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Pathogenesis of Cholestatic Liver Disease and Therapeutic Approaches

GIDEON M. HIRSCHFIELD,[*,‡] E. JENNY HEATHCOTE,[*,‡] and M. ERIC GERSHWIN[§]

[*]Liver Centre, Toronto Western Hospital, Toronto, Ontario, Canada; [‡]Department of Medicine, University of Toronto, Toronto, Ontario, Canada; and [§]Division of Rheumatology, Allergy and Clinical Immunology, University of California, Davis, California

Cholestatic liver disorders are caused by genetic defects, mechanical aberrations, toxins, or dysregulations in the immune system that damage the bile ducts and cause accumulation of bile and liver tissue damage. They have common clinical manifestations and pathogenic features that include the responses of cholangiocytes and hepatocytes to injury. We review the features of bile acid transport, tissue repair and regulation, apoptosis, vascular supply, immune regulation, and cholangiocytes that are associated with cholestatic liver disorders. We now have a greater understanding of the physiology of cholangiocytes at the cellular and molecular levels, as well as genetic factors, repair pathways, and autoimmunity mechanisms involved in the pathogenesis of disease. These discoveries will hopefully lead to new therapeutic approaches for patients with cholestatic liver disease.

Keywords: Primary Biliary Cirrhosis; Primary Sclerosing Cholangitis; Cholestasis; Ursodeoxycholic Acid.

Cholestatic liver diseases arise from impaired hepatobiliary production and excretion of bile, which cause bile constituents to enter the circulation. In these disorders, injuries to bile ducts or hepatocytes can lead to a range of clinical presentations, from isolated abnormalities in liver biochemistry, to liver failure or hepatobiliary malignancy; congenital, immunologic, structural (obstructive/vascular), and toxic factors can all contribute to disease (Figure 1 and Table 1). In response to injury, mature cholangiocytes and hepatocytes proliferate, which may lead to periductular fibrosis, biliary fibrosis, and cirrhosis. Disease progression and the efficacy of repair depend on etiology and the individual's response to injury. We review mechanisms and biliary pathophysiology of cholestatic liver disease, focusing on primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC).

Cell Biology of Cholestasis

Approximately 5% of cells in the liver are cholangiocytes; these ciliated epithelial cells line the biliary tree, an intricate network of interconnecting bile ducts that increase in diameter from the ducts of Hering to the extrahepatic bile ducts.[1,2] Cholangiocytes that line the large interlobular and major ducts are predominantly involved in secretory functions, whereas cholangiocytes that line smaller bile duct branches, cholangioles, and ducts of Hering have roles in inflammatory and proliferative responses.[3,4] Each cholangiocyte has a primary cilium that extends from the apical plasma membrane into the ductal lumen and regulates mechanosensory, osmosensory, and chemosensory functions.[5] Cilia detect and signal changes in bile flow and osmolality.[6]

Secretory Function and Bile Acid Transport

Cholangiocyte stimulation through paracrine and endocrine routes leads to secretion of water and biliary alkalinization with a net luminal flux of chloride and bicarbonate.[7] The process of integrating prosecretory (secretin, glucagon, vasoactive intestinal polypeptide, acetylcholine, bombesin) and antisecretory (somatostatin, endothelin-1) stimuli involves transmembrane adenylyl cyclases that regulate the concentration of intracellular 3′, 5′-cyclic monophosphate.[8–10] Basolateral (sinusoidal) and canalicular (apical) transport proteins move bile acids from sinusoidal blood into the canaliculus in a highly efficient, regulated enterohepatic circulation.[11,12] Nuclear receptors regulate transcription of genes that encode proteins involved in hepatobiliary transport systems, bile acid synthesis, bile acid detoxification,[13] and fibrogenesis.[14,15] These nuclear receptors include the farnesoid X receptor, pregnane X receptor, vitamin D receptor, and constitutive androstane receptor. Bile acids are internal-

Abbreviations used in this paper: AMA, antimitochondrial antibody; GI, gastrointestinal; IL, interleukin; MADCAM, mucosal addressin cell adhesion molecule; MDR, multi-drug–resistant; NK, natural killer; PBC, primary biliary cirrhosis; PDC-E2, E2 component of the pyruvate dehydrogenase complex; PFIC, progressive familial intrahepatic cholestasis; PSC, primary sclerosing cholangitis; Treg, T regulatory cell.

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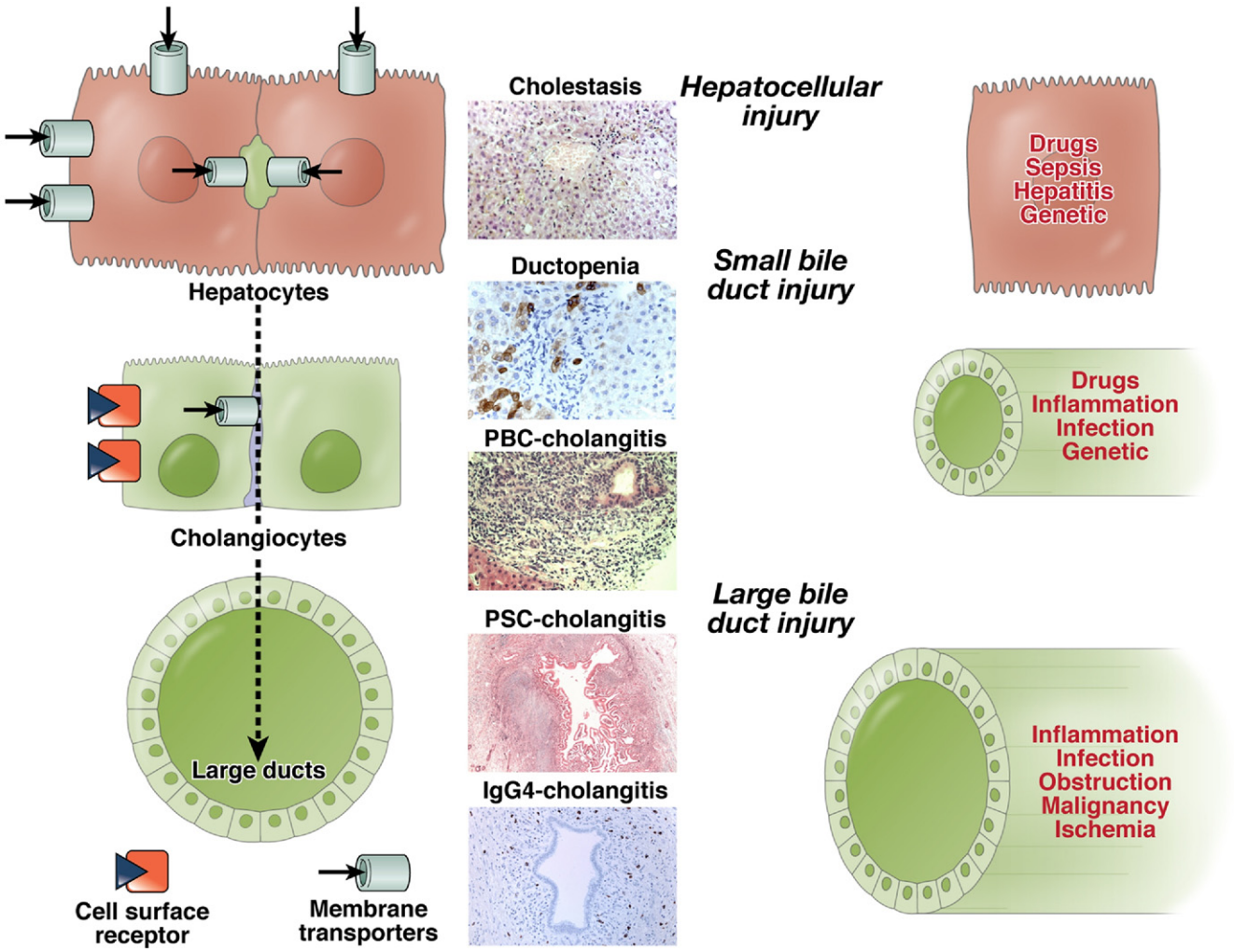

