The Cholangiopathies: Disorders of Biliary Epithelia

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The intrahepatic bile ducts comprise a complex 3-dimensional network of conduits within the liver lined by specialized epithelial cells called cholangiocytes. A major function of cholangiocytes is bile formation (Figure 1). Hepatocytes, the other epithelial cell type of the liver, produce primary or hepatic bile, which percolates through the intrahepatic bile ducts; during this journey, bile is modified by cholangiocytes via a series of secretory and absorptive processes that provide additional bile water (cholangiocytes secrete ~40% of daily bile production in humans) and alkalinity (Figure 2).1,2 To perform this task, biliary epithelia express an array of “flux molecules” (i.e., proteins involved in transport, such as channels, exchangers, transporters) on their plasma membranes (see Strazzabosco et al.3–5 for reviews on structure, topography, and function of cholangiocyte flux molecules).

Cholangiocytes also proliferate in response to endogenous or exogenous signals/stimuli and actively participate in inflammatory and reparative processes within the liver. Furthermore, cholangiocytes interact with the immune system and microorganisms and are involved in drug metabolism (Figure 1). To accomplish these multifaceted functions, cholangiocytes display morphologic and functional heterogeneity along the biliary tree. As shown on Figure 2, cholangiocytes lining large bile ducts (300–800 μm) participate in hormone-regulated bile secretion, whereas cholangiocytes lining small bile ducts (15–300 μm) possess proliferative capabilities and display considerable plasticity, being able to assume a “reactive phenotype” in disease conditions (see Marzioni et al.6 and Kanno et al.7 for recent reviews on cholangiocytes heterogeneity).

Cholangiocytes represent the primary cell target of a diverse group of genetic and acquired biliary disorders, which we have called collectively “cholangiopathies” (Table 1). Many of the cholangiopathies are, at their early stages, site restricted along the biliary tree. For instance, primary biliary cirrhosis (PBC), drug-induced cholangiopathies, and graft vs. host disease (GVHD) involving the liver affect primarily the small bile ducts. In contrast, primary sclerosing cholangitis (PSC) and cholangiocarcinoma mainly involve the large intra- and extrahepatic bile ducts. Thus, recognition of cholangiocyte heterogeneity along the biliary tree is necessary to understand better the cholangiopathies and, potentially, to devise novel, effective therapies.6,7 Because of their morbidity, mortality, need for liver transplantation, and overall cost to society, cholangiopathies are now recognized as an important group of liver diseases. Most cholangiopathies display a progressive course leading to cirrhosis and liver failure. To date, the cause of most cholangiopathies remains obscure. In this review, we summarize possible pathogenetic mechanisms involved in the cholangiopathies.

Possible Pathogenetic Mechanisms in the Cholangiopathies

The spectrum of cholangiopathies ranges from acquired conditions in which the cholangiocyte is damaged by disordered immunity (e.g., PBC, GVHD), infectious agents (e.g., cytomegalovirus, cryptosporidium), ischemia (e.g., posttransplant hepatic artery stenosis, chronic liver transplant rejection), and toxic compounds (e.g., drugs, toxins) to genetically transmitted or developmental diseases such as cystic fibrosis, Alagille’s syndrome, biliary atresia, and fibropolycystic diseases (Table 1).

Despite their heterogeneity, cholangiopathies share a number of basic pathogenetic mechanisms, albeit their contribution may differ in each entity. Moreover, the end result of most of the cholangiopathies is the loss of bile

Abbreviations used in this paper: CTGF, connective tissue growth factor; ET-1, endothelin-1; GVHD, graft vs. host disease; HGF, hepatocyte growth factor; NCAM, neural cell adhesion molecule; PBC, primary biliary cirrhosis; PDGF-BB, platelet derived growth factor-BB; PSC, primary sclerosing cholangitis; RNOS, reactive nitrogen oxide species; TGF-β2, transforming growth factor-β2.

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ductopenia lead to development of cholangiopathies (chronic interplay of cholestasis, biliary inflammation/fibrosis, and choleresis (i.e., increased bile flow). Nevertheless, in humans, the causes cholangiocyte proliferation, which, in the early stage, leads to apoptosis, necrosis) in bile ducts may result in biliary hyperplasia (e.g., disappearance of proliferating ducts). Because of the evidence supporting their generalizability, we focus here on the mechanism(s) of cholangiocyte proliferation as well as apoptotic death. We will also review the establishment of the portal inflammatory reaction and the consequent reparative/fibrotic changes and the ensuing potential for neoplastic transformation. Because almost all cholangiopathies are associated with qualitative/quantitative changes in bile production, we will also discuss the possible mechanisms causing cholestasis. All these processes result in a chronic inflammatory response promoted and modulated by the actions of cytokines, chemokines and other inflammatory mediators.

