the G1 phase of the cell cycle [20]. The expression of senescence-related markers is increased in BECs during early chronic rejection in chronic liver allograft and PBC. Cellular senescence of BECs is involved in impaired regeneration and eventual and progressive bile duct loss in PBC and ductopenic chronic rejection [21, 22]. A relatively insufficient proliferative response of BECs due to cellular senescence (see below) is also responsible for the progressive loss of bile ducts due to apoptosis.

Senescence-Associated Secretory Phenotypes. Accumulating evidence suggests that senescent cells remain metabolically active and play an important role in modulating the microenvironment around them by secreting cytokines, chemokines, growth factors, and profibrogenic factors [19]. For example, senescent BECs of PBC expressing CCL2 and CX3CL1 may be involved in the recruitment of monocytes and possibly T lymphocytes into portal tracts, around injured and senescent BECs, and thereby responsible for the development of immune-mediated cholangitis such as PBC [19, 23].

3.1.3. Biliary Epithelial Cell Renewal. The homeostasis of physiological and pathological biliary epithelia operates through a balance between cell loss and cell renewal. Cell loss in the biliary epithelium is mainly due to apoptosis or senescence and mostly regulated by the *bcl-2* family of proteins or senescence-associated factors such as *p16* and *p21*. The biliary epithelial cells of bile ductules or small bile ducts may be replenished by bile ductular cells or hepatic progenitor cells in the canal of Hering, though such processes may be unlikely in the intrahepatic large bile ducts. As mentioned, the peribiliary glands themselves or progenitor cells located in these glands may be involved in renewal of the biliary epithelium of intrahepatic large bile ducts and extrahepatic bile ducts and also proliferation of the epithelia lining these bile ducts [7, 8].

3.1.4. Biliary Epithelial Hyperplasia. Inhibition of the apoptotic or senescent process in the biliary epithelia may cause hyperplasia with an increased risk of neoplastic transformation [24]. Hyperplasia of lining epithelia of the septal and large bile ducts manifests as micropapillary projections or as a stratification of the epithelium with or without dilatation of the duct lumen. Peribiliary glands, intramural or extramural, also show hyperplasia and proliferation and participate in the secretion of neutral, carboxylated, and sulphated mucins into the bile duct lumen. When prominent, in particular with *Clonorchis sinensis* infections or hepatolithiasis, the term adenomatous hyperplasia or chronic proliferative cholangitis has been used. As for the proliferation and hyperplasia of bile ductules and small interlobular bile ducts, they appear tourtous and increase in their number in the portal tracts. Some of these lesions are included in the so-called ductular reactions [25].

3.1.5. Metaplasia. Several kinds of metaplasia are reported in the biliary epithelium of the intra- and extrahepatic biliary tree, usually in cases of chronic biliary diseases such as hepatolithiasis, parasitic cholangitis, and primary sclerosing cholangitis (PSC). *Gastrointestinal metaplasia* resembling pyloric glands and goblet cells is not infrequently seen in chronically inflamed large bile ducts and peribiliary glands. This change is associated with the aberrant expression of gastric type mucus core protein (MUC) 5AC and MUC6 and also intestinal type MUC2. Such gastric and intestinal mucin is involved in lithogenesis in hepatolithiasis [9]. The so-called intramural glands with a gastric pyloric gland-like appearance are increased in long-standing biliary diseases and may reflect invagination of the biliary epithelium with gastrointestinal metaplasia. *Goblet cells* are occasionally encountered among bile duct-lining cells and also in peribiliary glands. *Intestinal metaplasia* with Paneth cells may also be encountered in the peribiliary glands. The expression of other molecules in intrahepatic large bile ducts, such as REG I and trefoil factors, appears to be related to intestinal or gastric metaplasia. While *pancreatic acinar metaplasia* is also reported infrequently in PSC, its differentiation from heterotopic pancreatic acini is controversial. *Hepatocytic metaplasia* occurs in interlobular bile ducts and bile ductules in various pathological situations but remains of unknown significance. *Squamous metaplasia* is rarely encountered in long-standing inflammation of large bile ducts such as PSC or in the lining of biliary cysts.

3.1.6. Biliary Intraepithelial Neoplasm (BilIN). Chronic biliary diseases such as hepatolithiasis and PSC are occasionally complicated by cholangiocarcinoma. In such cases, dysplastic or early neoplastic lesions are known to precede the invasive cholangiocarcinoma. Such biliary epithelial lesions are known as dysplasia or atypical hyperplasia of the biliary epithelium and characterized by atypical, enlarged, and hyperchromatic nuclei, an increased nucleocytoplasmic ratio, and a loss of polarity [5, 26]. Usually either micropapillary or flat lesions affect a portion or the circumference of the bile duct. These lesions were proposed to be called biliary intraepithelial neoplasm (BilIN), and this terminology was recently adopted by WHO [26]. They are divided into three grades according to cellular and structural atypia; BilIN-1, BilIN -2, and BilIN -3. In BilIn-1, cellular/nuclear atypia are mild or moderate but not enough for overt malignancy, and cellular polarity is minimally disturbed and corresponding to low-grade dysplasia. In BilIN-2, cellular/nuclear atypia are evident but not marked enough for overt malignancy, and the disturbance of cellular polarity is mild or focal, corresponding to high-grade dysplasia. BlIN-3 shows cellular/nuclear atypia corresponding to overt malignancy, and cellular polarity is diffusely disturbed, corresponding to a so-called carcinoma in situ of the biliary tract [26]. BilIN-1, BilIN -2, and BilIN -3 are seen in both large intrahepatic and extrahepatic bile ducts, peribiliary glands, and gallbladder and considered to reflect a multistep neoplastic transformation of the biliary epithelium.

3.2. Basic Pathology of Bile Duct Damage. In the biliary tree, there are several types of bile duct damage such as cholangiopathies and cholangitis. Representative pathological features of the biliary tree are as follows.

3.2.1. Cholangitis and Its Classification. Cholangitis is characterized by biliary epithelial damage with inflammatory cell infiltration. Some cholangitis is also associated with ductal and periductal fibrosis. It occurs along the biliary tree, and the term cholangitis is used for inflammatory damage to bile ductules.

Cholangitis can be histologically classified into suppurative and nonsuppurative forms. *Suppurative cholangitis* implies the presence of numerous polymorphonuclear cells around and within the wall as well as within the lumen of the ducts. This may involve ducts of any size and is occasionally associated with abscess formation—cholangitic abscess. A microbial infection is often responsible, but the change also occurs in the presence of sterile bile, particularly after bile extravasation. The release of chemokines or cytokines is the likely cause in some cases.

"*Nonsuppurative cholangitis*" includes a spectrum of bile duct inflammation which may be granulomatous cholangitis, lymphoid cholangitis, fibrous cholangitis, and pleomorphic cholangitis according to the predominant type of inflammatory reaction present [27]. *Granulomatous cholangitis* almost always seems to be destructive. This type involving the interlobular bile ducts constitutes the hallmark of PBC and is also found in drug-induced liver disease and sarcoidosis. The other types can be either destructive or nondestructive. *Lymphoid cholangitis* refers to a close association between duct branches, usually interlobular bile ducts, and lymphocytic aggregates, which may show a follicular arrangement. This is found in PBC and PSC with concomitant bile duct destruction or in nonbiliary disorders, in particular autoimmune and viral hepatitis C. *Pleomorphic cholangitis* is associated with inflammatory cell infiltration. All other types of cholangitis are found in CAH, PBC, PSC, and other liver diseases. *Fibrous cholangitis (also called sclerosing cholangitis)* with evident ductal fibrosis develops as a consequence of long-standing bile duct inflammatory, obstruction, or ischemic injury; it can be obliterative or nonobliterative. BECs show variable damage. The former is characteristic of PSC, though, in our experience, it may be seen in acquired forms of sclerosing cholangitis too. BECs of obliterative type are actually lost in fibrous lesions, appearing as a fibrous core. Sclerosing cholangitis with bile duct obliteration suggests a diagnosis of PSC in adults.

3.2.2. Bile Duct Sclerosis. In long-standing sclerosing cholangitis and also in other biliary diseases such as ischemic cholangitis, the bile duct wall shows a marked deposition of collagen fiber (bile duct sclerosis). The affected bile ducts in sclerosing cholangitis show a marked increase in the number of c-kit receptor-expressing mast cells which secrete fibrogenic factors such as histamine, basic fibroblast growth factor (bFGF), and/or tumour necrosis factor-alpha (TNF-α). The biliary epithelium itself produces and secretes fibrogenic substances such as bFGF, transforming growth factor-beta (TGF-β), and platelet-derived growth factor (PDGF), as well as basement membrane proteins and extracellular matrix proteins. In biliary atresia, BECs of the affected bile ducts variably express mesenchymal markers such as vimentin and might have acquired phenotypes of mesenchymal cells, though distinct morphological epithelial mesenchymal transition (EMT) of biliary epithelium is hardly recognizable [28, 29]. In all forms of bile duct sclerosis, a marked attenuation of the peribiliary vascular plexus is seen within the sclerotic duct wall, but it remains unknown whether these changes are secondary to, or responsible for, the bile duct fibrosis.

