Role of Hepatic Foxp3 in biliary atresia

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List of Abbreviations

AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
AP	Alkaline phosphatase
BASM	biliary atresia splenic malformation
BA	Biliary atresia
CBC	Complete blood count
CBD	Common bile duct
CHD	Common hepatic duct
CMV	Cytomegalovirus
CRP	C- reactive protein
DISIDA	Diisopropyl iminodiacetric acid
ERCP	Endoscopic retrograde cholangiopancreatography
ELISA	Enzyme Linked Immuno Sorbent Assay
Foxp3	Transcription factor forkhead box protein3
JAG1	human Jagged1 gene
CXCR3+	the lymphocyte chemokine receptor
GIT	Gastrointestinal tract
GGT	Gamma glutamyl transferase
Hb	Hemoglobin
IG	Immunoglobulin
IL	Interleukin
IL-Ra	Interleukin -1 receptor antagonist
ICOS	a co- stimulatory receptor
IL-10	interleukin 10

IPEX	the X-linked syndrome of immunodysregulation, polyendocrinopathy, and enteropathy
IFN	Interferon
INR	International normalization ratio
LHD	Left hepatic duct
LP	Lipopeptide
LPS	Lipopoly saccharides
MCT	Median chain triglycerides
MBP	myelin basic protein
MHC	Major histocompatibilty complex
NK	Natural killer cells
PBC	Primery biliary cirrhosis
PFIC	Progressive familial interahepatic cholestasis
PMNs	Polymorphonuclear leucocytes
RHD	Right hepatic duct
TGF-b	transforming growth factor-β
SD	Standard deviation
T_R	T regulatory cells
Th3	T helper 3
Treg	regulatory T cells
TC	Ultrasonographic triangular cord
T4	Thyroid hormone, thyroxine
TH	T helper cells
TMS	Trimethoprim-Sulfamethoxazole
TMB	Tetramethyl benzidine
TPGS	Tocopherol polyethylene glycol succinate

TSH	Thyroid stimulating hormone
VDRL	Veneral Disease Research Laboratory
WBCs	White blood cells

Introduction

Neonatal jaundice may indicate cholestasis rather than a benign, physiological condition. Any four-week-old newborn with persistent jaundice should have a fractionated bilirubin screen to determine whether the hyperbilirubinemia is unconjugated or conjugated hyperbilirubinemia, a hallmark of neonatal cholestasis; it is pathological and requires further investigation. **(Pashankar and Schreiber, 2000).**

Biliary atresia is a condition of uncertain cause where part, or all, of the extra-hepatic bile ducts are obliterated by inflammation and subsequent fibrosis, leading to biliary obstruction and jaundice **(Kelly and Davenport, 2007).**

Biliary atresia is a rare disease of infancy, which has changed within 30 years from being fatal to being a disorder for which effective palliative surgery or curative liver transplantation, or both, are available **(Hartley et al, 2009).**

The term biliary atresia is imprecise because the anatomy of abnormal bile ducts in affected patients varies markedly. A more appropriate terminology would reflect the pathophysiology namely, progressive obliterative cholangiopathy. Patients may have distal segmental bile duct obliteration with patent extrahepatic ducts up to the portahepatis. This is a surgically correctable lesion, but it is uncommon. The most common form of biliary atresia, accounting for 85% of the cases, is obliteration of the entire extrahepatic biliary tree at or above the porta hepatis. This presents a much more difficult problem in surgical management. Most patients with biliary atresia (85-90%) have a postnatal onset; embryonic fetal onset presents at birth and is associated with other congenital anomalies within the polysplenia spectrum (biliary atresia splenic malformation (BASM) (**Kader and Balistreri, 2007**).

Biliary atresia is the end result of a destructive, idiopathic, inflammatory process that affects intra- and extrahepatic bile ducts, leading to fibrosis and obliteration of the biliary tract and eventual development of biliary cirrhosis. Biliary atresia is the most common cause of chronic

cholestasis in infants and children, and because of the high frequency of progression to end-stage liver disease, it is the most frequent indication for liver transplantation in the pediatric age group (**Balistreri et al, 2007**).

