

## Biliary Atresia: Current Concepts and Research Directions

### Summary of a Symposium

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**Biliary atresia (BA) is the end result of a destructive, inflammatory process that affects intra- and extrahepatic bile ducts, leading to fibrosis and obliteration of the biliary tract with the development of biliary cirrhosis. It is the commonest cause of chronic cholestasis in infants and children, and therefore is the most frequent indication for liver transplantation in this age group. The disease occurs worldwide, affecting an estimated 1 in 8,000 to 12,000 live births. At present, there is no specific therapy for BA; however, sequential surgical therapy begins with creation of a hepatoportoenterostomy (HPE); in those with end-stage liver disease, liver transplantation is indicated. Since most candidates are young children of small size, there is a shortage of size-matched donors for liver transplantation. At present, an increased awareness to ensure early diagnosis and development of methods to prevent progressive fibrosis are needed. These considerations are dependent on detailed studies of the pathogenesis of BA. Recent studies have focused on normal and altered bile duct morphogenesis and the role of various factors (infectious or toxic agents and metabolic insults) in isolation or in combination with a genetic or immunologic susceptibility in the etiology of BA. (HEPATOLOGY 1996;23:1682-1692.)**

Biliary atresia (BA), the most serious digestive disease affecting infants, is an idiopathic, localized, complete obliteration or discontinuity of the hepatic or common bile ducts at any point from the porta hepatis to the duodenum.<sup>1,2</sup> Obstruction of bile flow leads to cholestasis, progressive fibrosis, and, ultimately, cir-

rhosis. Until recently, BA was uniformly fatal; liver transplantation has altered the inevitability of that outcome, but at a "cost" to the patient, who must face the consequences of living with a transplanted organ, and to society in real dollars. Our understanding of the etiology and pathogenesis of BA has remained virtually unchanged for two decades. This disorder is of interest to all individuals involved in basic and clinical studies of diseases of the liver; the rapidly progressive fibroobliterative process may represent a paradigm for other forms of hepatobiliary injury, perhaps reflecting an inter-relationship between genetic predisposition and environmental exposure.

In September 1994, a symposium, sponsored by the National Digestive Diseases Advisory Board, focused on BA to address the pathogenesis and the clinical challenges presented by this disorder, including the need for rapid and precise diagnosis and improved management. The ultimate goal was to stimulate basic investigation of this enigmatic disease, addressing specific issues, such as: 1) the genetic, virologic, or immunologic basis for BA; 2) the optimal timely and precise methods of discrimination from other causes of neonatal cholestasis; 3) the natural history of BA; 4) the optimal use and timing of medical and surgical interventions (portoenterostomy and transplantation); and 5) the effect of preexisting liver disease or surgical complications (cholangitis, sepsis, peritonitis) on the outcome of transplantation. Because a small number of patients are seen in individual centers and patients are not managed in a uniform manner between centers, an accurate assessment of these issues is difficult. Therefore, a collaborative, multicenter study is needed; a hypothesis potentially subject to study in such a project is that BA is the phenotype of several underlying disorders.

#### THE CLINICAL CHALLENGE

Dr. Richard J. Grand (Tufts–New England Medical Center, Boston, MA) presented an overview of the unique clinical aspects of the disorder. The enigmatic nature of the disorder has perplexed physicians for over 100 years.

BA is a progressive, sclerosing, inflammatory process that affects the extrahepatic biliary tract, leading to

Abbreviations: BA, biliary atresia; HPE, hepatoportoenterostomy; BASE, biliary atresia splenic malformation syndrome; CDC, chenodeoxycholic acid; CMV, cytomegalovirus.

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**TABLE 1. Two Clinical Forms of BA**

Embryonic or fetal type (35%)	
1.	Early onset of neonatal cholestasis.
2.	No jaundice-free period after physiological jaundice.
3.	No bile duct remnants in hepatoduodenal ligament.
4.	Associated congenital anomalies (10-20% of cases).
Perinatal type (65%)	
1.	"Later onset" of neonatal cholestasis.
2.	Jaundice-free interval may be present after physiological jaundice.
3.	Remnants of bile duct structures found in hepatoduodenal ligament.
4.	No associated congenital anomalies.

NOTE. According to Schweizer<sup>3</sup> and Desmet.<sup>4</sup>

ductular luminal obliteration and cirrhosis. Patients with BA are subject to progressive and rapid development of end-stage liver disease due to the persistent intrahepatic inflammatory process. BA occurs in two clinical forms<sup>3,4</sup> (Table 1): 1) the embryonic or fetal type, and 2) the perinatal type; the latter form accounted for ~65% of all cases in one series,<sup>3</sup> but as high as 90% in other studies.<sup>1</sup> BA in both subtypes appears to be the result of the ongoing inflammatory process that produces complete or partial sclerosis of bile ducts (extrahepatic and intrahepatic). There is no difference in histological features of the liver in infants with and without congenital anomalies. A major challenge is to define the role of various laboratory tests, imaging methods, and biopsy samples in establishing the diagnosis, in particular, differentiating this disorder from various forms of intrahepatic cholestasis (idiopathic neonatal hepatitis).<sup>1</sup> The most reliable information is obtained by review of hepatic histopathology, followed by direct visualization of obliterated extrahepatic bile ducts (intraoperative cholangiography). A percutaneous liver biopsy has a diagnostic accuracy of ~95% if an adequate size sample, containing 5 to 7 portal spaces, is obtained.<sup>1</sup> Characteristic findings include ductular proliferation, canalicular and cellular bile stasis, and portal or perilobular edema or fibrosis. Many centers combine liver biopsy with hepatobiliary scintigraphy, with or without phenobarbital pretreatment. Use of endoscopic retrograde cholangiopancreatography to view the biliary tree presents challenges in infants, regardless of the quality of the optics, because of the lumen:tube ratio. Laparoscopy has no role; luminal patency, the key factor in diagnosing BA, is impossible to determine through a laparoscope.