Figure 1. Cholestasis and hepatobiliary injury. Etiologies of cholestatic liver disease related to the site of hepatobiliary insult. (*Left panel*) Bile production is a complex process that involves hepatocytes and cholangiocytes; a number of different bile acid transporters coordinate bile formation (reviewed by Zollner and Trauner[11]), with various cell surface receptors on cholangiocytes that regulate cholangiocyte secretion and function (reviewed by Glaser et al and Marzioni et al[30,31]). (*Middle panel*) Histologic representation of cholestatic liver disease illustrating bland hepatic cholestasis in drug injury, the small bile duct lymphocytic cholangitis of PBC, ductopenia as illustrated by immunostaining for keratin, the classic large bile duct involvement seen in PSC (obliterative cholangitis), and immunochemical staining showing IgG4-positive plasma cells surrounding a bile duct in IgG4-associated cholangitis (Histology images are courtesy of Dr M Guindi and Dr S Fischer, Toronto General Hospital, Toronto, Cananda). (*Right panel*) Cholestasis develops after injury to hepatocytes and small and large bile ducts.

ized across the basolateral membranes of hepatocytes by the Na^+/taurocholate cotransporter and organic anion transporting proteins (OATP2/OATP1B1); this transporter also mediates the hepatic uptake of many drugs.[16] Genetic polymorphisms in the gene encoding this transporter, *SLCO1B1*, associate with total bilirubin levels in healthy individuals[17] and development of statin-induced myopathy.[18] Active export into bile is mediated by the canalicular bile salt export pump (ABCB11)[19] and the canalicular conjugate export pump (MRP2).[20] MRP2 regulates canalicular excretion of organic anions, such as bilirubin. Formation of mixed micelles in bile results from the presence of bile acids, cholesterol, and phosphatidylcholine, and the phospholipid export pump, multi-drug– resistant 3 protein (MDR3), is actively involved in controlling the process.[21]

Cytokines and Receptors

The cholangiocytes in the smaller bile ducts, cholangioles, and ducts of Hering express receptors that allow proliferation in response to liver damage and participation in inflammatory responses.[22,23] These receptors mediate protection from pathogens (via signals from antimicrobial peptides or the Toll-like receptors 2, 3, 4, and 5, which bind to bacterial molecules, double-stranded RNA, gram-negative bacteria, and lipopolysaccharide[24]), antigen presentation (HLA molecules and costimulatory molecules[25–27]), leukocyte recruitment (adhesion molecules such as intercellular adhesion molecule 1, leukocyte factor antigen 3, and CD40, cytokines, and chemokines[28,29]), and leukocyte apoptosis. Reactive cholangiocytes also produce growth factors such as vascular endothelial

Table 1. The Characteristics of Common Cholestatic Syndromes

	Primary biliary cirrhosis	Primary sclerosing cholangitis	Drug induced cholestasis	Biliary atresia
Demographics	Female predominant; middle age onset; geographic hotspots; co-existent autoimmunity; Asymptomatic screening now most common mode of presentation	Men more than women; onset before 40 years old common; high prevalence of co-existent colitis; Identified commonly through asymptomatic screening in inflammatory bowel disease; may present with cholangitis	10% of drug induced liver injury is cholestatic; more common in elderly; jaundice and pruritus seen	Exclusively neonatal; Isolated in 65%–90%; Presents with persistent neonatal conjugated hyperbilirubinemia >14 days old
Key diagnostics	Anti-mitochondrial antibody (AMA) positive >95%; confirm immunofluorescence with specific AMA assay[a]	Cholangiographic appearance of stricturing and beading of intra- and/or extra-hepatic bile ducts; MRI largely supplanted ERCP as initial diagnostic modality	History of offending agent[b] and exclusion of alternative etiologies	Liver biopsy differentiates obstructive and hepatocellular causes of cholestasis, with 90% sensitivity and specificity; cholangiography if diagnostic doubt (patent biliary tree proximally and distally excludes biliary atresia)
Characteristic pathology	Granulomatous non-suppurative small duct cholangitis, ductopenia, ductal proliferation, interface hepatitis Biopsy is not required routinely for diagnosis or staging	Fibro-obliterative cholangitis, periductal fibrosis and inflammation, absence of bile ducts in some portal tracts and ductal proliferation in other portal tracts Biopsy is not required routinely for diagnosis or staging	Bland cholestasis or cholestatic hepatitis >Biopsy commonly performed to exclude alternative etiologies	Active inflammation with bile duct degeneration, a chronic inflammatory reaction with proliferation of both ductular and glandular elements, and fibrosis Biopsy commonly performed
Overlapping features	Anti-nuclear antibodies present in 50%[c] and possible overlap with autoimmune hepatitis (AIH) in <10%	Autoimmune serology frequently positive; pediatric onset AIH is accompanied by sclerosing cholangitis in 50% of cases; overlap with AIH in adults <10%.	Non-specific serology may be present	Embryonic form in 10%–35% with associated situs inversus or polysplenia/asplenia +/- other congenital anomalies
Natural history and treatment	Progressive biliary cirrhosis, portal hypertension and liver failure	Recurrent cholangitis, progressive biliary cirrhosis, portal hypertension and liver failure; malignancy (hepatobiliary/colorectal)	Usually self limiting but overt ductopenia may be observed	Biliary cirrhosis, liver failure
Treatment	Ursodeoxycholic acid (UDCA) 13–15 mg/kg/day effective in majority	No primary medical intervention presently; liver transplant effective rescue therapy (>50% require transplant within 15 years of symptoms)	No proven therapy other than cessation of drug	No primary medical therapy; Kasai porto-enterostomy and/or liver transplantation highly effective
Disease severity indices	Biochemical response to UDCA predicts disease progression and survival, thus identifying patients at risk of adverse outcome[d]	No early markers of outcome; Mayo PSC score and MELD of use in late disease	"Hy's rule": if both drug-induced hepatocellular injury and jaundice occur simultaneously, a mortality of at least 10% can be expected	If bilirubin is less than 2 mg/dL at 3 months post-surgery, then the chance of being transplant-free at 2 years of age is 84%
Key unmet need	Second line therapy for UDCA non-responders	Effective primary treatment; effective screening for cholangiocarcinoma; intervention for transplant recurrence; surrogate end points of disease progression	No effective therapy for progressive injury; predictive tools for preventing exposure to risk-prone patients	Early diagnosis to facilitate early surgery (<60 days old); no effective medial therapy
Diagnostic pitfalls	AMA identified in autoimmune hepatitis, acute liver failure and 0.5% of healthy individuals; isolated elevated alkaline phosphatase values occur in NAFLD	IgG4 associated autoimmune pancreatitis/sclerosing cholangitis responds to steroid therapy; ~10% of patients with PSC have elevated IgG4	Liver injury can present after the cessation of potentially injurious medicines	Differential diagnosis include Alagille syndrome, progressive familial intrahepatic cholestasis, alpha-1-antitrypsin deficiency, and cystic fibrosis

[a]The mitochondrial autoantigens have been cloned, sequenced, and identified as members of the 2-oxo-acid dehydrogenase pathway, including the E2 subunits of pyruvate dehydrogenase (PDC-E2), branched-chain 2-oxo-acid dehydrogenase (BCOADC-E2), and 2-oxo-glutarate dehydrogenase (OGDC-E2).
[b]Cholestatic liver injury is reported with many agents including, but not exclusiviely, estrogens, anabolic steroids, chlorpromazine, erythromycin, the oxypenicillins, tamoxifen, macrolides, ticlopidine, terfenadine, terbinafine, nimesulide, irbesartan, fluoroquinolones, cholesterol-lowering 'statins,' herbal remedies (greater celandine, glycyrrhizin, chaparral), amoxicillin-clavulanic acid and ibuprofen.
[c]Highly specific anti-nuclear antibodies are detected in ~ 50% of patients with PBC (gp210 and sp100).
[d]A number of biochemical predictors of treatment response are associated with histological progression and survival in patients treated with UDCA (eg, Barcelona, Paris and Toronto criteria respectively).

growth factor, endothelin-1, platelet-derived growth factor BB, transforming growth factor β2, and connective tissue growth factor.

