Biliary atresia (BA) is a relatively rare disease, which occurs at a rate of 0.5–3.0 cases per 10,000 live births, depending on the ethnicity of the patients[1]. BA results from a destructive inflammatory process of both the intra- and extra-hepatic bile ducts. The etiology of BA remains unknown, although BA has never been believed to be an inherited disorder[2]. Clinically, infants present with persistent jaundice and pale-colored stools, which may not always be acholic depending on the extent of biliary destruction. Patients may also have associated hepatosplenomegaly at presentation unless the diagnosis is made early. BA is progressive and leads to death in all patients by 2 years of age without surgical intervention[3]. It may be ‘curable’ if the disease is detected early, the patient undergoes appropriate hepatoportoenterostomy (i.e., the Kasai procedure) and has appropriate postoperative care. This may include corticosteroid therapy, although the efficacy of corticosteroids is still controversial[4]. Even when patients are diagnosed early and have successful bile drainage after a Kasai procedure, fibrosclerotic disease in the liver can occur. For patients diagnosed late or who have undergone an unsuccessful Kasai procedure, gradual scarring of the liver ensues, progressing to cirrhosis, and eventually leading to hepatic failure. Even in long-term survivors with excellent bile flow postoperatively, a sudden onset of obstructive liver dysfunction may occur. This can occur with or without associated bacterial cholangitis.

The overall survival rate for patients with biliary atresia after the Kasai procedure is approximately 50%[5]. With the advent of liver transplantation as a rescue procedure, the patient survival rate has risen to greater than 90%[5]. However, patients who undergo organ transplantation require lifetime immunosuppressive therapy that involves inherent risks. In addition, transplantation requires considerable medical effort and expense. In light of this, determining the underlying cause of BA will be a major medical breakthrough, paving the way for new treatment options and hopefully improved clinical outcomes. This report reviews the recent immunologic findings in BA, focusing on a role for maternal microchimerism (mMC) in its etiopathogenesis.

Porta hepatis morphology & histological findings of the liver
Gross inspection of a BA liver at laparotomy shows that the extrahepatic bile ducts have been obliterated and replaced by fibrous cords/masses.
Often, the lymph nodes in the porta hepatis appear swollen. Destruction of the extrahepatic bile ducts can occur on various levels. The type most commonly seen occurs with obstruction at the level of the hepatic ducts. Intrahepatic bile ducts are also affected, often appearing withered (Figure 1). When the porta hepatis becomes occluded, the gallbladder usually becomes atrophic. In some cases of BA, the gallbladder appears normal in size. In such cases, the extrahepatic bile ducts have a patent but slightly narrow common distal bile duct. In approximately 10% of BA patients, a cystic structure filled with bile is found in the porta hepatis. This is considered the ‘correctable type’. In such cases, the intrahepatic bile ducts are sometimes visualized by an intraoperative cholangiography performed through the cyst, but normal intrahepatic biliary trees are not observed. The cystic type can be antenatally detected by fetal ultrasonography. The earliest reported antenatal diagnosis, which was later confirmed as BA, was in the 16th week of gestation [6].

Microscopically, the resected fibrous mass at the porta hepatis includes interrupted remnants of bile ducts with degenerated and ulcerated epithelium and scarce lymphocyte infiltration [7]. Liver histology at the time of the Kasai operation shows expanded portal areas with infiltration of polymorphonuclear leukocytes, mainly macrophages [7] and mononuclear lymphocytes in biliary epithelial cells [8], and proliferation of bile ductules. In general, portoportal bridging fibrosis is infrequently seen with early diagnosis, while a portocentral bridging fibrosis is frequently a manifestation of advanced disease. In cases of BA diagnosed late, the degree of inflammatory cell infiltration is often less severe, while bile stasis and bile duct proliferation with plugging is more notable. However, the severity of fibrosis is not necessarily in proportion to the age at diagnosis [9]. Even a patient presenting after 3 months of age can have only a slight portoportal bridging with good bile flow after the Kasai operation. Based on this observation, it is speculated that the timing of the immune insult that triggers BA varies from case-to-case, during or after a critical period of bile duct development in utero.

