REVIEW

Pathophysiology of the intrahepatic biliary epithelium

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Abstract The intrahepatic bile duct epithelium modulates the fluidity and alkalinity of the primary hepatocellular bile from which it reabsorbs fluids, amino acids, glucose and bile acids, while secreting water, electrolytes and immunoglobulin A. The transport function of the intrahepatic biliary epithelium is finely regulated by a number of gastrointestinal hormones, neuropeptides and neurotransmitters that promote either secretion or absorption. The intrahepatic biliary epithelium appears to be a primary target in a broad group of chronic cholestatic disorders that represent an important cause of morbidity and mortality. The spectrum of cholangiopathies ranges from conditions in which a normal epithelium is damaged by disordered autoimmunity, infectious agents, toxic compounds or ischaemia, to genetically determined disorders arising from an abnormal bile duct biology, such as cystic fibrosis or biliary atresia. Probably as a result of the known heterogeneity in cholangiocyte function, different portions of the biliary tree appear to be preferentially affected in specific cholangiopathies. From a pathophysiological point of view, cholangiopathies are characterized by the coexistence of cholangiocyte loss (by apoptotic or lytic cell death) with cholangiocyte proliferation and various degrees of portal inflammation, fibrosis and cholestasis. These basic disease mechanisms are discussed in detail. Better understanding of cholangiocyte pathophysiology, in particular the immune regulation of cholangiocyte function, will help in designing newer genetic or pharmacological approaches to treat cholangiopathies. © 2000 Blackwell Science Asia Pty Ltd

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PHYSIOLOGY OF THE BILIARY EPITHELIUM

Bile formation requires the coordinated function of hepatocytes and intrahepatic bile duct epithelial cells (cholangiocytes). After its secretion by hepatocytes, during its route towards the duodenum, the primary bile is extensively modified by the bile duct epithelium which can secrete fluid, bicarbonate, chloride and immunoglobulin A (Ig)A or reabsorb glucose, bile acids, amino acids and electrolytes.1,2 The intrahepatic ducts form an extensive network inside the liver and are responsible for the production of approximately 30% of bile volume, a percentage that can be rapidly increased to meet changing physiological demands.3 Alkalination and fluidification of bile prevails in the intrahepatic portion of the biliary tree, while bile concentration and acidification mainly take place in the gall-bladder. The intrahepatic biliary tree is further separated into segments possessing different functional properties, with hormone-regulated bile secretion being confined to the interlobular and septal intrahepatic bile ducts.4 Diseases of the bile ducts show a clear predilection for specialized sections of the biliary tree (Fig. 1); for example, primary biliary cirrhosis (PBC) selectively involves the interlobular bile ducts, the section where secretin-stimulated secretion is supposed to be more active. In comparison, drug-induced ductopenia is restricted to the small cholangioles, suggesting that there may be functional differences between major ducts and cholangioles in terms of xenobiotic transport and metabolism. The transport function of the intrahepatic bile duct epithelium is finely regulated by a complex interplay of gastrointestinal hormones, neuropeptides and neurotransmitters that promote either net secretion or absorption. In response to secretin, the intrahepatic bile duct epithelium increases the hydration and alkalinity of hepatocellular bile by secreting Cl− and HCO3−.
Bicarbonate secretion is a major function of the biliary epithelium, being important, not only to meet digestive needs, but also to regulate biliary pH and thus the absorption of weakly lipophilic organic acids.

