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Update on investigations pertaining to the pathogenesis of biliary atresia

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Abstract

Biliary atresia is a devastating biliary disease of neonates that results in liver transplantation for the vast majority. The etiology of biliary atresia is unknown and is likely multifactorial, with components of genetic predisposition, environmental trigger and autoimmunity contributing to disease pathogenesis. This review highlights recent work related to investigations of disease pathogenesis in biliary atresia.

Keywords

Neonatal cholestasis; Autoimmunity; Genetic predisposition

Introduction

This review article on biliary atresia (BA) provides a summary of current data regarding the epidemiology, clinical manifestations, and theories of pathogenesis. Biliary atresia entails an inflammatory fibrosing lesion of intrahepatic and extrahepatic bile ducts of unknown etiology. As a result, the disease leads to biliary cirrhosis and the need for liver transplantation for survival in the majority [1]. If the etiology of BA is discovered, it could lead to treatment options that would delay or negate the need for liver transplant.

Epidemiology

The incidence ranges from 1:5000 to 1:19,000 births, and is dependent on geographic location [1–4]. Biliary atresia has been found to occur more frequently in Asian countries such as Taiwan and Japan in comparison to North America and Europe [4]. According to the National Birth Defects Prevention Study on BA risk factors (1997–2002), infants with BA were more likely to be preterm, born to non-Hispanic Black mothers, and were more likely to have been conceived in the Spring than the Winter [1]. There are three forms of BA: (1) non-syndromic BA (~ 85% of cases in United States), (2) syndromic BA with laterality defects and spleen anomalies (~ 10%), and (3) BA with at least one malformation but without laterality defects (~ 5%) [5]. Interestingly, a recent study showed that syndromic BA

occurred significantly less frequently in China compared to the Western world (0.5 vs. 6.5–10.2%, respectively) [6]. The reason for this is unclear but suggests that environmental or genetic factors may contribute to syndromic BA incidence.

At the time of diagnosis, a Kasai hepatoportoenterostomy (HPE) is performed to aid in the re-initiation of bile flow. Many factors have been proposed to predict outcome after HPE. According to a recent study from Britain, the 5-year survival rate without liver transplantation following HPE is determined by the number of procedures performed by the institution. Institutions performing more than five cases a year had an actuarial 5-year survival rate without transplantation of 61% compared to only 14% in institutions performing the procedure less frequently [7]. The age at HPE remains an important predictor of outcome. Patients who have received an HPE within the first 60 days of life are less likely to require a liver transplant [8]. If the HPE was performed by 45 days of life, it is estimated that up to five pediatric liver transplants could be avoided each year [9]. The total serum bilirubin level at 3-month post-HPE is a prognostic biomarker. Total serum bilirubin of ≥ 2.0 mg/dL at 3-month post-HPE was associated with poor weight gain, ascites, hypoalbuminemia, coagulopathy, and the need for liver transplantation [10]. Identifying therapies to increase bile flow in the first 3 months post-HPE may have a great impact on long-term outcomes.

Clinical manifestations

The majority of children with BA who are surviving with their native liver had problems directly related to biliary cirrhosis, including portal hypertension, poor growth, fat soluble vitamin deficiencies, and cardiomyopathy.

Portal hypertension (PHT) is present in the majority of patients with BA to a variable degree, due to impedance of portal venous blood flow in the setting of liver fibrosis and cirrhosis. The manifestations of PHT include spleno-megaly with hypersplenism, esophageal and gastrointestinal variceal bleeding, and ascites, with associated significant morbidity and mortality. A study from the Childhood Liver Disease Research Network (ChiLDReN; United States and Canada) characterized PHT in 163 children with BA with their native liver. Definite PHT (presence of complication of PHT or splenomegaly and thrombocytopenia) or possible PHT (presence of splenomegaly or thrombocytopenia only) was identified in 67% of subjects. The most common complication of PHT was variceal bleeding, occurring in 20% of subjects. The majority (62%) had only one episode of variceal bleeding in this retrospective study [11].

Children with BA have difficulty with growth due to fat malabsorption and increased metabolic rate in the setting of chronic liver disease. Infants with BA have an increased energy expenditure that is 29% greater than age-matched control [12]. In a large liver transplant database, approximately 40% of BA patients had growth failure prior to transplant [13]. Fat soluble vitamin (FSV) deficiencies inevitably occur in BA due to impaired bile flow and micelle formation. In a recent ChiLDReN study, BA patients' status post-HPE was evaluated for FSV deficiency while on supplementation. Although patients received a standardized commercially available formulation of FSVs daily, 58% of infants continued to

have FSV deficiencies. An inverse correlation was identified between FSV levels and total bilirubin; patients with a total bilirubin level of ≥ 2.0 mg/dL following HPE were found to be at greatest risk for FSV deficiencies [14]. Therefore, infants with BA should be on a high-MCT containing formula, and all infants and children should have serum fat soluble vitamin levels monitored routinely.