Repair and Regulation

Biliary proliferation contributes to the initiation and progression of liver fibrosis.[30,31] Cholangiocytes normally do not proliferate because they constitutively express the cyclin-dependent kinase inhibitors p27, Bbcl2, Bcl-xL, and Mcl-1. In response to liver damage, gastrointestinal (GI) and neuroendocrine hormones, and autocrine and paracrine signaling factors, cholangiocytes proliferate and acquire a neuroendocrine secretory phenotype. In the "ductular reaction," an expanded population of epithelial cells accumulates at the interface of the biliary tree and hepatocytes, along with proliferation of preexisting ductules, activation of progenitor cells, and the appearance of intermediate hepatocytes. The integrin αvβ6 is up-regulated in proliferating bile duct epithelia and promotes fibrogenesis via adhesion to fibronectin and autocrine/paracrine activation of transforming growth factor β1.[32]

Reactive cholangiocytes express receptors, such as the β1 and β2 adrenergic receptors, the M3 acetylcholine receptor, and serotonin 1A and 1B receptors, that bind neurotransmitters. Cholangiocytes can also directly secrete serotonin, which limits the growth of bile ducts through an inhibitory, autocrine loop.[33] Epithelial-mesenchymal transition is a process that might also occur during cholangiocyte proliferation and fibrogenesis.[34–36] Some epithelia-derived mesenchymal cells undergo a mesenchymal-epithelial transition, reverting to epithelial cells that ultimately become hepatocytes or cholangiocytes. The hedgehog signaling pathway appears to promote and regulate hepatic accumulation of immune cells that interact with cholangiocytes.

Cell Death

An unbalanced equilibrium between cholangiocyte death (via apoptosis or necrosis) and proliferation leads to duct loss and fibrosis. In advanced biliary disease, cholangiocytes lose the ability to proliferate[37]; in progressive ductopenia, there is more apoptosis than proliferation. Apoptosis contributes to duct loss and is induced by signals such as activation of death receptors, immune-mediated injury, oxidative stress, infections, and toxins. Apoptosis also promotes fibrogenesis, with apoptotic debris contributing to activation of hepatic stellate cells. Cholangiocytes are the primary epithelial source of tumor necrosis factor α in the liver, a proinflammatory mediator that activates caspase cleavage and apoptosis but also mediates cell survival via activation of the transcription factor nuclear factor κB. In vitro studies have shown that a combination of tumor necrosis factor α, interleukin (IL)-1, IL-6, and interferon gamma inhibits 3′, 5′-cyclic monophosphate–dependent ductal secretion. Nuclear factor κB, which is regulated by I-κB and its kinases (IKKs), regulates immune and inflammatory responses; it protects against cytokine-induced death and oxidative damage. Mice with disruptions in IKK1 and IKK2 or IKK1 and NEMO (nuclear factor κB essential modulator) develop jaundice and a fatal cholangitis, characterized by inflammatory destruction of small portal bile ducts.[38] The combined loss of IKK1-specific functions leads to biliary disease, a process proposed to result from changes to the regulation of tight junctions in biliary epithelial cells. Activation of the receptor tumor necrosis factor–related apoptosis-inducing ligand 2 and death receptor 5 induce cell death and mediate cholestatic liver injury.[39] Healthy cholangiocytes do not express tumor necrosis factor–related apoptosis-inducing ligand, but diseased cholangiocytes up-regulate it, which might control inflammatory responses by killing leukocytes that express the death receptors.

The Hepatic Lymphoid System

In healthy liver, lymphocytes are scattered throughout the parenchyma and portal tracts and include subpopulations from the innate and adaptive immune systems. Less than 10% of human intrahepatic lymphocytes are B cells; most are $CD5^+$ B1 cells that control innate and adaptive immunity.[40,41] T-cell populations, primarily $CD4^+$ helper (Th1 and Th2), $CD8^+$ cytotoxic, T-regulatory, and Th17 cells, have important roles in pathogenesis, along with hepatic macrophages. Nonparenchymal liver cells involved in immune system tolerance to liver include resident dendritic cells, liver sinusoidal endothelial cells, Kupffer cells, and hepatic stellate cells. These cells mediate immunosuppression by producing anti-inflammatory cytokines such as IL-10 and transforming growth factor β; they also express the inhibitor of T-cell activation, programmed cell death 1. IL-10, transforming growth factor β, programmed cell death 1, and cytotoxic T lymphocyte antigen can contribute to the immunosuppressive mechanisms of $CD4^+CD25^+Foxp3^+$ regulatory T cells, which appear to be converted in the liver from infiltrating naive $CD4^+$ T cells and/or effector $CD4^+$ T cells.

Immunologic Role of Cholangiocytes

In response to inflammation, cholangiocytes secrete cytokines and chemokines (eg, tumor necrosis factor α, IL-1, or interferon gamma) that recruit and activate immune cells, including T cells, macrophages, and natural killer (NK) cells.[23,42] Human cholangiocytes constitutively express and secrete chemotactic agents for neutrophils, monocytes, and T cells, including IL-8, IL-6, and MCP-1; under basal conditions, cholangiocytes express low levels of lymphocyte adhesion molecules. In normal livers, some cholangiocytes express HLA class I but not class II molecules. However, cholangiocytes from patients with PBC express HLA class II, but it is not known if costimulatory molecules for T-cell activation are present.

The presence of the adhesion molecule leukocyte factor antigen 3 on the cholangiocyte cell surface allows their interaction with CD2 on cytotoxic T lymphocytes and NK cells. T cells are also activated by CD40, which is expressed on cholangiocytes.[43] The CD40-CD40L and leukocyte factor antigen 2 (CD2)/leukocyte factor antigen 3 (CD58) complexes induce production of IL-12. Cholangiocytes also secrete and transport protective immunoglobulins. In bile, immunoglobulin (Ig) A has a role in biliary mucosal immune defense; by preventing the attachment of pathogens or their toxins to the cholangiocyte surface, it protects biliary ducts.[44] IgA is synthesized by plasma cells around bile ducts and secreted into bile after it binds to the polymeric Ig receptor located on the basolateral membranes of cholangiocytes.

Vascular Supply

Bile ducts are supplied with blood only from hepatic arteries; in contrast to hepatocytes, the biliary epithelium receives blood from a network of capillaries near the intrahepatic bile ducts, the peribiliary vascular plexus, which originate from the terminal branches of the hepatic artery. This specific vascular supply, lacking in canals of Hering and terminal cholangioles, accounts for the prevalent involvement of the interlobular bile ducts in ischemic injury.[45]

Mechanistic Insights From Genetic Studies

From the many genetic insights recognized in both common and uncommon cholestatic liver diseases, it is possible to appreciate a number of important pathophysiologic pathways likely relevant to cholestasis more generally.

