cmgh
CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

REVIEW

What Causes Biliary Atresia? Unique Aspects of the Neonatal Immune System Provide Clues to Disease Pathogenesis

Cara L. Mack

Section of Pediatric Gastroenterology, Hepatology and Nutrition, Children's Hospital Colorado, University of Colorado School of Medicine, Aurora, Colorado

SUMMARY

Biliary atresia is a devastating cholestatic liver disease of children of unknown etiology. Research pertaining to the immunopathogenesis of biliary atresia should focus on unique aspects of neonatal immunity that promote aggressive and ongoing inflammation and fibrosis early in life.

Biliary atresia (BA) is the most frequent identifiable cause of neonatal cholestasis, and the majority of patients will need liver transplantation for survival. Despite surgical intervention with the Kasai portoenterostomy, significant fibrosis and cirrhosis develop early in life. An increased understanding of what causes this inflammatory fibrosing cholangiopathy will lead to therapies aimed at protecting the intrahepatic biliary system from immune-mediated damage. This review focuses on studies pertaining to the role of the adaptive immune response in bile duct injury in BA, including cellular and humoral immunity. The neonatal presentation of BA prompts the question of what potential modifications of unique aspects of the neonatal immune system set the stage for the progressive biliary disease. This review also discusses the characteristics of neonatal immune response and the theories on how alterations of this response could contribute to the pathogenesis of BA. These include aberrant type 1 helper T-cell (T_H1) and T_H17 responses, deficiencies in regulatory T cells, activation of humoral immunity, and autoimmunity. To advance our understanding of the etiology of BA, future studies should focus on the unique aspects of the neonatal immune system that have gone awry. *(Cell Mol Gastroenterol Hepatol 2015;1:267–274; http://dx.doi.org/10.1016/j.jcmgh.2015.04.001)*

Keywords: Cholestasis; Adaptive Immunity; Neonatal Autoimmunity.

Biliary atresia (BA) is the most frequent identifiable cause of neonatal cholestasis, occurring in approximately 1 out of 12,000 live births in the United States and accounting for an estimated 350 new cases annually. It is most common in Taiwan (~1:5,600 live births) and occurs more frequently in females, Asians, and African Americans.[1] There are three types of BA: isolated BA (84% of cases), BA with at least one malformation but without laterality defects (6%; cardiovascular, gastrointestinal, or genitourinary defects), and BA splenic malformation, a syndrome associated with laterality defects and polysplenia or asplenia (4% to 10%).[2] In isolated BA, meconium and initial stools are normal in color, suggesting early patency of the ducts. However, within the first 3 months of age, the extrahepatic biliary tree becomes obstructed, and the pathology is consistent with an inflammatory fibrosing cholangiopathy. At diagnosis, the extrahepatic biliary remnant is removed, and a Kasai portoenterostomy is performed in an attempt to reestablish bile flow. This results in initial restoration of bile flow in up to two-thirds of patients if performed within 60 days of life.

Even with surgical intervention, significant fibrosis and cirrhosis develops early in life, and the majority of patients will need liver transplant for survival. Analysis of liver tissue from BA patients >4 years old after a Kasai portoenterostomy revealed that, despite resolution of cholestasis in 83% of patients, 100% of patients had fibrosis (Metavir stage >2) or cirrhosis.[3] On average, 20% of children with BA will enter adulthood with their native liver, and the vast majority of those patients will have evidence of chronic liver disease or cirrhosis.[4] An increased understanding of what causes the inflammatory sclerosing cholangiopathy of BA could lead to therapies aimed at protecting the intrahepatic biliary system from inflammatory-mediated damage and fibrosis.

The etiology of BA is unknown, and theories of its pathogenesis include perinatal virus infection targeting cholangiocytes, chronic inflammatory or autoimmune-mediated bile duct injury, and abnormalities in bile duct development.[5] A recent retrospective study of neonatal direct bilirubin levels obtained at 24 to 48 hours of life has shed light on the timing of the initial bile duct injury in BA.[6] In that study, neonatal direct bilirubin levels were obtained for all newborns in a single hospital between 2007 and 2010, and the infants who later developed isolated BA were compared with newborns from the same period who did not have BA. The BA newborns had mean direct bilirubin levels

Abbreviations used in this paper: Ag, antigen; APC, antigen presenting cell; BA, biliary atresia; BCR, B-cell receptor; CD, cluster of differentiation; GST, glutathione *S*-transferase; IFN, interferon; IL, interleukin; NK, natural killer; NSP4, nonstructural protein 4; RRV, Rhesus group A rotavirus; T_H, helper T cell; TNF, tumor necrosis factor; Treg, regulatory T cell; VP, viral protein.

2352-345X
http://dx.doi.org/10.1016/j.jcmgh.2015.04.001

that were significantly higher than those of the controls (1.4 ± 0.43 vs. 0.19 ± 0.075 mg/dL), suggesting that the bile duct damage began in utero. A leading hypothesis in the pathogenesis of isolated BA is that the cholangiocyte injury is initiated by a virus infection followed by an exaggerated autoinflammatory or autoimmune response that targets bile duct epithelia, resulting in progressive bile duct damage and obliteration.[7] In this theory, the virus infection is short lived, but the cholangiocytes within the entire biliary tree may continue to be damaged by aberrant autoimmune mechanisms. Cholangiocyte proteins may be seen as foreign because of alterations from the virus or exposure of previously sequestered antigens. Alternatively, the virus and epithelial proteins could have a high degree of sequence homology leading to antiviral and autoimmune responses that overlap, an autoimmune mechanism known as molecular mimicry.