Cardiomyopathy is a fairly new finding related to the chronic liver disease found in BA. This is characterized by hypertrophy of the left ventricle and septum, impaired relaxation of the left ventricle during diastole, hyperdynamic contractility of the left ventricle, prolonged QTc interval, and a reduction in cardiac reaction to stressors. A retrospective, single-center study compared echocardiogram findings of 40 pediatric patients with BA less than 2 years of age awaiting liver transplantation to 30 normal age-matched controls [15]. Over 70% of patients with BA (median age 8 months) awaiting liver transplant had echocardiogram abnormalities including increased thickness in the walls of the left ventricle and septum, increased left ventricular mass, or greater left ventricular shortening fraction. 30% of the BA patients had both cardiac structure and function abnormalities on echocardiogram. Furthermore, BA patients with cardiac abnormalities had a 30% longer length of stay in the pediatric intensive care unit following transplantation. Therefore, it is important to consider echocardiogram analysis on all BA patients with their native liver, especially prior to liver transplantation.

Theories of pathogenesis

There are many theories proposed to explain the etiology of BA, including genetic variants, toxins, virus infection, and autoimmune-mediated processes. This review summarizes these various theories and focuses on recent studies of pathogenesis. The compelling finding of mild direct hyperbilirubinemia at birth in infants who eventually went on to have BA begs the question as to whether BA is initiated in-utero [16].

Genetic influences

Using the advanced technology of genome-wide association studies (GWAS), a group from China genotyped nearly half a million single-nucleotide polymorphisms (SNPs) in BA and found a strong association of BA with the SNP rs17095355 on chromosome 10q24 [17, 18]. One gene in the region of this SNP is adducin 3 (*ADD3*). A study in the United States analyzing this genetic region confirmed an association of *ADD3* and BA [19]. *ADD3* is expressed in hepatocytes and biliary epithelia, and is involved in the assembly of spectrin-actin membrane protein networks at sites of cell-to-cell contact. Defective *ADD3* could result in excessive deposition of actin and myosin, contributing to biliary fibrosis. To date, there are no published GWAS studies on BA patients from the United States and Europe.

Genetic associations have been described most commonly in association with syndromic BA or BA with other anomalies. Multiple case reports have been published identifying BA associated with congenital ichthyosis vulgaris [20], hypoparathyroidism, sensorineural deafness, renal dysplasia syndrome (*GATA3* gene haplo-insufficiency) [21], and gastrointestinal luminal disorders (tracheoesophageal fistula and duodenal atresia) [22]. Recently, *FOXA2* haplo-insufficiency in conjunction with a polymorphism that decreases the expression of *NODAL* was identified in a patient with syndromic BA. The *FOXA2*

deletion is believed to have contributed to the patient's interrupted inferior vena cava and abdominal heterotaxy, while the *NODAL* polymorphism was theorized to contribute to the development of BA. On further evaluation of other patients with Syndromic BA, seven additional cases of *FOXA2* sequence changes with a polymorphic *NODAL* gene were identified [23]. These studies suggest that in the rare cases of syndromic BA or BA with other anomalies, a genetic mutation may contribute to defects in bile duct development.

Toxins

Recently, the group from Children's Hospital of Philadelphia reported on a new cholangiocyte toxin ("biliatresone") associated with BA [24]. Biliatresone was discovered in connection with outbreaks of BA in Australian livestock who had ingested plants containing the toxin. The group showed that biliatresone caused destruction of the extrahepatic biliary system in zebrafish. The toxin also caused loss of cilia in neonatal mouse extrahepatic cholangiocytes in culture, suggesting that toxin-induced ciliopathy contributes to the pathogenesis of BA. They went on to show that biliatresone decreased glutathione and SOX17, resulting in disruption of cholangiocyte apical polarity and loss of monolayer integrity [25]. Human neonatal bile duct explants treated with the toxin showed lumen obstruction and fibrosis. This intriguing and exciting discovery of a potential toxin as the inciting event in BA warrants further investigation.

