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Biliary Atresia and Th1 Function: Linking Lymphocytes and Bile Ducts

Commentary on the article by Mack *et al.* on page 79

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Biliary atresia is the most common cause of neonatal cholestasis. It results from a progressive fibroinflammatory cholangiopathy that leads to obliteration of the extrahepatic biliary system within weeks of birth. This obstruction leads to impaired bile flow, chronic cholestasis, reactive proliferation of intrahepatic bile ducts, and ongoing hepatocellular injury. When untreated, biliary obstruction will rapidly progress to end-stage cirrhosis, with growth failure and other consequences of chronic liver disease. Therefore, the initial challenge is to differentiate biliary atresia from other forms of neonatal cholestasis. Timely diagnosis is vital because the success of hepatic portoenterostomy in restoring bile drainage improves when surgery is performed before 3 mo of age. When surgery is effective in reducing the serum bilirubin level to <1 mg/dL within 3 mo after portoenterostomy, up to 53% of infants will have normal growth and minimal complications of cirrhosis (1,2). Unfortunately, this may represent only a small portion of all infants with biliary atresia; in most patients, liver disease progresses and requires liver transplantation for long-term survival. Thus, we are challenged to search systematically for pathogenic mechanisms of disease so that new medical therapies can be developed to stop disease progression.

The pathogenesis of biliary atresia is multifactorial (3–6). Although molecular mechanisms that regulate disease onset and progression are not known, patient- and animal-based studies have identified five mechanisms with potential pathogenic roles (Table 1). Collectively, these mechanisms support a "working model" in which a genetically susceptible subject undergoes destruction of the extrahepatic biliary system in response to environmental factors. Regardless of the triggering insult, the close association between inflammatory cells and injured bile ducts in infants with biliary atresia suggests that inflammatory cells may play a chief role in disease pathogenesis. Phenotypic characterization of these cells reveals important functional features of Kupffer cells and hepatic lymphocytes in biliary atresia. Kupffer cells may serve as antigen-presenting cells and produce IL-18, a cytokine that promotes a proinflammatory differentiation of lymphocytes (7–9). In agreement with this concept, some of the cells that infiltrate portal tracts in biliary atresia have been reported to consist of CD4+ (helper), CD8+ (cytotoxic), and CD56+ (natural killer) lymphocytes (7,10–12). Of interest, CD8+ lymphocytes were found in proliferated bile ducts of affected patients, although the cells did not express perforin or granzyme B, markers of functional activation (12). Further evidence supporting a functional commitment of lymphocytes is the unique hepatic transcriptional profile displaying the coordinated expression of genes that regulate proinflammatory differentiation of lymphocytes in infants early in the course of biliary atresia (13). The profile contained the overexpression of interferon-γ in affected infants, even when inflammatory infiltrates were similar to liver samples from age-matched infants with intrahepatic cholestasis, suggesting different activation states of similar cell types. In this issue of *Pediatric Research*, Mack *et al.* add a comprehensive spatial and functional dimension to the relationship between bile ducts and inflammatory cells in infants with biliary atresia.

To gain insight into whether the inflammation of the liver of infants with biliary atresia represents a primary or nonspecific (secondary) response to biliary injury, Mack *et al.* performed detailed immunohistochemistry and gene expression studies in livers of children with biliary atresia at the time of diagnosis (3–12 wk of age), age-matched diseased control subjects [neonatal giant cell hepatitis, choledochal cyst, and total parenteral nutrition (TPN)-induced cholestasis], and healthy normal control subjects. Immunohistochemical staining revealed a greater infiltration of portal tracts by CD4+ and CD8+ T lymphocytes as well as CD68+ (Kupffer) cells in biliary atresia than in diseased and normal control subjects, with no obvious differences in the number of cells stained with antibodies against CD20 and NK1 (markers of B and NK lymphocytes, respectively). Notably, these findings were associated with an increased production of proinflammatory cytokines (IL-2, IL-12, interferon-γ, and tumor necrosis factor-α) in livers of patients with biliary atresia when compared with control subjects, except for livers of patients with TPN-induced cholestasis. To explore the basis for the similar level of expression between biliary atresia and TPN-induced cholestasis, the authors determined the pattern of cytokine expression at the cellular level by *in situ* hybridization. They found a distinct anatomic distribution of cytokine expression, with a unique localization of

