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REVIEW

The immunopathology of human biliary cell epithelium

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Abstract Bile ducts lined with biliary epithelial cells, or cholangiocytes, are the main components of the biliary system in liver. Cholangiocytes participate in the production and transport of bile substances, as well as participate in immune responses. Cholangiocytes protect against pathogens by expressing toll-like receptors and antimicrobial peptides; act as antigen-presenting cells by expressing human leukocyte antigen molecules and costimulatory molecules; recruit leukocytes to the target site by expressing adhesion molecules, cytokines, and chemokines; and induce apoptosis of leukocytes to limit the immune responses. Several cholangiopathies result from dysfunctions of the biliary system. They can broadly be divided into autoimmune, genetic, infectious, drug, and ischemic-injury-induced categories. The pathogenesis of many of these cholangiopathies is unclear and treatment is limited. Further understanding of the complexity of the biliary system is critical for medical advancements in this field.

Keywords Biliary epithelium · Cholangiocytes · Cholangiopathy · Immune response

Abbreviations

CFTR	cystic fibrosis transmembrane regulator
DR4, DR5	death receptors 4 and 5
GVHD	graft-vs-host disease
hBD-1	human β-defensin 1
HLAs	human leukocyte antigen
IFN-γ	interferon γ
IRAK-M	interleukin-1 receptor-associated kinase M
LPS	lipopolysaccharide
PBC	primary biliary cirrhosis
PSC	primary sclerosing cholangitis
SC	secretory component
SIgA	secretory IgA
TLRs	toll-like receptors
TNF-α	tumor necrosis factor-α
TRAIL	TNF-related apoptosis-inducing ligand
UCDA	ursodeoxycholic acid

Introduction

The biliary system can be divided into extrahepatic and intrahepatic components [1]. The extrahepatic biliary tract is composed of the gallbladder, common hepatic duct, common bile duct, and cystic duct [2] (Fig. 1). Bile canaliculi, the canals of Hering (intrahepatic bile ductules), interlobular bile ducts, intrahepatic bile ducts, and the left and right hepatic bile ducts comprise the intrahepatic biliary tract [1]. Bile ducts lined with biliary epithelial cells, or cholangiocytes, are the main components of the biliary system. Cholangiocytes play a number of roles in the biliary system, such as contributing from 30% to 40% of total bile secretion, participating in bile acid reabsorption and drug metabolism, and mediating immune responses [3–5]. They compose

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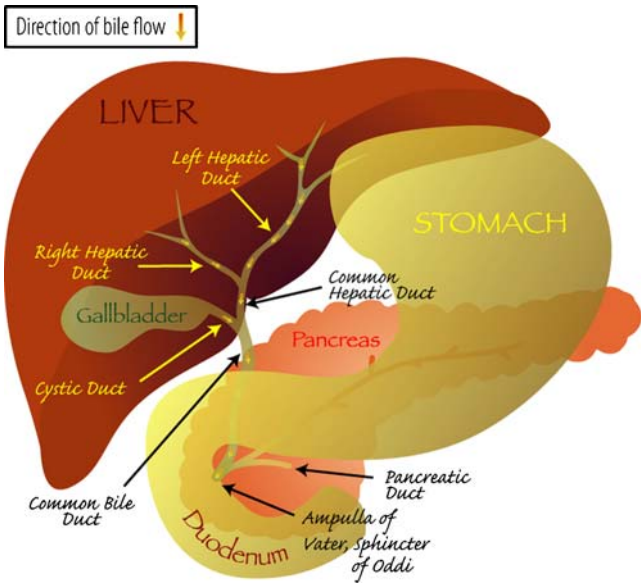

Fig. 1 The biliary system. The biliary tract is a system of ducts which carry bile from the liver to the gallbladder and the intestines. Bile is secreted by the hepatocytes and drains into bile canaliculi, which is connected to interlobular bile ducts via the canals of Hering. From there, bile flows from the intrahepatic bile ducts, which join the left and right hepatic ducts that merge to form the common hepatic duct. Bile in the common hepatic duct leaves the liver and joins the cystic duct that exits from the gallbladder, forming the common bile duct. Eventually, bile from the common bile duct merges with the pancreatic duct, which joins to form the ampulla of Vater that enters the duodenal lumen and aids in digestion. The sphincter of Oddi is located at the end of the ampulla of Vater and controls secretions into the duodenum

approximately 4–5% of liver mass and exhibit a special capacity to proliferate under disease conditions [6, 7]. Due to the exposure of the biliary tract to foreign antigens, cholangiocytes are equipped to respond through various immunological pathways. They also interact with a number of leukocytes in various fashions. However, a balance between inflammatory responses and tolerance is a key in mucosal environments. This review will cover the immunology of the cholangiocytes, as well as associated diseases.