Biliary atresia has been detected in 1/10,000-15,000 live births, idiopathic neonatal hepatitis in 1/5,000-10,000. Intrahepatic bile duct paucity appears much less commonly in about 1/50,000-75,000 live births.Pathways for pathogenesis of perinatal BA may develop when a perinatal insult, such as a cholangiotropic viral infection, triggers bile duct (BD) epithelial cell injury and exposure of self-antigens that elicit a subsequent immune response. The resulting inflammation induces apoptosis and necrosis of extrahepatic BD epithelium resulting in fibro-obliteration of the lumen and obstruction of the BD. Intrahepatic bile ducts can also be targets in the ongoing TH1 immune (autoimmune) attack and the cholestatic injury, resulting in progressive portal fibrosis and culminating in biliary cirrhosis. Embryonic BA may be the result of mutations in genes controlling normal bile duct formation or differentiation, which secondarily induces an inflammatory immune response within the common bile duct and liver after the initiation of bile flow at 11-13 wk of gestation. (**Kader and Balistreri, 2007).**

T-regulatory cells are immunosuppressive and limit chronic inflammatory diseases and antitumor immunity and have been implicated in the control of autoimmune response. Deficiency in the number of T-regulatory cells or in their functions can lead to autoimmunity, allergy and transplantation failure. CD25 and the forkhead box p3 (FOX P3 has been defined as markers of T-regulatory cells (**Christenssson M, 2009**).

Immune responses need to be constantly regulated. Abalancing act that ensures appropriate reactivity to pathogens while preventing the development of an unwanted autoimmune reaction. Autoimmune diseases arise when self-reactive T cells somehow overcome the usual restraining mechanisms leading to clonal expansion and activation **(Coffer and Burgering, 2004).**

Aim of the work

The aim of this work is to study and investigate the role of regulatory T-cells marked by Foxp3 in biliary atresia.

Anatomy of the biliary tract

Biliary tract pathology is commonly encountered and it can also present significant diagnostic and therapeutic challenges to the practitioner. One of the main challenges is attributable to the variability in the anatomy of the biliary system. The development of the liver and biliary system is a complex process that can lead to numerous anatomic variations. A thorough knowledge of this anatomy is essential in radiologic, endoscopic, and surgical approaches to the biliary system (**Bannister, 1995)**.

Embryology of the biliary system:

The biliary system and liver originate from the embryonic foregut. Initially, at week four, a diverticulum arises from the ventral surface of the foregut (later duodenum) cephalad to the yolk sac wall and caudad to the dilation that will later form the stomach. The development of the liver involves interplay between an endodermal evagination of the foregut and the mesenchymal cells from the septum transversum. The liver diverticulum initially separates into a caudal and cranial portion. The caudal portion gives rise to the cystic duct and gallbladder and the cranial portion gives rise to the intrahepatic and hilar bile ducts. As the cranial diverticulum extends into the septum transversum mesenchyme, it promotes formation of endothelium and blood cells from the mesenchymal cells. The endodermal cells differentiate into cords of hepatic cells and also form the epithelial lining of the intrahepatic bile ducts (Fig. 1) **(Clavien et al, 2000)**.