Cholangiocyte Proliferation

The cholangiocyte maintains mitotic capability throughout adult life. Proliferation of cholangiocytes and subsequent distortion of the biliary tree architecture is a common, but nonspecific, pathologic response in the early stages of cholangiopathies. A systematic approach to classify the different types of cholangiocyte hyperplasia has been proposed. In animals, biliary obstruction causes cholangiocyte proliferation that is associated in the early stages with increased ductal mass and elevated basal and secretin-induced ductal secretion, causing choleresis (i.e., increased bile flow). To date, however, a comprehensive understanding of the mechanisms by which putative molecules stimulate cholangiocyte proliferation is lacking. Evidence from animal studies indicates that, in addition to acetylcholine and PKA agonists, estrogens, interleukin-6 (IL-6), epidermal growth factor (EGF), 3-iodo-thyronine (T3), and selected bile acids are among potent agents promoting cholangiocyte proliferation. Moreover, in human studies, the hepatocyte growth factor (HGF) has a proliferative effect on biliary epithelial cells. In rodents, biliary bile acid depletion and repletion appeared to regulate cholangiocyte proliferation and ductal bile secretion via a phosphatidylinositol 3-kinase pathway. Of interest, 3-dimensional imaging studies in rats have shown that bile duct proliferation precedes hepatic artery neovascularization. Indeed, recent studies (Fabris L., and Strazzabosco M., personal communication) indicate that cholangiocytes secrete angiogenic factors (i.e., vascular endothelial growth factor [VEGF], angiopeitins 1 and 2) that, in addition to paracrine signalling to the endothelium, are capable of autocrine stimulation of cholangiocyte proliferation.

Apoptotic Death and Ductopenia

As cholangiopathies advance in human, ductopenia (i.e., a decrease in the number of bile ducts per portal tract) predominates over proliferation, leading to a state of vanishing bile ducts, a phenomenon that describes most, if not all, late-stage cholangiopathies (Figure 1). Progressive disappearance of bile ducts in conjunction with portal fibrosis accounts for the majority of complications seen in cholangiopathies. Ductopenia heralds the terminal stages of most cholangiopathies and is considered the result of cholangiocyte loss prevailing over cholangiocyte proliferation. Thus, the sequence of events leading to ductopenia in cholangiopathies is of considerable importance. The role of apoptotic phenomena (i.e., programmed cell death) in bile duct morphogenesis is already established. Apoptotic mechanisms also contribute to tissue regression after experimentally induced biliary hyperplasia (e.g., disappearance of proliferating ducts following the relief of temporary biliary obstruction). It is currently believed that, under normal homeostatic conditions, there is a balance between apoptosis of senescent, damaged/abnormal cholangiocytes and the...
proliferation of new cholangiocytes (Figure 1). To this end, ductopenia may result primarily from excessive apoptosis (because of immune-mediated processes, infections, or toxins) that dominates over cholangiocyte proliferation. On the other end of the spectrum, inhibition of apoptosis may lead to cholangiocyte hyperplasia that could facilitate malignant transformation of cholangiocytes (see section on Neoplastic Transformation).

The exact role of apoptosis in human cholangiopathies is still unresolved. It must be understood, however, that the study of cholangiocyte apoptosis is challenging, given the transitory events involved in programmed cell death and the fact that apoptotic cells may be eliminated by shedding into the bile duct lumen. The molecular mechanisms that dictate apoptosis involve initiation and execution pathways that are controlled by an array of apoptosis regulators. Perhaps the most important apoptotic initiator in cholangiocytes is the Fas receptor/Fas ligand pathway. Human cholangiocytes express the Fas receptor. Given that Fas ligand is constitutively expressed on T-cytotoxic lymphocytes, cholangiocyte apoptosis may be initiated by a T-cell Fas-mediated mecha-
Apoptotic (i.e., Bcl-2, Bcl-xL, Mcl-1, Bfl-1) and located sis, and inflammation-related carcinogenesis.25–29 In fact, proliferation to apoptosis, cytotoxicity, cholestasis, fibrosis, and regulators of apoptosis may be relevant in developing new therapies for cholangiopathies. In an elegant animal study, NCX-100 (a nitric oxide [NO]-releasing derivative of ursodeoxycholic acid [UDCA]) was shown to inhibit of caspase activity.23 This novel pharmacological approach may have therapeutic potential in selected cholangiopathies.

Apoptosis is also regulated by the B-cell lymphoma/leukemia 2 (Bcl-2) protein family members,24 which are either proapoptotic (i.e., Bcl-xL, Bax, Bad, Bak) or antiapoptotic (i.e., Bcl-2, Bcl-xL, Mcl-1, Bfl-1) and located within different cellular compartments.24 Of interest, the expression of Bcl-2 in cholangiocytes has been shown to be regulated by glutathione (GSH), a natural antioxidant. Indeed, GSH depletion has been associated with decreased Bcl-2 expression and enhanced apoptosis in a human cholangiocyte cell line.18

