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The Pathogenesis of Biliary Atresia: Evidence for a Virus-Induced Autoimmune Disease

Cara L. Mack, M.D.¹

¹Pediatric Liver Center and Liver Transplant Program, Section of Pediatric Gastroenterology, Hepatology, and Nutrition, The Children's Hospital, University of Colorado at Denver–Health Sciences Center, Denver, Colorado

Abstract

Biliary atresia is a mystifying cause of neonatal cholestasis, manifested by progressive inflammation and fibrosis of both the extrahepatic and intrahepatic bile ducts. It is a devastating disease that leads to cirrhosis and the need for liver transplantation in the majority of children. The etiology is unknown, and one theory is that it may involve a primary perinatal hepatobiliary viral infection and a secondary generation of an autoimmune-mediated bile duct injury. This review will outline the evidence from both human and murine studies supporting a potential cholangiotropic viral infection as the initiator of bile duct injury in biliary atresia and the role of the adaptive immune response and autoimmunity in progression of disease. Delineating the pathways of immune and autoimmune-mediated bile duct injury within biliary atresia could stimulate development of new medical interventions aimed at suppressing the specific immune response, decreasing the inflammatory damage to bile ducts, and delaying or negating the need for liver transplantation.

Keywords

Reovirus; rotavirus; cholangitis; adaptive immunity; neonatal immunity

Biliary atresia (BA) is a mystifying cause of neonatal cholestasis, characterized by progressive inflammation and fibrosis of both the extrahepatic and intrahepatic bile ducts. The incidence of BA is highest in Taiwan and French Polynesia with a frequency of 1 in 3000 live births.^{1,2} The incidence in the United States is approximately 1 in 12,000 live births, and it is estimated that 400 to 500 new cases are encountered annually. The majority (80%) of cases of BA are of the perinatal or acquired form, whereas the rarer embryonic or fetal form is associated with multiple congenital anomalies. In the perinatal form, these otherwise normal infants are presumably born with a patent biliary system that undergoes progressive inflammation and fibro-obliteration initiated by a perinatal insult.^{3,4} At the time of diagnosis (1 to 3 months of age), the extrahepatic bile ducts are partially or entirely obliterated with residual inflammatory cells present within duct remnants.⁵ The intrahepatic bile ducts at this point have an ongoing inflammatory response with lymphocytes surrounding and invading the ducts.⁶ At diagnosis, surgery is performed whereby the fibrotic extrahepatic bile duct is removed, the porta hepatis is carefully dissected, and a loop of small intestine is anastomosed to the porta (Kasai portoenterostomy) in an attempt to

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Address for correspondence and reprint requests: Cara L. Mack, M.D., Assistant Professor of Pediatrics, Pediatric Liver Center and Liver Transplant Program, Section of Pediatric Gastroenterology, Hepatology, and Nutrition, The Children's Hospital, University of Colorado at Denver–Health Sciences Center, 1056 E. 19th Avenue, Box B290, Denver, CO 80218.
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reestablish a conduit for bile flow. Despite surgical intervention, the intrahepatic bile duct inflammation and injury progresses at a variable but inevitable pace, leading to biliary cirrhosis in the majority of children. As a result, ~80% of patients with BA will require liver transplantation, accounting for half of all pediatric liver transplants.⁷ Only 15% to 20% of children with BA will enter adulthood with their native liver, and more than 95% of these patients have evidence of chronic liver disease or cirrhosis.⁸

A recent study estimated that \$77 million is spent each year on children for liver transplantation and the ensuing hospitalizations in the United States.⁹ This sum of money covers 0.2% of total health care expenditures related to children, even though these children represent only 0.0006% of the total pediatric population. Importantly, this disproportionate expenditure for liver transplantation in children could be cut in half if improved therapies for BA were developed that could abrogate or further delay the need for liver transplantation.