This review will focus on studies pertaining to the role of the adaptive immune response in bile duct injury in BA, including cellular (T cell) and humoral (B cell) immunity. The significance of T cells versus B cells in disease pathology cannot be easily separated, as the two arms of adaptive immunity are intertwined. T cells provide "help" for B-cell immunoglobulin isotype switching and antibody production. B cells function as antigen-presenting cells or producers of cytokines for T-cell activation. The neonatal presentation of BA prompts the question of what potential modifications of known unique aspects of the neonatal immune system set the stage for the progressive biliary disease. In this review I discuss the characteristics of the neonatal immune response and the theories of how alterations of this response could contribute to the pathogenesis of BA. Innate immune responses in the infant with BA also contribute to bile duct damage; these pathways will not be reviewed here, but key investigations are referenced.[8,9]

Adaptive Cellular Immunity: T-Cell Subsets

Adaptive cellular immunity involves the interaction of antigen-presenting cells with T cells, resulting in activation of T cells with the production of cytokines. Adaptive immune responses are triggered by repeat exposure to both pathogen and non-microbial antigens, resulting in highly specific memory T-cell activation. Aspects of adaptive cellular immunity that characterize the neonate include decreased frequencies and function of dendritic cells (antigen-presenting cells) compared with adults and T-cell responses that are skewed to a type 2 helper T cell (T_H2) profile, with the production of interleukin 4 (IL-4), IL-5, and IL-13. It is becoming clear that neonates are also capable of generating adult-like T_H1 responses (IL-2, interferon-γ [IFN-γ]) when the conditions for antigenic priming are optimized. Over 30 years ago, Hoffman et al described T-cell responses in neonatal mice and found that infection with a high dose of a murine leukemic virus (>1,000 plaque-forming units) led to nonprotective T_H2 responses and disease.[10] In stark contrast, a low exposure of virus (0.3 plaque-forming units) to the neonate induced a virus-specific T_H1 response with clearance of virus.[11] These studies have led to the hypothesis that BA pathogenesis could be related to low-dose neonatal virus infection with proinflammatory T_H1 immune responses. Multiple studies have since shown that neonates are able to mount fully mature T_H1 responses under certain circumstances, which increases costimulatory signals on antigen-presenting cells.[12–14] It can therefore be theorized that an abnormal skewing of the T-cell response in the neonate from the default T_H2 response to the inflammatory T_H1 response could be an early event that promotes ongoing T-cell-mediated bile duct injury in BA.

The predominant cellular immune response in BA at diagnosis encompasses activated $CD4^+$ and $CD8^+$ T cells within portal tracts that produce T_H1 cytokines (IL-2, IFN-γ, tumor necrosis factor α [TNF-α]) and macrophages secreting TNF-α.[15–17] These lymphocytes have been found invading between bile duct epithelia, resulting in degeneration of intrahepatic bile ducts.[18] With the aim of understanding whether the inflammation is nonspecific (bystander activation) versus antigen specific with expansion of clones of T cells, T-cell receptor characterization was performed. Analysis of the T-cell receptor variable region of the β-chain (Vβ) within BA liver and extrahepatic bile duct remnants revealed that the T cells were indeed oligoclonal in nature with a limited T-cell receptor Vβ repertoire, suggesting antigen-specific activation. The exact antigen(s) stimulating the clonal expansions remains a mystery that if solved will provide a wealth of information on the processes of T-cell-mediated bile duct injury in BA.[19]

T_H1 Cellular Immunity

To perform mechanistic studies of immune-mediated hypotheses, the Rhesus group A rotavirus (RRV)-induced mouse model of BA (murine BA) has been employed by many investigators. This model mimics many aspects of the human disease, including bile duct epithelial apoptosis, portal inflammation, intrahepatic bile ductule proliferation, and extrahepatic biliary obstruction. The main limitation of the mouse model is that the extrahepatic biliary fibrosis is minimal compared with humans and the biliary obstruction is mainly due to inflammation and edema.

Many investigators view the findings in the mouse model as representative of the early events in human BA. In murine BA the virus is cleared within the first 2 weeks of life, a time point when extrahepatic biliary obstruction is complete. The $CD4^+$ T_H1 cellular inflammatory environment found in murine BA recapitulates the human disease, and the progressive inflammatory destruction and obliteration of the bile ducts leads to death by 3 weeks of age.[20,21] In support of a T_H1 cytokine environment in BA mice, many investigators have described increased levels of chemokines that promote T_H1 cellular differentiation [chemokine (C-C motif) ligand 2, chemokine (C-C motif) ligand 5, C-X-C motif chemokine 10].[22,23] IFN-γ is a necessary cytokine in the pathogenesis of murine BA, as RRV-infected IFN-γ knockout mice are protected from developing biliary obstruction and have a dramatic increase in survival.[24]