Cholangiocytes and Inflammation

Cytokines and chemokines are thought to mediate many of the cellular events in the cholangiopathies, from proliferation to apoptosis, cytotoxicity, cholestasis, fibrosis, and inflammation-related carcinogenesis.25–29 In fact, most cholangiopathies are associated with significant inflammatory infiltrates in the portal spaces. For example, there is increased production of cytokines in the liver of PBC patients, including TNF-α30 and both T\(_{H1}\) (i.e., IL-2, IFN-γ) and T\(_{H2}\) (i.e., IL-4, IL-5, and IL-6) cytokines,31 with the T\(_{H1}\) pattern being predominant in advanced disease stages. These peptides and mediators act via both autocrine and paracrine pathways affecting cholangiocyte function. For instance, INF-γ promotes MHC class II expression in human cholangiocytes,32 affects the transport properties of cholangiocyte flux molecules, and stimulates NO production by cholangiocytes.27 Furthermore, IL-6 acts as a potent growth factor for rodent cholangiocytes.33 Finally, it is becoming increasingly apparent that cholangiocytes are not only targets for inflammatory mediators but also active contributors to liver inflammation and the ensuing reparative/fibrogenetic processes. Indeed, “reactive” cholangiocytes secrete proinflammatory and chemotactic cytokines and growth factors able to activate mesenchymal cells and matrix production (Figures 3 and 4).

The “Reactive” Cholangiocyte “Phenotype”

The presence of an increased number of cholangioles (i.e., the smallest of bile ducts) at the periphery of portal spaces is commonly seen in many forms of liver injury and is called the “ductular reaction.” These newly formed bile ducts are composed of “reactive” cholangiocytes, which abandon their differentiated epithelial phenotype, express adhesion molecules that are transiently present during ontogenesis such as the neural cell adhesion molecule (NCAM),34 turn on antiapoptotic genes (i.e., Bcl-2), and acquire the ability to produce proinflammatory and chemotactic mediators such as TNF-α, IL-6, IL-8, monocyte chemotactic protein-1 (MCP-1), cytokine-induced neutrophil chemoattractant (CINC) and NO (Figure 3). Reactive cholangiocytes also produce growth factors able to activate mesenchymal cells and matrix production such as human growth factor (HGF), platelet derived growth factor-BB (PDGF-BB), connective tissue growth factor (CTGF), endothelin-1 (ET-1) and transforming growth factor-β2 (TGF-β2)34,35 (Figure 3). These peptides and mediators enable the cholangiocyte to communicate extensively with other liver cells, including hepatic stellate cells (HSC), portal fibroblasts, inflammatory and endothelial cells (Figure 4). Interestingly, cholangiocytes do not normally produce these autocrine/paracrine factors. Thus, reactive cholangiocytes likely play an important role not only in reparative bile duct processes but also in the progression of chronic liver damage by acting as “the pace-maker of portal fibrosis,” as suggested earlier by Desmet.36 The histogenesis of reactive cholangiocytes has been long debated. Most likely, cholangiocytes are derived from

### Table 1. Classification of Cholangiopathies

<table>
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<td>Alagille’s syndrome</td>
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<td>Cystic fibrosis</td>
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<td>Fibropolycystic diseases (i.e., Caroli’s syndrome, congenital hepatic fibrosis, ADPKD, ARPKD, ADPLD)</td>
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<td>Immune-mediated</td>
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<td>Primary biliary cirrhosis</td>
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<td>Primary sclerosing cholangitis</td>
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<td>Hepatic allograft rejection</td>
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<td>Graft vs. host disease involving the liver</td>
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<td>Autoimmune cholangitis</td>
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<td>Infectious</td>
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<td>Bacterial cholangitis</td>
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<td>Parasitic cholangitis</td>
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<td>Fungal cholangitis</td>
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<td>Viral cholangitis (i.e., AIDS cholangiopathy)</td>
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<td>Drug-induced (i.e., Floxuridine-induced cholangiopathy)</td>
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<td>Vascular/Ischemic (i.e., postliver transplantation hepatic artery stenosis)</td>
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<td>Idiopathic</td>
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<td>Biliary atresia</td>
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<td>Sarcoidosis</td>
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<td>Idiopathic childhood/adulthood ductopenia</td>
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<td>Malignant</td>
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<td>Cholangiocarcinoma (i.e, bile duct adenocarcinoma)</td>
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ADPKD, Adult dominant polycystic kidney disease; ARPKD, Adult recessive polycystic kidney disease; ADPLD, Adult dominant polycystic liver disease.
liver progenitor cells that are interspersed between cholangioles and the canals of Hering. Recent work in humans has clarified that the canals of Hering extend into the proximal third of the hepatic lobule, and this compartment expands following liver injury.37–39 In rodents, progenitor cells generate either intermediate hepatocytes or reactive cholangiocytes that are activated when damage to the epithelial cells of the liver (i.e., hepatocytes or cholangiocytes) is combined with impaired epithelial regeneration. In humans, recent studies have indicated that the progenitor cell compartment is activated not only in fulminant hepatitis but also in chronic conditions such as alcoholic liver disease,40 nonalcoholic fatty liver disease,40 and chronic viral hepatitis.41

Cholestasis

Consistent with the role of cholangiocytes in bile production (Figure 1), chronic cholestasis (i.e., decreased bile production) is a universal manifestation of cholangiopathies. Cholangiocyte cholestasis may originate from (1) altered secretory responses induced by inflammatory mediators; (2) derangement of cholangiocyte plasma membrane flux molecules expression/function; (3) fibroin inflammatory obliteration of bile ducts; and/or (4) retention of hydrophobic bile acids causing hepatocyte apoptosis and necrosis resulting in hepatocellular cholestasis.