Inherited Cholestatic Syndromes

Alagille syndrome (arteriohepatic dysplasia or congenital deficiency in interlobular bile ducts) is characterized by chronic cholestasis, posterior ocular embryotoxon, butterfly-like vertebral arch defects, peripheral pulmonary artery hypoplasia or stenosis, and dysmorphic facial features.[46] This autosomal dominant multi-system disorder varies in phenotypic expression and is largely associated with mutations in *JAG1*, which encodes a ligand in the Notch signaling pathway. Less than 1% of patients have mutations in the receptor *NOTCH2*. By altering the expression of liver-enriched transcription factors, Notch signaling contributes to biliary tree development during ductal plate remodeling and controls transdifferentiation of hepatoblasts and mature hepatocytes into cholangiocytes.[47] In the course of cholestatic liver disease more generally, changes in the expression of Jagged1 and Notch have been described. Changes in cell-cell tight junction function and membrane fusion are also associated with cholestasis, leading to changes in the cytoskeletons and tight junctions of hepatocytes. Neonatal ichthyosis-sclerosing cholangitis syndrome, a rare autosomal recessive condition characterized by hypotrichosis of the scalp, scarring alopecia, ichthyosis, and sclerosing cholangitis, has been associated with changes in the gene that encodes the tight junction protein claudin-1,[48] whereas other even rarer multi-system disorders, including neonatal cholestasis, have been associated with defects in vesical fusion (eg, exocytosis).[49,50]

The progressive familial intrahepatic cholestasis (PFIC) syndromes 1, 2, and 3 are characterized by abnormal bile formation and associated with penetrant mutations in genes that encode proteins in the hepatocellular transport system.[51] PFIC1 and PFIC2 usually appear early in life (first few months), whereas onset of PFIC3 varies. PFIC1 and PFIC2 are each caused by impaired bile salt secretion; they are caused by defects in *ATP8B1* (which encodes FIC1) and *ABCB11* (which encodes the bile salt export pump protein), respectively. Changes in these 2 proteins are also associated with a recurrent cholestatic syndrome known as benign recurrent intrahepatic cholestasis. ATP8B1 is a type 4 P-type adenosine triphosphatase acting as a flippase for phosphatidylserine, such that atp8b1-deficient mice display an increase in the biliary extraction of cholesterol from the canalicular (apical) membrane of the hepatocyte. The ABCB11 gene changes in PFIC2 lead to changes in function of an adenosine triphosphate–dependent canalicular bile salt export pump, which is expressed at the hepatocyte canalicular membrane and acts as the major exporter of primary bile acids. As a result, there is decreased biliary bile salt secretion, decreased bile flow, and hepatic accumulation of bile acids. Functional mutations in *ABCB4*, which encodes MDR3, impair biliary phospholipid secretion and result in PFIC3. In healthy liver, MDR3 is present in the canalicular wall and phosphatidylcholine enters bile to complex with bile acids; it acts as a chaperone to prevent toxic membrane injury. Reduced biliary excretion of phosphatidylcholine leads to a high saturation index of biliary cholesterol and impairs protection of the bile duct epithelium from the detergent properties of bile acids. γ-Glutamyltransferase is present in large quantities in the canalicular and bile duct membranes (mostly canalicular); when bile ducts are damaged, γ-glutamyltransferase is released into the bile and subsequently serum. This explains the increase in γ-glutamyltransferase levels in PFIC3 syndromes but not in PFIC1 and PFIC2 syndromes, because bile is especially toxic to the canalicular membrane. In addition to patients with classic PFIC3, individuals with *ABCB4* mutations (and associated altered MDR3 function) are recognized to develop a wide spectrum of cholestatic diseases, including idiopathic adult biliary cirrhosis, low phospholipid-associated cholelithiasis syndrome, transient neonatal cholestasis, intrahepatic cholestasis of pregnancy, and drug-induced cholestasis.[21]

Cholangiocytes are the only epithelial cells in the liver to have cilia, and signals from the cilia regulate cholangiocyte development and function; developmental abnormalities of the portobiliary system, in association with fibrocystic disease of the kidneys, are known as "ciliopathy diseases" or "cholangiociliopathies."[52,53] The most frequent hepatic manifestations include congenital hepatic fibrosis (ductal plate malformation, abnormal portal veins, progressive portal fibrosis), Caroli's disease (saccular or fusiform dilations of the medium- and large-sized intrahepatic bile ducts), and polycystic liver disease (closed cysts that originate from biliary microhamartomas embedded in fibrous tissue and not in continuity with the biliary tree). Examples of disease include autosomal dominant polycystic kidney disease, caused by mutations in *PKD1* or *PKD2*, and autosomal recessive polycystic kidney disease, caused by mutations in *PKHD1*, which encodes fibrocystin. Autosomal dominant polycystic liver disease is characterized by the presence of only liver cysts; disease results from mutations in *PRKCSH*, which encodes hepatocystin (protein kinase C substrate 80K-H), or *Sec63*. Other rare, genetic, multi-system ciliopathies that involve the liver include nephronophthisis (a medullary cystic kidney disease), Bardet–Biedl syndrome, and Meckel–Gruber syndrome.

HLA and Cholestasis

With the association of the *HLA* locus repeatedly the most significant of any genetic observations for PBC,[54] PSC,[55] and drug-induced cholestasis,[56] there is a clear implication that cholestatic liver diseases are in part triggered in individuals with certain *HLA* alleles that are better at presenting peptides that resemble self-peptides to mature T cells. Studies of PBC from northern and southern Europe and North America have associated *HLA-DR8* (*DRB1*08*) with PBC,[57,58] whereas the only genomic study of patients with PSC to date found that HLA-B at chromosome 6p21 was most strongly associated with this disease.[55] Previous studies of PSC investigated markers of innate immunity near the HLA locus. KIR, inhibitory receptors on NK cells, bind HLA class I molecules; NK cells of patients with PSC had lower amounts of HLA-Bw4 and HLA-C2 (ligands for the KIRs 3DL1 and 2DL1, respectively) compared with controls. These findings indicate that NK cell activity is increased in patients with PSC (decreased inhibition).[59,60] In addition to the increased activity of NK cells through HLA-KIR interactions, genetic evidence indicates a role for the direct activation of NK cells through the *MHC class I chain* (*MIC*)-related genes. *MICA* and *MICB* lie in the HLA region; their products activate the receptor NKG2D on NK cells. The alleles *MICA*002* and *MICA*008* reduce the risk of PSC,[61] whereas a Norwegian study associated the alleles *MICA5.1* and *MICB24* with disease.[62]

Non-HLA Associations

In the genome-wide studies of PBC available to date, which were designed to discover novel non-HLA associations, disease was associated with the IL-12 signaling cascade and the *IL12A* and *IL12RB2* loci in particular. Other robust gene loci include *IRF5* (also associated with systemic lupus erythematosus and inflammatory bowel disease), *MMEL1* (also associated with rheumatoid arthritis), *17q12.21* (also associated with asthma), and *SPIB*.[54,63,64] Bigger cohorts will inevitably discover or confirm, at an appropriately robust statistical level of certainty, many more risk loci, some of which may already have been discussed (eg, *STAT4* and *CTLA4*). The findings, however, firmly confirm PBC as a classic autoimmune disease. For PSC, associations outside of the HLA locus remain more speculative, pending testing of larger cohorts, but include *Glypican 6* as well as loci at chromosomes 2q35 and 3p21, likely reflecting changes to genes encoding the G protein–coupled bile acid receptor 1 and macrophage-stimulating 1, respectively.[55] Whole genome sequencing of cohorts of patients with cholestatic liver disease will ultimately be required, because present genome-wide association testing will not identify the majority of genetic risk for disease. Such an approach is predicted to identify presently unknown rare mutations within genes encoding proteins with significant biological effect.