**Evolution of BA hypotheses**

Biliary atresia is thought to have two main types: an embryonal subtype, associated with congenital malformations, and a ‘perinatal’ subtype, which is associated with a progressive inflammatory destruction of both intra- and extra-hepatic bile ducts without congenital malformations. The ‘perinatal’ or ‘acquired’ subtype accounts for the vast majority of BA cases [10]. The embryonal type is most often associated with splenic involvement, and falls under the pseudonym BA splenic malformation (BASM) syndrome. This accounts for approximately 10% of BA patients [11].

Prior to the 1960s, BA was generally categorized as a birth defect of extrahepatic bile ducts as a result of failure in early organogenesis [3]. An etiological concept in the 1970s was monofactorial; Landing hypothesized that BA was secondary to viral hepatitis or an inflammatory process. He proposed that neonatal hepatitis, choledochal cysts and BA all had similar etiologies [12]. It was not until 1981, when Hadchouel et al. first reported activated humoral immunity in the degenerative bile ducts, that an immune-mediated mechanism occurring later in pregnancy was proposed as the pathogenesis [13]. In the 1990s, the prevailing hypothesis was that BA was the phenotype of several underlying disorders caused by infection, genes and environmental exposures [14]. Schreiber and Kleinman suggested in 1993 that the ‘acquired’ form may be the result of a ‘two-hit’ phenomenon dependent on genetic vulnerability to an environmental precipitating factor [15]. After Riepenhoff-Talty’s first report in 1993 on newborn mouse models using rhesus rotavirus group A [16], Petersen et al. followed the animal experiments and observed that antenatal infection of pregnant mice did not induce BA in their offspring and that an immunological gap was essential for virally induced BA [17]. He also suggested that maternally transmitted immunity is involved in this gap, protecting the pups from virus-induced BA. As these murine models show the extrahepatic, but not intrahepatic, bile duct obstruction with prestenotic dilatation [18], it is unclear whether these murine models reflect the true pathogenetic mechanism of BA. Moreover, a putative viruses, including reovirus, rotavirus and cytomegalovirus, have not yet been established as the cause of BA in humans.

**Immunological findings in BA**

In 1976, a symposium entitled “Immunodeficiency: its nature and etiological significance in human diseases” was held in Tokyo. Interestingly, Landing reported progressive loss of thymic epithelium (Hassall’s corpuscles) over 18 months of age in his autopsy series of BA [19]. These days there are fewer occasions for postmortem investigations, and the underlying cause of the disappearance of Hassall’s corpuscles in BA remains to be clarified. Hadchouel et al. in 1981 reported that IgM alone, or IgM and IgG, were deposited in the basement membrane of the glandular structures in the fibrous remnant of the porta hepatis of one third of 128 patients with BA [13]. Furthermore, based on the histologic similarities of the intrahepatic bile ducts in BA to other immune-mediated diseases such as graft-versus-host disease (GvHD), Muraji, in 1988, showed increased HLA-DR expression of biliary epithelial cell (BEC) from BA patients [20], suggesting the possibility of an immune-mediated mechanism in the pathogenesis of BA. Subsequent immunohistochemical studies throughout the 1990s suggest that BECs in normal liver do not express HLA class II molecules, while aberrant expression is found in the bile ducts of BA [21,22], in allografts during rejection, in GvHD and in primary biliary cirrhosis (PBC) [23].

Dillon et al. showed that the strong expression of ICAM-1 adhesion molecules on biliary ductal epithelium of BA was similar to the ICAM-1 expression seen in the end-stage processes of PBC and primary sclerosing cholangitis [24]. Functional studies demonstrate the ICAM-1 expression in BECs facilitates the binding of cytotoxic lymphocytes [25]. In addition, serum levels of ICAM-1 correlate with poor prognosis in BA [26]. Kobayashi et al. showed that the expression of costimulatory factor (B7) was found in the biliary epithelium, vessels and sinusoid in BA patients. Their data strongly support the concept that bile duct damage was due to an immune-mediated mechanism, which also causes an increase in vascular resistance secondary to damage of the endothelium [27].
This assertion is interesting in the sense that splenomegaly secondary to portal hypertension often exists at the time of diagnosis or portoenterostomy.