In immune-mediated cholangiopathies such as PBC and PSC, recent studies have shown that inflammation of bile ducts affects cholangiocyte transport function.26,42 In vitro, proinflammatory cytokines impair rat cholangiocyte cAMP-dependent Cl⁻ transport and bicarbonate transport without directly affecting AE2 or CFTR expression.26 Ca²⁺-dependent Cl⁻ channels and their stimulation by ATP are not affected by cytokine treatment, suggesting specific involvement of the cAMP/protein kinase A (PKA) signal transduction pathway. Indeed, cytokines impair adenylate cyclase activity via up-regulation of nitric oxide synthases (NOS2, iNOS) and formation of reactive nitrogen oxide species (RNOS). In

Figure 3. In pathologic conditions, cholangiocytes become “reactive.” Indeed, biliary epithelia abandon their differentiated epithelial phenotype, acquire neuroendocrine features, express adhesion molecules, and have the ability to produce and secrete proinflammatory and chemotactic mediators. “Reactive” cholangiocytes also produce growth factors able to activate mesenchymal cells and matrix production. Thus, “reactive” cholangiocytes likely play an important role not only in hepatic reparative processes but also in the progression of chronic liver damage and act as “the pace-maker of portal fibrosis.” (A) Transmission electron micrograph (TEM) showing the ultrastructural appearance of septal normal cholangiocytes immunolabeled using anti-egp35 antibodies (courtesy of Fabris et al.34). (B) TEM showing the ultrastructural appearance of “reactive” cholangiocytes immunolabeled from cholestatic human livers using anti-NCAM antibodies (courtesy of Fabris et al.34). M3 Ach-R, M3 acetylcholine receptors; NGF, nerve growth factor; NCAM, neutral cell adhesion molecule; ICAM-1, intracellular adhesion molecule 1; MHC II, major histocompatibility complex-class II molecules; TNF-α, tumor necrosis factor-α; IL-6 and IL-8, interleukin 6 and 8; MCP-1, monocyte chemotactic protein-1; CINC, cytokine-induced neutrophilic chemoattractant; HGF, human growth factor; PDGF-BB, platelet-derived growth factor-BB; CTGF, connective tissue growth factor; ET-1, endothelin-1; TGFB2, transforming growth factor-β2; NO, nitric oxide.
chronic cholestasis, accumulation of hydrophobic bile acids induces cholangiocyte proliferation, whereas the hydrophilic bile acid UDCA has a growth inhibitory effect via a protein kinase C (PKC-α) pathway.

**Portal Fibrosis**

In most cholangiopathies, an extensive fibrotic response takes place in the portal tracts (Figure 1).  

Fibrogenesis is a dynamic process that depends on the extent and duration of cholangiocyte damage. Hepatic stellate cells (HSC) are the main connective tissue-producing cells in liver and, during tissue repair, undergo activation from quiescent to highly proliferative myofibroblast phenotype. This process is regulated by the interplay of extracellular matrix components, polypeptide growth factors, cytokines, and other soluble mediators (Figure 4). Because fibrosis is the result of prolonged activation of tissue repair mechanisms, marked liver fibrosis is present in late-stage cholangiopathies. On the contrary, most cases of acute biliary obstruction resolve without fibrosis.

Several lines of evidence indicate that reactive cholangiocytes play an active role in stimulating the fibrogenic response, possibly by activating the usually quiescent portal fibroblasts and HSC (Figure 4). In animal models of liver fibrosis, studies show that cholangiocytes produce paracrine factors such as TGF-β, ET-1, PDGF-BB, TGF-β2, NO, and CTGF and are able to stimulate HSC activation and matrix production (Figure 4). Furthermore, cholangiocytes synthesize basement membrane proteins and collagen type IV. The close association between cholangiocyte proliferation and mesenchymal activation is also present in cholangiocarcinoma, a neoplasm that shows a strong desmoplastic reaction.

**Neoplastic Transformation**

Neoplastic transformation of cholangiocytes may lead to the development of either benign (i.e., adenomas, cystadenomas) or malignant (i.e., cholangiocarcinomas, cystadenocarcinomas) tumors. Among them, cholangiocarcinoma is the most serious, and known risk factors in man include (1) history of PSC; (2) use of thorium dioxide (i.e., Thorotrast); (3) anabolic steroid administration; (4) congenital fibropolycystic liver diseases such as choleddochal cysts and Caroli’s disease; and (5) chronic infestation of bile ducts with the parasitic flukes Opisthorchis viverrini and Clonorchis sinensis.

To date, the precise pathways responsible for the malignant transformation of cholangiocytes remain unknown. It is generally accepted, however, that chronic inflammation of bile ducts is a risk factor for the development of cholangiocarcinoma. This situation parallels that seen in the chronic inflammation of colonic mucosa that occurs in chronic ulcerative colitis, a well-known risk factor for adenocarcinoma of the colon. In humans, current evidence suggests that biliary dysplasia precedes the development of cholangiocarcinoma. Furthermore, a number of factors may facilitate the progression of chronic inflammation to malignant transformation.