The etiology of BA is unknown, and theories of pathogenesis include viral infection,^{10–13} autoimmunemediated bile duct destruction,^{3,14} and abnormalities in bile duct development.¹⁵ A current view of the pathogenesis of BA is that it may involve both a primary perinatal hepatobiliary viral infection and a secondary generation of an autoimmune-mediated bile duct injury. In this scenario, a perinatal infection with a virus that is tropic for the bile duct epithelia would cause initial cholangiocyte apoptosis or necrosis. Even though viral clearance would occur, persistent inflammation and injury to the bile duct epithelia ensues. The damaged bile duct epithelial cells may express previously sequestered “self-antigens that are recognized as foreign and elicit autoreactive T cell-mediated inflammation directed at duct epithelia. This mechanism of viral-induced autoimmunity is known as the *bystander activation pathway*.¹⁶ Alternatively, viral proteins that may be structurally similar to bile duct epithelia proteins could elicit autoimmunity based on *molecular mimicry*. This process entails T-cell activation in response to microbial antigens with subsequent cross-reactivity to self-antigens. The immune response leads to cholangitis, bile duct obstruction, and eventually biliary cirrhosis (Fig. 1).

For more than 2 decades, viruses have been implicated as a potential trigger for autoimmune diseases.^{17–19} An initial protective antiviral immune response may lead to a chronic autoaggressive immune response. Substantiation of molecular mimicry between a microbe and autoantigen as a cause of human autoimmune diseases has been documented for rheumatic heart disease,^{20,21} lupus,²² and multiple sclerosis.²³ The role of molecular mimicry has also been demonstrated in several murine models of virus-induced autoimmune diseases (Table 1).^{24–27} In this review, we will outline the evidence from both human and murine studies supporting a potential cholangiotropic viral infection as the initiator of bile duct injury in BA and the role of the adaptive immune response and autoimmunity in progression of disease.

VIRUSES AND BA

Investigations into the role of a viral infection of the biliary system as the initiating event in BA have produced conflicting results. A variety of viruses have been implicated, as outlined in Table 2.^{10–13,28–30} Reovirus, rotavirus, and cytomegalovirus have been studied most extensively, and evidence will be reviewed for each with regard to the etiology of BA.

Reovirus

HUMAN STUDIES—Reovirus is a double-stranded RNA (dsRNA) virus from the Reoviridae family. Human investigations of reovirus have focused on detection of antireovirus antibodies in blood and reovirus RNA in liver specimens of BA patients. In 1982, Morecki et al¹¹ used indirect immunofluorescence and identified antireovirus IgG

antibodies in the sera of 9 of 15 BA patients and none in the controls. A large follow-up study from this group looked at 167 cholestatic infants and identified antireovirus antibodies in 62% of BA, 52% of idiopathic neonatal hepatitis, and less than 12% in normal or other cholestatic disease controls.³¹ These early studies are limited by the fact that the majority of IgG in the first 6 months of life is maternal in origin due to transplacental acquisition and may not reflect a recent neonatal reovirus infection. To that end, Richardson et al³² identified antireovirus IgM in 20% to 40% of infants with a variety of cholestatic diseases including BA, idiopathic neonatal hepatitis, total parenteral nutrition (TPN)-related cholestasis, and α_1 -antitrypsin deficiency, suggesting that exposure to reovirus infection was not unique to BA.

At least 3 studies have analyzed liver or extrahepatic bile duct remnant tissue for the presence of reovirus RNA using reverse transcription–polymerase chain reaction (RT-PCR) techniques. Tyler et al¹² reported the detection of reovirus RNA (L1 genome segment) in snap-frozen liver, gall bladder, or bile duct remnants from 55% of BA specimens (average age, 2.2 months), 78% of choledochal cysts (CDCs), 21% of other neonatal liver disease controls, and 12% of normal liver autopsy cases. The prevalence of reovirus RNA in BA and CDC tissues was significantly greater than in diseased controls or autopsy specimens. In contrast, Steele et al³³ did not detect reovirus RNA (M3 genome segment) in formalin-fixed liver tissue from 14 BA patients. Technical differences that may have contributed to the opposing results found in these two studies include the fixation of tissue analyzed (frozen versus formalin-fixed) and the reoviral genome segment analyzed (L1 vs. M3). A recent study from Japan³⁴ also did not detect reovirus RNA (L1 genome) from BA or controls. The average age when hepatobiliary tissues were taken for analysis in the Japanese study was 8.7 months, in contrast with 2.2 months of age in the Tyler study. It is the author's opinion that attempts to detect viral infection in BA tissue after the first few months of life is futile because the virus is most likely cleared from the liver and biliary system within weeks to months of initial infection.

