

Molecular Alterations of Canalicular Transport Systems in Experimental Models of Cholestasis: Possible Functional Correlations

Michael Trauner

*Division of Gastroenterology and Hepatology, Department of Internal Medicine,
Karl Franzens University School of Medicine, Graz, Austria*

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The discovery of unidirectional, ATP-dependent canalicular transport systems (also termed "export pumps") for bile salts, amphiphilic anionic conjugates, lipophilic cations, and phospholipids has opened new opportunities for understanding biliary physiology and the pathophysiology of cholestasis. In addition, ATP-independent canalicular transport systems for glutathione and bicarbonate contribute to (bile acid-independent) bile formation. Canalicular excretion of bile salts and several non-bile acid organic anions is impaired in various experimental models of cholestasis. Recent cloning of several canalicular transport systems now facilitates studies on their molecular regulation in cholestasis. Although the picture is far from complete, experimental evidence now exists that decreased or even absent expression of canalicular transport proteins may explain impaired transport function resulting in hyperbilirubinemia and cholestasis. With the increasing availability of molecular probes for these transport systems in humans, new information on the molecular regulation of canalicular transport proteins in human cholestatic liver diseases is beginning to emerge and should bring new insights into their pathophysiology and treatment. This article gives an overview on molecular alterations of canalicular transport systems in experimental models of cholestasis and discusses the potential implications of these changes for the pathophysiology of cholestasis.

PHYSIOLOGY OF CANALICULAR TRANSPORT SYSTEMS

The hepatocyte is a polarized epithelial cell with distinct features of its basolateral (sinusoidal) and apical (canalicular) plasma membrane domains. The canalicular membrane contains about 10-15 percent of the plasma membrane surface area of the hepatocyte and comprises the bile canaliculus. Bile formation begins with formation of a primary secretion at the canalicular level ("canalicular bile") by both bile salt-dependent and -

^aTo whom all correspondence should be addressed: Michael Trauner, M.D., Division of Gastroenterology and Hepatology, Department of Internal Medicine, Karl Franzens University School of Medicine, Auenbruggerplatz 15, A-8036 Graz, Austria. Tel.: 011-43-316-385-2863 or 2648; Fax: 011-43-316-385-3062; E-mail: michael.trauner@kfunigraz.ac.at.

^bAbbreviations: ATP, adenosine triphosphate; cBAT/cbat, canalicular bile acid transporter; BSP, bromosulphophthalein; CBDL, common bile duct ligation; CSA, cyclosporin A; cMOAT/cmcoat, canalicular multispecific organic anion transporter; ectoATPase, Ca⁺⁺, Mg⁺⁺-ecto-adenosine triphosphatase; EE, ethinylestradiol; EHBR, transport deficient Sprague-Dawley rat; GSH, reduced glutathione; GSSG, oxidized glutathione; GY/TR⁻, transport-deficient Wistar rat; LPS, lipopolysaccharide; MDR/mdr, multidrug resistance genes; mRNA, messenger RNA; MRP, multidrug resistance-associated protein; MRP2/mrp2, canalicular conjugate export pump; P-gp, P-glycoproteins; spgp, sister of P-glycoprotein; V_{max}, maximal transport velocity. Please note that human genes and their products are capitalized, whereas rodent genes and their products are written in lower case.

independent mechanisms, followed by modifications along the bile ductules and ducts [1, 2]. Canalicular bile is formed by osmotic filtration of water and small electrolytes via the tight junctions in response to osmotic gradients generated by active transport systems located at the canalicular membrane. The major determinant of canalicular bile formation is the excretion of bile acids into the canaliculus (bile acid-dependent bile flow). In addition, other (non-bile acid) organic anions and cations and their conjugates also osmotically influence bile flow. Canalicular excretion of glutathione and bicarbonate constitute the major components of the bile acid-independent fraction of bile flow, although the contribution of bicarbonate occurs mainly at the level of the bile ductules, particularly when stimulated with hormones and neuropeptides [1, 3].