For example, chronic inflammation causes cytokines release from cholangiocytes and inflammatory cells. Proinflammatory cytokines (i.e., IL-6) can induce expression of the nitric oxide synthase (iNOS) in cholangiocytes during chronic inflammation. Then, iNOS generates the bioreactive molecule NO, which subsequently may result in...
in the formation of RNOS. In addition to causing DNA damage, NO and RNOS can directly inhibit DNA repair enzymes such as human 8-oxodeoxyguanosine DNA glycosylase 1 and block apoptosis via nitrosylation of caspases. These molecular and cellular events allow DNA damage to accumulate, potentially leading to the multiple mutations necessary for development of invasive bile duct cancer. Other human studies have shown that NO also promotes cancer progression by functioning as an angiogenic factor. Of interest, immunohistochemistry in human liver samples from PSC patients revealed de novo expression of iNOS and the presence of 3-nitrotyrosine (i.e., a reactive nitrogen oxide species) and 8-oxoguanine (i.e., a marker of oxidative DNA damage) formation in cholangiocytes compared with control livers. Strategies to inhibit NO generation during chronic inflammation of bile ducts or to scavenge RNOS may prove useful not only to improve bile secretion but also to decrease the risk of biliary malignancies.

Although the inflammatory environment contributes to malignant transformation, an array of molecular alterations occurs in cholangiocarcinomas. For example, overexpression of key molecules including cyclooxygenase (COX)-2, the receptor tyrosine kinases, c-erbB-2, c-Met (hepatocyte growth factor receptor) has been reported in cholangiocarcinomas. To this end, pharmacologic inhibitors of such molecules could have a therapeutic potential in bile duct malignancies. Other molecular modifications during malignant transformation of the bile ducts include K-ras mutations, loss of heterogeneity for p53 (tumor-suppression gene), inactivation of pathways involved in cell-cycle control, and increased expression of telomerase activity to sustain continuous cell replication. To date, the regulation of cell growth in normal and transformed cholangiocytes is being investigated using in vitro models.

**Interactions With Microorganisms**

The human biliary tree is sterile under normal conditions despite its communication with the small intestine, which is populated by an endogenous bacterial flora. The presence of IgA and low concentration of glucose in bile, in addition to mechanical barriers such as the sphincter of Oddi, likely support the sterility of the biliary tree. Disruption of the normal biliary architecture (i.e., strictures, stones) or congenital malformations of the bile ducts (i.e., Caroli’s disease) results in infection (i.e., cholangitis).

It has been postulated that cholangiopathies such as PBC and PSC may be caused by bacterial or viral invasion of the biliary tree; however, this remains speculative. Of interest, however, AIDS patients and patients affected by CD154 deficiency (X-linked immunodeficiency with hyper IgM) develop a cholangiopathy characterized by the cholangiographic findings similar to those seen in patients with PSC (i.e., localized strictures, segmental ectasias). Among the infectious agents isolated from the bile ducts of AIDS patients (i.e., Cryptosporidium, Microsporidium, Cytomegalovirus, Mycobacterium avium complex, and Cyclospora) Cryptosporidium parvum is the most common, and its interaction with cholangiocytes has been extensively investigated. In an in vitro model of cultured human cholangiocytes infected with C. parvum, the microorganism (1) attaches to the cholangiocyte membrane using specific parasite surface lectins; (2) enters the cholangiocyte via activation of actin-associated proteins and host cell cytoskeletal remodeling; (3) is cytotoxic to bystander-uninfected cholangiocytes adjacent to the infected ones via a Fas receptor/Fas ligand-dependent apoptotic mechanism; and (4) inhibits apoptosis in infected cholangiocytes by activation of the NF-kB survival pathway, thus maintaining the infectivity. Of interest, in animal models using immunodeficient mice infected with C. parvum, T-cell cytokines are necessary for induction of bile duct inflammation and sclerosis.

**Drug Metabolism**

Despite an increasing list of drugs that can cause nonsuppurative cholangitis and ductopenia, little is known about the pathophysiology of these events. Rodent cholangiocytes possess enzymes of both phase I and phase II metabolic pathways. Phase I or mixed function oxygenase enzymes such as the microsomal P450 system and aminopyrine-N-demethylases possess oxidative metabolic activities. Phase II metabolizing enzymes known as glutathione (GSH) redox cycle enzymes such as GSH-peroxidase, UDP-glucuronosyltransferase, and glutathione-S-transferase have conjugating properties. Interestingly, phases I and II enzymes are heterogeneous expressed along the human biliary tree (intra- or extrahepatic bile ducts) and gallbladder, an observation that may provide an explanation for the heterogeneous susceptibility of cholangiocytes to drugs and toxins. For example, damage of large, but not small, rat cholangiocytes in response to CCl₄ administration results in part from the presence of P4502E1 enzyme exclusively in large rodent cholangiocytes. In addition, many xenobiotics undergo a cholehepatic circulation, being either secreted through apical cholangiocellular MDR-1-like carrier proteins or taken up by the apical, Na⁺-dependent bile acid transporter (ASBT). Thus, elucidation of the transport mechanisms and metabolic pathways of cholangiocytes in a site-restricted fashion (i.e., small vs.
large bile ducts) may prove instrumental in understanding the pathophysiology of drug-induced cholangiopathies and in devising novel diagnostic and therapeutic approaches for biliary diseases.65