In the last decade our understanding of the molecular mechanisms underlying secretory/absorptive functions of cholangiocytes has increased tremendously (Fig. 2). Here we will briefly summarize current knowledge on the molecular physiology of cholangiocyte secretion; the reader is referred to a number of recent reviews for a more detailed description.1,5,6 A number of different ion carriers and channels have been identified: The basolateral Na+/H+ exchanger isoform 1 (NHE1), Na+/HCO3− symporter7 (or a Na+-dependent Cl-/HCO3− exchanger in humans)8 intrude HCO3− into the cell; Na+/K+-ATPase maintains the Na+ gradient and, together with K+ channels, determines the membrane potential difference. A Na+/K+/2Cl− cotransporter9 actively transports Cl− ions into the cell and appears to be a major determinant of fluid secretion. On the apical pole of the cell, a Na+-independent Cl-/HCO3− exchanger (AE2),7 extrudes bicarbonate into the bile, according to the intracellular pH and the in-to-out Cl− gradient imposed by a cAMP-stimulated low conductance Cl− channel (CFTR).10 Application of patch-clamp techniques has led to the identification of at least two types of Cl− channels in cholangiocytes: a Ca2+-dependent Cl− conductance and a Ca2+- and cAMP-independent high conductance Cl− channel inhibited by pertussis toxin which binds guanosine triphosphate (GTP)-binding proteins.11 More recent work has demonstrated the expression of apical isoforms of the Na+/H+ exchanger (NHE2),12 consistent with a role for intrahepatic cholangiocytes in Na+ reabsorption. Other carriers, such as the Na+-dependent glucose transporter (SGLT1),13 the glutamate transporter14 and the ileal bile acid transporter (iBAT)15 are expressed on the apical membrane of cholangiocytes and participate in the reabsorption of glucose, glutathione breakdown products and conjugated bile acids. Finally, a water-selective channel, aquaporin 1, located at both the apical and the lateral membrane, mediates osmotic water movement and may be regulated by secretin. In addition to CFTR, a Ca2+-activated Cl− channel (9) is present on the apical pole of the cell and may be activated by luminal purinergic nucleotides. (10) A basolateral K+ channel and Na+/K+-ATPase establish the membrane potential and Na+ gradient. Chloride uptake is mediated by Na+/K+/2Cl− cotransport (11). The intrahepatic biliary epithelium also plays a role in Na+-dependent reabsorption of taurocholate (TCA) via an apical carrier similar to the ileal bile acid transporter (12). iBAT, ileal bile acid transporter.

After binding to a G-protein-coupled receptor and stimulation of the cAMP/protein kinase A (PKA)-dependent signalling pathway,17 secretin, the principal stimulatory hormone, promotes Cl− efflux, HCO3− secretion and membrane vesicular transport, resulting in increased ductular cholecystosis.18 In addition to secretin, a number of gastrointestinal hormones and neuropeptides stimulate (e.g. vasoactive intestinal peptide (VIP)19 and bombesin20) or inhibit (somatostatin and gastrin) ductal secretion.21 A rich peptidergic plexus is present in the liver parenchyma; in particular, bombesin immunoreactivity is localized in the bile duct epithelium, from the small cholangiocytes to the extrahepatic tree.
the portal triads in close contact with the bile duct epithelium.\textsuperscript{22} Nerve fibres are also present in the wall of bile ducts; vagal (cholinergic) stimulation increases $\text{HCO}_3^-$ output after acetylcholine binding to $M_3$ muscarinic receptors on the basolateral membrane of cholangiocytes and potentiates the rise in cAMP induced by secretin.\textsuperscript{23} The effects of secretin and acetylcholine, including their net effect on secretion, are additive, an observation that may bear physiological relevance, as the former stimulates bile duct cells during periods of parasympathetic activity.\textsuperscript{23}