Virus infection

In 1974, Benjamin Landing first proposed that BA and other infantile obstructive cholangiopathies were caused by viral infection of the liver and hepatobiliary tree [26]. Multiple viruses including reovirus [27–37], rotavirus [38–41], and cytomegalovirus (CMV) [42, 43] have been proposed in the etiology of BA. The rotavirus-induced BA mouse model has proved to be exceptionally helpful in investigating the role of virus and inflammation in the pathogenesis of bile duct injury in BA. Attempts to identify these viruses in serum and liver tissue from infants with BA at the time of diagnosis have yielded conflicting results. The largest breadth of literature supporting a virus infection as an initiating event in BA pathogenesis pertains to CMV. Similar to reovirus and rotavirus, CMV can infect biliary epithelia as demonstrated by CMV inclusion bodies seen within bile duct epithelia [44–46]. CMV has been implicated in neonatal hepatitis [47], ischemic vasculopathy [48], and intrahepatic bile duct paucity [49]. In a recent study in China, CMV DNA was identified in 60% BA patients at the time of diagnosis [50]. A higher rate of jaundice, cholangitis, and degree of liver fibrosis after HPE in BA infants with CMV infection suggests that CMV infection may correlate with a worse prognosis [51]. Fischler's group from Sweden has shown higher prevalence of CMV antibodies in the mothers of BA infants, higher serum CMV-IgM levels in infants with BA, and greater amounts of immunoglobulin deposits on the canalicular membrane of the hepatocytes in infants with BA with ongoing CMV infection [43, 52]. Brindley et al. identified a significant liver memory T-cell response to CMV in 56% of BA patients compared with other liver disease controls, suggesting that the BA patients had been exposed to CMV previously [53]. Davenport et al. have defined a subgroup of BA patients based on CMV-IgM positivity and found that those with CMV at diagnosis had higher rates of jaundice, liver inflammation, and fibrosis, and need for liver transplant [54]. These studies suggest that up to 60% of BA patients have evidence for

perinatal CMV infection. It is plausible that the virus infection is short-lived, leading to inability to identify the virus in some cases. Nonetheless, virus infection of cholangiocytes may set the stage for an aberrant immune response targeting cholangiocytes and leading to progressive biliary injury and cirrhosis.

Innate and adaptive immune responses and autoimmunity

The vast majority of research on the pathogenesis of BA has focused on the contribution of the immune system to bile duct injury. Here, we provide a detailed summary of the evidence for abnormal innate and adaptive immune responses in the etiology of BA (Fig. 1).

Innate immunity

The innate immune system responds to infection or danger signals by producing a rapid, non-specific inflammatory response with the release of pro-inflammatory cytokines such as TNF- α , IL-1, and IL-6. Innate immune responses play a critical role in subsequent adaptive immunity. Cells of the innate immune system, including macrophages, neutrophils, dendritic cells, and natural killer (NK) cells, possess membrane bound Toll-like receptors (TLR), one of two receptors collectively known as pattern recognition receptors (PRR) [55].

Importantly, bile duct epithelial cells can also express PRRs [56]. PRRs recognize pathogen-associated molecular patterns (PAMPs) on or released by infected cells, which are conserved molecular patterns that are invariant among an entire class of pathogens. Examples of PAMPs include bacterial lipopolysaccharide (LPS), dsRNA, and single-stranded viral RNA (ssRNA). Each TLR subtype recognizes and binds to a particular set of PAMPs. For example, LPS is detected by TLR4, dsRNA by TLR3, and ssRNA by TLR7/TLR8 [57]. It has also been shown that endogenous ligands (danger signals) from necrotic cells, in addition to pathogens, can activate TLR signaling, of significant importance as a link between TLR activation and the development of autoimmunity [58]. PRR-PAMP interactions result in a synthesis and release of a variety of inflammatory mediators, culminating in pathogen, and sometimes host cell, death. In a number of disease models, the failure to regulate TLR signaling is associated with a chronic inflammatory disease.

The state of innate immunity activation has been investigated in BA. Saito et al. reported upregulation of TLRs 3, 7, and 8 in BA livers at diagnosis and TLRs 3 and 7 expression positively correlated with the need for transplant [59]. Huang et al. [60] also showed increased levels of TLR7 mRNA in BA livers and strong expression of TLR7 was noted on bile duct epithelia, Kupffer cells, and neutrophils. Because TLR7 ligation by ssRNA viruses subsequently activates type 1 interferons through the signaling molecule MxA, this pathway was interrogated. Significantly increased levels of MxA were found in BA samples, suggesting stimulation of type 1 interferons. Harada et al. [35] demonstrated TLR3 expression in bile duct epithelial cells from patients with BA. When the bile epithelial cells were stimulated by a synthetic analog of viral dsRNA that activates TLR3 signaling the cells produced MxA and interferon- β 1, upregulated the expression of TRAIL, and induced biliary apoptosis. Thus, bile duct epithelial cells have the capacity to play an active role in activating innate immunity through the dsRNA virus-TLR3 signaling pathway, leading to cholangiocyte apoptosis and obstruction.