**TABLE 2. Outcome of the HPE Procedure**

Age at Surgery (d)	% With Bile Flow	
	Kasai et al. <sup>5</sup>	Howard et al. <sup>15</sup>
<60	82	77
60-90	45	59
90-120	10	28

**TABLE 3. Relationship Between Biliary Luminal Size and Establishment of Bile Flow Following the HPE Procedure<sup>(12)</sup>**

Size of Ducts	Total Patients	No. of Patients With Bile Flow (%)
• >150 μm	12	11 (92)
• 50-150 μm	42	34 (81)
• No epithelial-lined structures	11	2 (18)

There is no effective therapy. The prognosis of untreated BA is extremely poor, with death from liver failure usually occurring within 2 years. Until Dr. Morio Kasai introduced the hepatoportoenterostomy (HPE) in 1959, no surgery had been effective for BA.<sup>5</sup> The "Kasai procedure" can restore bile flow in most infants, but is often not curative. With the HPE procedure, the timing of the surgery correlates with outcome.<sup>5,6</sup> In several series, it has been reported that bile flow has been re-established in ~80% of infants who were referred for surgery within 60 days after birth (Table 2).<sup>3-15</sup> After 60 days of age, the benefit of surgery, as determined by establishment of biliary flow and the presence of pigmented stools, gradually decreases. Additional cited predictors of a poor outcome are caucasian race, the severity of the intrahepatic biliary cholangiopathy, the presence of cirrhosis on initial biopsy, and absence of ducts at the level of the liver hilus.<sup>3,5,8-11</sup> The outcome directly correlates with the size of the bile duct remnants identified in the porta hepatis at surgery; bile duct profiles of >150 μm, lined with columnar epithelium, have been associated with a good surgical result (Table 3).<sup>12</sup> Schweizer found that prehilary bile duct structures of >400 μm were associated with a favorable prognosis.<sup>3,13</sup> Significant hepatocyte injury, as indicated by lobular disarray and giant cell transformation, has also been associated with a poor outcome. Following HPE, recurrent bouts of ascending bacterial cholangitis can contribute to the ongoing bile duct injury and can lead to re-obstruction following successful establishment of bile flow.

Recent studies have reported the long-term outcome during the 10-year period following the Kasai procedure (Table 4).<sup>6,7</sup> Approximately one third of patients with BA will require transplantation in the first 12 to 14 months, another one third by their teens, and the rest will live with some degree of liver disease. Among

**TABLE 4. Clinical Course Following the Kasai HPE Operation**

Outcome	Kasai et al. <sup>6</sup>	Laurent et al. <sup>7</sup>
Bile excretion	89%*	—
Jaundice cleared	62%	—
Jaundice-free survival	62%	53%
Ten-year survival	74%*	33%
Esophageal varices	29%	73%

\* Operation <60 days.

pediatric patients, BA accounts for ~50% of the indications for liver transplantation.<sup>14</sup>

In summary, early recognition of babies who have BA is critical for optimal intervention. Optimally, BA should be identified by the time of the first well-baby visit after discharge from the hospital. The importance of a prompt and precise diagnosis must be stressed to all pediatric health care providers. In the United Kingdom, an educational effort (the "Yellow Alert" campaign) has been established to indicate the importance of jaundice persisting after 14 days of age.<sup>16,17</sup>

### CLINICAL CLUES TO THE PATHOGENESIS

Dr. Frederick J. Suchy (Yale University, New Haven, CT) discussed the clinical clues that might lead to a better understanding of the pathogenesis of BA, emphasizing that in any disorder becoming manifest in the first weeks of life, it is essential to consider the possibility that genetic factors may be causal or contributory.

Although over 30 cases of the disease have been reported in 14 families, BA is not thought to be inherited in the majority of cases.<sup>1,15,18,19</sup> HLA-identical twins discordant for BA have been described in several reports.<sup>20,21</sup> Cases in stillbirths or in premature infants are very rare, suggesting a postnatal origin of the inflammatory process.<sup>1,15,18,19,22</sup> A significant increase in HLA-B12 has been found among BA patients without associated anomalies (23 of 47 [49%]), a rate 3.23 times that seen in controls.<sup>23</sup> The haplotypes A9-B5 and A28-B35 were also found more frequently.<sup>23</sup> Additional work focusing on immune factors that may predispose to disease of the intra- and extrahepatic bile ducts is warranted.<sup>24</sup>

BA occurs more commonly in girls than in boys.<sup>1,18,19</sup> Patients with the more common perinatal form of BA present at 1 to 2 months of age with persistent cholestasis; they are generally full-term and of normal birth weight. Growth and development are normal in the immediate postnatal period. Jaundice is then observed after a period of physiological (unconjugated) hyperbilirubinemia. The possibility of hepatobiliary disease must be considered in any neonate jaundiced beyond 14 days of age.<sup>16,17</sup> Stools are acholic at presentation, but early in the course, during the evolving process of bile duct obliteration, stools may contain some bile pigment.