Regulation of ductal secretion also takes place at the apical membrane of cholangiocytes through paracrine factors present in the bile. Among them, ATP, a potent secretagogue in a number of epithelia, is present in micromolar concentrations in bile\textsuperscript{24} where it is released by hepatocytes.\textsuperscript{25} Luminal ATP binds to $P_2Y_2$ purinergic receptors present at the apical pole of cholangiocytes and activates luminal $\text{Cl}^-$ efflux through $\text{Ca}^{2+}$-activated $\text{Cl}^-$ channels\textsuperscript{26} and basolateral $\text{HCO}_3^-$ influx via NHE-1.\textsuperscript{27} The effects of ATP and cAMP appear to be additive in terms of stimulation of $\text{HCO}_3^-$ efflux. Furthermore, cholangiocytes regulate the concentration of osmotically active compounds in bile. One such substance is glutathione, a tripeptide that represents both the major determinant of bile salt-independent bile flow and a major hepatic detoxifying mechanism.\textsuperscript{3} The apical membrane of cholangiocytes is enriched with $\gamma$-glutamyltranspeptidase, an ectoenzyme able to catalyze biliary glutathione to glutamate and cysteinylglycine; which are then taken up by a glutamate carrier and by a dipeptide transporter located at the apical pole of cholangiocytes, respectively (H Daniel, pers. comm. 1999), thus realizing an important cycle for the conservation of glutathione. Other important biliary constituents that are recycled by cholangiocytes are the conjugated bile acids which are taken up by an apical carrier similar to the ileal bile acid transporter.\textsuperscript{15,28} The physiological role of this carrier is still unclear, but it may act as a regulator of the final concentration of monomeric bile acids in bile. Apical uptake of glucose from the bile via SGLT1 may also regulate biliary osmolarity;\textsuperscript{13} in fact, as SGLT-1 transports approximately 210 water molecules for each $\text{Na}^+$ and glucose molecule taken up, this carrier may function as a molecular water pump.\textsuperscript{29}

Finally, cholangiocytes synthesize and secrete a number of peptides and mediators, such as interleukin (IL)-6,\textsuperscript{30} transforming growth factor-$\beta_2$ (TGF-$\beta_2$),\textsuperscript{31} endorphin-1,\textsuperscript{32} monocyte chemotactic protein-1 (MCP-1),\textsuperscript{33,34} platelet-derived growth factor (PDGF)-B chain,\textsuperscript{31} tumour necrosis factor-$\alpha$ (TNF-$\alpha$)\textsuperscript{35} and nitric oxide (NO),\textsuperscript{36} that likely enable the bile duct epithelium to communicate extensively with other liver cells, including hepatic stellate cells (HSC), portal fibroblasts and inflammatory cells (Fig. 3). Interestingly, the biliary epithelium does not produce these paracrine factors under normal conditions, but actively synthesizes them in many forms of acute and chronic liver injury. Thus, activated cholangiocytes are likely to play an important role, not only in reparative processes, but also in the progression of chronic liver damage in chronic cholestatic syndromes.

**Figure 3** Theoretical scheme for paracrine communications between cholangiocytes and non-epithelial cells in the liver. Cholangiocytes synthesize and secrete a number of peptides and mediators that are likely to enable the biliary epithelium to communicate extensively with other liver cells, such as hepatic stellate cells, portal fibroblasts and inflammatory cells, by stimulating the fibrogenic response. However, peptides and mediators, either released in the portal spaces by immune cells, macrophages (M6) and mesenchymal cells or produced by the epithelium itself, may have profound effects on epithelial cell function, inducing expression of molecules of major histocompatibility complex (MHC) class II antigen expression or affecting transport properties. MCP-1, monocyte chemotactic protein-1; NO, nitric oxide; ET-1, endothelin-1; FGF, fibroblast growth factor; TGF-$\beta_1$, transforming growth factor-$\beta_1$; PDGF, platelet-derived growth factor; HGF, hepatocyte growth factor; IFN-$\gamma$, Interferon-$\gamma$; CIV, collagen IV; CVXII: collagen XVII; IL$_7$, interleukin; TNF-$\alpha$, tumour necrosis factor-$\alpha$; MMP-1 matrix metalloproteinase-1; PMN, polymorphonuclear cell. Modified and adapted from Shuppan and Hahn.\textsuperscript{85}