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Table 1. *Potential mechanisms involved in the pathogenesis of biliary atresia*

Mechanism	Supporting data
Defect in morphogenesis	Development of jaundice soon after birth
	Coexistence of nonhepatic embryologic abnormalities
	Abnormal remodeling of the "ductal plate"
	Mutations in laterality genes (*CFC1*, *ZIC3*) in patients with biliary atresia and laterality defects
	Epigenetic factors: overexpression of regulatory genes in children with the embryonic form of biliary atresia (15)
	Inv mouse: model of biliary obstruction and situs inversus
Defect in prenatal circulation	Intrauterine devascularization results in abnormal extrahepatic bile ducts
Immunologic dysregulation	Increased expression of intercellular adhesion molecules
	Increased frequency of the HLA-B12, B8, or DR3 alleles
	Hepatic profile displaying a predominant Th1-like phenotype
Viral infection	Cytomegalovirus, reovirus, rotavirus, and other viruses detected in infants with biliary atresia
	Biliary obstruction in newborn mice infected with rotavirus
Toxin exposure	Time-space clustering of cases

Adapted from Ref. 3.

proinflammatory cytokines restricted to portal tracts in biliary atresia, whereas the expression was predominantly lobular in livers of patients with TPN-induced cholestasis. This disparate pattern of expression supports regional specificity for biliary atresia and points to a potential functional synergism of inflammatory cells targeting the biliary microenvironment.

The potential link between lymphocyte activation and bile duct injury generates new questions regarding disease pathogenesis. For example, why do lymphocytes undergo activation and target the biliary system? What triggers lymphocyte activation during the first few weeks of postnatal development in humans? Experimental approaches to answer these questions meet remarkable challenges in view of the rapid changes that occur in the liver soon after birth. Remarkable changes in maturation of hepatic enzymes and transport systems occur to meet the high metabolic demands that newborns face as they achieve independence from the materno-placental unit (14). Perhaps equally important are the changes in the cellular milieu of the liver, which loses the rich extramedullary hematopoietic lineage that is typical of the fetus. Whether and how these hematopoietic cells contribute to the pathogenesis of biliary atresia is not known, but their presence and the potential to undergo activation must be taken into account when analyzing the phenotype of inflammatory cells in livers of infants with biliary atresia. In other words, comparative analysis should be done with age-matched subjects. This requirement was met in the studies by Mack *et al.*, which compared livers from 3- to 12-wk-old infants who had biliary atresia with those of age-matched subjects who had other causes of cholestasis; normal control subjects were obtained from young children at 2–7 y of age. Thus, instead of searching for additional markers of lymphocyte differentiation in livers of affected patients, it would be prudent to focus future studies on the search for causative factors and molecular mechanisms that regulate the proinflammatory response in biliary atresia.

Translation of the findings by Mack *et al.* into patient care will require additional patient- and animal-based studies to establish a direct cause-and-effect relationship between lymphocyte function and bile duct injury. To facilitate future translational studies, the National Institutes of Health recently created the Biliary Atresia Research Consortium (BARC). The objective of BARC is to facilitate studies on the cause, pathogenesis, treatment, and outcome of infants with biliary atresia and other forms of neonatal cholestasis. The multicenter nature of BARC will increase access to patient enrollment from several geographical regions in the United States (www.clinicaltrials.gov/ct/show/NCT00061828?order=33) and facilitate initiatives to translate new knowledge from the laboratory into novel clinical approaches.

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