In the less common fetal (embryonic) type of BA, in which congenital malformations occur and cholestasis is present from birth, bile duct remnants are rarely identified at the time of exploratory laparotomy.<sup>3</sup> This form of atresia almost certainly begins prenatally and may have a different etiology than the disorder recognized several weeks after birth. Carmi et al. have reported associated anomalies in 51 of 251 patients with BA (20%).<sup>25</sup> The anomalies segregated into two major groups: one group was comprised of 15 cases (29%) with various combinations of anomalies within the laterality sequence (polysplenia, cardiovascular defects, asplenia, abdominal situs inversus, intestinal malrota-

tion, and anomalies of the portal vein and hepatic artery). A second major group included 30 cases (59%) with single or dual anomalies involving the cardiac, gastrointestinal, and urinary systems; these latter anomalies did not follow any recognizable pattern. A third group of 6 cases (12%) all had intestinal malrotation, some with preduodenal portal vein; these cases show some similarity to the laterality sequence group and may represent a more confined phenotypical result of faulty situs determination. BA within the laterality sequence might prove a suitable candidate for a major gene mutation. However, it is of interest that, although left-right asymmetry is genetically controlled, there have been no reported familial cases of BA with the laterality sequence. Teratogenic, infectious, and polygenic multifactorial causes might play a more significant role in BA associated with "nonsyndromic" organ system anomalies.

Davenport et al. examined the splenic malformation syndrome in association with BA; 23 of 308 infants (7.5%) with BA had polysplenia.<sup>26</sup> There were also 4 infants with other types of splenic malformation: 2 with double spleen and 2 with asplenia. The presence of other anomalies, such as situs inversus and portal vein anomalies in all the categories of splenic malformation, suggested that they formed part of a larger association, for which the authors proposed the term biliary atresia splenic malformation (BASE) syndrome. There was no difference in age of presentation or in biochemical liver tests before surgery between infants with BASE and those with BA alone. Four infants with BASE (15%) were born to mothers with diabetes, three who were insulin-dependent and one with gestational diabetes treated by diet alone. Actuarial "survival" of infants with BASE after initial corrective surgery was worse (indicated by death or transplantation) than that in the group without other anomalies. The authors concluded that BASE syndrome appears to be a distinct subgroup of infants with BA; this subgroup may have a different cause and tends to have a worse prognosis. The confounding effect of coincident anomalies (e.g., cardiovascular malformations), which are in themselves detrimental, remains unclear.

Numerous mechanisms have been proposed to account for the progressive obliteration of the extrahepatic biliary tree.<sup>1,15,18,19,22</sup> An ischemic or toxic origin of extrahepatic bile duct injury is unlikely. No abnormal toxic bile acid metabolite specific for the disorder has been identified. Congenital infections with cytomegalovirus, Epstein-Barr virus, or rubella virus have been found occasionally, but the presence of these common agents may be coincidental.<sup>1,15,18,19,22</sup> These will be discussed subsequently. The fibrous remnant on microscopic examination shows complete fibrous obliteration of at least a portion of the extrahepatic bile ducts.<sup>22,27</sup> Bile ducts within the liver extending to the porta hepatis are initially patent during the first weeks of life but are progressively destroyed.<sup>28</sup> The same process that damaged the extrahepatic ducts may be causal;

the noxious effect of biliary obstruction is a contributing factor.

Tan et al. have recently compared the developing biliary system of normal human embryos and fetuses with the resected extrahepatic biliary remnants from 205 cases of BA.<sup>11,29-31</sup> At the porta hepatis level, the primary biliary ductal plate underwent a specific sequence of remodeling, between 11 and 13 weeks' post-fertilization, resulting in the formation of large tubular bile ducts surrounded by thick mesenchyme. Luminal continuity with the extrahepatic biliary tree is maintained throughout gestation. Contrary to previous speculation, no "solid phase" was documented during the development of the extrahepatic bile duct. Examination of the biliary remnants in BA showed that the porta hepatis was encased in fibrous tissue with a variable pattern of obliteration of the common hepatic and common bile ducts. There were similarities on anti-cytokeratin immunostaining between the abnormal ductules within the porta hepatis in BA and the normal developing bile ducts during the first trimester. The authors proposed that BA may be caused by failure of the remodeling process at the hepatic hilum, with persistence of fetal bile ducts poorly supported by mesenchyme. They further postulated that, as bile flow increases perinatally, bile leakage from these abnormal ducts may trigger an intense inflammatory reaction, with subsequent obliteration of the biliary tree. It is more likely that an underlying infectious or immune injury interferes with the normal remodeling process at the hepatic hilum and with ductal plates within the liver. This area remains important for future research, particularly in regard to molecular mechanisms underlying developmental remodeling of the biliary tree.<sup>32,33</sup>

In summary, the clinical presentation, the reported predisposing genetic factors, and the pace of progression may offer clues to the pathogenesis of BA.

#### HPE AND LIVER TRANSPLANTATION IN BA

Dr. Frederick C. Ryckman (Children's Hospital Medical Center, Cincinnati, OH) discussed the role of surgical intervention for patients with BA.

When establishing the principles of surgical management for BA, it is appropriate to begin by reviewing the conclusions of the 1983 National Institutes of Health Consensus Conference on Liver Transplantation<sup>34</sup>: 1) HPE (the Kasai procedure) should be the primary surgical therapy for BA; 2) transplantation is appropriate therapy for patients with BA who fail primary HPE; 3) liver transplantation should be delayed as long as possible to permit maximum growth; 4) transplantation should be deferred until progressive cholestasis, hepatocellular decompensation, or severe portal hypertension supervene; and 5) multiple attempts to revise an unsuccessful Kasai procedure are not warranted, because they can make liver transplantation more difficult and dangerous. These conclusions form the basis for contemporary management.