3.2.3. Bile Duct Loss or Ductopenia. The balance of cell death or dropout due to apoptosis or necrosis and the regeneration of lining biliary epithelia is important for the maintenance of bile ducts, and apoptotic activity that exceeds the proliferative response of bile duct cells results in progressive ductopenia. Ductopenia is defined as a loss of bile ducts from the portal tract in which hepatic arterial branches and bile ducts of similar size run parallel. Thus, portal tracts without evident bile ducts indicate a loss of bile ducts. Immunostaining of biliary cytokeratins such as CK7 and CK19 is helpful for the recognition of bile ducts. Ductopenia is usually defined as the absence of interlobular bile ducts in at least 50% of portal tracts. Extensive ductopenia is usually associated with chronic cholestasis and biliary fibrosis. Ductopenia is typically found during chronic liver allograft rejection with chronic cholestasis and also the advanced stages of PBC.

3.2.4. Mucobilia and Hemobilia. Mucin is impacted in the duct lumen and this is occasionally marked, leading to leakage and extravasation with the formation of mucus lakes. Drainage of mucin from Papilla of Vater is also a clinical manifestation of mucobilia, as seen in intraductal papillary mucinous neoplasms of the pancreas. Mucobilia is usually found in the neoplastic bile ducts and nonneoplastic bile ducts of "intraductal papillary neoplasms of the bile duct" (formerly known as "biliary papillomatosis") or mucin-producing bile duct tumors [26, 30]. When such changes are encountered in nonneoplastic biliary diseases such as PSC and hepatolithiasis, usually microscopic neoplastic biliary lesions are found in the affected bile ducts.

In cases of hemobilia, impacted erythrocytes are encountered in bile duct lumens. Recent endoscopic or surgical biliary manipulations in association with a primary or secondary malignancy may be underlining diseases for hemobilia.

3.2.5. Ductular Reaction. To date, many pathological terms such as oval cell proliferation, intermediate cells, and atypical bile ductular proliferation have been used to describe the "increased ductule-like cells or clusters of small epithelial cells different from mature hepatocytes" in the portal tract or the periportal area. This is a reaction of the ductular phenotype, possibly but not necessarily of ductular origin, commonly seen in many kinds of acute and chronic

Table 1: Etiologic classification of cholangiopathy.

(1) Immune-mediated cholangiopathy
(2) Infectious cholangiopathy
(3) Genetic cholangiopathy
(4) Ischemic cholangiopathy
(5) Drug- or toxin-induced cholangiopathy

hepatobiliary diseases. Recently, an international working group proposed the term "ductular reaction" for this lesion [25]. "Ductular reaction" implies a reaction of ductular phenotype, possibly but not necessarily of ductular origin. The epithelial component of a ductular reaction may actually derive from several sources: not only from the proximal branches of the biliary tree but also from the circulation (often if not always from bone marrow) and from biliary metaplasia of hepatocytes. "Reaction" encompasses the complex of stroma, inflammatory cells, and other structures of diverse systems, all of which participate in the reactive lesion. Bile ductular reaction is usually characterized by increased numbers in the periportal and portal areas and a common and frequent process in a number of hepatobiliary diseases.

A ductular reaction itself is heterogeneous in its development and has many meanings. There are several reports that bile ductules are very reactive anatomical elements in the liver, and proliferated bile ductules are involved in the progression of various chronic liver diseases. Our recent studies showed that bile ductular cells in PBC, PSC, and also NAFLD may undergo cellular senescence, and these cells could produce and secrete biologically active molecules and thereby be involved in hepatic fibrogenesis and other pathologic features of the liver.

3.2.6. Ductal Plate Malformation. Ductal plate malformations (DPMs), which are different from reactive changes of bile ducts or ductules, develop as a result of a remodeling failure of the ductal plate followed by the development of intrahepatic bile ducts. DPMs are characterized by increased numbers of abnormal bile duct-like structures and show a bridge-like structure in the dilated lumen and bulbar protrusion of biliary epithelia [31]. DPMs are observed in congenital hepatic fibrosis and Caroli's disease, biliary atresia, and other fibropolycystic liver diseases.

4. Cholangiopathies

Diseases that mainly target the biliary tree (cholangiopathies) can be divided into several categories according to the pathogenetic mechanism involved (Table 1). However, in many cholangiopathies, more than one pathogenetic mechanism is operative.

4.1. Immune-Mediated Cholangiopathies. The biliary tree could be affected by immunological assaults, and lymphoplasmacytic infiltration is evident around the damaged bile ducts. Primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) are representative immune-mediated cholangiopathies [32]. Autoimmune pathogenesis is operative in PBC and PSC. There is a mixture of immunocompetent cells in the affected bile ducts, and CD3+, CD4+, and CD8+ T cells that bear the T-cell receptor α/β are predominant in PBC, supporting that Th1 immune response-predominant cytotoxicity and/or cytokine release are involved in the pathogenesis of the bile duct lesions of PBC. HLA-class II antigens are aberrantly expressed in the affected bile ducts of PBC, PSC, and chronic allograft rejection [33]. Biliary innate immunity is also involved in the pathogenesis of cholangiopathies in patients with PBC and biliary atresia (BA) [9]. BECs possess an innate immune system consisting of the Toll-like receptor (TLR) family and recognize pathogen-associated molecular patterns (PAMPs). In PBC, CD4-positive Th17 cells characterized by the secretion of IL-17 are implicated in the chronic inflammation of bile ducts, and the presence of Th17 cells around bile ducts is causally associated with the biliary innate immune responses to PAMPs. In BA characterized by a progressive, inflammatory, and sclerosing cholangiopathy, dsRNA viruses are speculated to be an etiological agent and to directly induce enhanced biliary apoptosis via the expression of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). Moreover, the epithelial-mesenchymal transition (EMT) of biliary epithelial cells is also evoked by the biliary innate immune response to dsRNA. In addition, intrahepatic small bile ducts and bile ductules are a main target in graft-versus-host disease and also hepatic allograft rejection. Recent studies showed that IgG4-related sclerosing cholangitis is also associated with altered immunity. Upregulation of regulatory T cells (Tregs) associated with Th2 predominance is reportedly important in the pathogenesis of IgG4-related sclerosing cholangitis [3]. The anatomical level of the biliary tree affected is different among these immune-mediated cholangiopathies. Interestingly, the peribiliary glands are also involved in PSC, graft-versus-host disease, and IgG4-related sclerosing cholangitis.

4.2. Infectious Cholangiopathies. The biliary tree is affected by several types of infectious diseases, such as bacterial, fungal, protozoan, parasitic, and viral cholangitis. Stagnation of bile due to biliary stenosis or obliteration is followed by bacterial cholangitis, frequently with sepsis or abscess formation. Parasitic infections are also reported in the biliary tract including the liver, and liver flukes such as *Clonorchis sinensis* and *Opisthorchis viverrini* are endemic in East Asia, particularly northern Thailand and some parts of Korea, and cholangiocarcinoma is a serious complication of parasitic cholangitis [26]. Hepatolithiasis is predominantly a disease of the Far East and is causally also related to infectious cholangitis, especially bacterial cholangitis [27, 34]. Mucin plays an important role in the development of hepatoliths, which are formed within the intrahepatic large bile ducts. Clinically, patients may present acutely with recurrent bacterial cholangitis and its possible complications, such as liver abscesses and septicemic shock, or with chronic complications, such as cholangiocarcinomas

and intraductal papillary neoplasms [35]. Pathologically, it is characterized by pigmented calcium bilirubinate stones within dilated intrahepatic bile ducts featuring chronic inflammation, mural fibrosis, and proliferation of peribiliary glands, without extrahepatic biliary obstruction. A transient viral infection such as type A rhesus rotavirus and type 3 reovirus is reported as an initiating mechanism of biliary atresia (BA), particularly perinatal type.

4.3. Genetic Cholangiopathies. Genetic alterations affecting the biliary tree manifest as biliary dilatation, bile duct paucity, obstruction, proliferation, stone formation, and so on. Caroli's disease with congenital hepatic fibrosis (CHF) is a representative genetic cholangiopathy. Some cases of biliary atresia (BA) also belong to this category. The former is characterized by multiple saccular dilatations of the intrahepatic bile ducts. Caroli's disease with CHF belongs to autosomal recessive polycystic kidney disease (ARPKD) with ductal plate malformation characterized by a disordered remodeling of the intrahepatic biliary tree [28]. Disordered cell kinetics, including the apoptosis of biliary epithelial cells (BECs), may be significantly related to ductal plate malformation, and laminin and type IV collagen levels were reduced in the basement membrane of intrahepatic bile ducts of ARPKD; such a reduction is an additional factor for the dilatation of bile ducts. Paucity of the intrahepatic bile ducts is a genetic cholangiopathy [36]. For example, Alagille syndrome with a mutation in a ligand for the Notch protein is characterized by paucity of intrahepatic bile ducts and other anomalies. Cystic fibrosis (CF) due to a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) is associated with focal biliary fibrosis, and the bile duct and ductules are filled with pink and amorphous secretions. Low phospholipid-associated cholelithiasis (LPAC) is characterized by a low biliary phospholipid concentration with symptomatic and recurring cholelithiasis, and LPAC syndrome is associated with mutations of the adenosine triphosphate-binding cassette, subfamily B, member 4 (ABCB4) gene encoding the hepatobiliary phospholipid translocator multidrug resistance protein 3 [37]. This causes recurrent cholelithiasis, continuous irritations of the biliary tract with cholangitis, chronic cholestasis, and even biliary cirrhosis.