Depletion of the $CD8^+$ T-cell subset was also associated with increased survival, and the $CD8^+$ T cells from BA mice were found to be directly cytotoxic to cholangiocytes in vitro.[25] A recent study by Zheng et al[26] further analyzed the $CD8^+$ T-cell response in murine BA. Multiple RRV nonstructural protein 4 (NSP4) constructs were created to assess which viral epitope was responsible for $CD8^+$ T-cell activation. A computer based program was utilized that predicted which NSP4 viral epitopes would most likely interact with $CD8^+$ T cells. A fusion protein composed of glutathione *S*-transferase and NSP4 (GST-NSP4), as well as $NSP4_{144–152}$ and $NSP_{157–170}$ epitopes, were all recognized by $CD8^+$ T cells and induced $CD8^+$ T-cell IFN-γ production, similar to that found with RRV stimulation. Injection of neonatal mice with GST-NSP4, and not other viral constructs, led to biliary obstruction similar to RRV-infected mouse pups. Liver $CD8^+$ T cells from $NSP4_{144–152}$, $NSP_{157–170}$, and GST-NSP4 injected mice that were cultured with bile duct epithelial cells led to direct epithelial cytotoxicity. The fact that the viral epitope-specific liver $CD8^+$ T cells also recognized proteins within bile duct epithelia and elicited cellular damage suggests molecular mimicry as a potential mechanism of autoimmune activation. The researchers concluded that NSP4 is a pathogenic immunogen that initiates the inflammatory response, resulting in bile duct epithelial injury in murine BA. Collectively, these studies suggest that $CD4^+$ T_H1 cells may activate $CD8^+$ cytotoxic T cells and that both subsets contribute to the biliary injury and obstruction in BA.

The murine BA model has been used to understand the role of cellular autoimmunity in bile duct injury. Periductal inflammation involves an influx of bile duct epithelial-specific IFN-γ–producing T cells.[27] This was determined based on liver memory T-cell activation when cells were cultured with a bile duct epithelial homogenate protein source. In vitro analysis revealed that inhibition of $CD4^+$ T cells, but not $CD8^+$ T cells, was associated with loss of IFN-γ production, identifying the $CD4^+$ T cell as the key cell type associated with bile duct-specific autoimmune activation. Adoptive transfer of the liver T cells from BA mice into immunodeficient recipient mice resulted in bile duct-targeted inflammation.[25,27] Similar to human BA, the exact identity of the bile duct antigens that are being targeted has not been elucidated.

T_H2 Cellular Immunity

There is a rare subgroup of BA patients who present with a biliary cystic form of atresia. In this anatomic variant, there is a role for T_H2-mediated bile duct injury in murine BA. Stat1 knockout mice, which generate only T_H2 cytokines and are unable to make T_H1 cytokines, develop a cystic biliary form of BA after RRV infection. The role of IL-13, an effector cytokine found to be important in T_H2-mediated diseases, was also analyzed. Stat1/IL-13 double knockout mice showed some protection from bile duct obstruction with improved survival, and it was concluded that IL-13 and T_H2 responses contribute to the pathogenesis of cystic BA.[28]

T_H17 Cellular Immunity

IL-17 has been implicated as a major pathogenic cytokine contributing to autoimmune-mediated diseases. Neonates have an enhanced ability to mount proinflammatory T_H17 responses, based on research showing that TLR-stimulated cord blood cells produce high levels of IL-6 and IL-23, necessary cytokines for T_H17 differentiation.[29] In addition, cultured cord blood $CD4^+$ T cells can generate significant amounts of IL-17.[30] In the neonatal setting of a fully mature T_H17 pathway, is it possible that an aggressive, persistent T_H17 response plays a role in bile duct damage in BA? A recent study in BA found that serum IL-17a and IL-23 levels were increased in BA patients compared with healthy age-matched controls.[31] In addition, the ratio of T_H17 cells/regulatory T cells (Tregs) was significantly higher in the peripheral blood of BA patients. BA liver tissue had increased mRNA expression of ROR-γt (IL-17 transcription factor), IL-17a, IL-1β, IL-6, and transforming growth factor β1, an increased number of IL-17a infiltrating cells, and a decreased ratio of Treg/$CD4^+$ T cells. This study implies that T_H17 inflammatory pathways dominate and overcome the regulatory T-cell response, contributing to biliary injury in BA. T_H17 cell-mediated immunity requires further investigation to determine the significance of IL-17 to BA pathogenesis.

Regulatory T Cells

The Treg subset of $CD4^+$ T cells is responsible for controlling immune responses to prevent "bystander damage" of healthy tissue and to prevent activation of autoreactive T cells. The Treg subset expresses the cell surface marker $CD25^{high}$ and the transcription factor forkhead box P3 (Foxp3).[32] Tregs inhibit cells involved in adaptive immunity (T- and B-cell responses) and innate immunity (macrophages, dendritic cells, and natural killer cells).[33,34] In human neonates, the percentage of Tregs in peripheral blood increases significantly in the first 5 days of life, reaching adult levels at that time.[35] Recent studies suggest that there is a significantly greater number of Tregs in cord blood compared with adult Tregs.[36] Furthermore, cord blood Tregs are highly functional and can suppress T-cell proliferation and T_H1 IFN-γ production, similar to adult Treg function.[37]

In neonatal mice, Tregs exit the thymus and travel to the spleen and lymph nodes on day 3 of life.[32,33,38] Thymectomy in 3-day-old neonatal mice results in a spectrum of organ-specific autoimmunity that can be prevented by reconstitution of the thymectomized animals early in life with normal adult Tregs.[38–40] Autoimmune disease may also develop when exogenous insults, such as virus infection, disrupt the maturation or functioning of Tregs. Morse et al[41] showed that murine T lymphotropic virus infection on day 1 of life (but *not* on day 7 or 28) led to decreased release of Tregs from the thymus and the development of autoimmune gastritis. Kobayashi et al[42] found that the administration of a poly I:C virus mimic into neonatal thymectomized mice resulted in worsening incidence and severity of autoimmune gastritis and was associated with a significant

decrease in the number of splenic Tregs. These studies reveal that neonatal viral infection can induce or exacerbate the propensity for autoimmunity due to Treg deficiencies, which sets the stage to study this mechanism of autoimmunity in the pathogenesis of BA.