The success of the Kasai HPE in the primary treatment of BA is influenced by several factors; the long-

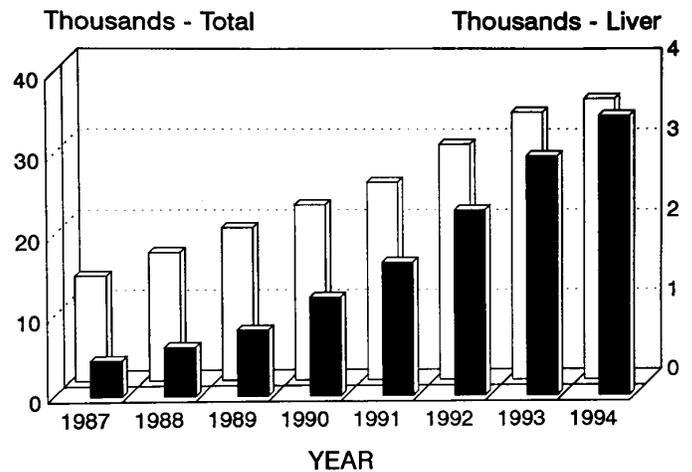


FIG. 1. Transplantation waiting list volume in the United States (1987-1994). During this interval, the number of patients awaiting liver transplantation increased from 449 patients to 3,500; total waiting list volume increased from 13,000 to 35,000 patients.<sup>92</sup> ■, liver; □, total.

term prognosis is directly related to the establishment of successful bile flow and the disappearance of jaundice, as discussed above. Ten-year survival rates of 73% and 92% have been reported for infants in whom jaundice has cleared.<sup>6,7,12,35-38</sup> In those patients in whom jaundice remains and bile flow is inadequate, the 3-year survival rate decreased to 20%.

While the coexistence of associated anomalies such as the polysplenia-malrotation syndrome or congenital heart disease has been associated with a more dismal prognosis, the presence of these anomalies does not, at the present time, exclude the infant from HPE. However, overall surgical results have been significantly worse in infants with this anomaly complex; therefore, these patients more frequently are candidates for transplantation.<sup>37</sup>

Approximately 65% of infants who have undergone primary HPE will ultimately require liver transplantation.<sup>2,39</sup> The remainder have varying degrees of medically manageable hepatocellular compromise and portal hypertension. Liver transplantation is necessary in infants with a failed HPE, manifest by progressive hepatocellular decompensation, refractory growth failure with hepatic synthetic dysfunction and the development of a coagulopathy, and intractable portal hypertension with recurrent gastrointestinal hemorrhage or hypersplenism. Patients with a "successful" HPE who remain jaundice-free for many years may also ultimately require transplantation because of the cumulative effects of repetitive episodes of cholangitis and progressive hepatocellular insufficiency.

The most significant limitation to transplantation in infants with BA is the availability of suitable donor organs. The success of liver transplantation has led to a rapid increase in the number of individuals awaiting transplantation; from 1987 to 1994, the liver transplant waiting list size has steadily increased (Fig. 1).

**LIVER DONORS 1978-1994**

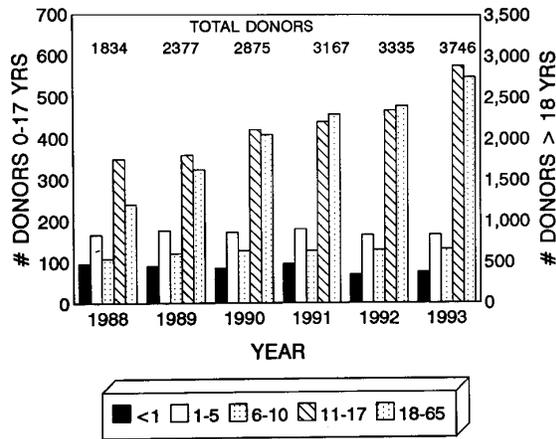


FIG. 2. Liver donor organ volume, 1988-1993, stratified by age of the donor.<sup>93</sup>

While the need for transplantation continues to grow, donor organ availability has not increased sufficiently to meet these demands (Fig. 2). This problem is intensified by the need for most BA recipients to undergo transplantation within the first 2 years of life, a time when size-matched donor organs are especially scarce. This discrepancy between organ donor need and supply has stimulated the development of surgical procedures to increase the potential donor organ pool for small children. These procedures include: 1) reduced-size liver transplantation, 2) split-liver transplantation, and 3) living-related donor transplantation.<sup>40</sup> Each of these procedures uses similar operative techniques to implant a functional segment from a larger donor liver into a small recipient. Survival rates for these procedures are equivalent to or exceed those for whole-organ transplantation. At the present time, reduced graft options supply donor organs for approximately half of the BA recipients in selected centers. Concurrent with the development of these procedures has been a significant decrease in waiting list mortality among patients with BA.<sup>39</sup>

The outcome for patients with BA who undergo liver transplantation is a favorable one; many centers now report 1-year survival rates approaching or equaling 90%.<sup>2,39,41</sup> Long-term mortality is quite limited, and patient lifestyles approximate those of nontransplanted siblings and peers. The high success rate is achieved through the complementary and sequential utilization of primary HPE in children with BA followed by transplantation when deemed necessary (Fig. 3).