4.4. Ischemic Cholangiopathies. Ischemic cholangiopathy is defined as focal or extensive damage to bile ducts due to an impaired blood supply [11]. Most causes of bile duct ischemia are iatrogenic, though some systemic vascular diseases also cause this type of cholangiopathy. This entity may be observed in various circumstances and is of clinical importance for practitioners involved in gastroenterology, oncology, abdominal surgery, and liver transplantation. Ischemic bile duct injury may occur when small hepatic arteries or the peribiliary vascular plexus are injured or when all possible sources of arterial blood supply are interrupted. Ischemic biliary injury may take the form of bile duct necrosis, bile leakage, biloma, bile duct fibrosis, or stenosis. Bile duct necrosis and bilomas develop predominantly where there is an abrupt and complete interruption of the arterial blood supply, for example, when HA thrombose in a liver transplant recipient. On the contrary, fibrous stenoses develop where there is progressive injury to the hepatic arterioles, for example, after several courses of intra-arterial chemotherapy. Cholangiographic findings include diffuse and multiple bile ducts lesions. Ischemic cholangiopathy is a serious complication during liver transplantation [4]. When biliary drainage or reconstruction is not possible or has failed, liver transplantation is the only potential cure.

4.5. Drug- or Toxin-Induced Cholangiopathies. Bile ducts, particularly interlobular bile ducts, are occasionally affected by drug-induced hepatobiliary damage, various bile duct injuries, various types of cholangitis, and bile duct loss (drug-induced cholangiopathy) [38]. This type of cholangitis is not infrequently associated with cholestasis. Some cases presenting with progressive ductopenia and cholangitis and prolonged cholestasis mimic PBC and also PSC. While the mechanism of drug-induced cholangitis remains speculative, immune-mediated processes including hypersensitivity may be operative. Some forms of drug-induced cholangiopathy develop after hepatic arterial infusion of floxuridine (FUDR) (floxuridine- (FUDR-) induced cholangiopathy). Ischemic changes to the peribiliary vascular plexus may be at least partly involved in this type of cholangiopathy. Although BECs have low metabolic activity compared with hepatocytes, cytotoxic or cytopathic bile duct injury has been produced experimentally or accidentally by toxic substances such as α-naphthylisothiocyanate, 4,4′-diaminodiphenylmethane, and paraquat (toxin-induced cholangiopathy) [18].

In conclusion, the anatomy and physiology of the biliary tree, basic injuries to biliary epithelial cells, basic forms of bile duct damage, and etiological classification of cholangiopathy were reviewed. This tutorial review will be helpful for better understanding cholangiopathies.

Conflict of Interests

The authors have no conflict of interests to declare.

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Review Article

Cholangiopathy with Respect to Biliary Innate Immunity

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Biliary innate immunity is involved in the pathogenesis of cholangiopathies in cases of biliary disease. Cholangiocytes possess Toll-like receptors (TLRs) which recognize pathogen-associated molecular patterns (PAMPs) and play a pivotal role in the innate immune response. Tolerance to bacterial PAMPs such as lipopolysaccharides is also important to maintain homeostasis in the biliary tree, but tolerance to double-stranded RNA (dsRNA) is not found. Moreover, in primary biliary cirrhosis (PBC) and biliary atresia, biliary innate immunity is closely associated with the dysregulation of the periductal cytokine milieu and the induction of biliary apoptosis and epithelial-mesenchymal transition (EMT), forming in disease-specific cholangiopathy. Biliary innate immunity is associated with the pathogenesis of various cholangiopathies in biliary diseases as well as biliary defense systems.

1. Introduction

Primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), and hepatolithiasis in adults and biliary atresia and choledochal cyst in infants are biliary diseases in which different anatomical levels of the biliary tree are specifically affected and characterized by cholangiopathy. The biliary tree, consisting of cholangiocytes, is a system of connecting ducts that drain the bile secreted by hepatocytes into the duodenum. Cholangiocytes provide the first line of defense in the biliary system against luminal microbes originating from the intestines via portal blood and duodenum [1]. In general, although human bile is normally sterile, it can contain bacterial components such as lipopolysaccharide (LPS), lipoteichoic acid, and bacterial DNA fragments, known as pathogen-associated molecular patterns (PAMPs) [2–5], and cultivable bacteria are detectable in bile of patients with biliary diseases [1, 6–8]. Enteric bacteria, in particular, may be responsible for the chronic proliferative cholangitis associated with hepatolithiasis [1, 6]. These findings indicate that cholangiocytes are exposed to bacterial PAMPs under physiological as well as pathological conditions.

Innate immunity was initially thought to be limited to immunocompetent cells such as dendritic cells and macrophages, but epithelial cells also possess TLRs and proper innate immune systems reflecting the specific micro-environment and function of each epithelial cell type. Recent studies concerning biliary innate immunity indicate that cholangiocytes express a variety of pathogen-recognition receptors such as Toll-like receptors (TLRs) [9, 10]. Infectious agents have been implicated in the etiology or progression of cholangiopathies including cholangitis, bile duct loss, and lithiasis as a trigger or aggravating factor. Notably, several enterobacteria and viruses are speculated to be primary or secondary factors for PBC, PSC, biliary atresia, hepatolithiasis, and chronic cholecystitis [2, 3, 11–15] (Table 1). Moreover, no microorganisms showing cholangiocyte-specific tropism have been identified, suggesting that an innate immune response specific to cholangiocytes rather than PAMPs is important in the pathogenesis of cho-lan-gi-op-a-thy. This review summarizes our current understanding of the biliary innate immune system against microbial infections including the various mechanisms employed by negative regulators and their associations with the pathogenesis of cholangiopathy in biliary diseases.

Table 1: Bacteria and viruses speculated to be etiologic factors in biliary diseases.

Primary biliary cirrhosis
Lipopolysaccharide
Lipoteichoic acid
Helicobacter
β-retrovirus
Propionibacterium acnes
Escherichia coli
Mycobacterium
Novosphingobium
Lactobacillus
Chlamydia
Biliary atresia
Reovirus
Rotavirus
Cytomegalovirus
Adenovirus
Enterovirus
Ebstein-Barr virus
Primary sclerosing cholangitis
Helicobacter
α-hemolytic streptococcus
Hepatolithiasis
Escherichia coli
Klebsiella
Streptococcus
Pseudomonas
Bacteroides
Clostridium
Campylobacter

2. Molecular Mechanisms of Biliary Innate Immunity

2.1. Expression of PAMP-Recognizing Receptors and Intracellular Adaptor Molecules. The TLR family are the best characterized cell surface receptors recognizing PAMPs, and 10 members (TLR1-10) have been identified in humans [16, 17]. The response to LPS is mediated by interaction with TLR4 in conjunction with the TLR4 accessory proteins MD-2 and CD14, triggering the transduction of intracellular signals followed by the activation of TLR-associated adapter proteins, myeloid differentiation factor 88 (MyD88), and IL-1 receptor-associated kinase- (IRAK-)1, leading to the activation of nuclear factor-κB (NF-κB) and then to the synthesis of antibiotics and proinflammatory cytokines. In contrast to bacterial PAMPs, dsRNA including viruses are recognized by TLR3, IFN-inducible helicase retinoic acid-induced protein I (RIG-I), and melanoma differentiation-associated gene-5 (MDA-5). The stimulation of these receptors by dsRNA transduces signals to activate transcription factor interferon regulatory factor 3 (IRF3) as well as NF-κB.

Table 2: Expression of Toll-like receptors in cultured human biliary epithelial cells (BECs), cholangiocarcinoma, and murine BECs.

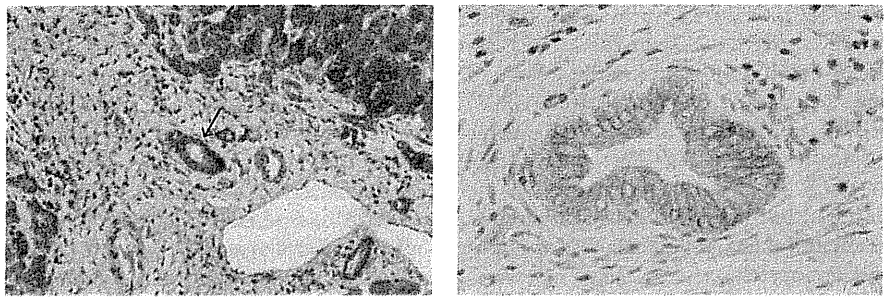
	Human		Murine
	BECs	Cholangiocarcinoma	BECs
TLR1	+ [19]		
TLR2	+ [19, 20]	+ [2]	+ [2]
TLR3	+ [19, 20]	+ [2]	+ [2]
TLR4	+ [19–21]	+ [2]	+ [2]
TLR5	+ [19, 20]	+ [2]	+ [2]
TLR6	+ [19, 20]		
TLR7	+ [19] / −*		
TLR8	+ [19] / −*		
TLR9	+ [19] / −*		
TLR10	+ [19]		

Blanks: no reports. *Our unpublished data. Parentheses denote reference numbers.

