations in 34% of the patients [25•]. Liver histology showed portal fibrosis with ductular reaction as well as strong macrophage infiltration of portal tracts without significant periportal and lobular necroinflammatory lesions or cholangitis; MDR3 immunostaining was decreased or absent. As discussed above for BSEP, it is not clear how heterozygous mutations produce substantial liver disease, unless one postulates the coexistence of mutations in as yet undefined, functionally related genes, or accepts the possibility that a decreased expression of MDR3 produced by the remaining normal allele cannot efficiently fulfill the demands for aminophospholipid transport. Possibly, the decreased expression of MDR3 acts as a susceptibility factor during physiologic (eg, pregnancy) or pathologic (eg, exposure to a hepatotoxin) conditions or stressors.

Extrahepatic Cholestasis: Biliary Atresia

Biliary atresia is the most common cause of cholestasis in neonates and the most common indication for pediatric liver transplantation. The clinical phenotype is produced by a fibrosing and inflammatory process that obstructs the lumen of extrahepatic bile ducts and disrupts the flow of bile into the duodenum. Bile duct abnormalities are also found within livers, typically with proliferation and plugging of the lumen by inspissated bile; variable degrees of portal inflammation, hepatocyte injury, and giant cell transformation coexist at diagnosis. Limited studies have addressed whether the liver pathology represents reactive changes secondary to insults that target primarily the extrahepatic bile ducts or whether it is also a primary site of injury. A recent paper shed some light on this question. Analyzing the liver histology and bile ducts from three newborns with the embryonic form of biliary atresia (ie, coexistence of biliary atresia and nonhepatic congenital malformations), the authors found that the livers were near normal at birth, but the neonates had biochemical and/or anatomic evidence of extrahepatic bile duct involvement [26]. This observation tilts the balance toward the extrahepatic duct as a primary site of initial injury, at least in those subjects with the embryonic form of disease.

Screening: Method and Potential Impact on Outcome

Age at diagnosis is one of the key factors influencing the short-and long-term response to surgical treatment (portoenterostomy), with the best outcome reported in younger infants. This finding was highlighted by a review of the

outcome of all infants diagnosed with biliary atresia in France between 1986 and 2002. Analysis of how age influenced outcome in 696 infants who had portoenterostomy showed a decrease in transplant-free survival with increasing age at surgery, with the best outcome reported for those infants≤30 days of age [27]. The authors estimated that if all portoenterostomies were performed before 46 days of age, 5.7% fewer liver transplants would be performed in patients less than 16 years old in France—potentially a great benefit to the patients and to society. Thus, one of the current challenges is to diagnose and treat infants as early as possible. One strategy for identifying patients at younger age is the use of stool color cards to identify at-risk infants by helping parents detect acholic stools and seek medical care even when other symptoms are not obvious. The use of this system throughout Taiwan resulted in an increase in the national rate of portoenterostomy before 60 days of age from 60% to 74.3% [28]. In addition, a greater percentage of infants showed improved bile flow 3 months after surgery (59.5% vs historical data of 37%). It will be important to test the impact of screening for biliary atresia with the stool color card in other regions. The approach might be useful in areas where the rate of early diagnosis is particularly low [29] by fostering community awareness and early referral to specialized centers, thus improving outcome by the timely diagnosis and surgical intervention. In 2006, a workshop sponsored by the National Institutes of Health reviewed opportunities for screening and identified stool color card programs and newborn testing for conjugated bilirubin levels as two promising methodologies that deserve prospective assessment [30].

Biomarkers of Disease

A low serum level of bilirubin 3 weeks after portoenterostomy has been consistently associated with improved survival with the native liver beyond 2 years of age [31, 32]. However, despite the relatively uniform clinical presentation with jaundice, acholic stools, and hepatomegaly, very little is known about the factors that influence the extent or severity of liver pathology and the basis for the variable rate of progression to end-stage liver disease. Examining histologic markers at diagnosis may be predictive of clinical outcome and prognosis. For example, investigators quantified the extent of portal fibrosis by applying a computerized system to liver biopsies stained with picrosirius red in 53 subjects with biliary atresia [33]. The researchers found that a low mean volume of fibrosis per number of periportal fields (Vfib score < 2.5%) had a strong predictive value for transplant-free survival with the native liver by 5 years of age. Interestingly, no association was present when fibrosis was quantified by the Ishak score. The use of a similar scoring system for fibrosis was also not predictive of long-term outcome in another study evaluating 47 liver biopsies obtained at diagnosis. Instead, this study found that extensive bile duct proliferation was associated with death or liver transplantation in the first year following portoenterostomy [34]. Although low levels of fibrosis or bile duct proliferation may reflect less severe disease at presentation, the negative predictive value for both variables was low. This limitation notwithstanding, the future quantification of candidate biomarkers in the liver or serum and key histopathologic features might discover novel approaches to stage the disease at presentation, predict long-term outcome, and design treatment strategies that take into account the stage of liver disease at the time of diagnosis.