Cholangiocytes protect against pathogens

Toll-like receptors

Toll-like receptors (TLRs) are key responders to pathogen-associated molecular patterns. Cholangiocytes from intrahepatic large bile ducts, septal bile ducts, interlobular bile ducts, and bile ductules express TLRs 2, 3, 4, and 5, which bind to ligands, such as bacterial molecules, double-stranded RNA, gram-negative lipopolysaccharide (LPS), and flagellin, respectively [8, 9]. Evidence for the maintenance of tolerance by cholangiocytes was demonstrated by the up-regulation of interleukin-1 receptor-associated kinase M (IRAK-M), a negative regulator of TLR signaling, in freshly isolated human intrahepatic biliary epithelial cells upon stimulation with TLR-2 and TLR-4 [10]. In fact, IRAK-M is expressed in cholangiocytes in the absence of TLR stimulation. However, in the presence of inflammatory cytokines, such as interferon γ (IFN-γ), cholangiocytes also activate the proinflammatory nuclear factor κB (NF-κB) pathway [8]. This points to the requirement of a coordinated inflammatory effort by multiple inflammatory mediators for the elicitation of immune responses in the biliary tract. In contrast, IRAK-M may be up-regulated as a result of natural negative feedback mechanisms upon TLR stimulation, as demonstrated by the increase in IRAK-M expression in TLR-stimulated macrophages [11]. In addition to TLRs, anti-microbial peptides are also crucial in mucosal defense.

Anti-microbial peptides

Defensins and cathelicidin are anti-microbial peptides belonging to the innate immune system [12]. They protect the mucosal barrier against gram-positive and -negative bacteria, mycobacteria, fungi, and viruses via mechanisms such as the disruption of microbial membranes. However, they also participate in adaptive immunity through the recruitment of $CD4^+$ T cells and immature dendritic cells [13]. Granulocytes express the highest density of defensins; however, cholangiocytes also produce these peptides in basal and diseased states [12, 14]. Specifically, human β-defensin 1 (hBD-1) is diffusely expressed in the cytoplasm of normal intrahepatic bile duct epithelium. However, hBD-2, although undetected in normal bile ducts, is expressed almost exclusively by diseased large bile duct epithelium in the liver. Cultured human cholangiocytes constitutively express hBD-1, while hBD-2 is up-regulated only upon IL-1β or tumor necrosis factor-α (TNF-α) treatment [14]. Cathelicidin is expressed by normal biliary epithelial cells, as well as hepatocytes. Moreover, bile salts and therapeutic bile salts, including chenodeoxycholic acid and ursodeoxycholic acid (UCDA), enhance cathelicidin expression through the farnesoid X receptor and the vitamin D receptor [15]. Again, the response of cholangiocytes to inflammatory cytokines reflects the significance of a concerted immune response. The ability of cholangiocytes to interact with the cellular immune arm is evidence of this concerted effort and important for maintaining mucosal integrity.

Cholangiocyte–leukocyte interactions

Adhesion molecules

Cholangiocytes interact with members of the immune system in a number of ways, such as the presentation of

foreign antigen, as well as the expression of leukocyte adhesion molecules. In basal conditions, normal cholangiocytes express low levels of lymphocyte adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1) and lymphocyte-associated antigen 3 (LFA-3). However, some studies report a lack of specific ICAM-1 immunohistochemical staining in normal livers [16]. ICAM-1 is critical for leukocyte migration to inflammatory sites. It binds to lymphocyte function-associated antigen-1 (LFA-1) and macrophage antigen-1 expressed on leukocytes such as neutrophils, macrophages, and lymphocytes [17]. Importantly, during an inflammatory response, cholangiocytes up-regulate their expression of these molecules. This is demonstrated via the addition of inflammatory cytokines such as TNF-α, IL-1, or IFN-γ to in vitro cultures [18]. Interlobular cholangiocytes from patients with primary biliary cirrhosis (PBC) exhibit increased expressions of ICAM-1, with a corresponding expression of LFA-1 on infiltrating lymphocytes [16]. LFA-3 on cholangiocytes can interact with CD2 molecules on cytotoxic and natural killer T cells [3]. Vascular cell adhesion molecule-1, which binds to very late antigen-4 on leukocytes, can also be up-regulated in cholangiocytes during inflammation [18]. Overall, the increase in leukocyte adhesion molecules facilitates tissue-specific migration by the slowing down of leukocyte circulation near the damaged epithelium, encouraging trafficking to the target site. The expression of antigen-presenting molecules on cholangiocytes also contributes to the local immune response.