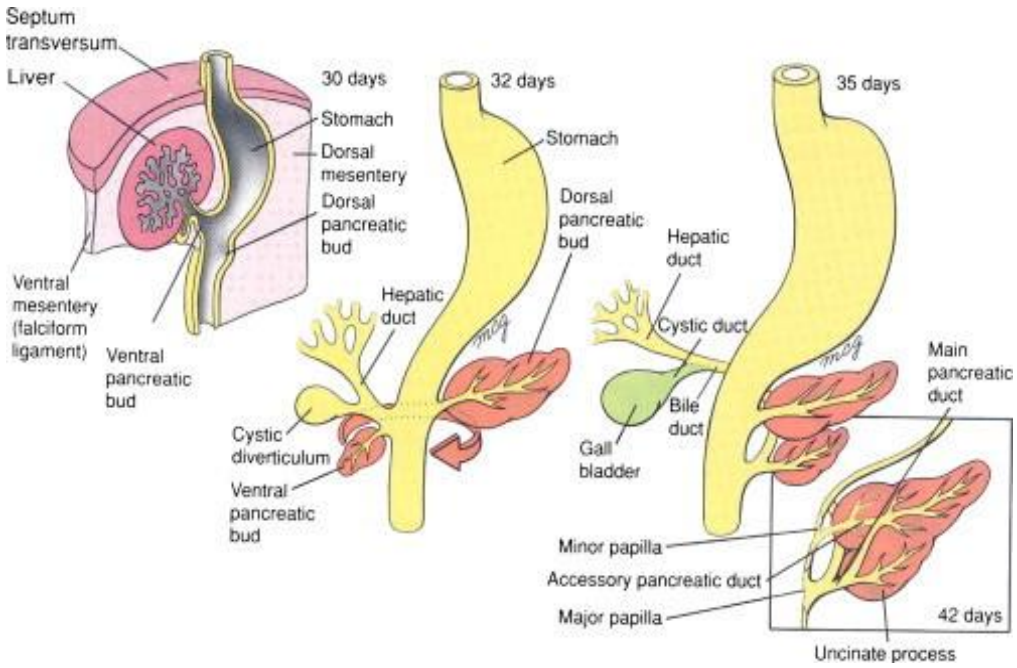

Figure (1): Development of the liver, gallbladder, bile ducts, and pancreas. **(Larsen, 1997)**.

The extrahepatic biliary system is initially occluded with epithelial cells but later it canalizes as cells degenerate. The stalk that connects the hepatic and cystic ducts to the duodenum differentiates into the common bile duct (CBD). Initially the duct is attached to the ventral aspect of the duodenum but when the duodenum undergoes rotation later on in development, there is repositioning of the CBD to the dorsal aspect of the duodenal wall **(Blumgart and Hann, 2006)**.

Overview of the liver and the biliary system:

Hepatocytes secrete bile into the bile canaliculi. Hepatocytes are surrounded by canaliculi on all sides except for the side adjacent to a sinusoid. The bile canaliculi are actually formed by the walls of the hepatocytes. Bile that is secreted by the hepatocytes flows through the canaliculi toward the center of the hepatic cords and drains into hepatic ductules that are lined by epithelial cells. The ductules then coalesce and drain into successively larger ducts. The segments of the liver are based on its biliary drainage **(Onishi et al, 2000)**.

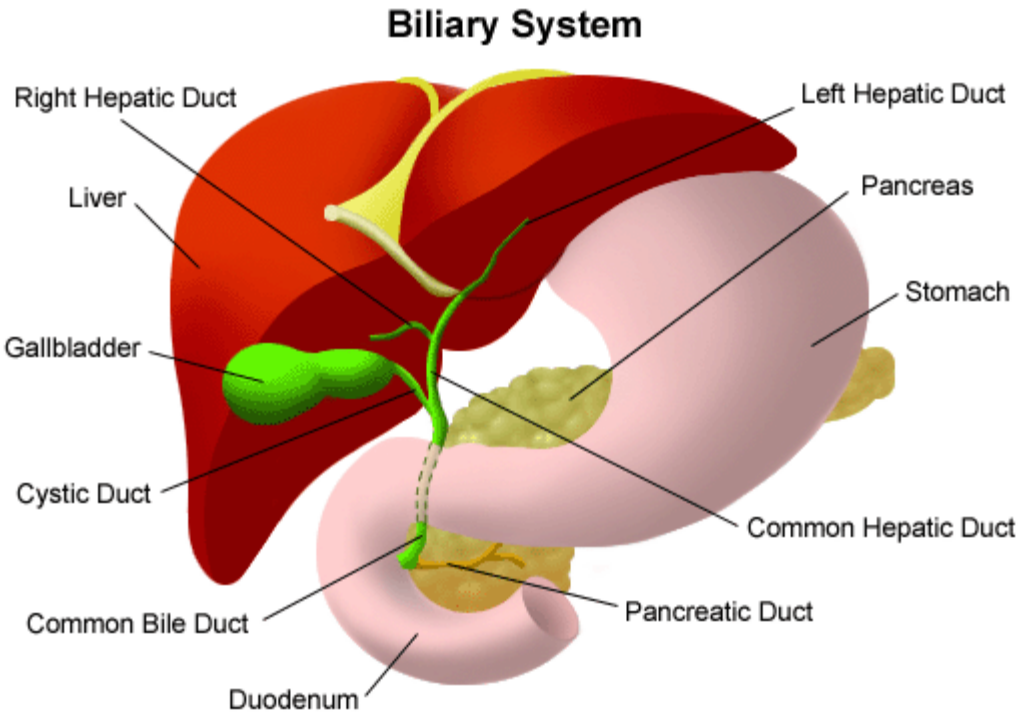

Figure (2): Liver and biliary system **(Sheila and James, 2002)**.