MURINE STUDIES—As early as 1968, Papadimitriou reported on the pathology of the biliary tract in murine reovirus serotype 3 infection.³⁵ Reovirus was found replicating within bile duct epithelial cells, resulting in epithelial necrosis. Serotype 3 reovirus neonatal infection resulted in “oily hair syndrome,” which was associated with a greasy appearance of the fur, failure to thrive, diarrhea, and jaundice on non-fur-bearing regions. In the chronic inflammatory phase of the disease (after 1 week), the extrahepatic bile duct was tortuous and edematous with a reduced lumen but without complete obstruction.³⁶ The intrahepatic portal tracts showed marked periportal inflammation.³⁷ Extensive damage to the biliary system has been related to tropism of certain strains of serotype 3 reovirus to the biliary system (T3 Abney strain), and not simply due to the amount of virus inoculated.³⁸ Furthermore, recent work by Barton et al³⁹ has shown that reovirus binding to a sialic acid coreceptor on ductal epithelial cells is essential for viral infectivity and generation of biliary disease.

In a spontaneous primate model, BA was observed in a 6-week-old jaundiced rhesus monkey.⁴⁰ Reovirus infection was suspected because of positive serum antibody titers for reovirus. The mother's titer was negative prior to conception and positive after the birth, providing circumstantial evidence for perinatal acquisition of the virus. At autopsy, the lumen of the extrahepatic bile duct was severely narrowed and surrounded by inflammation, edema, and fibrosis, mimicking human BA.

Rotavirus

HUMAN STUDIES—Rotavirus is also a dsRNA virus of the Reoviridae family. This virus has been shown to infect hepatocytes as well as bile duct epithelia.^{41,42} Riepenhoff-Talty et

al detected group C rotavirus RNA in 50% of infant BA liver specimens and none in other cholestatic disease controls.¹⁰ In comparison, Bobo et al⁴³ did not detect rotavirus (groups A, B, and C) RNA from infant BA liver specimens or controls.

MURINE STUDIES—Over the past decade, extensive research has used the rotavirus-induced murine model of BA. Riepenhoff-Talty et al⁴⁴ first described how rhesus group A rotavirus (RRV) neonatal infection led to biliary obstruction in mice with histologic features resembling human BA. Petersen et al^{45,46} performed subsequent thorough investigations verifying not only the reproducibility of the murine disease but also that it recapitulated human disease. This group also reported that only a short window of postnatal age for the RRV inoculation was permissive for the development of BA.⁴⁷ The highest incidence of biliary obstruction occurred when mice were inoculated with RRV at 12 hours of life (86% developed BA). The later the age at inoculation, the lower the incidence of biliary obstruction; infected adult mice did not develop any signs of biliary tract disease. This study suggested that differences in the immature neonatal murine immune system played a role in the ability of rotavirus to elicit biliary tract disease. Our group has also investigated this model and has shown that the majority of mice become jaundiced by 1 week after RRV infection, and that by 2 weeks of age the biliary obstruction is manifested by acholic stools, direct hyperbilirubinemia, and intense portal tract and extrahepatic ductal inflammation and obstruction.⁴⁸ The virus is no longer detectable in the liver at this time point; however, the portal and biliary inflammation persists and the mice die by 1 month of age.^{44,48} Many investigators have now analyzed various aspects of the immune system in the RRV-induced murine model of BA. These studies will be discussed below in reference to the specific arm of the immune system being investigated.

Recently, Allen et al⁴⁹ studied the effects of different strains of rotavirus on the development of BA. Five strains of rotavirus were tested and only two strains (RRV and SA11-FM) produced bile duct epithelial injury and biliary obstruction. This work parallels investigations of reovirus, lending credence to the importance of a specific viral infection of bile duct epithelia as the initiator of bile duct damage.

Cytomegalovirus

HUMAN STUDIES—The third virus that has received much attention is cytomegalovirus (CMV), which is a double-stranded DNA (dsDNA) virus of the Herpesviridae family. Similar to reovirus and rotavirus, CMV can also infect biliary epithelia as demonstrated by CMV inclusion bodies within bile duct epithelia.^{50–52} In 2005, two groups reported finding CMV DNA in 30% to 40% of frozen liver tissue specimens from infants with BA but not in other cholestatic disease controls.^{30,53} In contrast, Fischler et al⁵⁴ identified CMV DNA in 50% of BA specimens *and* in 43% of other cholestatic controls, suggesting that CMV infection was not unique to BA. Moreover, Jevon and Kimmick¹³ failed to identify CMV DNA from formalin-fixed tissue of BA patients, however there was no documentation of a positive control for CMV in the PCR reactions. To date, there are no literature reports of the use of CMV to generate BA in a murine model.