Infiltration of the liver with immune cells is also reported, including CD68+ cells (macrophage-related) [22], CD4+ lymphocytes, natural killer cells [22] and mast cells [28]. These hepatic infiltrations of various immune-related cells are reported to correlate well with the clinical outcome of BA patients, suggesting both innate and adaptive immune responses play a role in bile duct destruction in BA. With regards to macrophages, one important characteristic of macrophages is their capacity to secrete TNF-α, which may explain the aberrant expression of Fas Ligand mRNA [29] and the increased numbers of apoptotic cells in bile duct epithelia of patients with BA [30]. Mast cell populations seen in the portal tracts are reported to correlate with liver dysfunction in BA. However, it is unclear whether these inflammatory changes are the primary cause or a result of the underlying cholestasis.

In 2004, Mack et al., using fluorescent immunohistochemistry, analyzed the cells infiltrating liver tissues in the portal areas and revealed increases in CD4+, CD8+ and CD68+ cells in BA, compared with controls of neonatal hepatitis, total parenteral nutrition-related liver diseases and choledochal cysts [31]. They also analyzed the expression of cytokine mRNA by real-time (rt)-PCR to demonstrate the expression of IFN-γ, and showed CD4+ Th1 cell-mediated immunity to be a specific immune response in BA, not a secondary response to cholestasis. Shinkai et al. performed quantitative rt-PCR and immunohistochemistry to measure the relative levels of mRNA of CD4+, CD8+, IL-2 and TNF-α, and reported increased expression of IL-2 and increased CD8+ T-cell infiltration in liver biopsies from infants with BA, suggesting the possibility that IL-2 plays a role in the pathogenesis of BA [32].

In recent research on animal models of BA, rotavirus-infected newborn mice express IFN-γ from T cells in the liver, but when CD8+ cells are removed the obliteration of the bile ducts does not develop. This is in contrast to CD4+ cell elimination, which still leads to bile duct obliteration in rotavirus-infected newborn mice [33]. In these animal experiments, rotavirus-sensitized CD8+ cells attack rotavirus-infected BECs, causing normal cells to become apoptotic, but obstruction of intra- or extra-hepatic bile ducts was not observed. These animal experiments show that cell-mediated immunity is activated, attacking the BECs. A proposed mechanism of action is that initial virus-induced BECs become antigen-presenting cells, thereby initiating the T-cell immune response against the cells presenting viral antigens.

In 2004, Suskind et al., using FISH for X and Y chromosomes, and kinetic PCR for maternal HLA, demonstrated a higher presence of maternal cells in the livers of patients with BA compared with the livers of patients with neonatal hepatitis, which led to the hypothesis that mMC may trigger a series of immunological abnormalities in the bile duct epithelia [34]. The mMC in BA was confirmed by two other researchers [35,36]. Muraji et al. quantitatively demonstrated significantly larger numbers of maternal XX+ cells in the portal area and sinusoids of male patients with BA. In addition, they revealed these maternal cells to be CD45-CD8+ T-cell lymphocytes [37]. Figure 2, demonstrates XX cells in an enlarged lymph node at the porta hepatis, as well as in the liver histology obtained during the Kasai operation in a male BA patient. It is hypothesized that differentiated effector T cells of maternal origin have the potential to continuously damage the liver as seen in chronic GvHD. This phenomenon can explain the observation in BA that long-term survivors have episodes of liver dysfunction without bacterial cholangitis. Further clinical insight is provided from patients with severe combined immunodeficiency (SCID). Children with SCID have an increased occurrence of mMC, which can cause GvHD-like symptoms in their skin and liver [38]. In addition, experimental animal models of GvHD show both intrahepatic bile duct inflammation and scarring, as well as extrahepatic bile ducts fibrosis and sclerosis [39]. Whether the mMC is in fact instigating the inflammatory process in BA patients is still unknown; however, since tolerance is thought to play an important role during pregnancy, any failure in this tolerance-inducing system may bring about immunological conflict between mother and her fetus.[40]