HEPATIC BILE DUCTS:

The main right and left hepatic ducts emerge from the liver and unite near the right end of the porta hepatis as the common hepatic duct. The extrahepatic right duct is short and nearly vertical while the left is more horizontal and lies along the base of segment IV. The common hepatic duct descends approximately 3 cm before being joined on its right at an acute angle by the cystic duct to form the common bile duct. The common hepatic duct lies to the right of the hepatic artery and anterior to the portal vein in the free edge of the lesser omentum, **(Figure 2) (Standring, 2008).**

1-Right lobe bile duct anatomy:

The right hepatic duct (RHD) drains segments 5, 6, 7, and 8 of the liver. In the most common configuration, the union of posterior (6 and 7) and anterior (5 and 8) sectoral ducts forms the RHD. There is significant variation in the topographic configuration in which these sectoral ducts join one another. In addition, frequently one of the right sectoral ducts may drain

into the left hepatic duct (LHD). There is significant variability at the confluence of the right hepatic bile ducts. When there is a true RHD, the length of the duct may range from 2 to 25mm with an average length of 9mm **(Lindner et al, 1976)**.

2-Left lobe bile duct anatomy:

The left lobe is divided into left lateral and left medial sections that are separated by the umbilical fissure. Compared with the RHD, there is less anatomic variation of the LHD. The LHD courses horizontally at the base of segment 4 superior to the left portal vein. It then joins the RHD anterior to the portal vein bifurcation to form the common hepatic duct (**Northover and Terblanche, 1979)**.

3-Caudate lobe bile duct anatomy:

The caudate lobe (segment 1) is divided into a caudate lobe proper which located between inferior vena cava and the umbilical fissure and the caudate process which connects the caudate lobe to the right hepatic lobe. The biliary drainage of the caudate lobe can not be designated as solely part of the either the right hepatic lobe or the left hepatic lobe. The caudate lobe itself can be divided into right, left, and caudate process **(Figure 3)**. In 44% of cases, three separate ducts drained each part of the caudate lobe. In 26% of cases, the caudate process duct and the duct from the right portion of the caudate formed a common duct. In most cases, the caudate process duct drains into the RHD (85%) and the left part of the caudate lobe drains into the LHD (93%) **(Gunji et al, 2006)**.

Common bile duct anatomy:

The common bile duct is formed near the porta hepatis, by the junction of the cystic and common hepatic ducts, and is usually between 6 and 8 cm long. Its diameter tends to increase somewhat with age but is usually around 6 mm in adults. It lies anterior and to the right of the portal vein and to the right of the hepatic artery. It passes behind the first part of the duodenum with the gastroduodenal artery on its left, and then runs in a

groove on the superolateral part of the posterior surface of the head of the pancreas. The duct lies anterior to the inferior vena cava and is sometimes embedded in the pancreatic tissue. It may lie close to the medial wall of the second part of the duodenum or as much as 2 cm from it: even when it is embedded in the pancreas, a groove in the gland marking its position can be palpated behind the second part of the duodenum **(Standring, 2008).**

Hepatopancreatic ampulla (of Vater):

As it lies medial to the second part of the duodenum, the common bile duct approaches the right end of the pancreatic duct. The ducts enter the duodenal wall together, and usually unite to form the hepatopancreatic ampulla. Circular muscle usually surrounds the lower part of the common bile duct (bile duct sphincter) and frequently also surrounds the terminal part of the main pancreatic duct (pancreatic duct sphincter) and the hepatopancreatic ampulla (sphincter of Oddi). When all elements are present, this arrangement may allow for separate control of pancreatic and common bile duct emptying **(Standring, 2008).**

In some cases, the pancreatic duct and the CBD do not join and each enters the duodenum separately on the duodenal papilla (**Vellar, 2001)**.