Initially it was thought that the major driving force for the canalicular excretion of all biliary constituents was the intracellular negative membrane potential (-35 mV) generated by the Na^+ pump (Na^+/K^+ -ATPase) at the basolateral membrane. However, it has become evident that the membrane potential could only account for a 3-fold concentration gradient and thus is insufficient to explain the large gradients (ranging from 100-fold to as high as 1000-fold) observed for bile acids and certain anionic conjugates (e.g., cysteinyl leukotrienes). The discovery of unidirectional, primary active ATP-dependent transport systems at the canalicular membrane has opened new opportunities for understanding biliary physiology and the pathophysiology of cholestasis. These ATP-dependent transport systems belong to the ATP-binding cassette transporter superfamily and share common structural and functional characteristics [4, 5]. At least four ATP-dependent transport systems (also termed "export pumps") have been identified so far at the canalicular membrane (Figure 1, left panel): (i) A multidrug export pump (MDR1 P-glycoprotein) for hydrophobic cationic compounds (e.g., anticancer drugs, calcium channel blockers, cyclosporin A, various other drugs) [18, 19], and (ii) a phospholipid pump (MDR3 P-glycoprotein) which probably acts as a phospholipid flippase/translocase [20, 21]. The MDR1

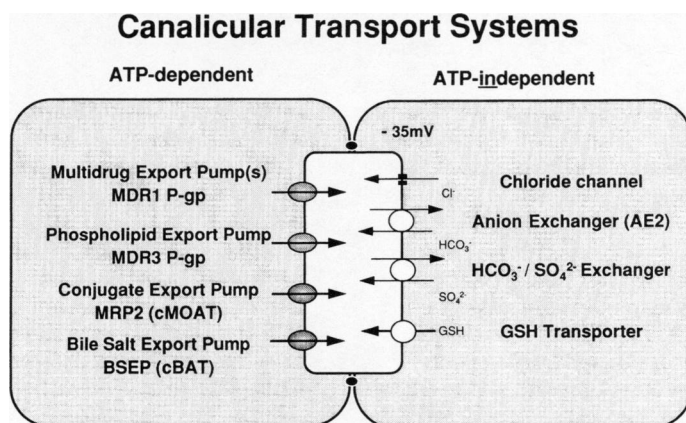


Figure 1. The canalicular membrane contains several ATP-dependent export pumps and ATP-independent transport systems. At least four ATP-dependent transport systems have been identified (left panel): The Multidrug Export Pump (MDR1 P-glycoproteins) mediates the canalicular excretion of bulky lipophilic cations (e.g., anticancer drugs). The Phospholipid Export Pump (MDR3 P-glycoprotein) probably functions as a phospholipid flippase and facilitates the biliary excretion of phosphatidyl choline. The Conjugate Export Pump (MRP2/cMOAT) mediates the canalicular excretion of divalent anionic conjugates (e.g., bilirubin glucuronides, cysteinyl leukotrienes, GSH-conjugates, divalent bile acid sulfates and glucuronides). The Bile Salt Export Pump (BSEP/cBAT) mediates the excretion of monovalent bile acids and has been functionally well characterized, although its molecular identity is still under investigation. In addition, the canalicular membrane also contains several ATP-independent transport systems (right panel), including a $\text{Cl}^-/\text{HCO}_3^-$ anion exchanger (AE2) for bicarbonate secretion and a canalicular GSH transporter.

and 3 P-glycoproteins (P-gp) are products of the multidrug-resistance (MDR) genes, and the MDR3 isoform is predominant in normal liver [19]. (iii) A conjugate export pump which has recently been cloned in rats [10, 11] and humans [12, 13] and was identified as the canalicular isoform of the multidrug-resistance-associated protein (MRP) and therefore designated as MRP2. This transport system is functionally also known as the canalicular multispecific organic anion transporter (cMOAT) [14] and mediates the canalicular excretion of a broad range of organic anions, most of which are amphiphilic anionic conjugates with glutathione, glucuronate and sulfate formed by phase II conjugation in the hepatocyte [14-17]. (iv) A bile salt export pump (canalicular bile acid transporter, cBAT) for monovalent bile salts, which functionally is well characterized [6-9] but has not been cloned yet.