NODs (i.e., NOD1 and NOD2) are also involved in the intracellular recognition of microbes through specific interactions with derivatives of pathogen-specific peptidoglycans [18].

The expression of TLRs in human and murine cholangiocytes and several human cholangiocarcinoma cell lines has been confirmed by several groups (Table 2), implicating the possible activation of biliary mucosal immunity against microbial infections [2, 19–23]. Cultured human and murine biliary epithelial cells (BECs) possess at least TLR1-TLR5, related molecules (MD-2, MyD88, and IRAK-1), RIG-I, and MDA-5 [2, 20, 23, 24]. Moreover, SV40-transformed human cholangiocytes expressed mRNAs for all ten human TLRs [19]. Immunohistochemistry has confirmed that intracellular adaptor molecules (MyD88 and IRAK-1) as well as TLRs (TLR1-TLR5) are diffusely distributed in the intrahepatic biliary tree of normal and diseased human livers, irrespective of anatomical level (Figure 1) [2, 20–22, 24, 25]. As for NODs, cultured human BECs and cholangiocytes in intrahepatic bile ducts constantly express the mRNA and protein of NOD2, but cultured BECs do not respond to the NOD2 ligand (muramyl dipeptide, MDP), indicating a suspicious functional expression (our unpublished data).

2.2. Recognition of PAMPs. In addition to the expression of TLRs in cholangiocytes and the biliary epithelium, the activation of TLRs has also been demonstrated during bacterial, viral, and parasitic infections. Stimulation with PAMPs including Pam3CSK4 (TLR1/2 ligand), MALP-2 (TLR2/6 ligand), peptidoglycan (TLR2 ligand), and polyinosinic-polycytidylic acid (poly(I:C), a synthetic analog of viral dsRNA, TLR3 ligand) induced the activation of NF-κB, a major transcription factor downstream of TLRs, in cultured human BECs [2, 20, 23]. In addition to bacteria, *Cryptosporidium parvum* (*C. parvum*), a protozoan parasite causing intestinal and biliary diseases, also activates both TLR2 and TLR4 in cholangiocytes to initiate epithelial host responses, accompanying the recruitment of these TLRs and ganglioside GM1 to membrane rafts [26]. Membrane rafts have been implicated in TLR activation in several other cell



(a) (b)

FIGURE 1: Immunohistochemistry for TLR3 in chronic hepatitis C (a) and TLR4 in primary biliary cirrhosis (PBC). The expression of TLR3 existing in endosomes is found in interlobular bile ducts (arrow in (a)) and hepatocytes in a cytoplasmic pattern. In contrast, TLR4 expression is highlighted in a membranous pattern (b).

types, including epithelial cells, following microbial infection [27]. Moreover, viral PAMPs such as double-stranded RNA (dsRNA) are also recognized by cultured BECs; cultured human BECs expressed nuclear transcription factors including NF-κB and interferon regulatory factor-3 (IRF-3) on stimulation with poly(I:C), a synthetic analog of viral dsRNA [23]. These findings indicate that human BECs possess functional PAMP-recognizing receptors and an innate immune system against viruses as well as bacteria.

In addition to microorganism components, several families of proteins originating from and produced by autocells are involved in the recognition of pathogens and the products released from injured or dying cells. In particular, endogenous factors including HMGB1, S100A8/S100A9, and heat shock proteins are known as damage-associated molecular patterns (DAMPs) [28], but a detailed analysis has not been conducted in cholangiocytes.

2.3. Response to PAMPs. As part of the host's defenses against infections, cholangiocytes secrete polymeric immunoglobulin A and produce several antibiotics against bacteria (lactoferrin, lysozyme, and defensins) [29–31]. Defensins, in particular, are key elements in innate immunity. Basic peptides activate against a broad spectrum of microbes including bacteria and fungus, defensins are divided into two types, α- and β-defensins. Human beta-defensins (hBDs) consisting of hBD1-hBD6 are produced by several epithelial cells including cholangiocytes and play an important role in the defense against mucosal infection. hBD1 distributes throughout the intrahepatic biliary tree and is detected in bile. Moreover, studies using cultured human BECs and SV40-transformed human cholangiocytes confirmed the constant production of hBD1 and also hBD3 [19, 22]. In contrast, hBD2 is not physiologically expressed in nondiseased livers and *de novo* expression is detected in bile ducts showing suppurative inflammation in patients with biliary diseases such as hepatolithiasis and biliary infections and also in their bile [22]. Moreover, the expression of hBD2 via the activation of NF-κB occurred on stimulation by PAMPs including LPS, *E. coli*, and *C. parvum* in cultured human BECs [19, 22]. This finding suggests that hBD-1 is constantly detectable in bile samples while it plays a role in the constitutive antimicrobial defense of the hepatobiliary system and hBD2 plays a role in the localized biliary defense in cases of biliary infection. In addition to defending against bacteria, cholangiocytes possess an innate immune system to fight viral infections, because cholangiocytes have TLR3, RIG-I, and MDA-5 recognizing dsRNA viruses such as Reoviridae (reovirus and rotavirus). Stimulation with poly(I:C), a synthetic analog of viral dsRNA, induces the activation of NF-κB and IRF3 and the production of key components of antiviral immunity, IFN-β1 and MxA [23]. In normal human liver tissues, small numbers of Kupffer cells, but no hepatocytes and cholangiocytes, exhibited MxA expression. In contrast, strong expression of the MxA protein was identified in Kupffer cells and cholangiocytes in patients with chronic liver diseases and fulminant hepatic failure [19]. These findings suggest that cholangiocytes participate directly in innate immunity and show a prompt response to pathogens without any help from immunocompetent cells such as macrophages.

In addition to antibiotics, cholangiocytes produce several inflammatory cytokines and chemokines such as IL-6, TNF-α, IL-8, fractalkine, monocyte chemotactic protein-1 (MCP-1), and CXCL16 [2, 19, 20, 32–37]. IL-6 has been demonstrated to increase DNA synthesis in human cholangiocytes *in vitro*, indicating increased proliferative activity [38]. IL-8 is closely associated with neutrophilic infiltration and its expression is found in cholangitis lenta which is usually encountered in septic patients and characterized by bile ductular proliferation, ductular cholestasis, and ductular epithelial damage [33, 39]. Chemokines produced in cholangiocytes as part of the biliary innate immune response could result in the recruitment and activation of T cells, macrophages, neutrophils, hepatic stellate cells, and NK cells to protect against biliary infection and also play an important role in bile duct-specific acquired immunity by forming periductal cytokine networks and migrating immunocompetent cells, thereby contributing to biliary mucosal defense and subsequent acquired immunity.

Cholangiocytes may also function as professional antigen-presenting cells (APCs) and contribute to the control of

inflammatory reactions [40]. Cultured murine BECs constitutively expressed low levels of MHC Class I and MHC Class II molecules, and these levels were significantly enhanced by IFN-γ stimulation and murine cytomegalovirus (CMV) infection [41]. Moreover, murine BECs infected with murine CMV showed a progressive cytopathic effect. In contrast, in cultured human BECs, CMV-infection augmented the expression of MHC class I but not MHC class II molecules [42]. These findings suggest that CMV affects the immunogenic potential of cholangiocytes.

TLR signals influence from fuctions of tight junctions in cholangiocytes by activating various intracellular signaling pathways. LPS disrupted the tight junctions of a rat BEC monolayer via a TLR4-dependent mechanism and LPS and *C. parvum* increased paracellular permeability by activating c-Src in rat and human BECs [43, 44]. Therefore, biliary innate immune reactions are involved in the functional regulation of tight junctions in cholangiocytes.