Pathogenesis: Candidate Genes

One working model of pathogenesis of disease proposes that the biliary atresia phenotype results from an interplay between environmental and genetic factors (Table 1) [35]. The contribution of genetic factors is based largely on the coexistence of congenital nonhepatic malformations, including laterality defects and polysplenia, in a subgroup of infants with biliary atresia. In support for the role of genes in pathogenesis of disease, obstruction of extrahepatic bile ducts and jaundice were reported in mice carrying the inactivation of the *Inversin* gene, which regulates laterality [36]. However, mutational analyses in children with laterality defects and biliary atresia failed to identify *Inversin* mutations. Other candidate genes have emerged from studies in patients and animal models. The first is *CFC1*, which encodes the CRYPTIC protein and regulates left-right axis determination. In a mutation survey of 10 unrelated patients with biliary atresia-splenic malformation syndrome, there was a 25% allele frequency for the c.433G>A variant, which resides in a highly conserved motif of the mammalian gene (vs 12.5% in the control population) [37]. In a separate study of children with biliary atresia (all clinical forms included), a +936C/T polymorphism in the vascular endothelial growth factor gene was more prevalent in 45 Taiwanese children with biliary atresia when compared with controls [38].

In animal-based studies, *Sox17* and *Lgf4* genes were found to play important roles in bile duct development. The first study under or overexpressed *Sox17*, a gene known to regulate endoderm lineage. Morphologic analyses of the hepatobiliary anatomy revealed that deletion of *Sox17* resulted in the loss of biliary structures and the presence of ectopic pancreatic tissue in the liver bud and common bile duct. When *Sox17* was overexpressed, pancreatic development was suppressed and ectopic biliary-like tissue

Table 1 Potential mechanisms involved in the pathogenesis of biliary atresia

Mechanism	Supporting data
Defect in morphogenesis	Development of jaundice soon after birth
	Coexistence of other embryologic abnormalities
	Abnormal remodeling of the "ductal plate"
	Inv mouse: model of biliary obstruction and situs inversus
	Polymorphisms in the *Jag1* gene
	Mutations in the *CFC1* gene (CRYPTIC protein) [37]
Defect in prenatal circulation	Vascular abnormalities
	Hepatic artery hyperplasia and hypertrophy
Toxin exposure	Time-space clustering of cases
Viral infection	Reovirus, rotavirus, CMV, HHV6, human papillomavirus detected in infants with biliary atresia
	Models of virus-induced injury to biliary tract in suckling mice
Immunologic dysregulation	Increased expression of intercellular adhesion molecules
	Infiltration of biliary structures by $CD4^+$, $CD8^+$, and NK [48••] lymphocytes and activated macrophages
	Prevention of experimental biliary atresia in mice by loss of $\alpha 2\beta 1$ integrin, IFNγ, CD8+ cells, NK cells [46••, 48••]
	Increased frequency of the HLA-B12 allele
	Expression of proinflammatory cytokines
	Oligoclonal expansion of lymphocytes
	Maternal chimerism [43]

CMV—cytomegalovirus; HHV—human herpesvirus; IFN—interferon; NK—natural killer
(*Adapted from* Bezerra [35])

was observed in regions that would typically house the pancreas [39•]. These experiments strongly suggest that the development of the extrahepatic biliary system is more aligned with molecular circuits controlling pancreatic, but not hepatic, development. The second study investigated the consequences of the inactivation of *Lgr4*, a gene encoding a leucine-rich repeat-containing G-protein—coupled receptor with a potential role in cell motility [40•]. In this study, the investigators showed that *Lgr4* inactivation resulted in the developmental arrest of the gallbladder primordium. Analysis of the hepatic hilum revealed a targeted loss of the cystic duct and gallbladder, although the intra-and extrahepatic ductular systems appeared intact. Both studies advance our understanding of the molecular regulation of gallbladder and bile duct development and raise the possibility that one or both genes may constitute susceptibility factors for biliary atresia.