**BASIC DISEASE MECHANISMS IN CHOLANGIOPATHIES**

A derangement in the physiological processes outlined constitutes the pathophysiological basis for cholangiopathies. This is a group of chronic, progressive, liver disorders, characterized by the clinical syndrome of chronic cholestasis, which affects the biliary tree and is
responsible for a significant morbidity and mortality among paediatric and adult populations.\textsuperscript{37,38} The spectrum of cholangiopathies ranges from conditions in which the biliary epithelium is damaged by (i) disordered immunity (PBC, graft vs host disease, primary sclerosing cholangitis); (ii) infectious agents (cytomegalovirus or Cryptosporidium infections); (iii) ischaemia (post-transplant hepatic artery stenosis, chronic transplant rejection); (iv) toxic compounds (many common drugs); or (v) genetically transmitted or developmental diseases arising from an abnormal bile duct biology (e.g. cystic fibrosis, Alagille syndrome and biliary atresia).

From a pathophysiological point of view, common to all cholangiopathies is the coexistence of cholangiocyte death (lytic or apoptotic), cholangiolar proliferation, various degrees of portal inflammation and fibrosis and qualitative/quantitative changes in bile production (cholestasis) (Fig. 4). These basic disease mechanisms are present, albeit to a different extent, in all kinds of cholangiopathies. Cytokines and other proinflammatory mediators released in close proximity to the biliary epithelium contribute to these processes through (i) stimulation of cholangiocyte apoptotic and proliferative responses;\textsuperscript{39} (ii) activation of fibrogenetic processes;\textsuperscript{40} (iii) induction of damage to the peribiliary circulation; (iv) induction of histocompatibility antigen expression;\textsuperscript{41} and (v) alteration of biliary epithelium transport function.

**Cholangiocyte death (lytic or apoptotic)**

A vanishing bile duct syndrome (i.e. a decrease in the number of bile ducts per portal tract) is the end result of most cholangiopathies. Ductopenia can be considered as the result of bile duct loss prevailing over bile duct proliferation. The mechanisms by which cholangiocytes die are not well known, but considerable interest is being placed on apoptotic phenomena, the role of which in bile duct morphogenesis is well established. In addition to being involved in ductal plate structure regression,\textsuperscript{42} apoptotic phenomena are also involved in tissue regression after induced hyperplasia (e.g. the disappearance of proliferated ducts after the relief of biliary obstruction).\textsuperscript{43} The role of apoptosis in cholangiopathies is, however, unclear and because of important methodological problems (apoptosis is a transient phenomenon and apoptotic bodies may be eliminated by shedding into the bile duct lumen), the question is difficult to address. Available morphometric studies indicate that in PBC, apoptotic and lytic cell deaths coexist. Apoptosis of cholangiocytes can be induced \textit{in vitro} by Cryptosporidium infection.\textsuperscript{44,45} The effects of cytokines are also unclear: TNF-\textalpha, a major proapoptotic cytokine, is not toxic to cholangiocytes,\textsuperscript{46} while IL-6, a proinflammatory cytokine and a known autocrine growth factor,\textsuperscript{30} protects cultured cholangiocytes from ceramide-induced apoptosis by changing the balance of apoptosis regulatory genes towards an antiapoptotic equilibrium (reduction of Bax, increase of bcl-2).\textsuperscript{47}

Hydrophobic bile acids can induce apoptosis in hepatocytes via a Fas ligand-independent CD95 (Fas) dimerization, induction of the mitochondrial transition pore and activation of cathepsin B,\textsuperscript{48} but whether bile acids can trigger apoptosis in cholangiocytes is presently unknown. The bile duct epithelium is a potential target for bile acid toxicity, as shown by the presence of extensive ductular damage and proliferation with portal inflammation and fibrosis in \textit{mdr}-2-deficient mice, an animal model for progressive familial cholestasis type III (MDR3 deficiency).\textsuperscript{49,50} In these conditions, the absence of phospholipids in the bile exposes the biliary epithelium to the unrestrained effects of high monomeric concentrations of bile acids. Under normal conditions, the biliary epithelium is capable of absorbing (passive, non-ionic diffusion, or active transport through iBAT), metabolizing and recirculating bile acids through the peribiliary circulation. Bile acids may damage the epithelium, promote bile duct cellular proliferation, or act as secretory signals. According to Alpini \textit{et al.}, expression of iBAT is restricted to major
ducts; if so, cholangioles would be more prone to the toxic effects of hydrophobic bile acids. Hydrophilic bile acids (e.g. ursodeoxycholic acid, UDCA), however, are of benefit in cholangiopathies such as PBC and CF. Recent data indicate that UDCA counteracts the apoptotic effects of hydrophobic bile acids on hepatocytes. It is unknown whether UDCA possess anti-apoptotic effects on cholangiocytes. Interestingly, Koga et al. reported a much lower rate of apoptosis in PBC patients treated with UDCA.

