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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

REVIEW

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

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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]