Pathogenesis: Liver Cell Phenotypes

Several lines of evidence suggest that the immune system plays a role in pathogenesis of bile duct injury (Table 1). One intriguing scenario that has received attention is maternal chimerism [41, 42]. A recent study found that double chromosome X-labeled cells reside in the sinusoids and portal tracts in male infants with biliary atresia more frequently than in normal controls and display CD8, a marker of cytotoxic lymphocytes [43]. Based on the presence of these cells in normal controls, it is not clear how they may be independently driving the immune response or whether their increase may reflect a more generalized lymphocyte expansion of all lineages (native and the occasional maternally derived cell) in response to the inflammatory process targeting the bile duct epithelium.

Cholangiocytes appear to undergo epithelial to mesenchymal transition (EMT), a process in which mature epithelial cells lose the expression of epithelial markers and acquire features of mesenchymal cells. Immunostaining of livers of infants with biliary atresia showed that cholangiocytes displayed both epithelial (eg, CK19) and mesenchymal (eg, vimentin, Snail) markers at diagnosis and at the time of liver transplantation [44]. A similar profile was also found in residual bile duct epithelium and peribiliary glands of extrahepatic bile ducts from infants with biliary atresia, and in cultured cholangiocytes stimulated with poly(I:C), a synthetic analog of viral double-stranded RNA [45]. Based on a functional link between EMT and tissue fibrosis, these findings implicate cholangiocytes in the production of extracellular matrix at diagnosis and during progression to end-stage liver disease.

Pathogenesis: Immunologic Mechanisms of Bile Duct Injury

The availability of a neonatal mouse model of rotavirus-induced biliary atresia has enabled studies to directly address mechanisms of bile duct injury. In this model, a single inoculation of rotavirus in the first 2 days of life produces an inflammatory obstruction of extrahepatic bile ducts and the atresia phenotype by 12 to 14 days, inducing inflammatory and molecular changes that recapitulate many features of the human disease. In this experimental model, cholangiocytes were recently shown to display high levels of $\alpha_2\beta_2$ integrin. When this integrin was blocked by specific antibodies, neonatal mice became resistant to rotavirus-induced experimental biliary atresia [46••]. Thus, the expression of integrins and other molecules may play key roles in disease susceptibility and initiation of bile duct injury. In keeping with this possibility, a cholangiocyte cell line and freshly isolated cholangiocytes were shown to express markers of antigen-presenting cells (eg, major histocompatibility complex [MHC]-I and II, CD40). However, despite their expression, cultured cholangiocytes were unable to function as competent antigen-presenting cells in T-lymphocyte proliferation assays [47•].

Cellular and molecular analyses of extrahepatic bile ducts are critical to understanding mechanisms of bile duct mucosa injury and lumenal obstruction in humans. However, these analyses are severely limited by the extensive fibrosis that is typically present at diagnosis. A potential approach to overcome this problem is to study the biliary system at different time points after rotavirus inoculation in the neonatal mouse model. Using this model, investigators purified mononuclear cells from extrahepatic bile ducts; they found that natural killer (NK) lymphocytes are the most abundant mononuclear cell type in normal extrahepatic bile ducts of neonates and undergo additional surge following rotavirus challenge [48••]. The importance of this surge was supported by the ability of virus-primed NK cells to recognize and kill cholangiocytes via the Nkg2d receptor. Notably, the timely removal of NK cells or blocking of Nkg2d-mediated attachment by specific antibodies prevented injury to the duct epithelium after rotavirus challenge. As a consequence, the duct wall displayed minimal inflammation, the duct lumen remained patent, and survival improved. Collectively, these experiments show that maintenance of mucosal integrity is critical to bile flow, and identify NK cells as initial effectors of duct injury by direct contact and killing of cholangiocytes (Fig. 2). Another important component of the study was the demonstration that NK cells also populate the portal tracts of human livers at diagnosis and express activation markers [48••]. These types of human-and mouse-based studies will advance our understanding of pathogenesis of disease and may identify therapeutic targets to block progression of disease.

Adjuvant Therapy for Biliary Atresia

The use of drugs that would reduce inflammation or suppress key inflammatory pathways has the potential to