3. Regulation of Biliary Innate Immunity

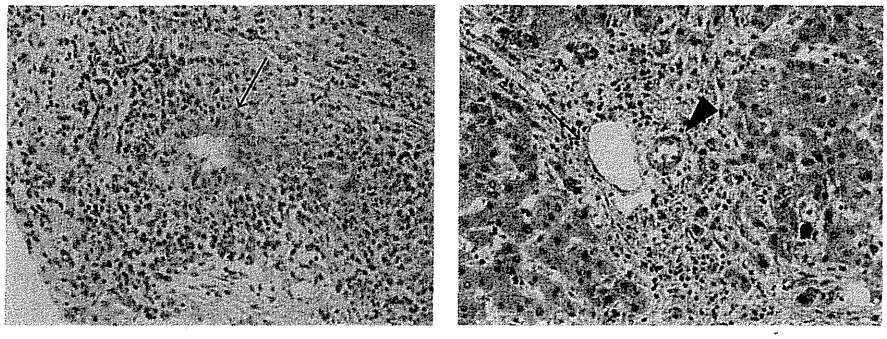
TLR signaling initiates adaptive immunity which then regulates the innate immune system to maintain mucosal homeostasis. The expression of TLRs in cholangiocytes is highly regulated, but its disruption has been associated with various hepatobiliary diseases. Infecting cultured human cholangiocytes with *C. parvum* induced a significant increase in TLR4 protein, a process that appears to be associated with the production of hBD2 [19]. T cell-derived inflammatory cytokines are known to participate in the regulation of TLR expression in several cells [45, 46]. The interactions of TLRs with Th1 cytokines, in particular, participate in the pathogenesis of inflammatory bowel diseases [47]. Cholangiocytes express receptors for cytokines such as IFN-γ, TNF-α, IL-4, IL-6, and IL17, and thus, are also the target of many periductal inflammatory mediators during biliary inflammatory diseases. A Th1-type cytokine, IFN-γ upregulates the mRNA expression of TLR2-TLR5 and accelerates the upregulation of PAMP-induced NF-κB activation in cholangiocytes, suggesting that a Th1-dominant peribiliary milieu leads to the increased susceptibility to PAMPs and the production of inflammatory cytokines and chemokines from BECs [20]. This impaired regulation of biliary innate immunity caused by the Th1-predominant milieu may be involved in the pathogenesis of cholangiopathy in biliary diseases including PBC [48]. In fact, upregulation of TLR4 and TLR9 in cholangiocytes has been reported in patients with PBC and PSC [25, 49].

Micro-RNAs play important roles in a wide range of biological events through posttranscriptional suppression of target mRNAs. Recent studies indicate that micro-RNA-mediated posttranscriptional pathways may be critical to host-cell regulatory responses to microbial infections. Cultured human BECs express let-7 family members which posttranscriptionally downregulate TLR4 expression and infections of *C. parvum* decrease the expression of let-7 resulting in the upregulation of TLR4 [50]. Moreover, microRNA-98 and let-7 suppressing cytokine-inducible Src homolog 2-containing protein (CIS, a suppressor of cytokine signaling family) at the translational level are expressed in cholangiocytes and LPS and *C. parvum* infections downregulate these mirco-RNAs, suggesting the regulation of the TLR-mediated biliary innate immune response [51].

The luminal surface of the bile duct is continually exposed to PAMPs via bile, but cholangiocytes physiologically do not elicit an inflammatory response. This lack of response to PAMPs, especially LPS, could be due to "endotoxin tolerance" and this system is important in preventing endotoxin shock in infections as well as maintaining homeostasis in organs [52]. As for negative regulatory systems of innate immunity, mechanisms compete with TLR binding and suppress intracellular TLR signaling using several molecules including extracellular soluble TLRs (sTLRs), single immunoglobulin IL-1-related protein (SIGIRR), IRAK-M (homolog of IRAK-1), MyD88s (inactive splice variant of MyD88), SARM (negative regulator of TRIF), Toll-interacting protein (Tollip), A20, SHIP (a PI3K inhibitor), and suppressor of cytokine signaling-1 (SOCS1) [52–58].

Our previous study using cultured BECs and cholangiocarcinoma cell lines revealed that the activation of NF-κB and the increased expression of TNF-α caused by stimulation with PAMPs including LPS are gradually attenuated with time and that pretreatment with LPS significantly inhibits the response to subsequent stimulation, suggesting an induction of LPS (endotoxin) tolerance [59]. Moreover, pretreatment with Pam_3CSK_4 (TLR1/2 ligand) effectively induced tolerance to subsequent stimulation with LPS (TLR4 ligand) [52, 59]. Among several negative regulators, the expression of at least IRAK-M and Tollip has been demonstrated in human cholangiocytes and treatment with LPS and Pam_3CSK_4 upregulates the expression of IRAK-M, but not Tollip. IRAK-M negatively regulates TLR signaling by inhibiting the activation of IRAK-1 and MyD88 [55]. Furthermore, immunohistochemistry using human liver tissue sections confirmed that IRAK-M is diffusely expressed in intrahepatic biliary trees in both normal and diseased livers. This negatively regulated mechanism of innate immune response is important to escape hypercytokine milieu and tissue injury caused by excessive innate immune responses.

In contrast, treatment with poly(I:C), TLR3 ligand, significantly enhanced NF-κB activity in fresh cultured BECs and pretreatment did not lead to tolerance to poly(I:C). [60] Levels of production of MxA and IFN-beta1 were also preserved. Therefore, TLR tolerance to a viral PAMP (poly(I:C)) is not found in BECs. Although IRAK-M mRNA expression was upregulated by stimulation with dsRNA (TLR3 ligand), no tolerance to the dsRNA was found in cultured BECs. This is reasonable because the intracellular signaling of TLR3 is a MyD88-independent pathway, that is, the dsRNA-related response is not affected by IRAK-M [17]. These findings suggest that cholangiocytes lining biliary trees are resistant to nonpathogenic commensal bacterial PAMPs, but not virus-derived dsRNA, maintaining the homeostasis of biliary innate immunity in physiological conditions. Moreover, the upregulation of IRAK-M expression on treatment with poly(I:C) is speculated to cause dsRNA-stimulated BECs to become resistant to TLR2- and TLR4-related PAMPs including LPS. Therefore, once cholangiocytes are infected



(a) (b)

FIGURE 2: Primary biliary cirrhosis (PBC). (a) Chronic nonsuppurative destructive cholangitis (CNSDC). Damaged bile ducts (arrow) and infiltration of mixed chronic inflammatory cells surrounding bile ducts are found. (b) Bile ducts have disappeared in the portal tract. Arrowhead and arrow denote artery and portal vein, respectively.

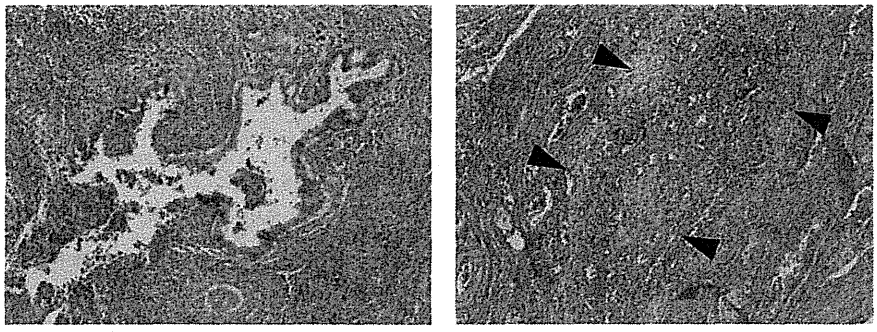
by a dsRNA virus, progressive destruction caused by the biliary innate response to dsRNA and resistance to bacterial infection continues until the virus is eliminated.

4. Disease-Specific Cholangiopathy Associated with Biliary Innate Immunity

4.1. PBC. PBC is characterized by the selective destruction and loss of interlobular bile ducts including chronic nonsuppurative destructive cholangitis (CNSDC) (Figure 2) [61]. The etiopathogenesis of PBC still remains speculative, but a high prevalence of vaginal and urinary tract infections and the presence of bacterial and viral components in bile and liver tissue and of the molecular mimicry of human and bacterial pyruvate dehydrogenase complex-E2 (PDC-E2, a major epitope of antimitochondrial antibody [AMA]) and xenobiotics are demonstrated (Table 1) [3, 5, 62–68]. Moreover, BECs translocate immunologically intact PDC-E2 to apoptotic bodies and create an apotope. The unique triad of BEC apotopes, macrophages from patients with PBC, and AMAs induces intense inflammatory cytokine production, providing a mechanism for the biliary specificity of PBC [69]. Innate immunity changes may be critical to the initiation and perpetuation of the autoimmune injury, as in the case of the enhanced response of immunocompetent cells (monocytes and memory B cells) as well as target BECs to infectious stimulation and environmental mimics [70, 71]. These findings suggest that the presence of microorganisms and the innate immune responses against them are involved in the pathogenesis, particularly cholangiopathy, of PBC.

In PBC, the expression of TNF-α and IL-6 was detected in cholangiocytes from the liver of patients with PBC, suggesting the result of some biliary response including a biliary innate immune response [72]. Several studies revealed that, compared with Th2, a Th1-dominant cytokine milieu is associated with the pathogenesis including bile duct injury in PBC [48]. Cholangiocytes possess the receptor for IFN-γ (Th1 cytokine) and IFN-γ upregulates the expression of TLRs and susceptibility to PAMPs in cholangiocytes, impairing the regulation of biliary innate immunity. Moreover, IL-4 (Th2 cytokine) and IFN-γ up- and downregulate the expression of peroxisome proliferator-activated receptor γ (PPARγ) showing anti-inflammatory activities in biliary innate immune response, respectively, in cultured human BECs [73, 74]. PPARγ is constitutively expressed in cholangiocytes of intrahepatic small bile ducts. PPARγ as well as IRAK-M, therefore, may also relate to the maintenance of biliary homeostasis as a tolerant regulator by attenuating inflammatory signals in cholangiocytes to commensal PAMPs in biles [73]. However, in PBC liver, PPARγ expression is significantly downregulated in the affected bile ducts as a Th1-dominant periductal cytokine milieu [73]. Moreover, the upregulation of TLR4 and TLR9 in cholangiocytes and of TLR3 and type I IFN signaling pathways in portal tracts and parenchyma are also found in PBC [24, 25, 49]. These finding indicate an increased susceptibility to PAMPs, suggesting an association with the pathogenesis of cholangiopathy in PBC.