In murine BA, RRV infection must take place in the first 48 hours of life to induce biliary disease, and the incidence of disease is highest when virus is administered in the first 24 hours of life.[43] The necessity of early age at the time of viral infection to generate disease leads to the question of whether this neonatal virus infection could alter the release of Tregs from the thymus or decrease their suppressive capacity in the periphery, thus allowing for pathogenic autoreactive T cells and inflammation to flourish, stimulating effector cell functions (Figure 1). Miethke et al[44] characterized the frequency of Tregs in neonatal mice and found that the liver and spleen of 3-day-old mice had significantly fewer Tregs compared with 7-day-old mice, similar to previous studies showing that Tregs begin migration to the periphery on day 3 of life. The Treg deficit in week 1 was associated with enhanced dendritic cell activation of natural killer (NK) cells, resulting in biliary injury and obstruction. Lages et al[45] showed that adoptive transfer of total $CD4^+$ T cells, but not Treg-depleted $CD4^+$ T cells, into RRV-infected mice was associated with increased survival and diminished $CD8^+$ T-cell cytotoxicity. Tucker et al[46] reported significant deficits in liver Treg frequencies as well as Treg suppressive function in BA mice. In addition, adoptive transfer of highly purified adult Tregs into RRV-infected BA mice prevented the development of biliary obstruction, dramatically increased survival and inhibited T_H1 cell-mediated biliary injury. These complimentary studies demonstrate that Treg frequency and function are diminished in BA; further analysis of the mechanisms