Cytokines and chemokines

Human cholangiocytes constitutively express IL-6, IL-8, and monocyte chemotactic protein-1 (MCP-1) [19, 20], which are important chemotactic agents for neutrophils, monocytes, and T cells. IL-6 and MCP-1 are increased upon TLR-4 stimulation with LPS in the absence of inflammatory cytokines. However, IL-8 expression is unaltered by TLR ligation [19]. Cytokines and chemokines secreted by cholangiocytes could recruit and activate immune cells such as T cells, macrophages, and natural killer cells to protect against biliary infection [21].

Antigen presentation

Human leukocyte antigen (HLA) classes I and II, human MHC molecules, are essential for antigen presentation to $CD8^+$ and $CD4^+$ T cells, respectively. In normal livers, HLA class I is expressed at a low frequency on cholangiocytes, while HLA class II molecules are not detected [22]. However, upon cytomegalovirus infection, HLA class I expression is significantly augmented, reflecting the role of $CD8^+$ T cells in viral responses [23]. PBC patients exhibit increased HLA class II expressions on injured cholangiocytes [24]. Despite the expression of HLA molecules on cholangiocytes in an inflammatory environment, costimulation is required for T cell activation. In vitro cultures of cholangiocytes in the presence of proinflammatory cytokines, such as IFN-γ and TNF-α increased the expression of HLA class II. However, the lack of up-regulation of CD80 or CD86 molecules (B7-1 and B7-2, respectively) resulted in the inability of these cholangiocytes to induce T cell responses in culture [25]. In contrast, B7-2 expression was found in damaged cholangiocytes of PBC and primary sclerosing cholangitis (PSC) patients [24, 26]. The expression of antigen-presenting-cell-related molecules on cholangiocytes suggests a capacity for antigen presentation, although limited by the requirement for the expression of costimulatory molecules. Similarly, the induction of apoptosis in infiltrating leukocytes is another method of controlling the inflammatory response in the biliary tract.

Apoptosis

There is controversial evidence concerning the expression of programmed-death (PD) ligands, other members of the B7 family, in cholangiocytes of diseased livers. PD molecules are expressed on leukocytes, which, when ligated, induce apoptosis in the leukocyte and may be another method of limiting the immune response [27, 28]. Ligation of TNF-related apoptosis-inducing ligand (TRAIL) receptors such as death receptors 4 and 5 (DR4, DR5) on target cells such as cancer and immune cells has also resulted in apoptosis [29, 30]. Although absent in normal conditions, the expression of TRAIL by diseased cholangiocytes in PBC and PSC may be an attempt by cholangiocytes to control the inflammatory responses in these diseases by targeting DR-expressing leukocytes [30]. Therefore, cholangiocytes are capable of antigen presentation in the appropriate environment, with the requirement of costimulatory molecules, as well as the expression of apoptosis-inducing molecules preventing unwarranted inflammatory responses.

The induction of apoptosis in cholangiocytes by leukocytes is also important for the clearance of pathogens and may play a role in the induction of cholangiocyte damage in biliary diseases. CD40, a member of the TNF receptor superfamily, is virtually undetected in cholangiocytes from normal livers but is up-regulated in inflammatory settings. It binds to the CD40L found on leukocytes such as T cells, B cells, and macrophages. In the case of PBC, infiltrating macrophages and T cells express the CD40L, with a more pronounced expression on macrophages [31]. In the same study, cultured cholangiocytes constitutively express CD40, which, upon ligation, increased expression of FasL and the transcription of NF-κB and activator protein 1. The ligation