**Fetal liver development & immunoembryology**

It is important to understand the development of the immune system as well as the bile ducts when discussing the pathogenesis of BA. During the fourth week of gestation, the liver arises as the hepatic diverticulum from the foregut, near the junction with the yolk sac. Lymphocyte progenitors appear in the fetal liver at the seventh to eighth week after conception, and begin migration from the fetal liver to the thymus during the ninth week [41]. A few MHC class II-positive cells are seen in the liver at 7–8 weeks of gestation. Around the eighth week of gestation, the liver precursor cells located around the largest portal vein branches become more strongly immunoreactive to the cytokeratins 8, 18 and 19, which are referred to as the ducal plate [42]. Hematopoietic stem cell markers, including c-kit, CD34 and CD33, are also expressed in the ducal plate. These early cytokeratin markers continue
system in the fetus, there is still much that is unknown and needs to be clarified, especially regarding maternal fetal tolerance. Recent work from Mold et al. showed that immune cells from the fetus at 18–22 gestational weeks had a significant tendency of inducing tolerance against maternal antigens, but, when regulatory T cells were depleted from these fetal immune cells, they showed a substantial proliferative response against maternal antigens, indicating the fetal immune system is already functional at this gestational age [44].

**BA & human leukocyte antigen**

Many autoimmune diseases tend to be associated with the specific allele of HLA. With regards to BA, there is a paucity of medical literature reporting common HLA types. Reports thus far include the B8-DR3 haplotype from Egypt determined by the lymphotoxicity test [45]. In Japan, however, HLA typing in 392 Japanese patients with BA who underwent liver transplant revealed the A24-B52-DR2 haplotype to be associated with BA at a higher than expected frequency. This phenomenon is known as linkage disequilibrium (LD) [46].

The maternal–fetal HLA-compatible condition is thought to prefer acceptance or tolerance of maternal cells [47]. A bidirectional HLA-compatible relationship occurs when an offspring’s inherited paternal antigen is identical to the noninherited maternal antigen. An increase in HLA compatibility between mother and child has been shown in some of the autoimmune disease states, such as scleroderma [48] and systemic lupus erythematosus [49], where mMC has been implicated in disease pathogenesis. Irie and Muraji recently analyzed the HLA compatibility between 57 BA patient–mother pairs and 50 control–mother pairs among those who underwent liver transplant. The HLA class I matching was significantly more frequent in BA pairs than controls [50].

**Ethnicity-dependent incidence of BA**

In 1965, Shim et al. reported a difference in incidence of BA among various racial populations living in Hawaii [1]. The incidence in the Japanese living in Hawaii was 0.8 per 1000 live births, similar to that of the Japanese in Japan (1.0 per 10,000 live births) [51]. The incidence in Caucasians was 0.6 ± 0.2, similar to that of the French (0.6 per 10,000 live births) [52] and that of the British [53]. The incidence of Filipinos in Hawaii is 2.0 ± 0.9, which is as high as that seen among French Polynesians (3.2 per 10,000 live births), while the incidence in Chinese living in Hawaii was 3.0 ± 1.6, which may be slightly lower in comparison to 1.7, a recent report from Taiwan [54].
Figure 3. Possible roles of maternal microchimeric cells in the pathogenesis of BA. Concerning maternal cells, only reported cell types were described in this figure. (1) Maternal effector T cells attack the fetal BEC, which falls into apoptotic cells. (2) Maternal effector T cells attack the fetal regulatory T cell, which enhances the autoimmune response. (3) Fetal effector T cells sensitized by maternal cells attack the adjacent fetal biliary epithelial cell, which falls into apoptotic cells. (4) Maternal cells are simply bystanders.