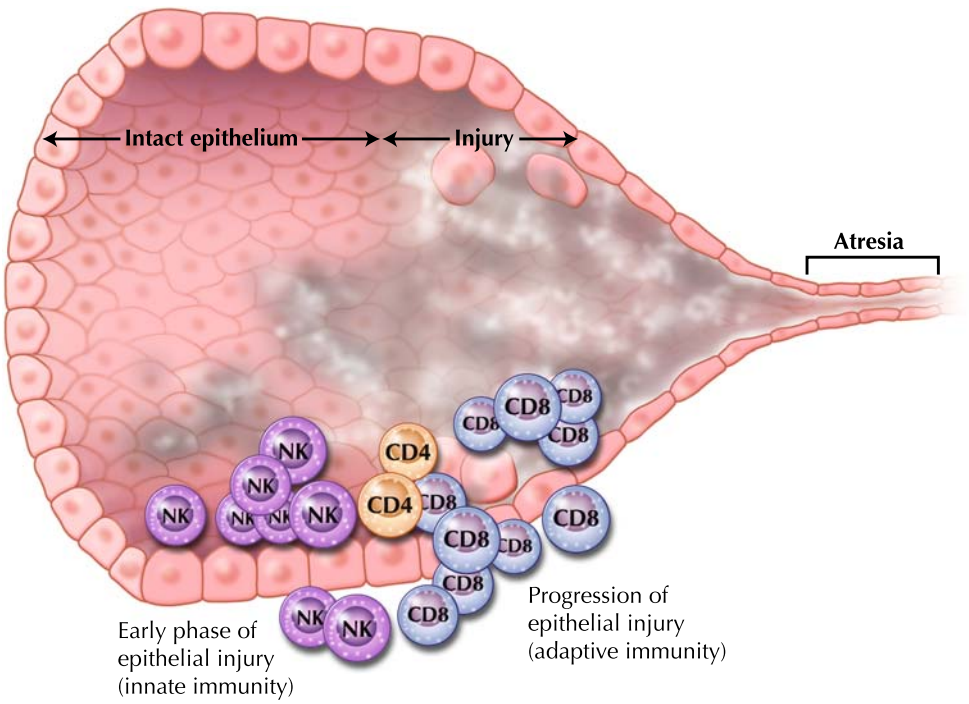

Fig. 2 Diagram depicting an invasion of the duct epithelium by inflammatory cells and progression to biliary atresia. New evidence links natural killer (NK) cells to the injury of cholangiocytes in an experimental model of rotavirus-induced biliary atresia [48••]. Following the disruption of epithelial integrity by NK cells, $CD4^+$ T cells populate the bile duct along with $CD8^+$ T cells, which lead to obstruction of the lumen and progression to atresia

decrease liver injury and improve outcome. Corticosteroids appeared promising based on initial uncontrolled trials that reported improved bile flow after portoenterostomy and long-term survival. Despite the encouraging initial reports, a randomized, double-blind, corticosteroid trial showed no difference in biliary flow or transplant-free survival between infants treated with corticosteroids and those receiving placebo, except for improvement in serum bilirubin levels when corticosteroids were administered to infants younger than 70 days of age at the time of portoenterostomy [49••]. An open-label study from another liver center reported that the use of corticosteroids (4 mg/kg/d beginning 7 days after portoenterostomy for 2 weeks, followed by weaning over 4 weeks) was associated with a decrease in serum bilirubin at 3 and 6 months after surgery, but did not lead to a significant change in transplant-free survival at 15 months after surgery [50]. A second open-label study, using 10 mg/kg/d at 1 to 5 days after surgery followed by 1 mg/kg/d during days 6 to 28, showed no differences in bilirubin at 6 months after portoenterostomy or transplant-free survival at 2 years [51]. Collectively, these studies raise questions about the potential benefit of corticosteroids and underscore the need for a study that is prospective in nature, double-blinded, and randomized, and appropriately sized to meet statistical stringency.

At some centers, a different adjuvant therapy after portoenterostomy in infants with biliary atresia is ursodeoxycholic acid (UDCA). In a cohort of 16 children following successful portoenterostomy, biochemical monitoring following the "on-off-on" use of the drug showed that treatment with UDCA was associated with lower levels of aspartate transaminase, alanine transaminase, and γGTP [52]. Although preliminary in nature, this study provides initial support for using UDCA to promote choleresis in children with biliary atresia.

Conclusions

Recent studies provide new insight into the molecular basis of clinical phenotypes, either identifying candidate genes as disease modifiers (eg, *ERManI* for A1AT deficiency and *CFC1* for biliary atresia) or creating a link between cholestasis-associated genetic mutations with hepatic neoplasia (for *ABCB11* gene). We now know that liver pathology may emerge in the new graft in children transplanted for end-stage cirrhosis secondary to progressive forms of FIC1 (PFIC-1) and BSEP (PFIC-2) deficiencies. For families with a history of previous infant(s) affected with neonatal iron storage disease, the development of disease in future offspring may be prevented or greatly minimized by the use of IV Ig during at-risk pregnancies. The field now looks ahead for strategies to facilitate the early diagnosis of infants with biliary atresia so that portoenterostomy can be performed in a timely fashion. Although we lack effective adjunct therapies to foster long-term survival with the native liver, we remain hopeful that new discoveries of pathogenesis of biliary atresia will identify potential therapeutic targets to block progression of disease.

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- • Of importance
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