In addition to these ATP-dependent transport systems, the canalicular membrane also contains several ATP-independent transport systems (Figure 1, right panel): Canalicular excretion of reduced glutathione (GSH) is driven by the membrane potential but the molecular identity of this transport system remains currently unclear. Yi et al. [22] reported isolation of a putative rat canalicular GSH transporter (RcGshT), but with the recent availability of the full *E. coli* genome it became apparent that the sequence of RcGshT is an *E. coli* gene and represents a cloning artifact [23, 24]. Canalicular bicarbonate secretion is mediated via the $\text{Cl}^-/\text{HCO}_3^-$ exchanger belonging to a family of anion exchangers (AE) which consists of several members, three of which (AE1, AE2, and AE3) are now well characterized [25]. AE2 is encountered in various tissues, including the liver and secretory epithelia, and plays a major role in mediating hepatic $\text{Cl}^-/\text{HCO}_3^-$ exchange. The AE2 protein is localized to both canaliculi (mainly in periportal hepatocytes) and the luminal side of both terminal and interlobar bile ducts [26], consistent with a role for HCO_3^- excretion

Table 1. Canalicular transport systems in humans and rodents

Name	Human	Rat/Mouse	Substrate(s)
I. ATP-dependent :			
Canalicular bile salt export pump (canalicular bile acid transporter)	BSEP (cBAT)	bsep (cbat)	Monovalent bile salts
Canalicular conjugate export pump	MRP2 (cMOAT)	mrp2 (cmoat)	Divalent amphiphilic anionic conjugates with glutathione, glucuronate, sulfate
Cation export pump	MDR1 P-gp	mdr1a and b P-gp	Bulky lipophilic cations (type II) (e.g., anthracyclines, vinca alkaloids, and others)
Phospholipid export pump	MDR3 P-gp	mdr2 P-gp	Phospholipids
Sister of P-glycoprotein	SPGP	spgp	Unknown (monovalent bile salts?)
II. ATP-independent:			
$\text{Cl}^-/\text{HCO}_3^-$ anion exchanger	AE2	ae2	HCO_3^- (in exchange for Cl^-)
Canalicular GSH transporter	—	RcGshT	Reduced glutathione (GSH)

and bile formation. In addition, a tertiary active $\text{HCO}_3^-/\text{SO}_4^-$ exchanger resides in the canalicular membrane [27], although its function in bile formation remains to be established. Table 1 summarizes the current nomenclature and functional features of ATP-dependent and -independent transport systems at the canalicular membrane in humans and rodents.

MODELS OF CHOLESTASIS AND CLINICAL CORRELATES

Cholestasis may result either from a functional defect in bile formation at the level of the hepatocyte (hepatocellular cholestasis) or a mechanical impairment in bile secretion and flow at the level of bile ductules or ducts (ductular/ductal cholestasis). Several experimental models of cholestasis in rats have been developed that simulate human cholestatic disease, such as sepsis-induced cholestasis (endotoxin [lipopolysaccharide, LPS]-treated rats), oral contraceptive-induced cholestasis/cholestasis of pregnancy (ethinylestradiol [EE]-treated rats), and extrahepatic biliary obstruction (common bile duct ligation [CBDL]) (Table 2). Despite their different etiology, each of these experimental models result in marked impairment of canalicular excretion of bile acids and various (non-bile acid) organic anions as demonstrated in isolated perfused rat livers, isolated rat hepatocytes, and isolated canalicular membrane vesicles. Interestingly, transport studies in canalicular membrane vesicles from cholestatic animals have revealed changes in the maximal transport velocity (V_{max}) without significant changes in the relative affinity (Michaelis constant, K_m) [28-30]. These changes in V_{max} would be consistent with a decrease in the number of functional transport proteins as a consequence of either decreased gene expression or targeting of the protein to the canalicular membrane as a general mechanism ultimately leading to cholestasis in these different experimental models. Cloning of various transport systems has precipitated the availability of molecular probes for several canalicular transport systems. Therefore, it has now become possible to investigate potential changes in the expression of hepatocellular (canalicular) transport systems in various experimental and clinical forms of cholestasis. In addition, knockout animals and naturally occurring mutants deficient in specific canalicular transport functions/systems have provided major insights in the physiological function of canalicular transport systems and their role in the pathogenesis of cholestatic liver disease.