The costs on a national basis for this therapy are difficult to calculate; however, some approximation can be reached. When the Kasai procedure is used as primary therapy, the cost of initial hospital admission and surgical care throughout the first year of life approximates \$17,500 to \$20,000. With an estimated incidence of BA of 1:10,000 live births, approximately 410 infants

with BA would be treated each year, an annual cost of \$7.6 million. From 1990 to 1993, 240 to 280 BA patients underwent transplantation each year. With an annual average of 260 transplantation procedures, and a hospital bill of \$225,000, the total expenditure for primary transplantation care is \$58.5 million. Of these patients, approximately 10% will require early retransplantation and 5% may require retransplantation for chronic rejection throughout their lifetime. In addition, immunosuppressive therapy and other medications may cost \$5,000 to \$10,000 per year per patient. Reviewing these statistics, it is clear that any and all efforts directed at early diagnosis and more effective primary therapy for BA will not only be beneficial to the patient, but also cost-effective.

In summary, in addition to prompt diagnosis, we should vigorously investigate the mechanisms governing growth failure and promote aggressive protocols to improve the growth and nutrition of these infants. We also must improve our organ donor resources to allow more timely transplantation, thus minimizing costs and complications. Efforts to address these problems will decrease morbidity and improve the long-term survival of children with this devastating disease.

**BASIC RESEARCH REGARDING THE PATHOGENESIS OF BA**

Dr. David Perlmutter (Washington University School of Medicine at St. Louis University) discussed current basic research efforts designed to address the pathogenesis of BA. For each of five major mechanisms (Table 5) that have been proposed in the literature, he briefly discussed current major research efforts directly addressing the problem of BA, as well as current research in related areas that may be relevant to the pathogenesis of BA.

First, there have been a number of studies examining the possibility that BA results from an occult viral infection. Several reports of time-space clustering of cases tend to support an infectious etiology.<sup>42,43</sup> Mo-

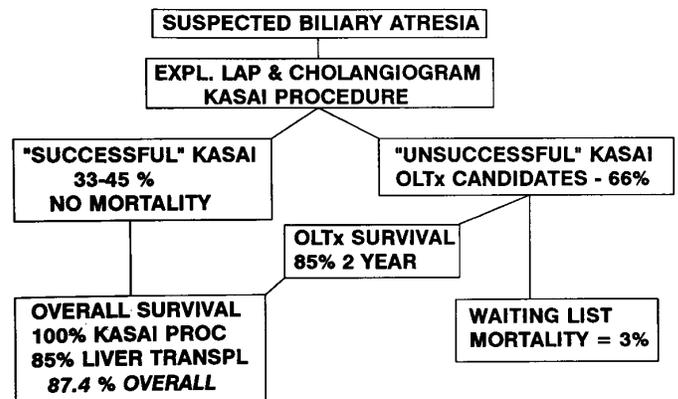


FIG. 3. Flow chart of BA management strategy, indicating success rates following sequential Kasai (HPE) procedure, followed, when needed, by liver transplantation.

TABLE 5. Etiology of BA

Proposed Mechanisms
1. Occult viral infection.
2. Environmental toxin exposure.
3. Defect in morphogenesis (tissue level, cell level).
4. Disorder of immunologic/inflammatory system (autoantibody; altered class I or II major histocompatibility complex expression).
5. Defect in fetal/perinatal circulation.

recki et al. found evidence for serological reactivity to reovirus type 3 in several children with BA,<sup>44</sup> and for localization of reovirus particles in the porta hepatis of one infant with BA.<sup>45</sup> It had been known for some time that this virus could cause an obliterative cholangiopathy in weanling mice.<sup>46,47</sup> However, several recent studies have not been able to substantiate a relationship between reovirus 3 and BA when comparing unselected populations of infants with BA, neonatal hepatitis, or other liver disorders with normal control infants.<sup>48-50</sup> Recent studies suggesting that prior injury to the liver, or perturbation of the liver, is necessary for susceptibility of murine liver cells to reovirus infection<sup>51</sup> also raise the possibility that reovirus (or, for that matter, any virus) may act in concert with other types of hepatobiliary injuries. Riepenhoff-Talty et al. have reported the development of extrahepatic biliary obstruction in newborn mice orally inoculated with group A rotavirus.<sup>52</sup> These investigators have also presented preliminary evidence for PCR amplification of group C rotavirus sequences from livers of patients with BA,<sup>53</sup> for immunoreactivity to group C rotavirus in serum of patients with BA, and for group C rotavirus particles in the stool of patients with BA (M. Riepenhoff-Talty, personal communication). Additional studies have been initiated to provide further evidence for a relationship between rotavirus and BA pathogenesis. There have also been sporadic reports of cytomegalovirus (CMV) infection in children with BA.<sup>54</sup> However, recent studies using PCR technology have detected CMV sequences in liver from many infants with neonatal hepatitis,<sup>55</sup> therein raising questions about the causal relationship between CMV infection and either BA or neonatal hepatitis.

Second, exposure of pregnant mothers to toxic substances has been considered in discussions on the pathogenesis of BA. Time-space clustering of cases also tends to favor a toxic etiology. Although there is no evidence in the literature for specific toxins that might cause BA in human infants, there is an intriguing report of a presumed toxic disorder in lambs and calves that resembles BA.<sup>56</sup> Harper et al. described two outbreaks of jaundice, failure to thrive, "white scours," and death within 4 weeks of birth for 300 cross-bred lambs and 9 cross-bred calves in New South Wales, Australia.<sup>56</sup> Bile ducts and gallbladder were absent, fibrotic, or atretic, and there was bile duct proliferation, inflammation, and fibrosis in the liver. An outbreak of this

illness occurred in 1988 when sheep management was changed and the ewes were restricted to a specific, flat property during pregnancy and lambing. In previous years, they had been unrestricted and could graze on a number of other properties. The flat property is located on the foreshores of a dam, and it is mostly under water, but intermittently exposed. During 1987 and 1988, the water level was particularly low. Interestingly, the only previously recorded outbreak of the illness occurred in 1964 on an adjoining property when water levels were also at a very low point. These observations suggested the presence of a phytotoxin or mycotoxin that could insult the fetal hepatobiliary tree, but extensive investigation of the weeds in this property did not reveal an abnormal growth.