PBC as a Model Autoimmune Disease

PBC is considered a model autoimmune disease because of the homogeneity of its phenotype among patients, its predominance in women, and its association with a specific autoantibody (against a mitochondrial protein).[65–67] It occurs in 0.1% of women older than 40 years of age[68–71] and is characterized by chronic small bile duct cholangitis. Previous epidemiologic studies have shown that having a first-degree relative with PBC, a history of urinary tract infections, a history of smoking, or use of hormone replacement therapies increased the risk of PBC.[68,71] Other autoimmune diseases develop in approximately 33% of patients with PBC (for example, scleroderma and Sjögren's syndrome), as do cholestasis or pruritus during pregnancy.[68,72–74] $CD4^+$ and $CD8^+$ T-cell and innate responses are involved in pathogenesis, following environmental insults in genetically susceptible individuals[75–77] (Figure 2). PBC is characterized by specific loss of tolerance to a ubiquitous mitochondrial antigen, the E2 component of the pyruvate dehydrogenase complex (PDC-E2)[78–80] (Figure 3). There are several mouse models of PBC[81–85] that have many genetic and environmental features of human disease; studies of these models indicate a role for regulatory T cells (Tregs) and autoreactive, $CD8^+$ T cells in disease induction and modulation. Limitations remain, and the mouse biliary system inevitably differs from that of humans; for exam-

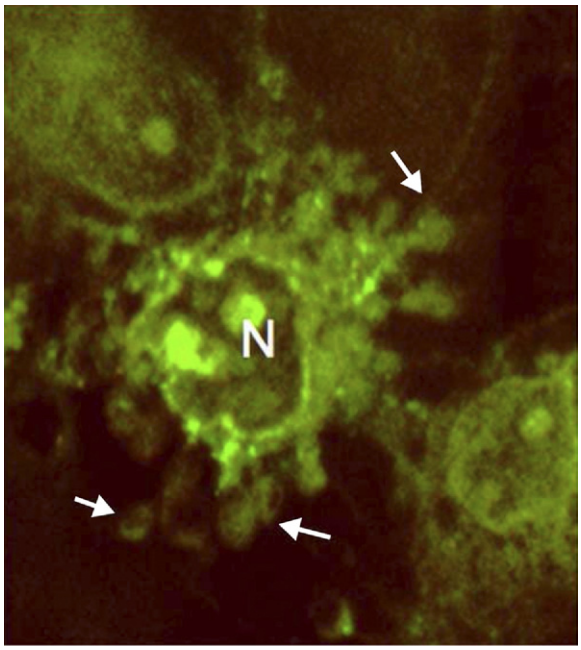

Figure 2. Apoptotic blebs and specificity in PBC. Immunohistochemical analysis of apoptosis-dependent anti-pyruvate dehydrogenase complex (PDC-E2) staining in biliary epithelial cells incubated with AMA-positive serum. PDC-E2 is a ubiquitous protein present in mitochondria of nucleated cells; biliary epithelial cells translocate intact PDC-E2 to apoptotic bodies and create an apotope, which is immunoreactive. N, nucleus.

ple, mice do not have polymeric IgA receptors.[81] The role of B cells in pathogenesis is also complex, but selective deletion of B cells reduces the cholangitis in mouse models.[81,83,84,86–88]

Treatment of patients with PBC is still not disease specific and has not changed for more than 10 years; the standard of care is therapy with the secondary bile acid ursodeoxycholic acid (UDCA).[89–93] The clinical consensus is that a biochemical response to UDCA delays progression of disease in most patients.[94–99]

Environmental Factors

The mitochondrial autoantigen PDC-E2 contains lipoic acid; PDC-E2 captures electrons to alternate oxidation and reduction of a disulfite bond within lipoic acid. In genetically susceptible individuals, drugs or chemicals that modify this disulfite bond might lead to loss of tolerance to PDC-E2. This protein is well conserved throughout evolution, so loss of tolerance could also result from an immune response against similar epitopes in bacteria (molecular mimicry).[100–103] Urinary tract infections are more frequent in patients with PBC than controls, and several bacterial strains have been proposed to lead to immune cross-reactivity with the mitochondrial protein; serologic and molecular analyses have indicated the xenobiotic-metabolizing, gram-negative bacterium *Novosphingobium aromaticivorans* as a good candidate.[104] *N aromaticivorans* was found in fecal samples from 25% of patients with PBC as well as in controls. PDC-E2 from this bacteria has high homology to human PDC-E2, and antimitochondrial antibodies (AMAs) in serum samples from patients with PBC have a 1000-fold stronger reaction against *N aromaticivorans* PDC-E2 than against *Escherichia coli* PDC-E2.[105,106] Notably, infection of mice with *N aromaticivorans* induces production of serum AMAs and development of cholangitis.[107]

In addition to bacteria, chemical xenobiotic agents might induce PBC. Some halogenated organic compounds can attach to specific epitopes of PDC-E2 and induce production of antibodies that have higher affinities for modified mitochondrial epitopes than for normal PDC-E2. One such agent is 2-octynoic acid, which is present in cosmetic products, including nail polish; in vitro and in vivo data indicate its role in autoimmune biliary disease.[108,109] AMAs have equal affinities for 2-octynoic acid and lipoic acid. NOD mice immunized with bovine serum albumin–conjugated 2-octynoic acid developed higher titers of AMAs than those injected with only bovine serum albumin and developed histologic features of PBC, including liver granulomas and portal infiltrates enriched in $CD8^+$ cells.[82]

Disease Specificity

It is not clear why patients with PBC develop defects specifically in the biliary system, but they might arise from specific features of cholangiocyte apoptosis.[76,77] PDC-E2 remains intact and immunogenic during biliary epithelial cell apoptosis; unlike in other apoptotic cells, it is not modified with glutathiol.[110] This allows biliary PDC-E2 to be exposed to dendritic cells and its epitopes to be presented to T cells in draining lymph nodes, leading to production of AMAs.[76,77] AMAs though are detected in patients with other autoimmune and nonautoimmune liver diseases, so loss of tolerance to PDC-E2 alone is not sufficient to cause autoimmune cholangitis.[111,112] The specific destruction of small biliary epithelial cells in patients with PBC might also arise from differences in expression of trefoils (protease-resistant peptides that are expressed in specific patterns throughout the normal GI tract, and which help protect epithelial cells) between small and large bile ducts. Small bile ducts do not produce intestinal trefoils in response to damage, so they might be more vulnerable than large bile ducts.[113]

Gender Disparities

The predominance of PBC in women is unexplained, but believed by some to result from changes in the X chromosome. Genes on the X chromosome maintain levels of physiologic sex hormones and immune responsiveness. Although genome-wide studies have not associated PBC with the X chromosome, age-dependent increases in monosomy X have been reported in the