**Lessons From Archetype Cholangiopathies**

**Obstructive Cholestasis and Experimental Bile Duct Ligation**

Obstructive cholestasis is frequent in clinical practice, the etiology of obstruction ranging from common causes like bile duct stones to rare malformative diseases such as biliary atresia.68 A useful animal experimental model of obstructive cholestasis is common bile duct ligation (BDL).69 Major changes noted in cholangiocytes after BDL include (1) induction of an intense ductal proliferative response (i.e., increase of cholangiocyte mass in liver); (2) stimulation of ion secretory properties (i.e., increase of basal and secretin-induced ductal bile secretion); (3) rapid establishment of portal fibrosis; and (4) over-expression of the apical, Na+-dependent bile acid transporter (ASBT), thus facilitating cholangiocyte uptake of potentially toxic bile acids from bile.

In the rat, during the first week following experimental BDL, there is up-regulation of growth factors (i.e., HGF, EGF) and chemokines (i.e., IL-6, CINC) and their receptors (i.e., cMet), along with increased cholangiocyte proliferation, periportal inflammatory cells/myofibroblasts infiltration, and rapid, vigorous fibrotic reaction. Throughout this early phase, increased expression of VEGF, basic fibroblast growth factor (bFGF), IL-6 receptor, and NF-κB activation are observed stimulating neoangiogenesis, resistance to apoptosis, and maintenance of liver hepatocyte mass.70 In the chronic phase (2nd to 12th week) following BDL, cholangiocyte proliferation decreases, and profibrogenic factors such as PDGF-BB, TGF-β2, and bFGF increase. During this chronic phase as a result of extensive cross talk between epithelial, mesenchymal, and endothelial cells, the portal tract is transformed into an expanding cholangiocyte/mesenchymal wedge that gradually remodels the liver architecture forming portal-portal septa that lead to biliary cirrhosis.

Overall, patients with biliary cirrhosis maintain good hepatocellular function. Nevertheless, over time, loss of hepatocyte function becomes evident, likely caused by bile acid-induced hepatocellular apoptosis and necrosis. An important growth factor in the proliferative response to BDL is IL-6. The increased mortality following BDL in IL-6 knockout mice seems to be due to an inability to maintain hepatocellular mass that, in these conditions, is also dependent on IL-6.13,55 Studies in NF-κB knockout mice indicate that activation of this transcription factor is also very important in protecting hepatocytes from bile acid-induced apoptosis.71

**Ductal Plate Malformations**

Congenital diseases of the intrahepatic bile ducts are caused by failure of the physiologic ductal plate remodeling during embryonic development of the biliary system and, therefore, are classified as ductal plate malformations (DPM). Morphologically, DPM are characterized by necroinflammatory destruction of intrahepatic bile ducts (e.g., fetal-type bile duct atresia) or by bile duct ectasia accompanied by a variable degree of portal fibrosis (e.g., fibropolycystic diseases, congenital hepatic fibrosis, Caroli’s disease).34

During ontogenesis, remodeling of the ductal plate requires sequential, regulated events, starting around the eighth gestational week when the primordial single layer ductal plate is formed by a phenotypic switch of the hepatoblasts located along the portal interface surrounding the portal vein branches. The primordial ductal plate is in part duplicated, whereas the remaining single layer portions are deleted by apoptosis. The duplicated structures dilate and form a tubular channel, which is incorporated into the portal mesenchyme and later undergoes a branching process to form the biliary tree. This branching process in the bile duct system may be hampered, resulting in persistence of embryonic biliary structures retaining a ductal plate phenotype. Factors controlling ductal plate remodeling are under investigation. Epithelial-mesenchymal interactions play a crucial role in inducing the differentiation process of ductal plate cells.53,72 Angiogenic factors are also likely to play a relevant role because DPM are frequently associated with abnormalities in the ramification pattern of vasculature, a basic morphologic feature known as pollard willow pattern.36 A prototypic example of DPM is the cholangiopathy associated with autosomal dominant polycystic kidney disease (ADPKD). The responsible genetic defects have been identified as mutations in the PKD1 and PKD2 genes encoding polycystin-1 and polycystin-2, respectively.73,74 Although polycystin-1 is a surface glycoprotein that mediates cell-cell and cell-matrix interactions, polycystin-2 represents a cation channel. These two proteins work in concert to regulate the growth and differentiation of tubular structures in the kidney and biliary tree. Epithelial cells lining the liver cysts show signs of immaturity, express adhesion molecules and a number of vascular growth factors that are reminiscent of ductal plate cells and are also important in stimulating the growth of the cysts (Fabris L., and Strazzabosco M.,
personal communication), the process that ultimately leads to clinical liver disease.