Macrophages function in both the innate and adaptive immune responses. Increased numbers of macrophages have been identified in the portal tracts in BA at the time of diagnosis and correlate with worse outcome [61–65]. Urushihara et al. [64] identified significantly increased number and size of Kupffer cells in the liver and increased serum IL-18. IL-18 (IFN- γ -inducing factor) is a macrophage-derived cytokine that works in concert with IL-12 to promote Th1 cell differentiation in the inflammatory setting. Our group also described dramatic increases in the number and size of macrophages infiltrating the portal tracts in BA, and showed that these cells were producing high levels of TNF- α [62]. Genetic polymorphism analysis of macrophage genes revealed significantly increased frequencies of *T/T* homozygosity within the CD14 promoter region, resulting in increased CD14 expression. CD14 is a macrophage cell surface glycoprotein that recognizes endotoxin (LPS) and activates TNF- α . This study found that the CD14 polymorphism correlated with poorer outcome. The authors concluded that exaggerated activation of macrophages through CD14 promoter polymorphisms resulted in excess stimulation of innate immunity and contributed to bile duct damage. A study from Turkey demonstrated an increased frequency of the macrophage migration inhibitory factor (MIF)-173C allele in BA patients [66]. MIF is a pleiotrophic lymphocyte and macrophage cytokine that plays an important role in innate immunity. Promoter polymorphisms of the MIF gene have been associated with over-production of MIF and increased susceptibility to chronic inflammatory diseases [67, 68].

Studies in the RV-induced mouse model of BA have demonstrated a role for NK cells in bile duct epithelial injury. NK cell numbers are increased in BA mice and promote chronic liver inflammation [69]. Depletion of NK cells or antibody blockade of their Nkg2d receptor immediately after birth prevented jaundice in newborn mice infected with RV. Similarly, Saxena et al. identified a significant increase in plasmacytoid dendritic cells (pDCs) in both the mouse model and in humans [70]. The pDCs produced IL-15 that activated NK cells, resulting in bile duct epithelial-targeted injury. Depletion of pDCs to RV-infected newborn mice prevented the development of BA. Taken together, all of these studies suggest that a sustained induction of the innate response, without the development of tolerance, results in chronic inflammation and injury to bile duct epithelia in BA.

T-cell immunity

Adaptive immunity entails immune responses that are stimulated by repeat exposure to a pathogen or non-microbial proteins (i.e., self antigens). Effector T cells in adaptive immunity produce cytokines that can directly damage cells or indirectly cause damage through activation of other immune cells. T-cell responses have been categorized based on the type of cytokines that are generated: Th1 responses involve IL-2, IFN- γ , and TNF- α , and Th17 responses involve IL-17. In the past decade, much attention has focused on the role of Th1 and, recently, Th17 cellular immunity in bile duct injury in BA. The rotavirus (RV)-induced mouse model of BA recapitulates the immune response found in the human disease, with portal tract CD4⁺ T cells producing IFN- γ and TNF- α , followed by CD8⁺ T-cell and macrophage infiltration [41]. Coinciding with the Th1 cellular cytokines identified, Leonhardt et al. [71] found that many chemokines associated with a Th1 response were upregulated in the mouse model, including CCL2, CCL5, and CXCL10 [IFN- γ inducible protein (IP)-10 chemokine]. With regard to the important role of IFN- γ in bile duct injury,

Shivakumar et al. [72] demonstrated that RV-infected IFN- γ knockout mice developed jaundice in a similar manner as the wild-type controls, however, the cholestasis resolved by 3 weeks of age in 77% of the knockout mice, compared with progression of disease in 75% of the wild-type controls. This study strengthened the contention that the immune response, and not the initial viral infection, was responsible for the progression of bile duct injury. In human BA, the predominant cellular immune response at diagnosis encompasses activated CD4⁺ and CD8⁺ T cells within portal tracts that produce Th1 cytokines (IL-2, IFN- γ) and macrophages that generate TNF- α [61, 62, 73, 74]. These lymphocytes have been found invading between bile duct epithelia, leading to degeneration of intrahepatic bile ducts [75]. The T cells are highly activated, expressing the proliferation cell surface marker CD71 and activation markers CD25 and LFA-1.63. Bezerra et al. [76], utilizing gene expression microarray techniques to analyze BA liver biopsies, observed upregulation of pro-inflammatory genes including IFN- γ at the time of BA diagnosis.