In summary, many viruses have been proposed to cause BA; however, there continues to be conflicting reports of the detection of viral infection at the time of diagnosis. Discrepancies may be related to techniques used to store tissue (fresh, snap-frozen vs. formalin-fixed), the viral genome segment analyzed, and the age of the patient at the time of analysis. Nonetheless, in the studies that identified molecular evidence of a pathogen, reovirus, rotavirus, or CMV were found in up to 50% of specimens tested. If one extrapolates from the murine data, it is possible that the virus is cleared quickly from the liver and biliary tract in the first few weeks of life. An interesting observation from these studies is the ability of

all three viruses to infect and damage bile duct epithelia, lending support to a primary cholangiotropic viral infection as the initiating event in the pathogenesis of BA.

ADAPTIVE IMMUNITY: CELLULAR IMMUNITY IN BA

Human Studies

Adaptive immunity entails immune responses that are stimulated by repeat exposure to a pathogen or nonmicrobial antigens. The defining characteristics of adaptive immunity include the exquisite specificity for distinct molecules and memory that evokes the ability to respond to repeat exposures. There are two types of adaptive immune responses: *cellular immunity* mediated by T cells, which produce cytokines, and *humoral immunity* mediated by B cells that produce antibodies. In the past decade, much attention has focused on the role of *cellular immunity* in bile duct injury in BA. Many investigators have shown that the portal tract infiltrates surrounding bile ducts are composed of both CD4⁺ and CD8⁺ T cells.^{55–59} These lymphocytes have been found invading between bile duct epithelia, leading to degeneration of intrahepatic bile ducts.⁶⁰ The T cells are highly activated, expressing the proliferation cell surface marker CD71 and activation markers CD25 and LFA-1.⁵⁷ Analysis of the T-cell receptor variable region of the α -chain (TCR V α) within BA liver and extrahepatic bile duct remnants revealed that the T cells were oligoclonal in nature with a limited TCR V α repertoire, suggesting that the T cells in BA are proliferating in response to a specific antigen(s).⁶¹

Activated effector T cells produce cytokines that can directly damage epithelial cells or indirectly cause damage through stimulation of other immune cells. T cells within the liver of BA patients have been shown to secrete the Th1 cellular cytokines IFN- γ , IL-2, and TNF- α , which was unique to BA and not found in other neonatal cholestatic diseases.⁵⁹ Similarly, Bezerra et al,⁶² using gene expression microarray techniques to analyze BA liver biopsies, observed upregulation of proinflammatory genes including IFN- γ and osteopontin. These studies to date are descriptive and not capable of clearly defining the role that T cells and cytokines may play in human BA bile duct injury. Therefore, mechanistic studies in the RRV-induced murine model of BA have been used to shed light on the role of cellular immunity in bile duct damage.

Murine Studies

The RRV-induced murine model of BA recapitulates the immune response found in the human disease. We have shown that portal tract CD4⁺ T cells produced IFN- γ and TNF- α 1 week after RRV infection, followed by CD8⁺ T cell and macrophage infiltration by 2 weeks of age.⁴⁸ Coinciding with the Th1 cellular cytokines identified, Leonhardt et al⁶³ recently found that many chemo-kines (cytokines that stimulate leukocyte movement from the blood to the diseased tissue) associated with a Th1 response were upregulated in the murine model, including CCL2, CCL5, and CXCL10 [IFN- γ inducible protein (IP)-10 chemokine]. Similarly, Carvalho et al⁶⁴ reported increased gene expression of IFN- γ -induced chemokines CXCL9 and CXCL10 at 1 week after RRV infection. With regard to the important role of IFN- γ in bile duct injury, Shivakumar et al⁶⁵ demonstrated that RRV-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 (IFN- γ), and not the initial viral infection, was responsible for the progression of bile duct injury. The roles of IL-12⁶⁶ and TNF- α ⁶⁷ in the progression of murine BA have also been analyzed through the use of cytokine knockout mice. In contrast with the IFN- γ studies, the lack of either of these cytokines failed to alter the progression of the bile duct injury and obstruction.