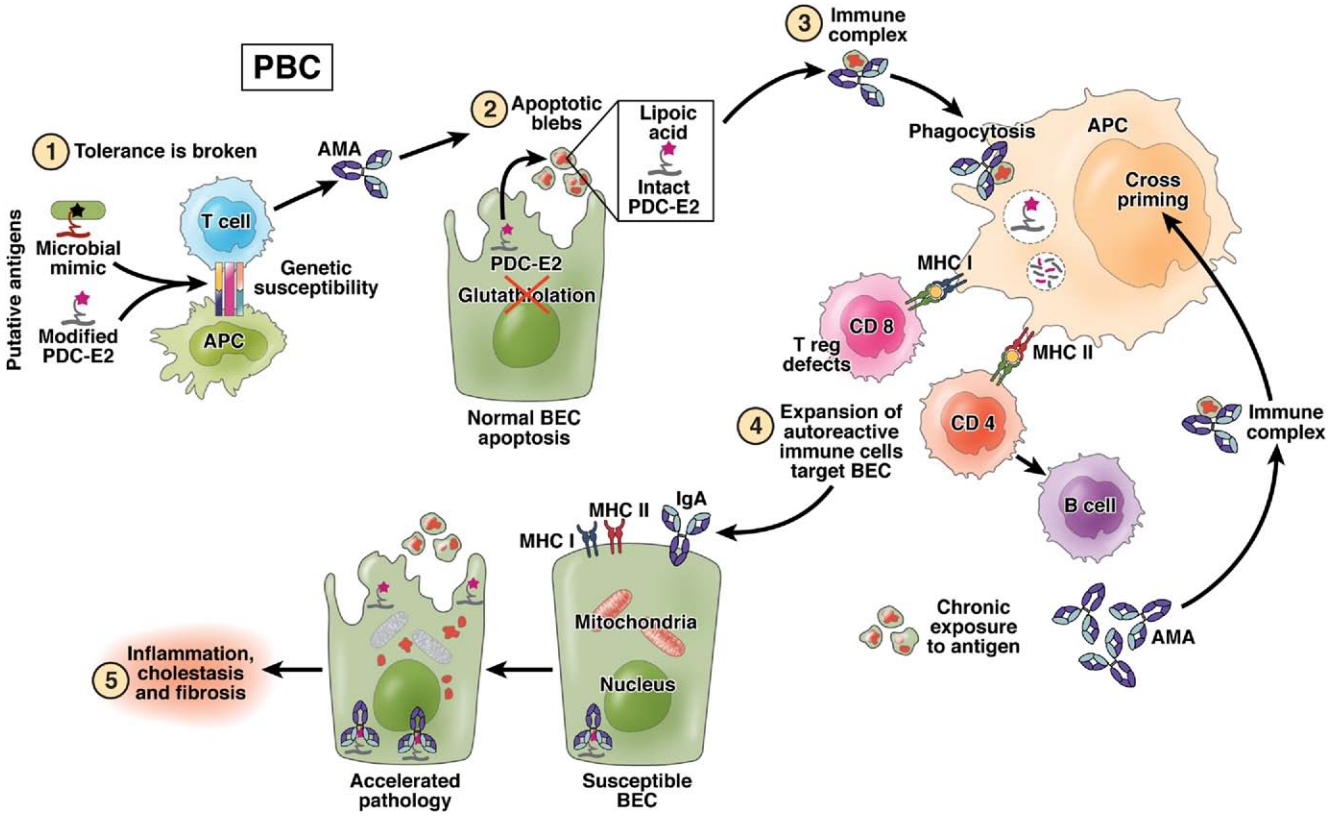

Figure 3. Pathogenic model of PBC. The mitochondrial antigen, PDC-E2, which is in biliary epithelial cell apoptotic blebs, is presented to immune cells. Immune complexes form, leading to expansion of autoreactive immune cells that target the biliary epithelia and cause chronic inflammation, cholestasis, and fibrosis. Genetic and environmental factors determine risk for this disorder. APC, antigen-presenting cell; MHC, major histocompatibility complex.

peripheral white blood cells of women with PBC. Loss of the X chromosome has been reported to be more common in women with PBC than in women without the disorder.[114,115] There are little data on epigenetic factors that contribute to cholestatic liver disease, but sex-specific epigenetic factors might be involved.[116]

Adaptive Responses in PBC

In patients with PBC, the presence of autoantibodies against PDC-E2 is accompanied by a 100-fold increase in hepatic, antigen-specific $CD4^+$ T cells and a 10-fold increase in antigen-specific $CD8^+$ T cells.[117–122] These specific T cells are present even when the AMAs are not detected in serum.[123] Among patients who carry the *HLA DR4*0101* allele, there is a single epitope (a 163–176 amino acid sequence that encompasses the lipoic acid–binding residue of the inner lipoyl domain of PDC-E2) recognized by autoreactive, proinflammatory, $CD4^+$ T cells. Autoreactive $CD8^+$ T cells have also been characterized; the HLA class I–restricted epitope, a 159–167 amino acid sequence, is near the epitopes recognized by $CD4^+$ T cells and AMAs. As for $CD4^+$ autoreactive T cells, the use of tetramer technology has shown a 10-fold higher frequency of PDC-E2 159–167 specific $CD8^+$ T cells in diseased liver tissues compared with peripheral blood, particularly in patients with early-stage disease. Autoreactive $CD8^+$ T cells from patients with PBC specifically kill cells that express PDC-E2; they also produce interferon gamma rather than the anti-inflammatory cytokines IL-4 and IL-10.

T-helper cell subpopulations include Tregs and Th17 cells.[124,125] The proinflammatory, IL-17–secreting subgroup mediate host defense and autoimmunity; reductions in numbers of $CD4^+$ $CD25^{high}$ regulatory T cells are likely to contribute to autoimmunity, chronic viral infection, antitumor immunity, and allergy. These T cells are able to suppress inappropriate immune responses and regulate cellular immune responses. Studies in animals have shown that transfer of T cells that lack the $CD4^+$ $CD25^{high}$ population into athymic nude mice results in T-cell–mediated autoimmunity. In diseased liver samples, more than 15% of T cells in areas of inflammation are Tregs that express the forkhead family transcriptional regulator box P3 (FoxP3). These Tregs are recruited by specific chemokines; CXCR3 appears to mediate recruitment via the hepatic sinusoidal endothelium, whereas ligands for CCR4 are secreted by dendritic cells that have been recruited to sites of inflammation.[126] In patients with PBC, $FoxP3^+$ Tregs have been found in lymphoid infiltrates that localized to the portal tracts; significantly

lower proportions of $CD4^+$ $CD25^{high}$ Tregs were observed in patients and their family members.[127,128]

The role of Th17 cells in cholestatic liver disease is not yet clear. Increased numbers of IL-17^+ cells have been reported in liver tissues from patients with PBC and IL-2 receptor α knockout mice, which develop autoimmune biliary disease. Clusters of IL-17^+ cells were also observed in portal tracts of diseased liver.[129,130]

Innate Responses in PBC

In addition to T cells, PBC is mediated by other cells in the innate immune system.[131–134] Patients with PBC have granulomatous inflammation, increased production of polyclonal IgM, hyperresponsiveness to CpG oligodeoxynucleotides, increased numbers of NK cells, and increased responsiveness to cytokines. Bacterial and viral pathogen-associated molecular patterns induce innate immune responses through cell surface binding to Toll-like receptors and via lipopolysaccharide and lipoteichoic acids that are in bile; Toll-like receptors have been found in cultured human biliary epithelial cells. The downstream effects of biliary epithelial cell Toll-like receptor signaling mediate biliary injury and might involve activation of nuclear factor κB, which regulates transcription of proinflammatory factors (IL-6, tumor necrosis factor α) and leads to chemokines (IL-8, fractalkine/CX3CL1). Fractalkine can exist in membrane-bound and soluble chemotactic forms; it is produced by several types of epithelial cells, regulates cell adhesion, and is a chemoattractant for cells that express its receptor (CX3CR1), such as $CD8^+$ and $CD4^+$ T cells. In patients with PBC, CX3CL1 expression is up-regulated in injured bile ducts; $CD4^+$ and $CD8^+$ cells that express CX3CR1 are found in portal tracts and within the biliary epithelial layer of injured bile ducts.[135]

Immune Consequences of Cholestasis

Bile acid homeostasis is regulated to a large extent at a transcriptional level via nuclear receptors that regulate overlapping genes, including not only those related to bile acid synthesis, transport, and detoxification but innate and adaptive immunity as well.[13] Therefore, during cholestasis, retained bile acids signaling through a myriad of nuclear receptors could conceivably modulate immune responses. One specific example is the farnesoid X receptor, with data supporting a role for the bile acid sensor farnesoid X receptor in regulating the activation of liver NKT cells,[136] as well as being a negative modulator of nuclear factor κB–mediated hepatic inflammation.[137] Other nuclear receptors with similar potential bridging roles between cholestasis and the immune response include the glucocorticoid receptor, peroxisome proliferator-activated receptor, and vitamin D receptor.