The mechanisms leading to cell damage in immune-mediated cholangiopathies are poorly known. Whether antimitochondrial auto-antibodies have a pathogenic role in PBC is also unknown. The finding that AMA antibodies react against the apical membrane of cholangiocytes in PBC patients suggests that their molecular target is either a mistargeted/truncated form of pyruvate-dehydrogenase complex-E2 (PDC-E2), immune complexes or a cross-reactive epitope derived from an extrinsic (bacterial?) molecule. If this were the case, its presentation by major histocompatibility complex (MHC) class II molecules would allow activation of CD4+ cells and, consequently, of the invading effector CD8+ cells. However, as B7 costimulatory molecules seem not to be expressed during the early stages of the disease, it is presently unclear to what extent cholangiocytes may act as professional antigen-presenting cells. The presence of IgA antimitochondrial antibodies (AMA) in bile of PBC patients and the observation that, while PDC-E2 is an ubiquitous antigen, damage is restricted to biliary cells (i.e. to cells able to transport IgA transcellularly) have prompted the hypothesis that IgA AMA may penetrate the cell and induce liver 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. However, this sequence of events remains highly speculative.

Cholangiocyte proliferation

Cholangiocytes possess latent mitotic capabilities and proliferate following a number of liver injuries (ductular reaction). This is an important step in the development of chronic liver damage, as ductular reaction is considered as the pace-maker of portal fibrosis. In PBC, as in several other cholangiopathies, destruction of interlobular bile ducts is associated with a vigorous proliferation of bile ductules; atypical ductules at the border of the cirrhotic nodules show neuroendocrine features and transiently express phenotypic features that are present during fetal bile duct development, such as expression of the anti-apoptotic protein bcl-2 and of the neural adhesion molecule (NCAM; L Fabris et al. submitted). These cells are negative for the proliferative marker Ki67, which is consistent with the origin of atypical ductules from metaplastic hepatocytes. Following cholestatic injury, it seems that hepatocytes from the limiting plate undergo ductular metaplasia, in a manner similar to the ontogenetic response of hepatoblasts that differentiate into primordial cholangiocytes when in contact with the undifferentiated mesenchyma surrounding the portal vein terminations. The mechanisms responsible for bile duct cell proliferation and/or induction of metaplasia of portal hepatocytes into cholangiocytes, are not well known and their elucidation is an area of major effort. A number of growth factors, such as epidermal growth factor (EGF), hepatocyte growth factor (HGF) and IL-6 in humans and insulin-like growth factor-1, EGF and HGF in rats have been shown to stimulate cholangiocyte growth in vitro. A role for bile acids has been postulated and awaits experimental demonstration. The role of inflammatory mediators, locally released cytokines and changes in extracellular matrix should also be investigated. These studies will be facilitated by the ability to isolate NCAM-positive cells from diseased livers (L Fabris et al. submitted).