In addition to Th1 and Th2 cells, a third pathogenic type, Th17 cells, are involved in the pathogenesis of chronic inflammatory diseases. Human Th17 cells are characterized by the production of IL-17 (IL-17A and IL-17F) and IL-6, IL-1β, and IL-23 (TGF-β instead of IL-1β in mice) are critical for driving the differentiation of naïve T cells into Th17 cells and maintaining or stabilizing the functions of Th17 cells [75, 76]. In inflammatory hepatobiliary diseases including PBC, IL-17-positive mononuclear cells are scattered at the interface areas, particularly showing interface hepatitis [32]. In PBC, moreover, the periductal accumulation, particularly around cholangitis including CNSDC accompanying the expression of IL-6, IL-1β, and IL-23 p19, of IL-17 positive cells is found, suggesting that the Th17-related peribiliary cytokine milieu is involved in the histogenesis of the sustained cholangiopathy of PBC [32, 77]. Moreover, an *in vitro* study using cultured human BECs revealed that bacterial PAMPs (LPS and Pam3CSK4) induced the production of Th17-inducing and -maintaining cytokines (IL-6, IL-1β, and IL-23 p19) [32]. These results indicate that biliary innate immunity plays a role in the induction and maintenance of Th17 cells in the periductal area in cases of PBC and the differentiation into Th17 cells in periductal dendritic



(a) (b)

FIGURE 3: Transverse section of extrahepatic biliary remnants in biliary atresia. (a) Distorted common bile duct showing luminal occlusion with surrounding fibroplasia and inflammatory cells. (b) The common bile duct has disappeared leaving a fibrous scar (arrowheads).

cells and macrophages. Th17 cells are part of the mucosal host defense system and also propagate and modulate the cholangiopathy in PBC.

Our recent study revealed that Langerin-positive Langerhans cells (LCs) are dominantly scattered around or within biliary epithelial layers of the damaged bile ducts in PBC. Moreover, experiments with cultured human BECs showed that an LC-attracting chemokine, macrophage inflammatory protein-3α, was produced by cholangiocytes in response to cytokines (IL-1β, TNF-α, and IL-17) and PAMPs [78]. Therefore, LCs existing around or within biliary epithelial layers are important as periductal antigen-presenting cells in PBC and the migration of LCs into bile ducts is closely associated with the periductal cytokine milieu and biliary innate immunity in PBC.

4.2. Biliary Atresia. Biliary atresia characterized by a progressive sclerosing obstruction of extrahepatic bile ducts (Figure 3), is a common infant biliary disease and subdivided to embryonic and perinatal types based on the clinicopathogenesis. Little is known about the etiology and pathogenesis of biliary atresia, but studies using human materials and a virus-infected rodent model suggest an association with Reoviridae (type 3 reovirus and type C rotavirus) having dsRNA, although conflicting results also have been reported [12, 79–81]. Imbalanced cell kinetics caused by enhanced apoptosis in cholangiocytes lining extrahepatic bile ducts is speculated as an important mechanism in obstructive cholangiopathy [23, 82, 83]. Human cholangiocytes are sensitive to tumor necrosis factor-related apoptosis-inducing ligand- (TRAIL-) and Fas- (CD95-)mediated apoptosis [20, 23, 84]. Moreover, because Reoviridae show epitheliotrophysm, the innate immune response against viruses is speculated to be directly associated with epithelial injury and death in biliary atresia. Our previous study demonstrated that stimulation with poly(I:C) induced the activation of NF-κB and IRF-3, followed by the production of antiviral IFN-β1 and also enhanced apoptosis via production of TRAIL [23]. Moreover, in biliary atresia, cholangiocytes lining the remnants of extrahepatic bile ducts diffusely and constantly expressed TLR3 and showed an enhancement of TRAIL and single-stranded DNA- (ssDNA-)positive apoptosis accompanying the activation of NF-κB and IRF-3 [20, 23]. A significant increase of TLR7 and antimicrobial peptide hepcidin and MxA at the mRNA and protein levels, was found in patients in the early stage of biliary atresia [85–87]. Therefore, cholangiocytes not only directly participate in the antiviral innate immune response, but also play a role in the generation of apoptotic responses to infected cells. Moreover, as described above, because the innate immune tolerance of dsRNA is lacking in cholangiocytes, the biliary damage caused by the biliary innate immune response continues until the virus disappears and directly forms the histogenesis of obstructive cholangiopathy in biliary atresia [60].

As the histogenesis of sclerosing lesion, the epithelial-mesenchymal transition (EMT) of cholangiocytes has been speculated to be associated with periductal fibrosis and portal fibrosis in biliary atresia [88–91]. Fundamental to EMT is a loss of normal epithelial features such as cell-to-cell adhesion molecules, the gain of mesenchymal phenotypes, and the acquisition of a fibroblast-like (spindle) morphology with cytoskeletal reorganization [92]. As mentioned above, although the biliary innate immune response to dsRNA reduces the viability of cultured human BECs via TRAIL-mediated apoptosis, the rate of cell death is approximately 70% [23]. The cells that evade apoptosis show a gradual loss of epithelial markers, CK19 (biliary-type cytokeratin in liver) and E-cadherin, and increased expression of a mesenchymal marker S100A4 (also known as fibroblast-specific protein 1) and an essential transcription factor for EMT, Snail, via increased susceptibility to transforming growth factor-β1 (TGF-β1) and the production of basic fibroblast growth factor (bFGF), demonstrating the occurrence of biliary EMT [23]. Because EMT confers resistance to apoptotic effects in fetal rat hepatocytes [93], biliary EMT is thought to be a survival mechanism and associated with an incomplete induction of apoptosis caused by the biliary innate immune response. In fact, *in vivo* studies reveal that mesenchymal markers (vimentin and S100A4) and Snail are expressed but CK19 and E-cadherin are not in cholangiocytes lining the remnants of extrahepatic bile ducts and peribiliary glands of biliary atresia [91, 94], suggesting that the occurrence

of EMT in cholangiocytes is associated with an incomplete induction of apoptosis caused by the biliary innate immune response and that these surviving cells play a role in the sclerosing cholangiopathy of biliary atresia without inducing tolerance until the clearance of the virus.

5. Conclusion and Perspectives

Biliary innate immunity consisting of an organ-specific system is important for the mucosal immunity in intrahepatic and extrahepatic bile ducts and also associated with the pathogenesis of several cholangiopathies in biliary diseases. We speculate that biliary innate immunity is solely associated with the etiology of biliary diseases as the initial event and that the presence of causative microorganisms is not necessary in the pathogenesis of cholangiopathy caused by a subsequent acquired immunity. It is mandatory to understand the molecular basis underlying the immunophysiology and immunopathology of cholangiopathy in terms of innate as well as acquired immunity.

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Review Article

IgG4 Cholangiopathy

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IgG4 cholangiopathy can involve any level of the biliary tree which exhibits sclerosing cholangitis or pseudotumorous hilar lesions. Most cases are associated with autoimmune pancreatitis, an important diagnostic clue. Without autoimmune pancreatitis, however, the diagnosis of IgG4-cholangiopathy is challenging. Indeed such cases have been treated surgically. IgG4-cholangiopathy should be diagnosed based on serological examinations including serum IgG4 concentrations, radiological features, and histological evidence of $IgG4^+$ plasma cell infiltration. Steroid therapy is very effective even at disease relapse. A Th2-dominant immune response or the activation of regulatory T cells seems to be involved in the underlying immune reaction. It is still unknown why IgG4 levels are specifically elevated in patients with this disease. IgG4 might be secondarily overexpressed by Th2 or regulatory cytokines given the lack of evidence that IgG4 is an autoantibody.

1. Introduction

IgG4-related disease is a unique systemic inflammatory condition characterized by tumorous swelling of affected organs and high-serum IgG4 concentrations [1–3]. Autoimmune pancreatitis is a prototype of IgG4 disease as Hamano et al. described in a landmark paper in 2001 [4]. Further studies have confirmed that IgG4-related disease can involve a variety of organs including the salivary glands (chronic sclerosing sialadenitis) [5, 6], lacrimal glands (Mikulicz's disease) [7, 8], and retroperitoneum (retroperitoneal fibrosis) [9, 10]. The bile duct lesion is called IgG4-related sclerosing cholangitis (IgG4-SC) [11] or IgG4-associated cholangitis [12, 13] (the former is used hereafter). Since we reported that IgG4-SC is a distinct entity which should be differentiated from primary sclerosing cholangitis (PSC) [11], clinical and pathological features have been clarified [12, 13]. In this paper, we describe the concept, pathology, differential diagnosis, and pathogenesis of IgG4-SC.