Biliary Function

The secretin pathway is an important trophic factor in biliary growth and function,[138] and in cholestatic disorders an impaired response to secretin can be shown, which is improved in patients with PBC treated with UDCA.[139] The impaired ability to induce alkalinization and dilution of canalicular bile upon stimulation by secretin and several neuropeptides in PBC has been associated with altered regulation of cholangiocyte AE2 (SLC4A2; Cl^-/HCO_3^- anion exchanger) and NHE (SLC9A3; Na^+/H^+ exchanger) function[140,141] as well as loss of the expression of inositol 1,4,5-triphosphate receptors involved in $Ca2^+$-mediated bicarbonate secretion.[142] An impaired generation of a bicarbonate-rich choleresis also modifies normal biliary epithelial responses to microbes, while maintaining an alkaline pH near the apical surface of hepatocytes and cholangiocytes additionally prevents the uncontrolled membrane permeation of protonated, and potentially toxic, glycine-conjugated bile acids.[143] Therapeutically, the effects of UDCA and dexamethasone on AE2 gene expression have been elucidated and the combination of UDCA and dexamethasone, but not UDCA or dexamethasone alone, increases the expression of liver-enriched alternative messenger RNA isoforms AE2b1 and AE2b2 and enhanced AE2 activity.[144] In mice with widespread AE2 gene disruption,[85] splenomegaly, increased production of IL-12 p70 and interferon gamma, expanded $CD8^+$ T-cell populations, and underrepresented $CD4^+FoxP3^+$/regulatory T cells are seen. Additionally, most of these mice test positive for AMA and one third develop an extensive portal inflammation with $CD8^+$ and $CD4^+$ T lymphocytes surrounding damaged bile ducts, akin to human PBC. Mutations in the AE2 gene in patients with PBC are not clearly associated with disease onset,[145] and it remains to be seen whether disease progression rather than onset is modulated by biliary transporter function more generally, because presently there is little appreciated about determinants of response to UDCA.[146]

PSC: The Black Box of Cholestatic Liver Disease

PSC is a chronic inflammatory large duct cholangiopathy that results in fibrotic strictures and dilatations of the intrahepatic and extrahepatic bile ducts.[147–149] PSC predominates among men and is frequently associated with inflammatory bowel disease with specific features (rectal sparing, right-sided disease, and backwash ileitis).[150] In these patients, PSC causes cholangitis, secondary biliary cirrhosis, and progression to liver failure. Additionally, there is an ominous increased risk of malignancy, both within the hepatobiliary tree and in the bowel, if associated with inflammatory bowel disease.[151] However, little is known about the pathogenesis of PSC (Figure 4). There is an increased prevalence of autoimmunity in these patients and their relatives; although there is evidence to support genetic predisposition to this disease, it is likely that nongenetic factors have a larger role than in PBC. Many characteristics can mimic the

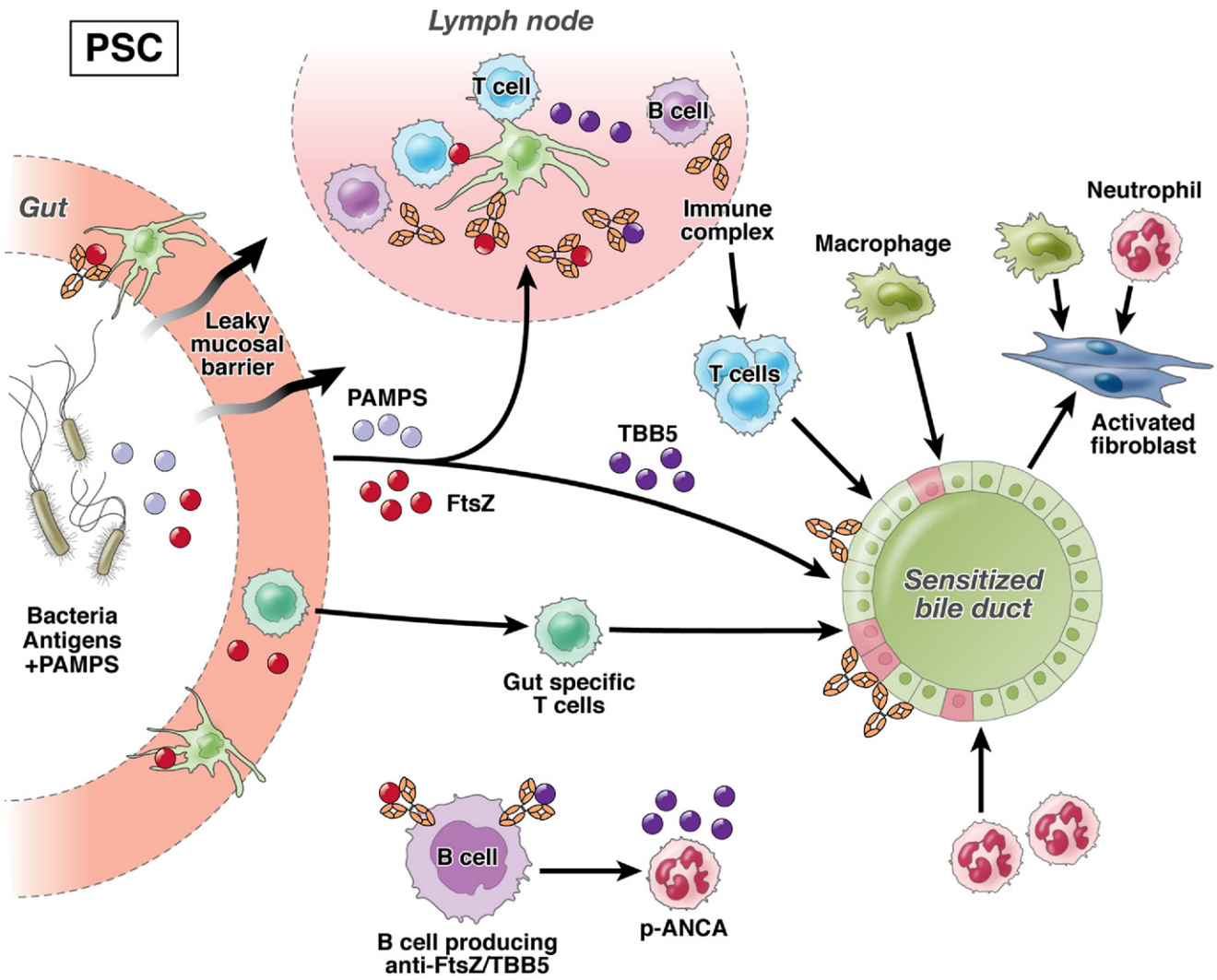

Figure 4. Pathogenic model of PSC. Although there are animal models of PSC, pathogenesis is still poorly characterized in contrast to PBC. Sensitized bile ducts are damaged by different immune cells that are activated in the GI tract and lymph nodes. p-ANCA, perinuclear antineutrophil cytoplasmic antibodies; TBB5, beta-tubulin isotype 5.

histologic and radiologic features of PSC, including autoimmunity, ischemia, infection, and toxins,[152,153] indicating common pathways to biliary injury that can arise from heterogenous primary insults. In PSC, the primary injury is not to hepatocytes but to medium- and large-sized bile ducts, with cholangiography showing aptly the resultant intrahepatic and/or extrahepatic biliary disease as localized or multifocal strictures with intervening segments of normal or dilated ducts. Histologically, concentric periductal fibrosis (onion skinning) occurs and progresses to narrowing and obliteration of small bile ducts. Cholangiocytes become activated to express adhesion molecules, inflammatory and profibrogenetic cytokines and receptors, and growth factors that stimulate production of extracellular matrix and accumulation and proliferation of periductal myofibroblasts. Drugs that disrupt this fibro-obliterative process are an important goal for new therapies.

Etiologic Factors

The lymphocytes that infiltrate the livers of patients with PSC are primarily T cells comprising $CD4^+$ Th-1 phenotype (portal infiltrate) and $CD8^+$ T cells (lobular infiltrate).[42,154–156] There are many $\gamma\delta^+$ T cells; the percentage of $\gamma\delta^+$ T cells in peripheral blood of patients with PSC is greater than that in healthy controls. The infiltrating T cells express the IL-2 receptor and CD45RO, indicating that they have an activated memory phenotype. Although the inflammatory infiltrate in patients with PSC mostly comprises T cells, other cell types, including NK cells, macrophages, B cells, and biliary epithelial cells, are likely to contribute to immunopathogenesis. Innate immune system activation might also be a primary event in PSC; the disease might be triggered or enhanced by bacteria (and pathogen-associated molecular patterns) that enter the portal circulation through an inflamed permeable intestine. IgGs against bil-

iary epithelial cells have been found in the sera of some patients with PSC; the binding of such antibodies to biliary epithelium induces production of proinflammatory cytokines and up-regulation of Toll-like receptors.[157]