Recent literature suggests that Th17 responses are as important as Th1-mediated inflammation in BA. IL-17 is a potent inflammatory cytokine implicated in disease pathogenesis for many autoimmune conditions. In the RV-induced mouse model of BA, Klenmann et al. showed that $\gamma\delta$ -T cells were high producers of IL-17 and blocking IL-17 resulted in decreased liver inflammation and serum bilirubin levels. Furthermore, liver tissue from BA patients at diagnosis had significantly increased levels of IL-17 mRNA [77]. Lages et al. showed that CD4⁺ T cells were primarily responsible for IL-17 production and IL-17 stimulated macrophage influx and biliary injury in the mouse model [78]. Analysis of human tissue reveals that high expression of IL-17 producing T cells was associated with need for liver transplant. Hill et al. found high levels of Th17 cells in portal tracts of BA patients at diagnosis and the number of Th17 cells positively correlated with serum bilirubin levels, suggesting that IL-17 was directly responsible for bile duct injury [79].

The inciting event that triggers the Th1 and Th17 inflammatory responses in humans is unknown, and theories include virus infection and bile duct-targeted autoinflammatory or autoimmune responses. The strongest evidence for autoimmunity has been gained from mouse studies, where autoreactive T cells targeting bile duct epithelia have been identified [80, 81]. In vitro analyses demonstrated significant increases in liver T cells from BA mice that generated IFN- γ in response to either RV or self-bile duct epithelial antigens [80]. In addition, adoptive transfer of liver T cells from BA mice into immunodeficient recipients led to bile duct-specific inflammation and injury [80, 81]. This induction of bile duct pathology occurred in the absence of detectable transferred virus, suggesting that bile duct antigens were the target of the T cells. Further investigations aimed at determining the mechanisms (molecular mimicry or bystander activation) of the apparent virus-induced autoimmunity are necessary.

B-cell immunity

B cells function as antigen presenting cells and as immunoglobulin producers in chronic inflammatory conditions. Periductal immunoglobulin deposits along the basement membrane of bile duct epithelia have been identified in humans [82] and in the RV-induced mouse model of BA [80]. Lu et al. identified α -enolase antibody as an autoantibody reactive

to cytosolic proteins within bile duct epithelia in the mouse model and in human BA sera. This 48-kD enzyme shares amino acid sequence similarities with RV-encoded proteins, suggesting a role for molecular mimicry [83]. Furthermore, RV-infected B-cell knockout (Ig- α -/-) mice are protected from developing BA, suggesting an important role of B cells in disease pathogenesis [84].

Autoimmunity

In humans, only circumstantial evidence exists for the role of autoimmunity in BA pathogenesis. Circumstantial evidence for categorizing a disease as autoimmune in nature includes the following criteria, followed by data in BA. (1) A familial increased incidence of autoimmunity: in a recent analysis of epidemiologic factors associated with BA, 44% of BA patients had a family member with an autoimmune disease [5]. (2) lymphocytic infiltrate of the target organ, especially with restricted T-cell receptor variable regions of the beta chain (TCR-V β) usage: Multiple studies have identified lymphocytic infiltrates surrounding and invading both the intrahepatic ducts and extrahepatic biliary remnant [61, 62, 73–75]. Furthermore, antigen-specific T-cell immunity involves oligoclonal expansion of T cells expressing similar TCR-V β s. Analysis of TCR-V β s within BA liver and extrahepatic bile duct remnants revealed that the T cells are oligoclonal in nature, with a limited TCR-V β repertoire, suggesting antigen-specific activation [85]. The CD4+ TCR expansions were limited to V β -3, -5, -9, and -12 T-cell subsets and the CD8+ TCR-V β expansions were predominantly V β -20. Nucleotide sequencing of the expansions confirmed that each identified V β subset was composed of oligoclonal populations of T cells, suggesting proliferation in response to specific antigenic stimulation. (3) Statistical association with human leukocyte antigen genotype or aberrant expression of HLA class II antigens on the affected organ: normal bile duct epithelium expresses HLA class I but not class II, which is usually present only on professional antigen presenting cells and vascular endothelium. Bile duct epithelium from BA patients aberrantly expresses MHC class II, with strong expression of class II HLA-DR on liver bile duct epithelia [86, 87]. One of the strongest genetic associations with autoimmunity is with the HLA genes; however, to date, there is no clear HLA predominance in BA. A Japanese study found a significant association between BA and HLA-DR2 as well as a linkage disequilibrium with a high frequency of HLA-A24-B52-DR2 [88]. In contrast, a large study in the United States, performed through the ChiLDReN network, utilized high-resolution genotyping of all HLA class I and class II alleles and failed to identify an HLA predominance in BA [89]. (4) Favorable response to immunosuppression. At the time of HPE, immunosuppressive therapy has been investigated, with the goal of establishing long-term bile flow. A recent meta-analysis of randomized trials and observational studies pertaining to steroids in BA encompassed all studies between 1969 and 2010 and included 233 BA patients [90]. There was no significant difference in the effect of steroids on serum bilirubin levels at 6-month post-HPE or in delaying the need for early liver transplantation. A prospective, randomized, double-blinded, placebo-controlled trial of high-dose corticosteroid therapy post-HPE in BA showed no significant improvement in bile drainage at 6 months with corticosteroid use. Importantly, steroid treatment was actually associated with earlier onset of serious adverse events and, therefore, is not recommended for routine use post-HPE [91].