ADPATIVE IMMUNITY: HUMORAL IMMUNITY IN BA

Human Studies

Humoral immune responses are initiated by the recognition of antigens by B cells that, in the effector phase, secrete antibodies specific for the antigen. Cellular and humoral immunity may be synergistic in that antibody responses to protein antigens require CD4⁺ T cells that recognize the antigen and activate B cells. Scant information is available regarding the potential role of humoral immunity in the pathogenesis of BA. More than 3 decades ago, Hadchouel et al⁶⁸ visualized immunoglobulin deposits in the biliary remnants of BA. Immunoglobulin (Ig)M deposits were found in 25 specimens and both IgM and IgG in an additional 19 of 128 remnants analyzed. The immunoglobulin deposits localized along the basement membranes of the bile duct epithelia, suggesting that the antigen reactivity was epithelial in origin. Based on what is known regarding virus infection of bile duct epithelia, an alternative interpretation is that the immunoglobulin deposits are reacting with viral proteins on epithelia.

To date, there has been limited investigation of the presence of autoantibodies in BA. A preliminary report by Vasiliauskas et al⁶⁹ showed that 10 of 11 patients with BA had higher levels of IgM antineutrophil cytoplasmic antibodies (ANCAs) compared with other liver diseases. Burch et al⁷⁰ examined autoantibodies in the mothers of children with BA to test the hypothesis that maternal transfer of autoantibodies might be involved in bile duct injury. Low-titer anti-Rho antibodies were more prevalent in mothers of infants with BA and idiopathic neonatal hepatitis than in controls; however, low-titer antinuclear antibodies were more common in mothers of infants with liver diseases in general.

Murine Studies

Humoral immunity in the RRV-induced murine model of BA has recently been demonstrated by immunoglobulin deposits (IgG) surrounding intrahepatic bile ducts.^{65,71} Furthermore, Western blot analysis of sera from RRV-induced BA mice identified antibodies reactive to multiple proteins within a bile duct epithelial homogenate, suggesting the presence of autoantibodies specific to bile duct epithelia.⁷¹ Further analysis of the targets of these immunoglobulins may shed light on the antigens triggering this immune response.

AUTOIMMUNITY AND BA

In 1957, Rose and Witebsky formatted a list of criteria used to define an autoimmune disease,⁷² which was updated in 1993 based on knowledge gained from molecular biology and hybridoma technology.⁷³ Three lines of evidence can be assembled to establish that a human disease is actually autoimmune in origin (Table 3). Following is an outline of the criteria for autoimmunity with reference to evidence of autoimmunity in BA.

1. Direct Proof

Human disease is reproduced in a normal recipient by direct transfer of autoantibody—Such instances would be rare; however, transplacental transmission of pathogenic IgG autoantibodies from the mother to the fetus have been identified in neonatal myasthenia gravis and neonatal Graves' disease.⁷³ To date, there is no direct proof of autoimmunity in BA.

2. Indirect Proof

Reproduction of autoimmune disease in experimental animal models by adoptive transfer of autoantibodies or auto-antigen-specific T cells from diseased mice into immunodeficient animals as well as identification of the

autoantigen—In support of the role of autoimmunity in BA, we have recently demonstrated that autoreactive T cells specific to bile duct epithelia are present in RRV-induced BA mice and are sufficient to result in bile duct inflammation.⁷¹ In vitro analyses (ELISPOT) demonstrated significant increases in liver T cells from 2-week-old RRV-induced BA mice that generated IFN- γ in response to either rotavirus antigen or self-bile duct epithelial antigens. Furthermore, adoptive transfer of liver T cells from RRV-induced BA mice into naïve syngeneic severe combined immunodeficiency (SCID) recipients led to bile duct-specific inflammation and injury. 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 mechanism (molecular mimicry or bystander activation) of the apparent virus-induced autoimmunity are ongoing.