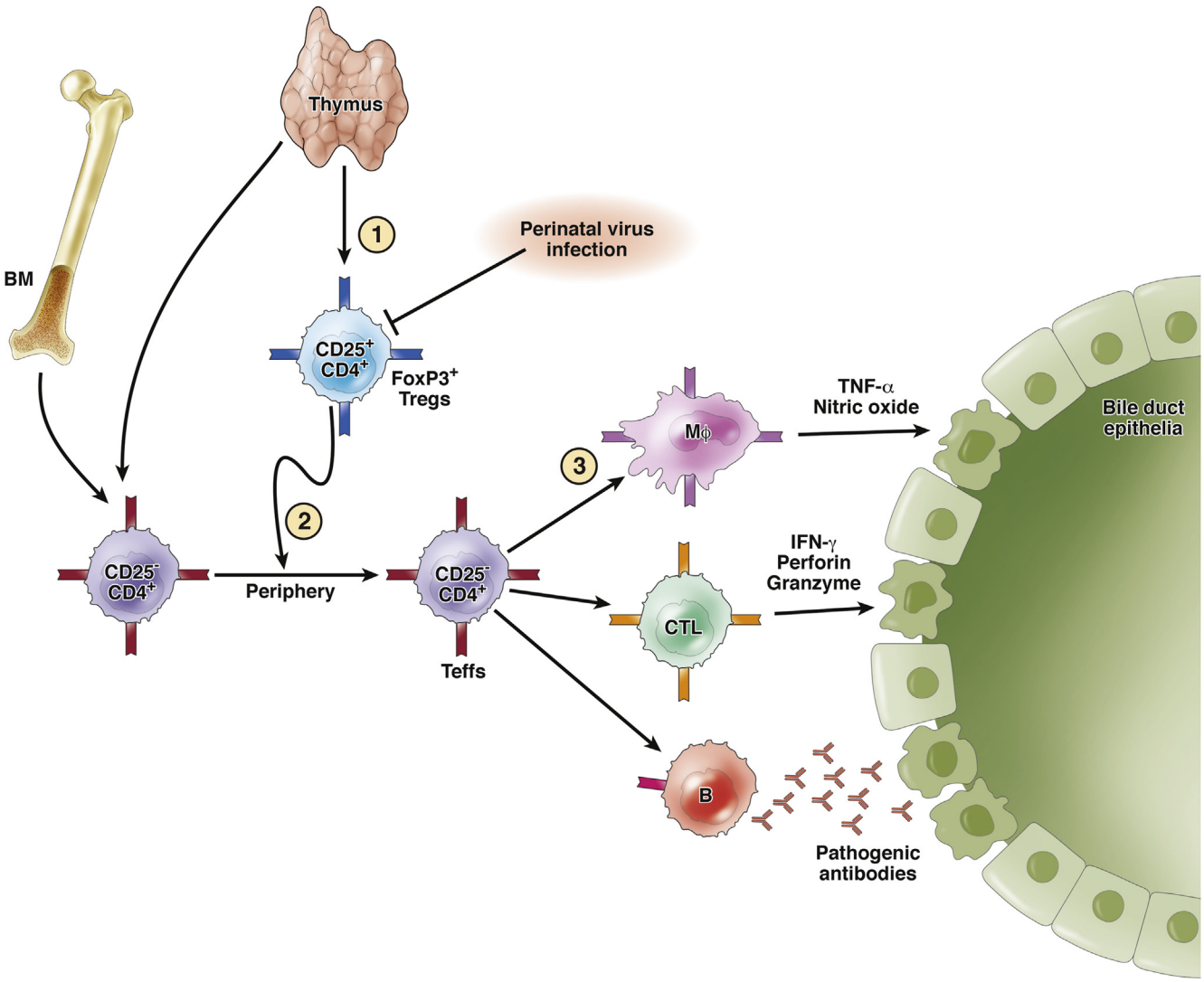

Figure 1. Hypothetical model of the role of Treg deficits in BA pathogenesis. Neonatal virus infection in the genetically predisposed individual may (1) alter the release of Tregs from the thymus or (2) decrease their regulatory capacity in the periphery, allowing for autoreactive $CD4^+$ T-effector cells (Teffs) to flourish and (3) activate macrophages, cytotoxic $CD8^+$ T cells (CTLs), and autoantibody-producing B cells, leading to progressive bile duct epithelial injury.

contributing to the Treg deficiencies is warranted. Future therapies aimed at enhancing Treg numbers and suppressive capabilities in BA may lead to protection of the intrahepatic biliary tree from ongoing damage.

Adaptive Humoral Immunity and B Cells

Humoral immune responses are initiated by interaction of antigen with the B-cell receptor (BCR) and direct cell contact with $CD4^+$ T cells and/or Toll-like receptor ligands. The BCR is composed of a membrane-bound form of IgM (binds antigen [Ag]) and the signal transduction moiety Ig-α/Ig-β that is necessary for activation. The engagement of BCR by Ag leads to activation and proliferation of Ag-specific B-cell clones that differentiate into either plasmablasts or germinal center B cells, which then give rise to plasma cells or memory B cells.[47] In neonatal immunity, murine studies demonstrate that predominantly IgM antibody responses to *T-cell–independent* antigens such as plant lectins, polysaccharides, and polymerized proteins are well established by 2 to 3 weeks of age. In contrast, *T-cell–dependent* IgG antibody responses begin at ~2 weeks of age and reach peak levels by 6 to 8 weeks of age.[48–50]

In humans, nearly all the IgG at birth is maternally derived and reaches a nadir between 1.5 and 3.0 months of age. At this point, IgG synthesis by the newborn begins; by 18 months of age, the maternally derived IgG is absent. Peak IgG levels are not attained until 5 years of age. On the other hand, IgM levels rise rapidly during the first months of life, attaining 75% of adult levels by 1 year of age. Pertinent to the potential pathogenesis of BA is a landmark study on the presence of IgM autoantibodies to defined self molecules in newborn humans.[51] Termed "natural antibodies," these neonatal natural IgMs react to a selective set of self antigens, many of which are among the target self antigens associated with autoimmune diseases. A subset of B cells, "B-1" cells, are in high frequency in the fetal liver and in neonates and are the main producers of these natural antibodies.[52] Evidence exists that the IgM natural antibodies may actually inhibit autoimmunity through facilitating clearance of apoptotic cell debris.[53,54] In contrast, it has been theorized that based on the presence of some major disease-associated self antigens within the IgM natural antibody repertoire, pathologic autoimmune disease could arise through a lapse in the regulation of otherwise benign, "natural" autoimmunity. One such cause of this lapse in regulation could be a perinatal virus infection, as theorized in BA.

To date, published work on humoral immunity in BA has been limited to characterization studies, such as those describing periductal immunoglobulin deposits.[55] Over 30 years ago, Hadchouel et al[56] described both IgM and IgG immunoglobulin deposits along the basement membrane of bile duct epithelia in the extrahepatic biliary remnant of 34% of infants with BA. Similar ductal epithelial immune deposits have been reported in the mouse model of BA.[24,27] The target antigen(s) that these immunoglobulins are complexing with is unknown and could include self-bile duct epithelia proteins or viral proteins present within infected epithelia.

Liu et al[57] performed immunoblot analysis of sera from BA mice and discovered autoantibodies reactive to cytosolic proteins within bile duct epithelia. One such protein was identified as α-enolase, and significant elevations of α-enolase autoantibodies were uniquely present in BA mouse sera. One possible theory to explain the increase in production of autoantibodies in the setting of previous virus infection is that there is molecular mimicry between virus and self proteins. In this study, a high degree of sequence homology between enolase and rotavirus proteins VP4 and VP8 were identified, and the anti-enolase antibodies bound to both enolase and rotavirus, suggesting molecular mimicry as a mechanism of the autoimmune response. In addition, high levels of IgM and IgG α-enolase autoantibodies were detected in ~40% of infants and children with BA.[57] Interestingly, anti-enolase antibodies have been found in other autoimmune diseases including autoimmune liver diseases, suggesting that this antibody may be a nonspecific marker of autoimmunity.[58,59] Future research centered on identifying potentially pathogenic, bile-duct specific autoantibodies should be pursued.