Infectious triggers continue to cause speculation, with some, albeit weak, supportive data (eg, *Helicobacter pylori*).[158] Sclerosing cholangitis, however, occurs in immunosuppressed patients with cryptosporidial infections, and autoimmune pancreatitis/sclerosing cholangitis has been associated with antibodies against a peptide that has homology to a plasminogen-binding protein of *H pylori*.[159] Patients with PSC often have perinuclear antineutrophil cytoplasmic antibodies, as do patients with other autoimmune liver diseases. In autoimmune liver disorders, perinuclear antineutrophil cytoplasmic antibodies react with β-tubulin isotype 5 (as an autoantigen) and its bacterial precursor protein FtsZ, indicating an abnormal immune response to intestinal microorganisms.[160]

Mouse models of PSC are limited; the only promising model has a targeted deletion of the mouse biliary transporter MDR2.[161,162] A sclerosing cholangiopathy is also encountered in adult patients with cystic fibrosis, raising the question of a role of cystic fibrosis transmembrane regulator variants in PSC.[163,164] The association between cystic fibrosis transmembrane regulator mutations and human PSC is not clear, but the G-protein–coupled bile acid receptor 1 (also known as TGR5) has been identified as a potential candidate gene in PSC.[55] This molecule is involved in bile acid–induced fluid secretion in biliary epithelial cells and colocalizes with cystic fibrosis transmembrane regulator and the apical sodium-dependent bile salt uptake transporter, suggesting a functional coupling of TGR5 to bile acid uptake and chloride secretion.[165] Intriguingly, it has also been identified in cholangiocyte cilia, where it might conceivably couple biliary bile acid concentration and composition to ductular bile formation.[166]

Adhesion Molecules and PSC

The hepatic vascular bed is a unique low-flow environment, with regulated and leukocyte lineage-specific recruitment, trafficking, and positioning important to the pathogenesis of immune-mediated liver injury. Chemokines and their receptors have specific roles in PSC[167–172]; memory lymphocytes develop during colitis and express receptors that direct them to the GI tract and liver. Normally, the GI endothelium expresses the adhesion molecule mucosal addressin cell adhesion molecule 1 (MADCAM1), which is absent from other vascular beds, and the chemokine CCL25, which is restricted to the small bowel. In PSC, hepatic inflammation up-regulates hepatic MADCAM1 and CCL25 and increases recruitment of mucosal T cells. Such memory cells recirculate between the liver and GI tract but

Table 2. Therapeutic Interventions in PBC and PSC

	Drug	Type	Relative effectiveness	Comments
PBC	UDCA (13–15 mg/kg/day)	Bile transport	++++	Standard of care
	Budesonide/Prednisone	Immunosupressive	+	Speculated of use if AIH overlap features
	Azathioprine/Mycophenolate Mofetil	Immunosupressive	−	No evidence of benefit
	Methotrexate	Immunosupressive	+/−	Biochemical response in some but randomized data lacking
	Cyclosporine	Immunosupressive	+	Side effect profile significant
	Colchicine	Cell replication	+	Potential for further study
	Rituximab	Anti B cell	NA	Animal models show B cell depletion deleterious
	INT-747	Farnesoid X receptor agonist/Bile transport	Phase II	Biochemical response in Phase II studies; pruritus as major side effect
	Bezafibrate	PPARα and *p* ligand	++	Biochemical response but no randomized data
	Fenofibrate	PPARα ligand	++	Biochemical response but no randomized data
	Penicillamine	Copper chelation	−	No benefit
	Tetrathiomolybdate	Copper chelation	+/−	Single study report
	Combivir	Anti viral	−	Controversial disease mechanism with no overt evidence in favor of retroviral hypothesis
	Moexipril	Angiotensin II synthesis blocker	+	Pilot data
	Tamoxifen	Estrogen blocker	+	Pilot data
PSC	UDCA (variable, including high dose)	Bile transport	+/−	High dose therapy reported as deleterious, potentially due to lithocholic acid elevations
	INT-747	Farnesoid X receptor agonist/Bile transport	NA	No clinical data
	Minocycline	Antibiotic/immunosupressive	+/−	Pilot studies
	Prednisone	Immunosupressive	+	No evidence of benefit overall but subgroups potentially helped, eg, IgG4 elevations
	Vancomycin	Antibiotic	NA	Pilot studies
	Anti-TNF	Immunosupressive	−	No benefit

can become deleteriously activated by hepatic antigens. Twenty percent of T cells that infiltrate the liver in patients with PSC are $\alpha_4\beta_7{}^+$CCR9$^+$ memory/effector T cells that produce interferon gamma; these are present at low frequencies in other liver diseases. $\alpha_4\beta_7$ and CCR9 are only colocalized on lymphocytes that are activated in the GI tract by dendritic cells there. GI-associated dendritic cells upregulate GI homing receptors on B cells via a mechanism that depends on the vitamin A metabolite retinoic acid. Liver dendritic cells do not migrate to the GI tract in experimental models, indicating that the $\alpha_4\beta_7{}^+$CCR9$^+$ T-cell infiltrate that occurs during PSC is primed in the GI tract.

$\alpha_4\beta_7$ and CCR9 expression is functionally relevant because MADCAM1 and CCL25, which are absent from normal liver, are present on hepatic endothelium in liver diseases associated with colitis; $\alpha_4\beta_7{}^+$CCR9$^+$ lymphocytes from livers of patients with PSC bind MADCAM1 and respond to CCL25 in adhesion and migration assays. In addition to a specific role for CCL25 in PSC, the CC chemokines CCL21 and CCL28 and the CXC chemokines CXCL9 and CXCL10 are involved in the recruitment of T cells into the portal tract in patients with PBC or PSC; in this regard biliary epithelial cells are recognized to produce many chemokines.

Therapeutic Options Beyond UDCA

Although management of patients with cholestatic liver disease has advanced significantly, UDCA is the only treatment available (Table 2); immunosuppressive agents to date have generally not been effective. Although the efficacy of UDCA is debated, it is largely accepted for most patients with PBC and obstetric cholestasis, whereas the effects of UDCA in patients with PSC are limited, if present at all.[75,149,173,174] UDCA is a major primary bile acid in some species of bears, whereas in humans it is a minor secondary product formed by 7β-epimerization of chenodeoxycholic acid in the GI tract. The choleretic effect of UDCA and its ability to make the bile acid pool more hydrophilic accounts for its beneficial properties. UDCA also stabilizes hepatocyte membranes, increases defense against oxidative stress, and inhibits apoptosis.[175]

Obeticholic acid is a novel chenodeoxycholic acid derivative; unlike UDCA, it is a farnesoid X receptor agonist.[176] Activation of these nuclear receptors reduces bile acid synthesis, induces phases I and II bile acid hydroxylation and conjugation, stimulates export of alternative bile acids, and limits hepatocellular bile acid import. Phase 2 studies in PBC are ongoing. Clinical trials are also planned for a modification of UDCA; 24-norursodeoxycholic acid is a side chain–modified ursodeoxycholic acid derivative that has therapeutic effects in experimental cholestasis.[177] 24-Norursodeoxycholic acid is resistant to amidation and can undergo cholehepatic shunting and directly stimulate cholangiocyte secretion; these cause hypercholeresis (mainly HCO_3^-) that protects the liver from cholestatic injury.[178]

Conclusions

Cholestatic liver disease is the consequence of many hepatobiliary insults. A variety of pathways contribute to liver tissue damage and the reparative response, so new therapies might be developed to target pathways that mediate these processes. As for rheumatoid arthritis, multiple sclerosis, and inflammatory bowel disease, new therapeutic approaches based on rational and biological insights from recent research could change dramatically the outcomes for patients with cholestatic liver disease.

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Reprint requests

Address requests for reprints to: M. Eric Gershwin, MD, Division of Rheumatology, Allergy and Clinical Immunology, University of California at Davis School of Medicine, 451 Health Sciences Drive, Suite 6510, Davis, California 95616. e-mail: megershwin@ucdavis.edu; fax: (530) 752-4669.

Conflicts of interest

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