A defect in morphogenesis of the extrahepatic biliary tract has been proposed as a third type of mechanism for the pathogenesis of BA. This hypothesis is appealing when considering the coexistence of another anomalies, particularly anomalies of visceral organ symmetry, in 10% to 30% of infants with BA.<sup>25,26</sup> Yokoyama et al. have recently reported anomalies of visceral organ symmetry, including complete abdominal situs inversus, severe jaundice, and death within the first week of life, in transgenic mice that have a recessive insertional mutation in the proximal region of mouse chromosome 4.<sup>57</sup> Although it is not mentioned in the report, most of these animals have abnormal development of the common bile duct (P. A. Overbeek, personal communication). Anomalies included mirror-image left-right inversions of stomach, spleen, and liver; some had polysplenia, preduodenal portal vein, intestinal malrotation, and dextrocardia. The mutation, now called the *inv* mutation, results in a situs abnormality in 100% of homozygous mutant mice as contrasted to other models of left-right asymmetry, such as the *iv* mutation<sup>58,59</sup> and the *Ft* mutation,<sup>60</sup> which affect 50% of homozygous mutant mice. Two other loci have been linked to situs abnormalities in humans: the Kartagener gene<sup>61</sup> and a gene on the X chromosome.<sup>62</sup> Jaundice and abnormalities in the biliary tree have only been observed in the *inv* mouse. Presumably, the gene mutated in this *inv* mouse ordinarily directs a critical phase in the morphogenetic program for establishing visceral symmetry and for early development of the extrahepatic biliary tree. This gene has not yet been isolated, and, therefore, the mechanism by which it specifies left-right axis determination is not known. However, Sklar has proposed an interesting model in which chromosomal inversion at this locus leads to specification of the inverse symmetry.<sup>63</sup>

Although most infants with BA and associated anomalies of visceral symmetry have polysplenia, there are several reports of BA in association with asplenia.<sup>26</sup> In a recent study by Roberts et al., the orphan homeobox gene *Hox 11* has been shown to control morphogenesis of the spleen.<sup>64</sup> *Hox 11* is ordinarily expressed in the splenic anlage arising from the splanchnic mesoderm. Targeted disruption of *Hox 11* results in mice with asplenia. Because there are no other anomalies of

splanchnic derivatives in *Hox 11*-deficient mice, a mutation of the *Hox 11* gene is unlikely, by itself, to cause BA. However, the mechanism by which tissue-specific and developmental stage-specific expression of *Hox 11* is induced in the splenic anlage may be relevant to the mechanism involved in extrahepatic biliary duct morphogenesis, and to a specific defect in the subgroup of infants with BA and splenic anomalies.

An interesting series of observations about the morphogenesis and differentiation of intrahepatic bile ducts has been made by Desmet et al.<sup>4</sup> Using human liver from different stages of fetal development and immunostaining with anticytokeratin antibodies specific for bile duct epithelial cells, these investigators have shown that bile ducts arise from the mesenchyme surrounding portal vein radicles. Presumed primitive hepatic precursor cells differentiate into a single layer of cytokeratin-staining cells, and then form a double layer. At focal points, these cells then scatter and remodel as a single layer around a lumen. In livers from some infants with BA, there is evidence for an arrest in remodeling such that lumens are not formed. The arrested structure has been called a "ductal plate malformation." These observations are especially interesting now that specific molecular mechanisms for lumen formation have been discovered. Recent studies have shown that hepatocyte growth factor/scatter factor, via interaction with its receptor, the *c-met* oncogene, mediates differentiation of mesenchymal tissue into epithelial cells, scattering and remodeling of these epithelial cells in a manner that results in formation of a lumen.<sup>65-67</sup> It would, therefore, be interesting to examine the role of hepatocyte growth factor (HGF) and *c-met* in the pathogenesis of BA, at least in the subgroup of BA patients in whom there is "ductal plate malformation." However, it is unlikely that there is a structural abnormality in hepatocyte growth factor or *c-met* in BA because these molecules probably play a role in lumen formation in many tissues which are unaffected in BA. Perhaps there is an abnormality in tissue-specific and/or developmental stage-specific induction of hepatocyte growth factor and *c-met*, i.e., during the critical period for mesenchymal-epithelial signaling in the hepatobiliary anlagen.<sup>68</sup> Perhaps related growth factors and receptors, such as the *c-sea* oncogene,<sup>69</sup> can provide overlap for the functions of HGF and *c-met* in other tissues.

Failure to remodel the embryonic ductal plate could also result from a defect in the structure or activity of one of the intracellular adhesion systems. Adhesion molecules are likely to be critically important in the scattering of mesenchymal cells, in their differentiation and reorganization into a single-layer epithelium that surrounds the lumen. Expression of the E-cadherin molecules, cell-cams of the carcinoembryonic antigen family, proteins of the zonula occludens, and desmosomes is induced at specific stages and locations during liver development.<sup>70</sup>

Although morphogenesis of the biliary tree is likely to be dependent on the determination of the biliary

**TABLE 6. Immunostaining for Adhesion Molecules in Liver of Patients with BA and Five Control Patients**

	BA (n = 6)		Control* (n = 5)	
	Hepatocytes	Biliary Epithelial Cells	Hepatocytes	Biliary Epithelial Cells
ICAM-1	+++	+++	+++†	—
VCAM-1	+++	+	+++†	—
ELAM-1	—	—	—	—

Abbreviations: +, presence of positive staining; number of + indicates the intensity of staining<sup>75</sup>; ICAM-1, intracellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule; ELAM-1, endothelial leukocyte adhesion molecule.