B cells are not only responsible for production of antibodies, but also play a key role as professional antigen-presenting cells (APCs), with subsequent T-cell activation. Naive neonatal APCs have limited ability to activate T cells due to low levels of major histocompatibility complex class II and costimulatory molecules.[60] However, neonatal mice exposed to low levels of replicating virus display increased antigen-presentation capabilities, with subsequent T_H1 cell activation and cytotoxic T-cell function.[61] Perhaps a similar virus-induced APC activation is occurring in the neonate with BA. To assess the importance of B cells in BA pathogenesis, Feldman et al[62] used mice deficient in Ig-α (Ig-$\alpha^{-/-}$) that have loss of BCR expression and function, resulting in defective B-cell antigen presentation and immunoglobulin production. RRV-infected Ig-$\alpha^{-/-}$ mice had dramatically increased survival and lack of bile duct obstruction. Significantly decreased numbers of liver $CD4^+$ T cells, NK T cells, and NK cells and macrophages were observed in RRV-infected Ig-$\alpha^{-/-}$ mice compared with wild-type mice. Similar to other B-cell depletion studies,[63–66] the RRV-infected Ig-$\alpha^{-/-}$ mice had increased levels of Tregs, suggesting a link between B-cell activation and Treg inhibition. In addition, lack of T-cell activation in RRV-infected Ig-$\alpha^{-/-}$ mice was demonstrated based on markedly decreased production of IFN-γ and TNF-α from $CD4^+$ T cells and IFN-γ from $CD8^+$ T cells. This implies that without B-cell antigen presentation, the T cells are not activated, which suggests a possible mechanism of protection from disease. B cells appear to play a critical role in the RRV-induced mouse model of BA, and future studies aimed at deciphering the specific role of antigen presentation and production of pathogenic autoantibodies are necessary to understand the impact of B cells in disease pathogenesis.

Summary

Biliary atresia is a devastating disease wherein the vast majority of patients require liver transplantation for

survival. It is critical to grasp the immunopathogenesis of BA in order to provide future therapies that control the intrahepatic biliary inflammation and prevent subsequent fibrosis. Evidence exists for a key role of both arms of the adaptive immune response in bile duct injury. The neonatal presentation of BA provides a clue to disease pathogenesis. Early events that impact the neonatal immune system (ie, perinatal virus infection) may alter the immune response and promote a progressive inflammatory or biliary autoimmune disease. To advance our understanding of the etiology of BA, future studies should focus on those unique aspects of the neonatal immune system that have gone awry, as detailed throughout this review.

References

1. Mack CL, Feldman AG, Sokol RJ. Clues to the etiology of bile duct injury in biliary atresia. Semin Liver Dis 2012; 32:307–316.
2. Schwarz KB, Haber BH, Rosenthal P, et al. Childhood Liver Disease Research and Education Network. Extrahepatic anomalies in infants with biliary atresia: results of a large prospective North American multicenter study. Hepatology 2013;58:1724–1731.
3. Lampela H, Kosola S, Heikkilä P, et al. Native liver histology after successful portoenterostomy in biliary atresia. J Clin Gastroenterol 2014;48:721–728.
4. Kumagi T, Drenth JP, Guttman O, et al. Biliary atresia and survival into adulthood without transplantation: a collaborative multicentre clinic review. Liver Int 2012; 32:510–518.
5. Bezerra JA. Potential etiologies of biliary atresia. Pediatr Transplant 2005;9:646–651.
6. Harpavat S, Finegold MJ, Karpen SJ. Patients with biliary atresia have elevated direct/conjugated bilirubin levels shortly after birth. Pediatrics 2011;128:e1428–e1433.
7. Mack CL. The pathogenesis of biliary atresia: evidence for a virus-induced autoimmune disease. Semin Liver Dis 2007;27:233–242.