3. Circumstantial Evidence

Based on distinctive clinical clues including:

A. Association with other autoimmune diseases in the same, *individual or the same family*—There is a lack of investigative studies addressing the potential association of BA and other autoimmune diseases within the patient or patient's family. The occurrence of de novo autoimmune hepatitis after pediatric liver transplantation is highest among BA patients; however, the majority of pediatric transplant recipients have BA.^{74,75}

B. Lymphocytic infiltration of target organ, especially if there, *is a restriction in T-cell receptor variable gene usage*—Numerous investigators have identified lymphocytic infiltrates surrounding both the intrahepatic ducts and extrahepatic biliary remnant.^{55–60} Antigen-specific T-cell immunity involves oligoclonal expansion of T cells expressing similar T-cell receptor variable regions of the α -chain (TCR V α). Our laboratory has recently shown that CD4⁺ and CD8⁺ T cells from BA patients are oligoclonal in nature.⁶¹ Fluorescent-activated cell sorter (FACS) analysis revealed V α subset expansions of CD4⁺ and CD8⁺ T cells from the liver or bile duct remnant in all 6 patients with BA and in only 1 of 6 controls. The CD4⁺ TCR expansions were limited to V α 3, 5, 9, and 12 T-cell subsets, and the CD8⁺ TCR V α expansions were predominately V α 20. Nucleotide sequencing of the expansions confirmed that each identified V α subset was composed of oligoclonal populations of T cells, suggesting proliferation in response to specific antigenic stimulation. Future studies will be required to identify the specific antigen(s) responsible for T-cell activation and bile duct injury.

C. Statistical association with a particular major histocompatibility complex (MHC) haplotype or aberrant expression of MHC class II antigens on the affected organ—Human MHC molecules are called human leukocyte antigens (HLAs), and the strongest genetic associations with autoimmunity are with these HLA genes. Aberrant expression of HLA-DR molecules on liver bile duct epithelia of BA patients has been described previously.^{76,77} HLA associations with the occurrence of BA have also been reported with conflicting results. A European study analyzed the HLA genotype as well as polymorphisms in the IL-1 gene family, IL-10 promoter, and TNF- α promoter genes within 101 children with BA and found no significant differences compared with controls.⁷⁸ In contrast, a Japanese study of 392 BA patients found significant association between BA and HLA-DR2 as well as a linkage disequilibrium with a high frequency of HLA-A24-B52-DR2.⁷⁹ A smaller study from Egypt also found significant increases in both HLA B8 and DR3 in BA compared with controls.⁸⁰ To date, no such HLA analysis has been performed in the United States, and a large, multicentered study of HLA types (high-resolution genotyping) in BA is warranted.

D. Favorable response to immunosuppression—At the time of Kasai portoenterostomy, immunosuppressive therapy has been used with the goal of establishing long-term bile flow. In 2001, Dillon et al⁸¹ retrospectively analyzed 25 patients treated with corticosteroids and found that 76% were jaundice-free at a mean followup time of 50 months. Meyers et al⁸² performed a retrospective review of 28 infants with BA and found that patients who received steroids had a 92% overall survival with their native liver compared with 38% survival in patients who did not receive steroids. In 2004, the Japanese Biliary Atresia Society was surveyed regarding the use of corticosteroids after Kasai surgery.⁸³ Two-hundred eight of 222 patients analyzed received steroid therapy with an overall jaundice-free survival rate with the native liver of 58% in the steroid group and 35.7% in the nonsteroid group ($p = 0.05$). Another study out of Japan analyzed differences in jaundice-free survival with the native liver between patients who did not receive steroids, patients who received steroids at varying doses, and patients who received steroids at the time of Kasai surgery as well as steroid bursts in the first 2 weeks after surgery if stools became acholic.⁸⁴ A significant increase in survival was seen in the steroid group that received steroid bursts (90.9%) compared with the no-steroid group (58.3%). The mean time to become jaundice-free was 33.3 ± 6.4 days in the steroid burst group and 82.6 ± 29.1 days in the no-steroid group ($p < 0.05$). The authors concluded that large-dose prednisolone therapy with stool color monitoring of bile flow had positive impact on outcome after Kasai surgery. Recently, Escobar et al⁸⁵ retrospectively reviewed the outcome of 21 BA patients after Kasai surgery who received steroids versus 22 BA patients who did not. A normal postoperative bilirubin was achieved at 6 months in 76% from the steroid group compared with 37% of the untreated patients ($p = 0.01$); however, there was no significant difference in the need for liver transplantation later in life. Limiting these studies are their retrospective nature, use of historical steroid-free controls, and lack of control for confounding factors such as surgical expertise or the use of antibiotics or other agents that may promote bile flow. A prospective, randomized, double-blinded, placebo-controlled trial of corticosteroid therapy at the time of portoenterostomy is currently being conducted in the United States.⁸⁶

CONCLUSION

BA is a devastating disease of neonates that leads to cirrhosis and the need for liver transplantation in the majority of children. The etiology of BA is unknown; however, evidence is accumulating that supports the theory of an initial viral trigger that is followed by an aberrant autoimmune-mediated attack on bile duct epithelia. Delineating the pathways of immune and autoimmune-mediated bile duct injury within BA should stimulate development of new medical interventions aimed at suppressing the specific immune response, decreasing the inflammatory damage to bile ducts, and altering the need for liver transplantation.