\* Includes one patient from each of the following diagnostic categories: TPN cholestasis, choledochal cyst, viral hepatitis, metastatic CA, and TTP.

† With inflammation.

ductular epithelial cell lineage, there is still relatively limited information on the molecular/cellular basis by which this lineage is established. Several recent studies have examined biliary epithelial cell lineage relationships in terms of transcription factors<sup>71</sup> and cell surface lectins,<sup>72</sup> but more extensive investigation will be necessary to clearly define multipotent precursor stem cells and intermediate cell types in the biliary epithelial cell lineage.

Fourth, an abnormality in the immune and/or inflammatory response in patients with BA has been discussed. The most compelling evidence comes from recent studies suggesting an increase in the frequency of the HLA-B12 allele in infants with BA as compared with controls.<sup>23</sup> The increase in HLA-B12 was most evident in infants with BA who do not have other associated congenital anomalies, making an immune mechanism even more plausible. However, this study was performed in a small number of patients, and the HLA-B12 allele was also the most common class I major histocompatibility complex allele in the control population. An increase in frequency of a particular HLA haplotype does not by itself imply altered immune function. Because of linkage disequilibrium displayed by genes within the major histocompatibility complex, it is possible that other genes that map into this region of chromosome 6 and are coinherited with the HLA-B12 allele are truly associated with BA. Several studies have examined the possibility that biliary ductular epithelial cells are susceptible to immune attack because of abnormal expression of HLA class I molecules at the cell surface,<sup>73,74</sup> or are susceptible to inflammatory attack because of abnormal expression of inflammatory adhesion molecules, such as intracellular adhesion molecule-1, on the cell surface<sup>75</sup> (Table 6). In each of these cases, a primary injury that triggers altered cell surface HLA class I or intracellular adhesion molecule-1 expression has not been proposed or conceptualized.

Fifth, vascular insults during fetal hepatobiliary development have been implicated in the pathogenesis of BA. In a recent study by Ho et al., thickened, tortuous,

and dilated arteries were identified in the biliary remnants of infants with BA.<sup>76</sup> These observations were taken as evidence for an arteriopathy accompanying the disorder. We are not aware of any ongoing research program directly addressing this pathogenic mechanism in BA or research in a related area that may be relevant to BA.

In summary, most of our current information about BA suggests that it is a heterogeneous disorder. Perhaps it should be considered a common phenotype of several different disorders. Careful segregation of patients based on clinical characterization and application of the tools now available to examine infectious, toxic, morphogenic, and immune/inflammatory etiologies should allow us to identify at least several of the underlying pathogenic mechanisms that determine the BA phenotype.

#### **PATHOGENESIS OF TISSUE INJURY IN BA**

Dr. Ronald J. Sokol (The Children's Hospital, Denver, CO) presented a proposal for the pathogenesis of tissue injury in patients with BA, cautioning that, until the specific processes involved in the initiation and perpetuation of bile duct and liver injury in this condition are understood, it will not be possible to develop new therapeutic interventions.

Although previous investigations have suggested a developmental, infectious, immune/inflammatory, or vascular cause of this condition, there is a paucity of information about the cellular and molecular mechanisms by which the bile ducts and liver become irreversibly damaged and fibrotic in patients with BA. It is clear that both the extrahepatic bile duct and the intrahepatic bile ducts are involved in this process, with the ultimate result of hepatocyte injury and dysfunction, collagen deposition and cirrhosis. A proposed paradigm by which these interrelated events may be explained encompasses three primary pathogenetic processes: bile duct injury, cytokine activation, and accumulation and toxicity of hydrophobic bile acids (Fig. 4). Although little is known about the relative contribution of each of these processes to the pathology of BA, basic knowledge in these areas continues to grow.

The primary pathology in BA involves acute and chronic inflammation of the extrahepatic and intrahepatic bile ducts, which appears to precede the development of periductular fibrosis and, eventually, luminal obliteration. Unfortunately, little is known about the nature of the T-cell inflammatory infiltrate and the cytokines, proteases, or radical species participating in this epithelial injury. In fact, little is known in general about the cell biology of bile duct injury, with the exception of immunologic processes that participate in the pathogenesis of primary biliary cirrhosis, another obliterative cholangiopathy. It has been proposed that in primary biliary cirrhosis, an initial unknown noxious agent (e.g., viruses, cytokines, or ischemia, among others) damages biliary epithelia and causes shedding of bile duct epithelial antigens, which are then processed by dendritic cells and macrophages in the portal tract.<sup>77</sup>

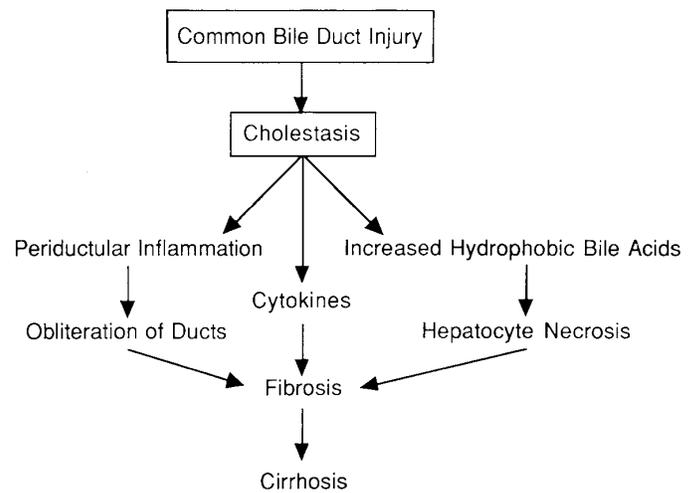


FIG. 4. Paradigm of events proposed to be involved in the generation of liver injury and hepatic fibrosis in BA.