These observations lend support to a genetic susceptibility to BA. Taking into account the hypothesis that BA is associated with mMC, and mMC is more likely to occur when the inherited paternal antigen is more often identical to the noninherited maternal antigen, the ethnic diversity in the incidence of this disease may be explained by the parents’ allele disparity for common phenotypes, or the degree of HLA diversity found in a genetic pool of each ethnicity. However, there is discordance in studies.
in monozygotic twins [55,56], even though they have an identical genetic background (including the same HLA compatibilities to their mothers). These observations strongly indicate that genetic vulnerability, including HLA compatibility to their mothers, is not by itself the only factor that triggers the development of BA. This may in fact point to BA being the result of a ‘two-hit’ phenomenon, as hypothesized by Schreiber and Kleinman in 1993, dependent on both a genetic vulnerability as well as an environmental precipitating factor [18].

**Expert commentary & 5-year view**

A new paradigm termed ‘materno–fetal immune disease (MFID)’ has encompassed the role of chimeric cells in several diseases, including juvenile idiopathic inflammatory myopathies [57], juvenile dermatomyositis [58] and neonatal lupus congenital heart block [59]. BA might be another MFID if mMC cells are proven to instigate an attack on the fetal BECs causing BA. But there remain many unanswered questions including, what is the functional capacity of chimeric maternal cells, and whether the immunologic tolerance induced in the fetal immune system also makes maternal cells tolerant. In fact, stem cell transplantation between the mother and the child provides more successful engraftment with a reduced risk of acute GvHD than one between father and child [60]. These studies indicate that mMC cells are a ‘double-edged sword’. More studies are needed to determine whether or not maternal chimeric cells are just innocent bystanders or effectors T cells attacking BECs of patients (e.g., GvHD or in doing out alloimmune response) (Figure 3). We will have to wait for further investigation of the fetal immunoembryology to answer these questions.

First, if mMC is a common phenomenon, then why is BA a relatively rare disease? Does there need to be a ‘third hit’ for disease to develop. What is the balance between tolerogeneity and immunogenicity of mMC in BA and healthy newborns? What role do regulatory T cells have in BA? What is the role of innate immunity in BA, which is suggested to play a more important role in the fetal immune response than adaptive immunity.

Second, investigations in large patient cohorts examining HLA compatibility between BA patients and their mothers must be verified with a larger population of BA patients in various ethnic groups. Why is BA in monozygotic twins rare, even though they have the same immunogenetic compatibility to their mothers?

Third and final, why are other antigen-presenting tissues such as the skin and intestines not involved in BA? These questions and many more are required to be answered to help determine the role of mMC in BA.

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The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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**Key issues**

- The etiopathogenesis of the ‘perinatal’ phenotype of biliary atresia is considered immune-mediated biliary epithelial damage.
- The disease mechanism currently proposed for the perinatal form is that initial virus-induced biliary epithelial cells become antigen-presenting cells, instigating an immune-mediated T-lymphocyte injury.
- A recent novel hypothesis proposed is that maternal microchimeric cells may trigger a series of immunological abnormalities in bile duct epithelia in the perinatal form, causing a materno–fetal immune disease.
- This materno–fetal immune disease hypothesis supports the concept of a ‘two-hit’ phenomenon, dependent on genetic vulnerability to environmental precipitating factors for the perinatal form of biliary atresia.
- The ethnic diversity in the incidence of this disease may be explained by parents’ allele disparity for common phenotypes, or the degree of disequilibrium of HLA linkage inherited in each ethnicity.

**References**

Papers of special note have been highlighted as:

- of interest
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Biliary atresia: a new immunological insight into etiopathogenesis


• Precious description that patients with biliary atresia (BA) have a possible defect or aftermath of immunological abnormality. Additional validations are awaited.


• First report of quantification and phenotype determination of maternal chimeric cells existing in the liver of patients with BA.


• Important in that patients with severe combined immunodeficiency disease (SCID) clinically showed the graft-versus-host disease (GvHD) occurring due to transplacentally engrafted maternal cells, supporting the possibility of GvHD as the cause of BA.


• Report of animal models of GvHD across minor histocompatibility that develop biliary epithelial damage of the extrahepatic bile ducts.


• Updates the information about the critical windows in the development of the human immune system, which will provide basic knowledge about when to consider the possible timing of the insult causing BA.


Perspective


First documentation of maternal HLA class II compatibility in men with systemic lupus erythematosus.


First work to suggest patients with BA have an immuno-genetically biased relationship against their mothers.


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