Portal inflammation and fibrosis

Most cholangiopathies are associated with significant amounts of inflammatory infiltrate in the portal spaces; few studies, however, have addressed the pattern of cytokines produced in the liver in the course of immune-mediated cholangiopathies. Results are equivocal and the interpretation is hampered by differences in the experimental protocols. Studies in PBC have shown increased production of both T helper type 1 (Th1) cells (IL-2, interferon-$\gamma$ (IFN-$\gamma$)) and T helper type 2 (Th2) cells (IL-4, IL-5 and IL-6) cytokines; the Th1 pattern being predominant in later stages of the disease. Tumour necrosis factor-$\alpha$ was also shown to increase in PBC. An emerging concept is that bile duct epithelial cells are active participants in inflammatory diseases and, in pathological conditions, secrete proinflammatory and chemotactic cytokines, such as IL-6, TNF-$\alpha$, IL-8 and MCP-1 together with growth factors able to activate mesenchymal cells and matrix production (endothelin-1, PDGF, TGF-$\beta$), or to decrease matrix production and leucocyte adhesion (NO; Fig. 3). These peptides and mediators, either released in the portal spaces by immune cells, macrophages and mesenchymal cells or produced by the epithelium itself, may have profound effects on epithelial cell function; for example, IFN-$\gamma$ promotes MHC class II antigen expression by biliary cells, affects the transport properties of the epithelium and stimulates induction of NO production by cholangiocytes. In contrast, IL-6 acts as a potent growth factor for biliary cells. Interestingly, in septic patients, cholestasis is associated with the pathological picture of cholangitis lenta in which proliferation of marginal ductules coexists with evidence of inspissated bile in bile ductular structures at the edges of the portal tracts.

Compared with hepatocellular cholestasis, which is not associated with progressive fibrosis, in chronic cholangiopathies there is an extensive fibrotic response in the portal tracts. Fibrogenesis is a dynamic process that depends on the extent and duration of parenchymal damage: the persistence of the original noxa causes a prolonged activation of tissue repair mechanisms that
leads to tissue fibrosis. Hepatic stellate cells are the main connective tissue producing cells and, during tissue repair, undergo a process of activation from the quiescent to the highly proliferative myofibroblast phenotype. This activation, which is also characterized by an increased synthesis of collagen types I and III, follows a complex interplay between extracellular matrix components, polypeptide growth factors, cytokines and other soluble mediators, (Fig. 3). As outlined, most cholangiopathies are associated with the proliferation of marginal ducts. Several lines of evidence suggest that cholangiocytes play an active role in stimulating the fibrogenic response, possibly activating the usually quiescent portal fibroblasts and HSC. In chronic liver diseases, in situ hybridization and immunohistochemical studies show that cholangiocytes produce paracrine factors such as TGF-α, MCP-1, PDGF-B and TGF-β2, which are able to stimulate HSC activation and matrix production. Furthermore, bile duct cells synthesize basement membrane proteins and collagen type IV. The close association between bile duct proliferation and mesenchymal activation is also present in cholangiocarcinomas, neoplasias that frequently show a strong desmoplastic reaction. It is still unclear whether the changing epithelial phenotype directly induces an alteration of the mesenchymal cells or if the changes in the extracellular matrix induce the phenotypic shift in the biliary epithelium. Modulation of the fibrogenic response may be helpful in avoiding obliteration of the bile ducts and in slowing the evolution to biliary cirrhosis.

**Cholestasis**

The role of the biliary epithelium in bile production is such that, while damage to the biliary epithelium will result in the reduced flow of bile, impaired fluid secretion by cholangiocytes will induce qualitative changes in the biliary fluid that may predispose the biliary epithelium itself to damage from other concurrent events. Cholestasis may originate from the fibro-inflammatory obliteration of bile ducts, from mutations in transport proteins, or from altered secretory responses induced by inflammatory mediators.