These antigens are presented to the T-cell receptor, and activation of T cells ensues. These sensitized T cells differentiate into cytotoxic T cells and secrete cytokines (e.g., interferon gamma) that cause aberrant expression of class II major histocompatibility complex on biliary epithelial cells and endothelial cells, resulting in presentation of self-antigens to the immune system. Altered class I and II major histocompatibility complex expression increases antigenicity of these target biliary epithelial cells to which sensitized T cells react and result in nonsuppurative destructive cholangitis and endothelial cell injury. The final consequence of these events is destruction of the interlobular bile ducts. As mentioned, the observation of clustering of certain HLA types in infants with BA<sup>23</sup> supports the notion that an immune mechanism may be involved in bile duct injury in a proportion of patients with this disease. More complete, prospective evaluation of the immunobiology of BA compared with other neonatal liver diseases will help to clarify the possible "autoimmune" nature of this disease.

The cellular and molecular events involved in the development of hepatic fibrosis and cirrhosis, the ultimate outcome in BA, are receiving wide attention. It is now clear that the lipocyte (stellate cell, fat-storing cell, Ito cell) is the predominant cellular source of collagen in the injured liver.<sup>78</sup> Lipocytes respond to various signals (transforming growth factor  $\beta$ , platelet-derived growth factor, oxidant stress) released during cellular injury by undergoing activation and proliferation, and by increasing transcription and synthesis of collagen type I.<sup>79</sup> Recent studies have shown that bile duct epithelial cells express messenger RNA for various cytokines involved in fibrogenesis.<sup>80</sup> It is thus postulated that direct signaling from injured biliary epithelia in BA may trigger the fibrotic process. Therefore, the cytokine profile and the role of activation of lipocytes in BA are other areas that deserve investigation, inasmuch

as several approaches to interrupt collagen deposition are being investigated.<sup>81</sup>

A third pathogenetic factor is the effect of hydrophobic bile acids; in elevated concentrations, these bile acids are hepatotoxic in numerous mammalian species, including man.<sup>82,83</sup> Furthermore, it has been shown that chenodeoxycholic acid (CDC) and its conjugates are increased in the liver of humans with cholestatic disease.<sup>84</sup> Therefore, it has been proposed that the accumulated CDC (or other toxic bile acids) in cholestasis may be an important factor producing hepatocyte injury.<sup>85</sup> The hepatocyte may, in turn, release additional factors that stimulate fibrosis. CDC has been shown to cause both hepatocyte necrosis (irreversible loss of metabolic functions and integrity of plasma membrane) at high concentrations and hepatocyte apoptosis (programmed cell death) at low concentrations.<sup>82,86</sup> Either of these processes can result in loss of biliary epithelial cells and hepatocytes. It is thought that the effect of bile acids on mitochondrial function (altered oxidative metabolism and release of oxygen free radicals) may be a key factor in the induction of hepatocyte necrosis in cholestasis.<sup>83,87</sup> Indeed, it has been shown that the activity of respiratory complexes I and III are reduced in hepatic mitochondria from bile duct-ligated rats<sup>88</sup> and in mitochondria exposed to toxic bile acids.<sup>89</sup> Recently, increased generation of hydroperoxides has been demonstrated in rat hepatocytes and rat liver mitochondria exposed to CDC or its conjugates.<sup>83</sup> Finally, reduced concentrations of hepatic and mitochondrial antioxidants (vitamin E, glutathione, ubiquinone) have been demonstrated in liver from bile duct-ligated rats.<sup>90,91</sup> Thus, it is proposed that the sequence of mitochondrial injury, oxidant stress, adenosine triphosphate depletion, increased cytosolic free calcium, and activation of degradative hydrolases in the cell may be the mechanism responsible for CDC-induced hepatocyte injury.<sup>83,87</sup> Interruption of the events in this pathway may prove to be a possible therapeutic approaches to attenuating the liver injury and fibrosis in BA.

Although advances in our understanding of cellular injury and the induction of fibrosis in liver disease are emerging at a rapid rate, the differences in these processes between the neonate and the older child or adult have not been adequately explored. For instance, how does the neonatal immune system recognize and respond to injured biliary epithelial cells compared with that in the adult? Do neonatal lipocytes respond to cytokines in the same manner as their adult counterpart? Does the relative antioxidant deficiency of the neonate predispose to more striking oxidant damage and its consequences? How does the immaturity of neonatal bile acid metabolism impact on hepatic injury during cholestasis? These and other areas will need to be investigated to understand the unique biology of neonatal bile duct injury and possible interventional strategies.

#### SUMMATION

Extensive systematic characterization of patients with BA in a multicenter study will enhance the likeli-

hood of (1) defining subpopulations within the BA phenotype; (2) understanding the natural history; (3) providing critical information about clinical care of patients within the context of current therapeutic options; and (4) establishing a basis for investigation of the underlying etiology. These are crucial steps in the attempt to develop rational therapeutic strategies, targeted to interrupt the progressive bile duct obliteration, and might allow achievement of the ultimate objective—to prevent the initiation of this process.

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