of CD40 also led to a three- to fourfold increase in the apoptosis of cultured cholangiocytes, which is Fas-dependent. Thus, the binding of CD40 on cholangiocytes by inflammatory leukocytes may cause apoptosis, leading perhaps to the clearance of infectious agents. DR5 is constitutively expressed by cholangiocytes in normal conditions. It is significantly up-regulated in cholangiocytes of some biliary diseases, which may result in their apoptosis via ligation by TRAIL expressed by activated infiltrating leukocytes [30]. Thus, apoptosis is an important mechanism in the control of immune responses and may contribute to cholangiocyte-related diseases. In addition to the biliary ducts, periductal connective tissue that loosely surround bile duct walls containing nerve bundles, lymphatics, and blood vessels also play an important role in the biliary system [2].

Transport of secretory IgA

The transport of IgA to the bile duct lumen is a critical component of humoral immunity in the biliary tract and other mucosal surfaces. Bile contains approximately twice the concentration of secretory IgA (SIgA) compared to that found in upper intestinal fluid [32]. IgA found in bile is mainly in the form of SIgA, which is composed of two IgA molecules, a peptide J chain, and a secretory component [33]. The secretory component (SC), or polymeric immunoglobulin receptor, is mainly expressed on cholangiocytes composing the bile duct walls of auxiliary ducts off the main duct. These molecules are present on the luminal surface, perinuclear spaces, endoplasmic reticulum, and endocytic vesicles of chonlangiocytes, although faint expression can be found on the basolateral membrane [32]. Polymeric IgA produced by plasma cells binds to the SC on the basolateral side of cholangiocytes and is transported to the luminal surface, where the SC is cleaved and secreted along with the polymeric IgA [34]. SCs act as a protective component against proteolytic digestion of the IgA in the gastrointestinal tract, and its carbohydrate residues anchor the SIgA to mucus [33]. SIgA functions in a number of ways to protect the biliary tract. For example, it can directly bind and neutralize bacterial toxins. Either in a specific or a non-specific manner, SIgA can bind to bacteria and prevent their adhesion to the mucosal membrane [33]. Additionally, IgA has been demonstrated to neutralize intracellular microbes and their products during its transit through mucosal epithelium. Immune complexes of IgA and foreign antigen in the lamina propria may also be transported to the lumen via SCs, excreting pathogens to a proteolytic mucosal environment [35]. The immunological roles of cholangiocytes are summarized in Table 1.

Cholangiopathies

There are a number of cholangiopathies involving autoimmune, genetic, infectious, drug-induced and ischemic injury, malignant, and idiopathic etiologies [36, 37]. Autoimmune cholangiopathies include diseases such as PBC, PSC, autoimmune cholangitis, allograft rejection, and graft-versus-host disease (GVHD). Alagille syndrome, cystic fibrosis, and fibropolycystic diseases are primarily considered genetic cholangiopathies. Infectious cholangiopathies include bacterial, fungal, parasitic, and viral cholangitis. Floxuridine-induced cholangiopathy is an example of drug-induced cholangiopathy, while post-liver transplantation hepatic artery stenosis represents an ischemic-injury induced cholangiopathy. Other cholangiopathies include cholangiocarcinomas and those with unknown etiologies, such as biliary atresia, sarcoidosis, and idiopathic adult/childhood ductopenia.

Table 1 The immunological roles of cholangiocytes

Cholangiocyte expression	Function	Mechanism
TLRs 2, 3, 4 and 5	Recognize pathogens	
IRAK-M	Maintain tolerance	Negative regulator of TLR signaling
PD, TRAIL	Limit immune response	Induce apoptosis of leukocytes
IL-6, IL-8, and MCP-1	Chemotactic	Recruitment of immune cells to protect against infection
Defensins (hBD-1, hBD-2), Cathelicidin	Anti-microbial, chemotactic	Disrupt microbial membranes; recruit CD4+ T cells and immature dendritic cells
ICAM-1, LFA-3, VCAM-1	Cholangiocyte–leukocyte interaction	Leukocyte migration to inflammatory sites
HLA class II molecules	Antigen presentation	
CD80, CD86, CD40	Costimulation of T cells	

TLRs Toll-like receptors, *IRAK-M* interleukin-1 receptor-associated kinase M, *PD* programmed death, *TRAIL* TNF-related apoptosis-inducing ligand, *MCP-1* monocyte chemotactic protein-1, *ICAM-1* intercellular adhesion molecule 1, *LFA-3* lymphocyte-associated antigen 3, *VCAM-1* vascular cell adhesion molecule-1, *HLA* human leukocyte antigen