Complete biliary obstruction is rarely seen in clinical practice; this condition is associated with vigorous proliferation of well-differentiated cholangiocytes, a phenomenon that may have a compensatory function. In fact, available information suggests that, in contrast to hepatocytes where the basolateral sodium-taurocholate transporter polypeptide (ntcp) is decreased, chronic extrahepatic cholestasis does not impair ion transport in cholangiocytes. Rather, the CFTR Cl channel is more highly expressed, and the choleretic response to secretin administration is much higher, partly as a result of an increased expression of the secretin receptor gene. These findings might explain the well-known occurrence of colourless bile enriched with water and electrolytes after bile duct ligation. Increased secretin receptor gene expression and Cl/HCO₃ exchanger activity and increased choleretic responses have also been reported in other conditions associated with proliferation of differentiated cholangiocytes, such as alpha-naphthyl-isothiocyanate intoxication and partial hepatectomy.

The role of altered ion transport systems in the pathogenesis of cholangiopathies is demonstrated in cystic fibrosis, a frequently occurring genetic disease in which mutations in CFTR lead to impaired cAMP-dependent Cl transport and to pulmonary, pancreatic and liver disease. In contrast, in hepatocytes, in which a number of genetically determined defects in membrane transport systems (such as Dubin–Johnson syndrome, progressive familial intrahepatic cholestasis, benign recurrent intrahepatic cholestasis, etc.) have been identified, CF-associated liver disease is the only known liver disorder affecting cholangiocyte transport proteins. Clinical liver decompensation is infrequent, but bile ductal cell damage can also be demonstrated in asymptomatic cases, well before hepatocellular damage appears. Cystic fibrosis results from a number of mutations in the gene encoding for CFTR, a low conductance Cl channel which in the liver is restricted to cholangiocytes and mediates the choleretic effects of secretin. In addition to its role as a cAMP/PKA activated Cl channel, CFTR also participates in the regulation of other membrane transport proteins, such as Na⁺ channels (EnaC), K⁺ channels, outward rectifying Cl channels, and Cl/HCO₃ exchangers. The CFTR also appear to regulate cellular secretion of ATP, intracellular vesicle acidification and processing and trafficking of certain proteins. In other epithelia CFTR may be permeable to HCO₃ and allow a substantial bicarbonate conductance. For example, studies in the duodenal mucosa of CFTR (−/−) mice suggest a dual pathway for HCO₃ secretion: one component is electrogenic via CFTR-mediated HCO₃ conductance, a second component is electroneutral via CFTR-dependent Cl/HCO₃ exchange and is closely associated with carbonic anhydrase activity, an enzyme that is highly expressed in duct cells. Indeed, cholangiocytes isolated from CF patients undergoing liver transplantation show reduced cAMP-stimulated Cl efflux and impaired bicarbonate secretion via Cl/HCO₃ exchange (A Zsembery et al., submitted). Although the molecular basis of the CF is reasonably well understood, the pathogenesis of liver damage in CF is not entirely clear; we speculate that in the presence of a mutated CFTR, reduced bile hydration and alkalinity precipitates a series of events that damages the biliary epithelium, such as retention of cytotoxic bile acids and xenobiotics and a reduction in natural defences against microbiological pathogens. In response to epithelial damage, release of inflammatory mediators and cytokines leads to chronic portal inflammation, that, depending on the immunogenetic background of the individual and other concurrent factors, may lead to pathognomonic focal biliary cirrhosis (Fig. 5). Furthermore, the lower incidence of CF liver disease, with respect to pancreatic damage, suggests that in the liver, other secretory mechanisms and signals may play a vicarious role in conditions in which the cAMP-mediated secretory pathway is impaired. As
Defective CFTR \( \rightarrow \) HCO\(_3\)\(^{-}\) and Cl\(^{-}\) secretion

Changes in biliary pH and hydration

Portal inflammation

Liver diseases

\( \uparrow \) Innate immunity*  
\( \downarrow \) Reduced biliary clearance

\( \uparrow \) Exposure to pathogens  
Hydrophobic bile acids  
Toxic compounds

Figure 5  Current working hypothesis for the pathogenesis of liver diseases in cystic fibrosis patients. Reduced bile hydration and alkalinity precipitates a series of events able to damage the biliary epithelium: reduced biliary clearance increases the exposure to pathogens and toxic compounds. Furthermore, as hypothesized in the respiratory epithelium, the altered volume and quality of bile may impair the epithelium’s innate immune system. This is the first line of defence against pathogenic insult and includes the integrity of the epithelium, the continuous flow of bile and the secretion of yet to be identified multiple substances with anti-inflammatory and antimicrobial properties. In the airways epithelium this includes lysozymes, phospholipase A\(_2\), defensin, cathelicidin, complement and immunoglobulin A.