8. Shivakumar P, Sabla GE, Whitington P, et al. Neonatal NK cells target the mouse duct epithelium via Nkg2d and drive tissue-specific injury in experimental biliary atresia. J Clin Invest 2009;119:2281–2290.
9. Saxena V, Shivakumar P, Sabla G, et al. Dendritic cells regulate natural killer cell activation and epithelial injury in experimental biliary atresia. Sci Transl Med 2011; 3:102ra94.
10. Hoffman PM, Ruscetti SK, Morse HC. Pathogenesis of paralysis and lymphoma associated with a wild mouse retrovirus infection: age and dose related effects in susceptible laboratory mice. J Neuroimmunol 1981; 1:275–285.
11. Garcia AM, Fadel SA, Cao S, Sarzotti M. T cell immunity in neonates. Immunol Res 2000;22:177–190.
12. Vekemans J, Amedie A, Ota MO, et al. Neonatal BCG vaccination induces adult-like IFN-gamma production by $CD4^+$ T lymphocytes. Eur J Immunol 2001;31: 1531–1535.
13. Ausiello CM, Urbani F, la Sala A, et al. Vaccine- and antigen-dependent type 1 and type 2 cytokine induction after primary vaccination of infants with whole-cell or acellular pertussis. Infect Immun 1997;65:2168–2174.
14. Adkins B. Neonatal immunology: responses to pathogenic microorganisms and epigenetics reveal an immunodiverse developmental state. Immunol Res 2013; 57:246–257.
15. Davenport M, Gonde C, Redkar R, et al. Immunohistochemistry of the liver and biliary tree in extrahepatic biliary atresia. J Pediatr Surg 2001;36:1017–1025.
16. Mack CL, Tucker R, Sokol RJ, et al. Biliary atresia is associated with $CD4^+$ T_H1 cell-mediated portal tract inflammation. Pediatr Res 2004;56:79–87.
17. Bezerra JA, Tiao G, Ryckman FC, et al. Genetic induction of proinflammatory immunity in children with biliary atresia. Lancet 2002;360:1653–1659.
18. Ohya T, Fujimoto T, Shimomura H, Miyano T. Degeneration of intrahepatic bile duct with lymphocyte infiltration into biliary epithelial cells in biliary atresia. J Pediatr Surg 1995;30:515–518.
19. Mack CL, Falta MT, Sullivan AK, et al. Oligoclonal expansions of $CD4^+$ and $CD8^+$ T-cells in the target organ of patients with biliary atresia. Gastroenterology 2007; 133:278–287.
20. Petersen C, Biermanns D, Kuske M, et al. New aspects in a murine model for extrahepatic biliary atresia. J Pediatr Surg 1997;32:1190–1195.
21. Mack CL, Tucker RM, Sokol RJ, Kotzin BL. Armed $CD4^+$ effector cells and activated macrophages participate in bile duct injury in murine biliary atresia. Clin Immunol 2005;115:200–209.
22. Leonhardt J, Stanulla M, von Wasielewski R, et al. Gene expression profile of the infective murine model for biliary atresia. Pediatr Surg Int 2006;22:84–89.
23. Carvalho E, Liu C, Shivakumar P, et al. Analysis of the biliary transcriptome in experimental biliary atresia. Gastroenterology 2005;129:713–717.
24. Shivakumar P, Campbell KM, Sabla GE, et al. Obstruction of extrahepatic bile ducts by lymphocytes is regulated by IFN-gamma in experimental biliary atresia. J Clin Invest 2004;114:322–329.
25. Shivakumar P, Sabla G, Mohanty S, et al. Effector role of neonatal hepatic $CD8^+$ lymphocytes in epithelial injury and autoimmunity in experimental biliary atresia. Gastroenterology 2007;133:268–277.
26. Zheng S, Zhang H, Zhang X, et al. $CD8^+$ T lymphocyte response against extrahepatic biliary epithelium is activated by epitopes within NSP4 in experimental biliary atresia. Am J Physiol Gastrointest Liver Physiol 2014; 307:G233–G240.
27. Mack CL, Tucker RM, Lu BR, et al. Cellular and humoral autoimmunity directed at bile duct epithelia in murine biliary atresia. Hepatology 2006;44:1231–1239.
28. Li J, Bessho K, Shivakumar P, et al. T_H2 signals induce epithelial injury in mice and are compatible with the biliary atresia phenotype. J Clin Invest 2011;121: 4244–4256.
29. Kollman TR, Levy O, Montgomery RR, Goriely S. Innate immune function by Toll-like receptors: distinct responses in newborns and the elderly. Immunity 2012; 37:771–783.