2. Spectrum of IgG4 Cholangiopathy

The relationship between IgG4-SC and autoimmune pancreatitis is summarized in Table 1. IgG4-SC can manifest as diffuse sclerosing cholangitis or a hilar pseudotumourous mass [11]. The former should be differentiated from PSC, whereas the latter radiologically resembles hilar cholangiocarcinoma [14]. Of note is that most case of IgG4-SC are associated with autoimmune pancreatitis. A study by the Mayo Clinic found that only 4 of 53 patients (7.5%) with IgG4 cholangiopathy had cholangitis without autoimmune pancreatitis [13]. Whether or not IgG4-SC can involve only peripheral small bile ducts like small-duct PSC is an interesting issue. Given that a recent study revealed that peripheral IgG4 cholangiopathy was always associated with large duct lesions [15], it seems safe to assume that IgG4-SC predominantly affects large bile ducts, which are detectable by cholangiographic or radiological examinations.

Recent papers have introduced IgG4-related autoimmune hepatitis [16], which accounts for 3% of cases of type 1 autoimmune hepatitis in the Japanese population [17]. The term IgG4 hepatitis should be only used for patients who do not have radiological biliary abnormalities and are found to have chronic hepatitis based on liver biopsies. Given that one cases of IgG4 hepatitis was complicated later by sclerosing cholangitis during followup [17], IgG4 hepatitis might also belong to a spectrum of IgG4 cholangiohepatitis.

3. Clinical Features and Autoantibodies

In our experience, patients with IgG4-SC usually present with obstructive jaundice due to a pancreatic head mass (autoimmune pancreatitis) or severe biliary stricture [12, 13]. Other patients are sometimes discovered to have IgG4-SC during a workup for other IgG4-related conditions such as sialadenitis, retroperitoneal fibrosis and kidney lesions. Weight loss or new-onset diabetes mellitus due to pancreatitis is another potential symptom.

Patients with IgG4 disease share serological abnormalities irrespective of the organ of origin. There is no doubt that elevated serum IgG4 levels are the most specific indicator. Other sensitive but not specific markers include hyper γ-globulinemia (observed in 50% of patients), hyper IgG (60–70%), antinuclear antibodies (40–50%), rheumatoid factor (20%), and eosinophilia (15–25%) [18, 19]. Autoantibody against SS-A (Ro) or SS-B (La), antimitochondria antibody, and antineutrophilic cytoplasmic antibody (ANCA) are all exceptional (<5%) [18, 19].

Studies on autoimmune pancreatitis have provided further data on autoantibodies which might participate in the pathogenesis. Antibodies against lactoferrin (LF) and carbonic anhydrase (CA) II are frequently detected in cases of autoimmune pancreatitis (73% and 54%, resp.) [20]. Interestingly, a strong positive correlation between increases in serum IgG4 levels and anti-CA-II antibody levels has been reported [21]. Anti-CA-IV, another autoantibody, was detected in 10 of 29 (34%) patients with autoimmune pancreatitis [22]. Given that LF and CAs are expressed in some exocrine organs, these autoantibodies may be related to systemic manifestations of IgG4-related disease. Of note is that autoantibodies of the IgG4 subclass have not been detected in patients with IgG4-related disease so far.

4. Diagnosis

4.1. Surgical Cases. It is not difficult to make a diagnosis of IgG4-SC if surgically resected specimens are available. The gold standard for the diagnosis of IgG4-SC is histology including characteristic features on H&E and extensive infiltration by $IgG4^+$ plasma cells on immunostaining. Pathological features can be summarized as follows: (1) diffuse lymphoplasmacytic infiltration, (2) storiform fibrosis, (3) obliterative phlebitis, (4) eosinophilic infiltration, and (5) numerous $IgG4^+$ plasma cells [11, 23]. Features unusual for IgG4-SC are neutrophilic infiltration with or without abscesses, xanthogranulomatous change, and mucosal erosive change. Obliterative phlebitis is a finding characteristic for IgG4-related disease irrespective of the organ affected. We speculate that endothelium may express chemotactic factors, but this has not been examined so far.

4.2. Patients with Autoimmune Pancreatitis. Serology, imaging, other organ involvement, and biopsy need to be considered for the diagnosis of nonsurgical cases. Given that most patients (>90%) with IgG4-SC have autoimmune pancreatitis, it seems most important to examine changes in the pancreas. Autoimmune pancreatitis is radiologically suspected in most cases, and the diagnosis can be confirmed by the serological examination of IgG4. Histological detection of $IgG4^+$ plasma cells is usually not necessary for cases showing typical radiological features (sausage-like diffuse swelling, peripancreatic capsule-like rim, and irregular narrowing of the pancreatic duct) [24] and high-serum IgG4 levels. But, if there are any unusual features on imaging or IgG4 levels are not elevated, biopsies should be considered to detect $IgG4^+$ plasma cells. Most institutions use 135 or 140 mg/dL as a cut-off point for serum IgG4 levels, with more than 300 mg/dL being highly specific for IgG4-related disease [4, 25].

4.3. Patients without Autoimmune Pancreatitis. It is challenging to diagnose IgG4-SC not associated with pancreatitis. In fact, most patients have been surgically treated for suspected biliary malignancy [13]. In our experience, detecting $IgG4^+$ plasma cell is recommended even if the patients have high-serum IgG4 concentrations. Three potential approaches have been proposed to detect infiltration by $IgG4^+$ plasma cells. Vater's ampulla biopsy is least invasive and technically easiest, and especially useful for patients discovered endoscopically to have ampullary swelling [26–29]. Another potential approach is a liver needle biopsy which can detect $IgG4^+$ plasma cells infiltrating into peripheral small portal tracts [15, 30, 31]. This is particularly useful for patients with intrahepatic biliary abnormalities on cholangiograms, but not useful for patients with only intrapancreatic bile duct stricture [15]. The last choice is a bile duct biopsy, the usefulness of which might depend on the ability and experience of the endoscopists [29]. The biggest advantage of this method is that not only $IgG4^+$ plasma cells but also other histological features such as storiform fibrosis and eosinophilic infiltration are detectable [29]. The specificity and sensitivity of the diagnosis by these three biopsies are summarized in Table 2. Normally, more than 10 $IgG4^+$ plasma cells are used as the diagnostic threshold for biopsy samples.

4.4. Steroid Trial. There is no international consensus regarding diagnostic steroid trials for IgG4-related disease. Some institutions use steroid trials for the diagnosis [32–34], whereas other groups, especially from Japan, are against doing so [35]. Given that IgG4-SC needs to be differentiated from malignant tumours (cholangiocarcinoma) different from other typical autoimmune diseases, much attention should be paid to diagnostic steroid trials.

5. Differential Diagnosis

5.1. PSC. IgG4-SC must be differentiated from PSC given that the steroid responsiveness is completely different. These two entities are histologically distinct. Large bile ducts affected by IgG4-SC show transmural inflammation with storiform fibrosis and obliterative phlebitis (Figure 1), whereas inflammation is more extensive on the luminal side with erosion or xanthogranulomatous changes in PSC [11]. Clinically, a young age (<40 years) and history of

Table 1: Disease spectrum of IgG4 pancreato-cholangiopathy and differential diagnosis.

IgG4-related pancreato-cholangiopathy	Differential diagnosis
Autoimmune pancreatitis without bile duct involvement	Pancreatic cancer
Autoimmune pancreatitis with IgG4 cholangitis	Pancreatic cancer and cholangiocarcinoma
IgG4-related sclerosing cholangitis	Primary sclerosing cholangitis
IgG4-related sclerosing cholangitis with hilar pseudotumor	Hilar cholangiocarcinoma