already discussed, a candidate mediator is biliary ATP, which binds to luminal P2Y\(_2\) receptors, activates apical Ca\(^{2+}\)-dependent Cl\(^{-}\) channels other than CFTR, and increases the activity of NHE-1, possibly resulting in stimulation of HCO\(_3\)\(^{-}\) efflux.\(^{27}\) In fact, our own data show that, in cholangiocytes isolated from CF livers, an increase in intracellular Ca\(^{2+}\) concentration, induced by administration of ionomycin leads to parallel activation of Cl\(^{-}\) channels and HCO\(_3\)\(^{-}\) extrusion. These results indicate that, in CFTR-deficient cells, secretion of bicarbonate can be restored by alternative activation of intracellular Ca\(^{2+}\)-dependent Cl\(^{-}\) channels, an observation that may have therapeutic relevance.\(^{81}\)

The molecular pathogenesis of cholestasis in immune-mediated cholangiopathies remains unclear. Cholestasis may be due to obliteration of bile ducts, but it is usually present well before the onset of ductopenia and it can be seen even in the absence of canaliculic dilatation and bile plugs. It has been suggested that alterations in biliary electrolyte transport may contribute to the pathogenesis of cholestasis in primary disorders of bile ducts. For example, defective expression of the gene coding for the AE-2 anion exchanger and reduced immunoreactivity for the apical anion exchanger have been reported in patients with PBC.\(^{82,83}\) Changes in cholangiocyte transport function may also be secondary to the inflammatory condition. In contrast to experimental cholestasis, where inflammation is minimal, most human cholangiopathies are associated with portal inflammation and to the release of a number of proinflammatory cytokines that may have profound effects on epithelial cell function, and even alter its transport properties.\(^{84}\) For example, endotoxin administration decreases bile flow, an effect that is inhibited by antibodies against TNF-\(\alpha\). Our own data show that mixtures of different proinflammatory cytokines (IL-6, IFN-\(\gamma\) and TNF-\(\alpha\)) inhibit cAMP-dependent fluid secretion and Cl\(^{-}\)/HCO\(_3\)\(^{-}\) activity in polarized rat cholangiocytes.\(^{86}\) These data are of interest because they demonstrate that cholangiocyte secretory function is deeply affected by the inflammatory microenvironment in the absence of duct obliteration and advocate the use of anti-inflammatory agents in cholangiopathies.

CONCLUSIONS

The role of the biliary epithelium in the pathogenesis of biliary tract disease is just beginning to be unveiled. The emerging idea is that cholangiocytes are active participants, rather than innocent bystanders, in the development of cholangiopathies. Whether genetically determined, induced by extrahepatic causes or by inflammatory mediators, the epithelium will respond to the different noxae via apoptotic or lytic cell death programmes, by activating mitosis and producing a vast array of mediators that enable paracrine communication with non-parenchymal and inflammatory cells and by changing its transport capability. Reduced fluid and electrolyte transport by cholangiocytes (ductular cholestasis) is a central step because it will predispose the biliary epithelium to further damage. The continuing inflammatory/fibrotic/cholestatic response will have a key role in the progression of the disease. The field of immune regulation of cholangiocyte function will prove to be very rewarding in the future, particularly in view of the potential to pharmacologically block the action of specific cytokines. Furthermore, better understanding of cholangiocyte physiology will help in the design of newer genetic or pharmacological agents that are able to target specific cholangiocyte functions and are likely to provide effective and safe therapeutic strategies in the near future.

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Pathophysiology of the biliary epithelium


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