Table 2. Experimental rat models of intrahepatic and obstructive cholestasis

Model	Clinical correlate
Endotoxin, inflammatory cytokines (e.g., TNF- α , IL-1 β)	Cholestasis of sepsis, total parenteral nutrition, cholestatic hepatitis (viral, alcoholic)
Hormones (ethinylestradiol, estradiol-17 β -glucuronide)	Oral contraceptive-induced cholestasis, cholestasis of pregnancy
Drugs (chlorpromazine, cyclosporin A, diclofenac, α -naphthylisothiocyanate)	Drug-induced cholestasis
Common bile duct ligation	Extrahepatic biliary obstruction

ALTERATIONS OF CANALICULAR TRANSPORT SYSTEMS IN CHOLESTASIS

Since transport processes through the canalicular membrane generally represent the rate-limiting step in bile formation, it may not be surprising that impairment of canalicular transport systems plays a major role in the pathogenesis of cholestasis. Impaired canalicular excretory function will result in reduction of bile flow (pathophysiological cholestasis) and in hepatocellular and systemic retention of potentially toxic endogenous and exogenous biliary constituents (clinical cholestasis). Defects in transporter system expression represent an important subset of mechanisms which can cause cholestasis. While many of these changes may be regarded as secondary phenomenon to the retention of biliary constituents within hepatic parenchyma, and thus a consequence rather than a cause of cholestasis, others, in particular genetic defects, may be considered to be the primary initiating cholestatic event. Nevertheless, reduced or even absent expression of a gene or its product once cholestasis is established, may provide an explanation for the ongoing functional impairment in bile formation. The following section attempts to correlate functional and molecular alterations of canalicular transport systems in experimental models of cholestasis (Table 3) and outlines potential implications of these changes for the pathophysiology of cholestasis. Furthermore, molecular changes of canalicular transporters in human cholestatic disorders are discussed whenever possible, although this information is still very sparse.

Bile salts

The canalicular excretion of monovalent bile salts is mediated by a canalicular bile acid transporter (cBAT/cbat) whereas the excretion of divalent sulfated and glucuronidated bile salt conjugates is mediated by the conjugate export pump (MRP2/mrp2) (see below). Functional studies in isolated canalicular membrane vesicles have clearly demonstrated inhibition of ATP-dependent transport of monovalent bile salts in various experimental models of cholestasis induced by LPS [29, 30], EE [28], cyclosporin A (CSA) [31]. Since the V_{\max} of ATP-dependent canalicular bile acid transport is diminished in most of these models of cholestasis [28-30], it could be expected that the expression of cbat is down-regulated by these cholestatic agents/injuries. However, the information on the molecular expression of canalicular bile acid transporter(s) in these forms of cholestasis is

Table 3. Expression of canalicular transport systems in experimental models of cholestasis.

Transporter	Endotoxin	Ethinylestradiol	Biliary obstruction
Mrp2	mRNA ↓ protein ↓	mRNA ↔ protein ↓	mRNA ↓ protein ↓
P-gp	protein ↔	protein ↔	mRNA (mdr1a,b)↑ protein ↑
Spgp	protein ↓	N. D.	protein ↓
Ecto-ATPase	mRNA ↔ protein ↔	mRNA ↔ protein ↔	mRNA ↔ protein ↔

↓ decreased; ↔ unchanged; ↑ increased; N.D. = not determined.

limited since the “definitive” canalicular bile acid transporter (cBAT/cbat) has not been cloned yet, although several candidate genes have been identified: In a preliminary report, down-regulation of the sister of P-glycoprotein (spgp) [32] was demonstrated in LPS-induced cholestasis and CBDL in rats [33]. This finding could be of interest, since spgp is exclusively expressed in hepatocytes at the canalicular membrane to a similar extent as the *mdr2* isoform of P-gp [32, 33] and some authors consider the spgp a candidate for the canalicular bile acid transporter [34]. Alternative candidate genes include the (yet unidentified) mammalian equivalent of a yeast gene (*BAT1p*) encoding an ATP-dependent transporter for taurocholate in yeast vacuoles [35], and a group of 150 to 200-kDa plasma membrane proteins overexpressed in a rat hepatoma-derived HTC cell line resistant to glycocholic acid [36, 37].

The expression of the canalicular enzyme and putative bile acid transporter $\text{Ca}^{++}\text{-Mg}^{++}$ -ecto-ATPase [38-40] was decreased after LPS and TNF- α administration in one study [29], but not in two others [41, 42]. In addition, ecto-ATPase expression also did not change during EE-induced cholestasis [43] and CBDL [44]. However, the true role of ecto-ATPase in canalicular bile acid transport remains controversial, since the sequence of this protein predicts a single membrane-spanning domain and the ATPase activity is oriented toward the extracellular rather than the intracellular domain and can be uncoupled from transport activity [39, 40], which does not conform to current expectations for an ATP-dependent in-to-out transport protein [16, 45].