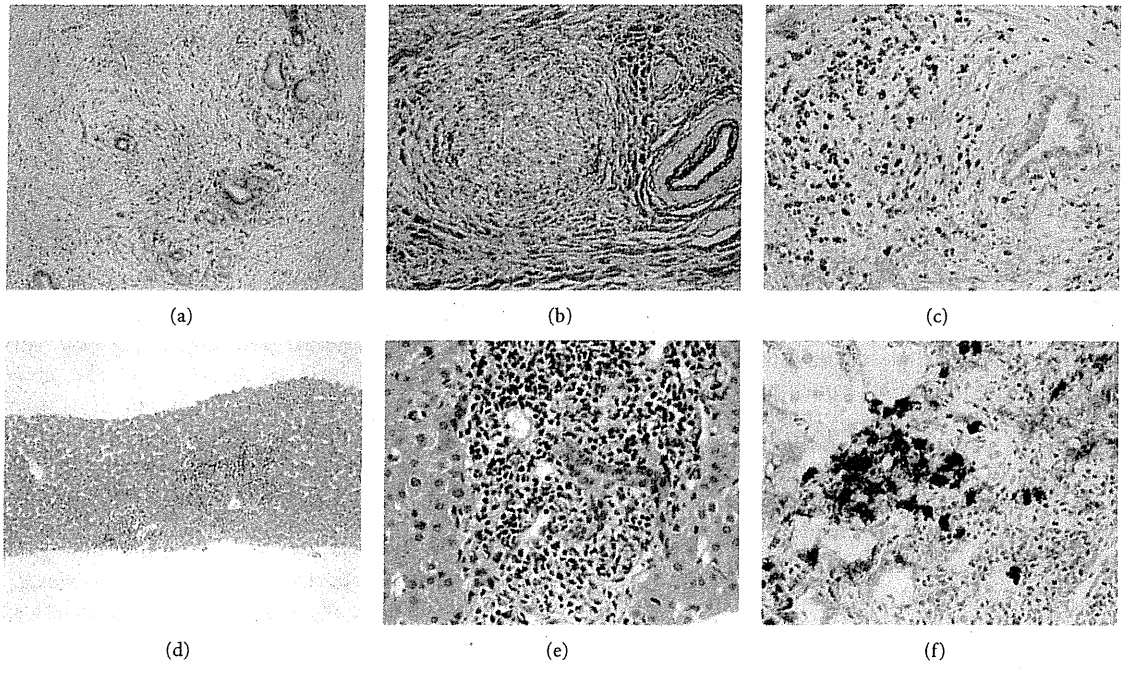

Figure 1: Histopathology of IgG4-related sclerosing cholangitis. The surgical specimens show diffuse inflammatory cell infiltration with fibrosis involving peribiliary glands (a), obliterative phlebitis (b), and infiltration of many $IgG4^+$ plasma cells (c). The liver needle biopsy reveals portal inflammation (d), bile duct damage (e), and $IgG4^+$ plasma cells (f).

Table 2: Sensitivity and specificity of detection of $IgG4^+$ plasma cells ($\geq$10 cells/high power field) by ampullary, liver, and bile duct biopsies.

	Sensitivity	Specificity	Reference
Ampullary biopsy	80%	100%	[26]
	67%	100%	[27]
	53%	100%	[28]
	52%	91%	[29]
Liver needle biopsy	24%	100%	[30]
	60%	100%	[31]
	26%	100%	[15]
Bile duct biopsy	52%	97%	[29]

inflammatory bowel disease are features suggestive of PSC, whereas IgG4-SC is more likely in patients with other sclerosing lesions including autoimmune pancreatitis and retroperitoneal fibrosis. Serologically, IgG4 levels are most useful, but it should be noted that 9% of PSC patients show elevated IgG4 levels [36]. Positivity for ANCA is suggestive of PSC. Eosinophilia is similarly detectable in both diseases. Nakazawa et al. reported that PSC and IgG4-SC can be differentiated based on a detailed examination of cholangiograms [37]. Liver biopsy is also useful. Infiltration of $IgG4^+$ plasma cells or the presence of periportal "fibroinflammatory nodules [30]" in needle biopsy samples is suggestive of IgG4-SC (Figure 1), whereas ductopenia and periductal concentric fibrosis are more commonly seen in PSC [15, 31]. It is still unclear whether or not PSC can be differentiated from "burned-out" IgG4-SC.

5.2. PSC with Many $IgG4^+$ Plasma Cells. Zhang et al. [38] examined tissue infiltration of $IgG4^+$ plasma cells in explanted liver with PSC. Twenty-three of 98 livers (23%) showed more than 10 $IgG4^+$ cells per HPF which might be less specific given that most IgG4-related lesions show more than 100 $IgG4^+$ cells/HPF in surgical specimens. In addition, another group revealed that 2 out of 41 (5%) explanted livers with PSC showed more than 100 $IgG4^+$ cells/HPF [39]. These two studies suggested that explanted livers with PSC can sometimes show moderate degrees of $IgG4^+$ cell infiltration

(>10 cells/HPF) and rarely exhibit marked infiltration (>100 cells/HPF) around large bile duct lesions. Importantly, the other histological features of these cases were not typical of IgG4-SC but consistent with PSC [38, 39]. The histological diagnosis of IgG4-SC is not enough just based on the number of $IgG4^+$ plasma cells.

5.3. Follicular Cholangitis. This is a rare disease entity characterized by numerous lymphoid follicles around hilar or perihilar bile ducts [40, 41]. Most patients reported so far underwent surgical resection on suspicion of hilar cholangiocarcinoma. Follicular cholangitis is different from PSC in that the inflammatory cell infiltration is more extensive and biliary epithelial damage is not conspicuous. $IgG4^+$ plasma cell infiltration or obliterative phlebitis is usually not conspicuous, different from IgG4-SC [40].

5.4. Hilar Cholangiocarcinoma. In our experience, hilar cholangiocarcinoma is the most important and difficult differential diagnosis of IgG4-SC in the clinical field, particularly for patients without autoimmune pancreatitis. Radiological features of IgG4-SC sometimes resemble those of hilar cholangiocarcinoma [14]. Serum IgG4 levels can be mildly elevated in patients with cholangiocarcinoma, but titers of more than 300 mg/dL are highly suggestive of IgG4-SC. As described above, histological examination to detect $IgG4^+$ cell infiltration is needed for patients without autoimmune pancreatitis.

6. Treatment

IgG4-SC responds dramatically to steroid therapy the same as other IgG4-related lesions. This is one significant difference from PSC [12, 13]. At the moment, it is difficult to conclude the recommended dose and duration of steroid therapy for IgG4-SC because of a lack of published data. A Japanese study on autoimmune pancreatitis recommended an initial dose of 0.6 mg/kg/day, which was then reduced to a maintenance dose over a period of 3–6 months [42]. In that study, disease relapses appeared to be reduced but not eliminated by maintenance treatment with low-dose steroid [42]. Rituximab is a potential treatment for steroid-resistant autoimmune pancreatitis or IgG4-SC [43].

7. Pathogenesis

Recent papers have provided data suggesting that the Th2-type immune response is activated in IgG4-related disease including IgG4-SC [44–48]. Quantitative real-time PCR using RNA extracted from frozen tissue of affected organs including bile ducts revealed significantly higher ratios of IL-4/IFN-γ, IL-5/IFN-γ, and IL-13/IFN-γ in IgG4-related disease tissues than in tissues from patients with classical autoimmune diseases [49]. Lymphocytes expressing IL-4 were clearly demonstrated by *in situ* hybridization. Recent papers also showed that peripheral blood mononuclear cells collected from patients with IgG4-related disease produced predominantly Th2-type cytokines such as IL-4, IL-5, IL-10, and IL-13 after T-cell stimulation.

Interestingly, the number of regulatory T-cells (Tregs) is characteristically increased in both tissue and blood of patients with IgG4-related disease. Our investigation revealed that the mRNA expression of forkhead box P3 (Foxp3, a Tregs-specific transcriptional factor) was higher in IgG4-related disease than in classical autoimmune diseases. Two regulatory cytokines, IL-10 and transforming growth factor-β (TGF-β), are significantly overexpressed [49, 50]. Furthermore, $CD4^+CD25^+Foxp3^+$ Tregs could be detected within affected tissues by immunohistochemistry, in numbers significantly higher than in autoimmune and nonautoimmune disease controls. The number of $Foxp3^+$ cells was significantly correlated with the number of $IgG4^+$ plasma cells in IgG4-related cholangitis [51]. Miyoshi et al. examined the number of Tregs in the blood and reported that the number of $CD4^+CD25^{high}$ Tregs was significantly higher in patients with AIP than in patients with chronic pancreatitis and was correlated with the level of IgG4 in serum [52]. The number of naïve Tregs was significantly decreased. They speculated that hyporeaction of naïve Tregs might be involved in the development of IgG4-related disease, whereas hyperreaction of $CD4^+CD25^{high}$ Tregs could reflect IgG4-related disease progression [52].

The possible involvement of *H pylori* in the pathogenesis of AIP was reported in 2005 [53]. Gastric *H. pylori* infection triggers AIP in genetically predisposed subjects via molecular mimicry between human CA-II and alpha-carbonic anhydrase of *H. pylori* [54]. Frulloni et al. found that 94% of patients with AIP had antibodies against a plasminogen-binding protein of *H. pylori* [55]. The amino acid sequence of the plasminogen-binding protein exhibited homology with that of the ubiquitin-protein ligase E3 component n-recognin 2, an enzyme expressed in pancreatic acinar cells. However, the involvement of *H. pylori* in the pathogenesis of other IgG4-related lesions has not been reported so far.

8. Conclusion

IgG4-SC is a unique cholangiopathy which should be differentiated from classical PSC or biliary malignancy. An underlying immune response might be mediated by predominantly Th2 or regulatory cytokines.

Abbreviations

ANCA: Antineutrophilic cytoplasmic antibody
CA: Carbonic anhydrase
IgG4-SC: IgG4-related sclerosing cholangitis
PSC: Primary sclerosing cholangitis
TGF: Transforming growth factor
Tregs: Regulatory T cells
Foxp3: Forkhead box P3.