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ABBREVIATIONS

ANCAs	antineutrophil cytoplasmic antibodies
BA	biliary atresia
BDE	bile duct epithelia

CDC	choledochal cyst
CMV	cytomegalovirus
FACS	fluorescent-activated cell sorter
ELISPOT	enzyme-linked immunosorbent spot
HLA	human leukocyte antigen
IFN	interferon
Ig	immunoglobulin
IL	interleukin
IP-10	IFN- γ inducible protein-10
LFA-1	lymphocyte function-associated antigen-1
MHC	major histocompatibility complex
RRV	rhesus rotavirus
RT-PCR	reverse transcription–polymerase chain reaction
SCID	severe combined immunodeficiency
TCR γ	T-cell receptor variable region of the γ -chain
TNF	tumor necrosis factor
TPN	total parenteral nutrition

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Figure 1.

Proposed model of viral-induced, T cell-mediated autoreactivity leading to bile duct epithelial injury in BA. Viral infection of bile duct epithelia (BDE) (1) causes initial injury to the cells. Virus particles phagocytosed by macrophages or dendritic cells (2) are presented to naïve T cells in local lymph nodes where activation and IL-2 stimulated proliferation of virus-specific CD4⁺ T cells ensues (3). These activated CD4⁺ T cells travel back to the original site of exposure and elicit T-cell effector functions (4) including IFN- γ -induced macrophage stimulation and activation of cytotoxic CD8⁺ T cells. The macrophages release TNF- α , nitric oxide (N.O.), and reactive oxygen species while the CD8⁺ T cells release granzyme and perforin, together resulting in further BDE injury through apoptotic or necrotic pathways. Previously sequestered or altered BDE antigens released from this initial injury are now phagocytosed by macrophages or dendritic cells and presented to autoreactive T cells (5), causing further activation of this T cell-mediated immune cascade (6) and progressive destruction of BDE.

Table 1**Murine Models of Virus-Induced Molecular Mimicry in Autoimmune Diseases**

Virus	Viral Epitope Mimic	Self-Epitope Mimic	Disease
Adenovirus	E1B protein	Thyroglobulin	Thyroiditis ²⁴
Encephalomyelitis virus	VP2 protein	Myelin (proteolipid protein)	Multiple sclerosis ²⁵
Herpes simplex virus	UL6 coat protein	Corneal antigens	Keratitis ²⁶
Coxsackievirus B4	VP1 protein	Cardiac myosin	Myocarditis ²⁷

Table 2**Putative Viruses in the Pathogenesis of BA**

Reovirus ^{11,12}
Rotavirus ¹⁰
Cytomegalovirus ^{13,29}
Human papilloma virus ²⁸
Human herpesvirus 6 ²⁹
Epstein-Barr virus ³⁰

Table 3**Defining Criteria for Autoimmune Diseases**

Direct proof	Human disease is reproduced in a normal recipient by direct transfer of autoantibody
Indirect proof	<p>A. Reproduction of autoimmune disease in experimental animal models by adoptive transfer of autoantibodies or autoantigen specific T cells from diseased mice into immunodeficient animals</p> <p>B. Identification of the autoantigen(s) Based on distinctive clinical clues including</p>
Circumstantial evidence	<p>A. Association with other autoimmune Diseases</p> <p>B. Lymphocytic infiltration of target organ, especially if there is a restriction in T-cell receptor variable gene usage</p> <p>C. Statistical association with a particular MHC haplotype or aberrant expression of MHC class II antigens on the affected organ</p> <p>D. Favorable response to immunosuppression</p>

Based on data from Rose NR, Bona C. Defining criteria for autoimmune diseases (Witebsky's postulates revisited). *Immunol Today* 1993;14:426–430.

MHC, major histocompatibility complex.