Amphiphilic anionic conjugates

The biliary excretion of various anionic conjugates (e.g., bilirubin diglucuronide, GSH-conjugates, cysteinyl leukotrienes, oxidized glutathione (GSSG), dibromosulphothalein (BSP), divalent bile acid glucuronides and sulfates) is also decreased in several models of intrahepatic (LPS, EE) and extrahepatic cholestasis (CBDL) [28, 30, 46-50]. Again, these functional studies have revealed decreased V_{max} without changes in K_{m} , consistent with a change in the number of functional transporters for these substrates. Recent studies have demonstrated that the expression of the rat canalicular conjugate export pump (*mrp2*) is profoundly decreased by LPS, EE, and CBDL at the protein level [51]. *Mrp2* steady-state mRNA levels are also markedly decreased in LPS and CBDL animals, but remain unchanged in EE-induced cholestasis. The findings in LPS-treated rats and CBDL suggest that the reduction of *mrp2* protein levels may result at least in part from a decrease in steady-state mRNA-levels, probably due to a reduction in the rate of gene transcription. Although the half-life of the *mrp2* protein is currently unknown, the rapid disappearance of the protein within 16 hr after LPS suggests either that the half-life of this protein is normally short or that it is rapidly degraded during LPS-induced cholestasis. In contrast to LPS and CBDL, *mrp2* steady-state mRNA levels did not decline in EE-treated rats, suggesting that the reduction of *mrp2* protein levels in this form of cholestasis occurs exclusively by posttranslational mechanisms which remain to be determined.

Decreased *mrp2* expression may provide a molecular mechanism explaining impaired excretion of a broad range of endogenous and exogenous organic anions and thereby many biochemical features of cholestasis, including conjugated hyperbilirubinemia and impaired BSP and indocyanine green clearance. The effects on *mrp2* expression are most pronounced in LPS-induced cholestasis, consistent with elevations of (mostly conjugated) serum bilirubin as the key-feature of sepsis-associated cholestasis (“jaundice of sepsis”) [52, 53]. Preliminary findings from our laboratory suggest, that inflammatory cytokines such as tumor necrosis factor- α and interleukin-1 β decrease *mrp2* mRNA and protein levels in primary rat hepatocytes *in vitro*, suggesting that the effects of LPS may be mediated through these proinflammatory cytokines. The clinical implications of these findings may not only be restricted to the pathogenesis of cholestasis of sepsis. Several other liver

diseases such as total parenteral nutrition-induced cholestasis, alcoholic hepatitis and liver cirrhosis are associated with significant portal vein and systemic endotoxemia and/or elevated levels of inflammatory cytokines [54-57]. In addition, down-regulation of *mrp2* may have broader implications for the pathogenesis of intrahepatic cholestasis, since biliary glutathione (GSH) excretion and thereby bile acid-independent bile flow is diminished in mutant rats with congenitally absent *mrp2* protein (see below). Thus, down-regulation of *mrp2* expression may represent a primary event in the pathogenesis of intrahepatic cholestasis by reduction of bile acid-independent canalicular bile flow. Finally, decreased *mrp2* expression and function during cholestasis may also have important implications for the metabolism and excretion of various potentially toxic endo- and xenobiotics [17, 58]. Drugs such as ampicillin and ceftriaxone are directly excreted into bile by *mrp2* [14-16], and their biliary excretion may be impaired during cholestasis [59, 60].