30. Black A, Bhaumik S, Kirkman RL, et al. Developmental regulation of T_H17 cell capacity in human neonates. Eur J Immunol 2012;42:311–319.
31. Yang Y, Liu YJ, Tang ST, et al. Elevated T_H17 cells accompanied by decreased regulatory T cells and cytokine environment in infants with biliary atresia. Pediatr Surg Int 2013;29:1249–1260.
32. Sakaguchi S. Naturally arising $CD4^+$ regulatory T cells for immunologic self-tolerance and negative control of immune responses. Annu Rev Immunol 2004;22:531–562.
33. Shevach EM. Regulatory T cells in autoimmunity. Annu Rev Immmunol 2000;18:423–449.
34. Campbell DJ, Koch MA. Phenotypic and functional specialization of $FOXP3^+$ regulatory T cells. Nat Rev Immunol 2011;11:119–130.
35. Grindebacke H, Stenstad H, Quiding-Järbrink M, et al. Dynamic development of homing receptor expression and memory cell differentiation of infant $CD4^+CD25^{high}$ regulatory T cells. J Immunol 2009;183:4360–4370.
36. Nettenstrom L, Alderson K, Raschke EE, et al. An optimized multi-parameter flow cytometry protocol for human T regulatory cell analysis on fresh and viably frozen cells, correlation with epigenetic analysis, and comparison of cord and adult blood. J Immunol Meth 2013;287:81–88.
37. Godfrey WR, Spoden DJ, Ge YG, Baker SR, et al. Cord blood $CD4^+CD25^+$-derived T regulatory cell lines express FoxP3 protein and manifest potent suppressor function. Blood 2005;105:750–758.
38. Asano M, Toda M, Sakaguchi N, Sakaguchi S. Autoimmune disease as a consequence of developmental abnormality of a T cell subpopulation. J Exp Med 1996; 184:387–396.
39. McHugh RS, Shevach EM. Cutting edge: depletion of $CD4^+CD25^+$ regulatory T cells is necessary, but not sufficient, for induction of organ-specific autoimmune disease. J Immunol 2002;168:5979–5983.
40. Bagavant H, Thompson C, Ohno K, et al. Differential effect of neonatal thymectomy on systemic and organ-specific autoimmune disease. Intern Immun 2002; 14:1397–1406.
41. Morse SS, Sakaguchi N, Sakaguchi S. Virus and autoimmunity: induction of autoimmune disease in mice by mouse T lymphotropic virus (MTLV) destroying $CD4^+$ T cells. J Immunol 1999;162:5309–5316.
42. Kobayashi Y, Murakami H, Akbar SM, et al. A novel and effective approach of developing aggressive experimental autoimmune gastritis in neonatal thymectomized BALB/c mouse by polyinosinic:polycytidylic acid. Clin Exp Immunol 2004;136:423–431.
43. Czech-Schmidt G, Verhagen W, Szavay P, et al. Immunological gap in the infectious animal model for biliary atresia. J Surg Res 2001;101:62–67.
44. Miethke AG, Saxena V, Shivakumar P, et al. Postnatal paucity of regulatory T cells and control of NK cell activation in experimental biliary atresia. J Hepatol 2010; 52:718–726.
45. Lages CS, Simmons J, Chougnet CA, Miethke AG. Regulatory T cells control the CD8 adaptive immune response at the time of ductal obstruction in experimental biliary atresia. Hepatology 2012;56:219–227.
46. Tucker RM, Feldman AG, Fenner EK, Mack CL. Regulatory T cells inhibit T_H1 cell-mediated bile duct injury in murine biliary atresia. J Hepatol 2013;59:790–796.
47. Goodnow CC, Vinuesa CG, Randall KL, et al. Control systems and decision making for antibody production. Nat Immunol 2010;11:681–688.
48. Press JL. Neonatal immunity and somatic mutation. Intern Rev Immunol 2000;19:265–287.
49. Astori M, Finke D, Karapetian O, Acha-Orbea H. Development of T-B cell collaboration in neonatal mice. Int Immunol 1999;11:445–451.
50. Holladay SD, Smialowicz RJ. Development of the murine and human immune system: differential effects of immunotoxicants depend on time of exposure. Environ Health Perspect 2000;108:463–473.
51. Merbl Y, Zucker-Toledano M, Quintana FJ, Cohen IR. Newborn humans manifest autoantibodies to defined self molecules detected by antigen microarray informatics. J Clin Invest 2007;117:712–718.
52. Nguyen TG, Ward CM, Morris JM. To B or not to B cells-mediate a healthy start to life. Clin Exp Immunol 2012; 171:124–134.
53. Baumgarth N. The double life of a B-1 cell: self-reactivity selects for protective effector functions. Nat Rev Immunol 2011;11:34–46.
54. Duan B, Morel L. Role of B-1a cells in autoimmunity. Autoimmun Rev 2006;5:403–408.
55. Fischler B, Woxenius S, Nemeth A, Papadogiannakis N. Immunoglobulin deposits in liver tissue from infants with biliary atresia and the correlation to cytomegalovirus infection. J Pediatr Surg 2005;40:541–546.
56. Hadchouel M, Hugon RN, Odievre M. Immunoglobulin deposits in the biliary remnants of extrahepatic biliary atresia: a study by immunoperoxidase staining in 128 infants. Histopathology 1981;5:217–221.
57. Lu BR, Brindley SM, Tucker RM, et al. α-Enolase autoantibodies cross-reactive to viral proteins in a mouse model of biliary atresia. Gastroenterology 2010;139:1753–1761.
58. Terrier B, Degand N, Guilpain P, et al. Alpha-enolase: a target of antibodies in infectious and autoimmune disease. Autoimmun Rev 2007;6:176–182.
59. Lundberg K, Kinloch A, Fisher BA, et al. Antibodies to citrullinated-enolase peptide 1 are specific for rheumatoid arthritis and cross-react with bacterial enolase. Arthritis Rheum 2008;58:3009–3019.
60. Hayakawa K, Tarlinton D, Hardy RR. Absence of MHC class II expression distinguishes fetal from adult B lymphopoiesis in mice. J Immunol 1994;152:4801–4807.
61. Siegrist CA, Saddallah F, Tougne C, et al. Induction of neonatal T_H1 and CTL responses by live viral vaccines: a role for replication patterns within antigen presenting cells? Vaccine 1998;16:1473–1478.
62. Feldman AG, Tucker RM, Fenner EK, et al. B cell deficient mice are protected from biliary obstruction in the rotavirus induced mouse model of biliary atresia. PLoS One 2013;8:e73644.
63. Yu S, Maiti PK, Dyson M, et al. B cell- deficient NOD.H-2h4 mice have $CD4^+CD25^+$ T regulatory cells that inhibit the development of spontaneous autoimmune thyroiditis. J Exp Med 2006;203:349–358.

64. Stasi R, Cooper N, Del Poeta G, et al. Analysis of regulatory T-cell changes in patients with idiopathic thrombocytopenic purpura receiving B cell-depleting therapy with rituximab. Blood 2008;112:1147–1150.
65. Hamel KM, Cao Y, Ashaye S, et al. B cell depletion enhances T regulatory cell activity essential in the suppression of arthritis. J Immunol 2011;187:4900–4906.
66. Sfikakis PP, Souliotis VL, Fragiadaki KG, et al. Increased expression of the FoxP3 functional marker of regulatory T cells following B cell depletion with rituximab in patients with lupus nephritis. Clin Immunol 2007;123:66–73.

Received February 16, 2015. Accepted April 7, 2015.

Correspondence
Address correspondence to: Cara L. Mack, MD, Section of Pediatric Gastroenterology, Hepatology and Nutrition, Children's Hospital Colorado, University of Colorado School of Medicine, 13123 East 16th Avenue, Mailstop B290, Aurora, Colorado 80045. e-mail: cara.mack@childrenscolorado.org; fax: (720) 777-7277.

Conflicts of interest
The authors disclose no conflicts.

Funding
This study was funded by National Institutes of Health grants NIDDK R01 DK094937–01A1 (to C.L.M.).