Important genetic advances have also been made in autosomal recessive polycystic kidney disease (ARPKD). Investigators have discovered mutations in \textit{PKHD1}, a gene encoding for a large, receptor-like protein called \textit{fibrocystin} that is involved in renal collecting and biliary ducts differentiation in a similar fashion to polycystins.\footnote{75}

An important recent breakthrough was the demonstration that fibrocystin and polycystin are located in the cilia of rodent cholangiocytes. These molecules are properly positioned to function as mechanoreceptors able to regulate the secretory activities of the biliary epithelium and the expression of proteins involved in cell differentiation.\footnote{76–78} Mutations in the human homologue of fibrocystin are also present in congenital hepatic fibrosis (CHF). Recently, an autosomal dominant polycystic liver disease (ADPLD) phenotype has been described, in which cysts are confined exclusively within the hepatic parenchyma and shown to be genetically distinct from ARPKD.\footnote{79} ADPLD has been linked to the \textit{PRKCSH} gene on chromosome 19, which encodes for a 59-kilodalton protein called \textit{hepatocystin} that may be involved in controlling the cell growth or differentiation.\footnote{79}

**Cystic Fibrosis-Associated Cholangiopathy**

Although several genetic defects of hepatocyte flux molecules are known to cause intrahepatic cholestasis, cystic fibrosis-associated cholangiopathy is the only known inherited disorder affecting a cholangiocyte flux molecule. Cystic fibrosis (CF), a disease of recessive inheritance, results from a number of mutations in the gene encoding for CFTR, a low conductance cAMP-activated \textit{Cl}⁻ channel (Figure 2). In the liver, CFTR expression is restricted to cholangiocytes and participates in the choleretic effects of secretin.\footnote{80} In CF patients, CFTR deficiency leads to pulmonary and pancreatic disease and, less commonly, to liver disease. Clinical hepatic decompensation is infrequent, albeit cholangiocyte damage can be demonstrated in asymptomatic cases well before hepatocellular damage appears. Thus, CF constitutes a model disease in which altered cholangiocyte \textit{Cl}⁻ transport causes biliary pathology.

Lack of CFTR has a major impact on cell function. In addition to its role as cAMP/PKA activated \textit{Cl}⁻ channel, CFTR also participates in the regulation of other membrane transport proteins, such as \textit{Na⁺} channels (EnaC), \textit{K⁺} channels, outward rectifying \textit{Cl}⁻ channels (ORCC), and \textit{Cl}/\textit{HCO₃} exchangers (AE2). CFTR also appears to regulate cellular secretion of ATP, intracellular vesicle acidification, processing, and trafficking of certain proteins, including secretion of mucins. The impact of CFTR dysfunction on \textit{HCO₃⁻} secretion is key to understanding the CF pathogenesis, as recently suggested by Quinton.\footnote{81} In addition to an electrogenic component because of CFTR-mediated \textit{HCO₃⁻} conductance, an electroneutral pathway requires the coordinated activity of an \textit{Cl}⁻/\textit{HCO₃⁻} exchange and is closely associated with carbonic anhydrase, an enzyme highly expressed in cholangiocytes.\footnote{3}

Studies in cholangiocytes isolated from CF patients undergoing liver transplantation show reduced cAMP-stimulated \textit{Cl}⁻ efflux and impaired \textit{HCO₃⁻} secretion.\footnote{82} The resulting reduced bile hydration and alkalinity likely precipitates a series of events that damage cholangiocytes, including retention of cytotoxic bile acids that may lead to biliary cirrhosis. Alternative secretory mechanisms and signals, such as purinergic activation of apical \textit{Ca²⁺}-dependent \textit{Cl}⁻ channels, can restore \textit{HCO₃⁻} secretion in CF cholangiocytes in vitro.\footnote{82} These observations provide a theoretical framework to development of novel treatments of pancreatic and biliary complications in patients with CF.

**Multidrug Resistance-3 Deficiency**

In humans, the multidrug resistance-3 gene (MDR-3; in mice the equivalent gene is termed \textit{mdr}-2) encodes for a phospholipid flippase and is expressed on the apical membrane of hepatocytes. Defects in MDR-3 cause a wide spectrum of human liver diseases ranging from familial cholestasis to intrahepatic lithiasis to cryptogenic biliary cirrhosis.\footnote{83} Cholangiocytes are able to absorb, recirculate, and metabolize bile acids through specific bile acid transporters\footnote{67} (Figure 2) and the periductular capillary plexus.\footnote{84} The cholangiocyte is also a potential target for bile acid toxicity as shown by the presence of extensive ductular proliferation with portal inflammation, fibrosis, and cell damage in the \textit{mdr}-2-deficient mouse, an animal model for progressive familial cholestasis type III in man (MDR3 deficiency). Accurate morphologic studies in \textit{mdr}-2-deficient mice have shown biliary changes resembling those seen in human sclerosing cholangitis.\footnote{85,86} In these conditions, absence of phospholipid secretion into bile due to lack of hepatocyte \textit{mdr}-2 exposes cholangiocytes to the unrestrained effects of high monomeric concentrations of bile acids. Altered biliary epithelium tight junctions and ruptures of the basal membrane permit leakage of bile acids into the portal tract with induction of portal inflammation and fibrosis.\footnote{86}