Autoimmune cholangiopathies

Autoimmune cholangiopathies are characterized by the involvement of autoreactive elements of the immune system in the destruction of the biliary tract. PBC is histologically identified by portal inflammation and leukocyte infiltration to intrahepatic bile ducts, eventually leading to their destruction. The destruction of intrahepatic bile ducts eventually leads to the accumulation of toxins, resulting in liver cirrhosis and failure [38–40]. A key diagnostic element of PBC is the presence of anti-mitochondrial antibodies against members of the 2-oxo acid dehydrogenase family, particularly the E2 subunit of the pyruvate dehydrogenase complex [41]. Other unique characteristics of PBC are the high levels of serum IgM and the high prevalence among women in their fifth decade of life [38]. The pathogenesis of PBC is currently unknown; however, the involvement of genetic factors, xenobiotics, and microbes has been suggested [41–45]. Recently, murine models of PBC have been identified, including those exhibiting regulatory T cell defects [46, 47]. The main treatment for PBC is the administration of UCDA, which is efficacious up to 10 years [38].

PSC is characterized by the chronic destruction of medium to large intrahepatic and extrahepatic bile ducts leading to eventual hepatic cirrhosis or failure [37]. Cholangiocarcinoma is developed in 10–30% of PSC patients [48]. The presence of lymphocytic infiltrations, the association with other autoimmune diseases such as inflammatory bowel disease, and the presence of autoantibodies suggest an autoimmune etiology. Unlike PBC, PSC is a male-dominant disease. Although serum anti-nuclear antibodies, anti-cardiolipin antibodies, anti-smooth-muscle antibodies, anti-thyroid peroxidase antibodies, and rheumatoid factor can be found in PSC patients, atypical perinuclear-staining, anti-neutrophil cytoplasmic antibodies are the most prevalent despite their presence in other autoimmune diseases [49]. However, there is disagreement as to the autoimmune nature of the disease due to the presence of bacterial and viral antigens and the induction of TLRs by anti-cholangiocyte antibodies [49].

Transplant complications such as liver allograft rejection and GVHD often result in cholangitis. Acute allograft rejection occurs between 5 and 30 days after liver transplantation and involves inflammation of portal or terminal hepatic veins and the destruction of bile ducts. Inflammatory infiltrates include mainly lymphocytes, but also neutrophils and eosinophils [50]. Chronic allograft rejection (CR) can occur as early as 2 weeks to 2 months after liver transplantation [51]. In CR, the loss of small bile ducts less than 60 μm in diameter results primarily from the senescence of these cholangiocytes. Interestingly, a compensatory proliferation of cholangiocytes resulting from injury is not observed [52]. CR also involves damage to the terminal hepatic venules, zone-three hepatocytes, portal tract hepatic arterioles, large perihilar hepatic artery branches, and large perihilar bile ducts [51].

Hematopoietic stem cell transplantation (HSCT) can often result in GVHD with the targeted destruction of small bile duct epithelium [53]. Although most cases of HSCT-induced GVHD-cholangitis (L-GVHD) result from allogeneic donors, it has been observed in patients receiving autologous and syngeneic HSCT [53, 54]. Acute GVHD usually occurs within 1 month of transplantation and involves the skin, gastrointestinal tract, and liver [50]. In the liver, destruction of interlobular bile ducts is the primary observation. On the other hand, chronic GVHD that occurs 80–100 days after transplantation is characterized by symptoms similar to those found in autoimmune connective tissue disorders, including Sjögren's syndrome, scleroderma, and cholestatic liver disease. Liver pathology resembles that of acute L-GVHD. T cells are the predominant effector cells inducing apoptosis via perforin and granzyme. Similar to CR, cholangiocyte regeneration is not as frequently observed as in PBC and PSC. L-GVHD eventually leads to bile duct loss and rarely to fibrosis. One major difference between CR and L-GVHD is the lack of damage to the arterial system in the liver [50]. Although genetic factors may play a role in many of these cholangiopathies, direct genetic involvement has been identified in a number of diseases involving the biliary tract.