Immune dysregulation

The regulatory T-cell (Treg) subset of CD4⁺ T cells is characterized by the Foxp3 transcription factor and is responsible for controlling T-cell-mediated immune responses to prevent “bystander damage” of healthy cells. In addition, Tregs are necessary to prevent activation of autoreactive T cells. In BA, deficits in the number or function of Tregs would allow for inflammation to flourish unchecked. Peripheral blood Treg quantification of BA infants at diagnosis revealed significant deficits in Treg frequencies in BA patients compared to controls, with the most marked deficits in those BA patients who were positive for CMV. In the mouse model of BA, Miethke et al. [92] reported that RV-infected neonatal mice had decreased numbers of Tregs 3 days after infection compared to mice that were 7 days old at the time of infection, suggesting that infection at birth was associated with the inability to produce a functional Treg response. Adoptive transfer of normal Tregs into RV-infected neonatal mice resulted in increased survival and decreased bile duct-targeted inflammation [93, 94]. Novel therapies aimed at expanding Treg populations in BA patients may prove to be beneficial.

A known cause of Treg dysfunction is due to epigenetic modification of Foxp3. Epigenetic modifications involve functional changes to the genome without altering the DNA sequence. Many factors affect epigenetics including environmental triggers and viruses. One mechanism of epigenetic regulation involves increased methylation of DNA. DNA hypermethylation causes nucleosomes to pack tightly together and transcription factors such as Foxp3 cannot bind to DNA, resulting in decreased gene expression. DNA hypermethylation of Foxp3 was recently reported in BA infants and children, as well as in the mouse model of BA [95]. Hypermethylation of Foxp3 was associated with decreased number and suppressive function of Tregs in the BA mouse model. In contrast, DNA hypomethylation has also been implicated as playing a role in autoimmune diseases [96] and in inhibiting lymphocyte differentiation [97]. Dong et al. [98] assessed the DNA methylation patterns within CD4⁺ T cells from BA patients, and found that certain genes were hypomethylated, including DNA methyltransferase (DNMT1), DNMT3a, and methyl-DNA-binding domain (MBD1). Importantly, the IFN- γ gene promoter region was also hypomethylated in BA CD4⁺ T cells and IFN- γ mRNA expression levels were significantly increased. The authors concluded that methylation changes in CD4⁺ T cells result in unchecked production of IFN- γ , contributing to bile duct injury in BA.

In conclusion, BA is a devastating disease of infancy, with a significant morbidity and the need for liver transplantation in the vast majority for survival. The etiology of BA is not known; however, the current research suggests interplay of genetic predisposition, virus trigger and progressive autoimmunity, culminating in bile duct injury, fibrosis, and biliary cirrhosis. A clear understanding of the key players associated with bile duct epithelial injury will provide the framework for future targeted therapeutic interventions aimed at protecting the intrahepatic biliary system from ongoing injury.

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Fig. 1.

Immunopathogenesis of biliary atresia. Transient virus infection of cholangiocytes results in activation of the innate immune system and bystander damage of cholangiocytes. This results in a chronic inflammatory state, with bile duct-specific autoreactive T cells stimulating downstream effector cells, resulting in ongoing biliary injury