**PBC**

PBC is a chronic, progressive, cholestatic liver disease because of immune-mediated destruction of bile ducts. PBC affects primarily middle-aged women and presents with a cholestatic pattern of liver enzymes, usually a 2- to 3-fold elevation of alkaline phosphatase and mild increases of AST and ALT.\footnote{87} To date, the
pathogenesis of PBC remains unclear. We refer the reader to recent comprehensive reviews about PBC pathogenesis, clinical presentation, and natural history of the disease.87–90

Here, we focus on the involvement of cholangiocytes in the pathogenesis of PBC. Evidence suggests that altered immunity induced by putative infectious agents91,92 and/or xenobiotics93–96 is a likely pathogenetic pathway in PBC. As a result, both autoantibodies and reactive T cells are produced. Serum positivity of antimitochondrial antibodies (AMA) with specificity for the inner lipoyl domain of pyruvate dehydrogenase complex subunit 2 (PDC-E2)97,98 is highly specific for PBC, and, whereas PDC-E2 antigen is present in all cell types, only secretory epithelia, and particularly cholangiocytes, are affected in PBC. The role of cholangiocytes in biliary secretion of IgA and the finding of IgA-PDC immune complexes in the bile of PBC patients may provide a clue to explain this tissue specificity. In patients with PBC, AMA react against the apical membrane of cholangiocytes,99 suggesting that their molecular target is either a mistargeted or truncated form of PDC-E2 immune complexes or a cross-reactive epitope derived from an extrinsic molecule. If this was the case, its presentation by MHC class II molecules would allow activation of CD4+ T cells and, consequently, of the invading effector CD8+ T cells. However, because B7 costimulatory molecules seem not to be expressed during the early stages of the disease, it is unclear to what extent cholangiocytes may act as professional antigen-presenting cells. Studies have reported a heterogeneous response of AMA that react with the apical region of cholangiocytes, suggesting that their molecular target is either a mistargeted or truncated form of PDC-E2 immune complexes or a cross-reactive epitope derived from an extrinsic molecule. If this was the case, its presentation by MHC class II molecules would allow activation of CD4+ T cells and, consequently, of the invading effector CD8+ T cells. However, because B7 costimulatory molecules seem not to be expressed during the early stages of the disease, it is unclear to what extent cholangiocytes may act as professional antigen-presenting cells. Studies have reported a heterogeneous response of AMA that react with the apical region of cholangiocytes, thus making the hypothesis of cross-reaction because of molecular mimicry less appealing.100 Furthermore, the bile of PBC patients contains AMA belonging to the IgA class, and damage is restricted to cells able to transport IgA transepithelially. These observations have prompted the hypothesis that IgA AMA may penetrate the cholangiocyte and induce damage by preventing the correct targeting of PDC-E2 and, thus, interfere with mitochondrial function, an effect that may also result in apoptotic cell death.101,102 This sequence of events remains speculative and awaits additional experimental evidence.

**Proposed Pathogenetic Pattern of Cholangiopathies and Future Directions**

In the last decade, we have witnessed substantial progress in understanding the cholangiocyte structure and function because of the development and implementation of novel experimental models. Also key to this progress was the now widely accepted concept that the cholangiocyte is a dynamic participant in normal liver function and not simply a component of passive conduits for delivery of hepatic bile to the intestine (Figures 1 and 2). Furthermore, we now appreciate the relevance of cholangiocyte as the cell target of cholangiopathies. Although the mechanisms underlying the development of acquired cholangiopathies are still emerging, we can propose a common pathogenetic pathway. Figure 5 illustrates a conceptual platform for the proposed pathogenetic model(s) of acquired cholangiopathies. In this working hypothesis, the putative initial insult of biliary epithelial cells may be an interaction with an endogenous or exogenous substance and/or microorganism. The host’s initial response is perhaps an inflammatory reaction. It is anticipated that, in most cases, the inflammatory response is resolved, leading to resolution of the insult/damage to the biliary tree. Derangement of the host’s response is, however, likely dependent on putative genetic susceptibility, and other yet unknown factors may result in perpetuating this initial inflammatory response, leading to chronic inflammation of bile ducts and ultimately to the development of cholestasis, bile duct proliferation/ductopenia, and biliary/hepatic fibrosis, including potential malignant transformation of bile ducts. An interplay of these abnormal phenomena characterizes the clinical presentation and natural history of most cholangiopathies. OLT, orthotopic liver transplantation.
advanced complications of acquired cholangiopathies (Figure 5).

Future research toward the presumed interaction of environmental elements(s) with the genetically programmed host responses will almost certainly shed light on the pathogenesis of cholangiopathies. Possessing such knowledge will provide us with the springboard to develop preventive and therapeutic strategies for the cholangiopathies.

References