Genetic cholangiopathies

Alagille syndrome (AGS) involves mutations in genes of the Notch pathway, in particular, *Jagged1* (JAG1) or NOTCH2. Clinical features of AGS include cholestasis, cardiac disease, ocular abnormality, skeletal abnormality, and characteristic facial features [55]. The paucity of intrahepatic bile ducts is the most prominent feature of AGS and is due to defects in the development of the biliary tree, not bile duct destruction [56]. Similar to HSCT GVHD and liver allograft rejection, cholangiocyte reactivity or proliferation in response to damage is lacking in AGS. This may be due to a defect in hepatobiliary cells in their capacity to differentiate into biliary progenitors [56].

In cystic fibrosis (CF), mutations in the CFTR gene result in multi-organ diseases. Although lung disease is primarily responsible for morbidity and mortality in CF, liver disease is diagnosed in approximately one third of all CF patients, with a potential for underreporting due to diagnostic difficulty [57]. The CFTR molecule is critical for the secretion of bicarbonate by cholangiocytes being present on the apical surfaces of biliary epithelium and gallbladder epithelium, while it is absent on hepatocytes.

The dysregulation of biliary secretions results in the production of bile with increased viscosity, which slowly progresses to plugged bile ducts, periportal fibrosis, and multilobular cirrhosis [37]. UCDA treatment has been demonstrated to improve liver biochemistry, liver histology, hepatic excretory function, biliary drainage, and essential fatty acid status [57]. Another group of genetically induced cholangitic diseases is the fibropolycystic disease family. The disease family includes Caroli's disease, congenital hepatic fibrosis, Von Meyenburg complex, autosomal dominant polycystic kidney disease, autosomal recessive polycystic kidney disease, and mesenchymal hamartoma [58]. The common characteristics of these diseases are the dilation of intrahepatic bile ducts and fibrosis. Fibropolycystic diseases have also been described as examples of ductal plate malformation, or the lack of remodeling by ductal plates into tubular bile ducts. For example, Caroli's disease affects the formation of larger bile ducts while the Von Meyenburg complex involves interlobular bile ducts [59]. Another category of biliary diseases is infectious cholangiopathies.

Infectious cholangiopathies

Fungal infections have been associated with sclerosing cholangitis-like symptoms. For example, infection by *Cryptococcus neoformans*, which can be found in pigeon excrement and soil, resulted in dilation of bilateral intrahepatic bile ducts, mass-like lesions in portal tracts resembling cholangiocellular carcinoma, and biliary obstruction [60]. The presence of *C. neoformans* was found in bile cultures and demonstrated in biopsies in areas of granulomatous inflammation and multinucleated giants cells. Candida infections can lead to bead-like deformation of the intra- and extrahepatic bile duct system, resembling secondary sclerosing cholangitis [61, 62]. Bacterial infections of the biliary tract can result in biliary obstruction, which may increase ductular pressures, causing the release biliary bacterial contents systemically [63]. Bacteria in the biliary tract can deconjugate bile acids and bilirubin, as well as hydrolyze phospholipids, resulting in epithelial damage and stone/sludge formation.

The biliary tract is also subject to parasitic infections by organisms such as liver flukes and roundworms. Examples of liver flukes include *Clonorchis sinensis* and *Opisthorchis viverrini*, while roundworms such as *Ascaris lumbricoides* can enter the biliary system via the ampulla of Vater [64]. Fluke infections begin with the ingestion of encysted larva, which are released in the stomach, penetrate to the peritoneal cavity, and enter the liver. Once in the liver, these flukes tunnel through the liver parenchyma until they reach the lumen of bile ducts, where they reside for years, feeding on bile. As they mature, liver flukes move from small bile ducts to larger ducts and the gallbladder, causing chronic inflammation resulting in thickened bile duct and gallbladder walls. *Ascaris lumbricoides* infections result in intrahepatic stones, recurrent pyogenic cholangitis, cirrhosis, cholelithiasis, pancreatitis, and cholangiocarcinoma [64].

Drug and ischemic-injury induced cholangiopathy

Drug-induced cholangiopathies often result in cholestasis. These cholestatic diseases may be divided into hepatocellular cholestasis and ductular/ductal cholestasis, as reviewed previously [65]. Hepatocellular cholestasis refers to dysfunctions in hepatocyte canalicular bile secretion, while ductular/ductal cholestasis results from the obstruction of bile ducts. In hepatocellular cholestasis, canaliculi dilation and the appearance of cytoplasmic granules in hepatocytes may be observed. Additionally, cholestatic hepatitis, a more severe manifestation of hapatocellular cholestatis, hepatocyte necrosis, inflammatory infiltration by mononuclear, polymorphonuclear, and eosinophils can occur. Ductular/ductal cholestasis usually results in inflammatory infiltration of ducts, edema, and cholangiocyte abnormalities. Treatments involving sex hormones, anabolic steroids, and sex hormone antagonists may result in hepatocellular cholestasis without liver necrosis. Drugs such as phenothiazines, anticonvulsants, anti-microbial agents, antirheumatic drugs, anti-thyroid drugs, cardiovascular drugs, anti-cancer drugs, immunosuppressants, and anti-depressants may cause cholestatic hepatitis. Examples of drugs causing ductular/ductal cholestasis include azathioprine (immunosuppressant), barbiturates, clindamycin (antibiotic), phenytoin (antiepileptic), and sulpiride (antipsychotic). Patients undergoing metastatic liver disease that have been treated with hepatic arterial infusion chemotherapy using floxuridine (FUDR) may develop sclerosing cholangitis and intrahepatic or extrahepatic strictures, potentially leading to biliary cirrhosis [66]. The effects of FUDR may be directly due to drug toxicity or via ischemic mechanisms caused by damage to common bile duct arterioles [65]. Post-liver transplantation hepatic artery stenosis can also cause bile duct strictures due to bile duct infection, viral infection, ischemic injury, persistent rejection, and blood group incompatibility [67].

Cholangiopathies with unknown etiologies

Several cholangiopathies present enigmatic etiologies, such as biliary atresia, sarcoidosis, and idiopathic adult ductopenia. Biliary atresia is a neotatal disease characterized by the inflammation and obstruction of the extrahepatic and intrahepatic bile ducts. Chronic biliary inflammation results in bile duct damage and loss and liver fibrosis [37]. Eighty

percent of biliary atresia cases in the Western countries are "acquired" after birth, while the remaining cases exhibit the "embryonic" form, which usually is presented with other congenital anomalies [68]. Intrahepatic bile ducts are infiltrated by lymphocytes, macrophages, and eosinophils. Macrophages compose a major fraction of leukocytic infiltrates, and their frequency correlates with poorer prognosis. Several hypotheses have been proposed as to the etiology of the disease, including viral infection, autoimmunity, and genetic mutation (particularly with the embryonic form). Further interdisciplinary efforts would be required to reveal the etiology of this disease in order to provide effective treatments. Similarly, the pathogenesis of sarcoidosis is unknown despite its recognition over a century ago [69]. Sarcoidosis is a systemic granulomatous disease, which affects all organs, with the lungs being the most frequently afflicted. A recent study demonstrated over 20% of 1,436 patients with sarcoidosis exhibited abnormal liver tests [70]. Of the available biopsy samples, 85% exhibited portal inflammation, 50% showed bile duct depletion, and 26% had signs of cirrhosis. Idiopathic adult ductopenia is a rare disease characterized by the absence of interlobular bile ducts in over 50% of portal tracts, adult onset, normal cholangiograms, a lack of anti-mitochondrial antibodies, and the absence of inflammatory bowel disease [71]. There is a 2:1 male-to-female ratio and the prognosis is variable. Genetic factors have been investigated due to evidence of familial clustering. Liver transplantation, immunosuppression, and UCDA have been successful in various patients; however, significant investigation would be required to identify actual mechanisms of this enigmatic disease [70].

Dysfunctions of the biliary system result in a number of devastating diseases. Unfortunately, a large number of etiologies in these diseases remain unclear. Further study of the functions and mechanisms of the biliary system under both normal and disease states will be required for the elucidation of the pathogenesis of these diseases, resulting in improved treatment development strategies.

Conclusion

The biliary system is a complex network of tissues/organs, as well as intestinal flora, coordinating functions such as digestion, detoxification, immunological defense, and elimination of waste [72]. Disruptions in this delicately balanced system may result in a range of diseases. Therefore, careful and thorough investigations considering the entire biliary system may eventually shed light on many enigmatic cholangiopathies. Although useful, animal models should be carefully considered due to differences in the composition of the biliary tract. Further study of the heterogeneity of the biliary epithelium is also important due to the specific targeting by various diseases.

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