Mutant Groningen Yellow (GY)/transport deficient (TR^-) and Eisai hyperbilirubinemic (EHBR) rats have a congenital defect in *mrp2* function characterized by absent canalicular transport for various endogenous and exogenous organic anions and are the rat model for the Dubin-Johnson syndrome in humans [14]. Cross-breeding studies among the individual mutant rat strains resulted in jaundiced offspring in either case, indicating that GY/TR^- and EHBR rats have an identical (autosomal recessive) defect. Recently, the molecular basis for this hereditary defect in GY/TR^- rats has been identified as a single-nucleotide deletion in the *mrp2* gene resulting in a frame shift which leads to the introduction of an early stop codon (at amino acid 401) [10]. In EHBR rats, a single nucleotide substitution (but not deletion) also results in the introduction of a premature stop codon (at amino acid 855) [61]. Both mutations lead to absence of the *mrp2* protein from the livers of GY/TR^- and EHBR rats, respectively [10, 11]. The untranslated *mrp2* mRNA is rapidly degraded explaining the very low to absent mRNA levels of *mrp2* in these rats. Recently, additional ATP-dependent canalicular conjugate export systems such as MRP-like proteins (MLP 1 and 2) preserved in mutant EHBR rats have been identified [62] and await further molecular characterization in models of cholestatic liver disease. In addition, ATP-independent, membrane potential-dependent transport routes for some *mrp2*/cacoat substrates (e.g., bilirubin glucuronide) are preserved in the mutant rats [16]. Taken together, these transport systems may explain residual canalicular conjugate transport under conditions with congenitally absent (mutant animals) or down-regulated *mrp2* (cholestasis).

The human homologue of the canalicular conjugate export pump (*MRP2*) has also recently been cloned [12, 13] and the *MRP2* protein is absent in the Dubin-Johnson syndrome [63] as a result of a mutation of the human *MRP2* gene [64]. This syndrome is characterized by conjugated hyperbilirubinemia and a selective abnormality in the biliary excretion of a range of endogenous (bilirubin, coproporphyrin isomer series I) and exogenous anionic conjugates (BSP, ICG conjugates, the oral cholecystographic agent iopanoic acid), and is the human counterpart to the defect in GY/TR^- and EHBR rats [14, 65]. However, patients with Dubin-Johnson syndrome are usually considered to be hyperbilirubinemic rather than cholestatic since there are no histological or biochemical signs of cholestasis. Whether bile flow is decreased in Dubin-Johnson syndrome patients, is not known.

Organic cations

The expression of P-glycoproteins (P-gp) at the canalicular membrane is dramatically increased in CBDL and α -naphthylisothiocyanate-induced cholestasis [51, 66, 67] but remains essentially unchanged in other forms of cholestasis induced by LPS and EE [51], although one group has described a slight decrease in canalicular P-gp content (by 15 percent) in EE-induced cholestasis [67]. In rat liver, the increase in P-gp in CBDL and α -naphthylisothiocyanate-induced cholestasis is mainly due to increased *mdr1a* and *1b* gene expression which is mainly accomplished through enhanced gene transcription [66]. In

addition, the P-gp content of the canalicular membrane appears to be also regulated through biliary excretion of P-gp. Interestingly, P-gp release into bile is markedly impaired in CBDL, which could contribute to the increase in the hepatic P-gp content [67]. The resulting higher level of the biliary cation efflux pump *mdr1* P-gp may facilitate elimination of potentially toxic biliary constituents and thus prevent or limit hepatocellular damage in cholestasis. Indeed, the increased expression of *mdr1* P-gp results in increased biliary excretion of the *mdr1* substrate vinblastine [67].

Knockout mice for the various *mdr1* isoforms have allowed an assessment of their role in hepatobiliary transport physiology and the pathogenesis of cholestasis. *Mdr1a*(-/-) knockout mice have normal bile flow and biliary organic cation excretion is only modestly affected, since compensatory up-regulation of hepatic *mdr1b* expression occurs [68, 69]. Subsequently, *mdr1a/1b* (-/-) double knockout mice have been developed to control for compensatory (over-) expression of *mdr1b* Pgp in the liver [70]. These animals are fully viable and, again, have normal bile flow, but biliary cation excretion is markedly impaired. The consequences of a *mdr1* defect may not become evident, unless exogenous toxins (that normally are eliminated via *mdr1* P-gp) are ingested with the food chain [69]. *Mdr1* substrates such as CSA inhibit ATP-dependent canalicular bile acid [31, 71] and organic anion transport [71] in the rat, and accumulation of such substrates may indirectly lead to cholestasis. Similarly, the phenotype of a human *MDR1* defect would be expected to remain undetected unless challenged with a *MDR1* substrate (e.g., under therapeutic conditions). Therefore, it could be possible that some forms of drug-induced hepatocellular cholestasis should occur in patients with such a defect.

Phospholipids

Biliary excretion of phospholipids is decreased in cholestasis induced by EE [66, 72] and CSA [72], but is markedly increased in the first days following CBDL [66] and decreases after prolonged CBDL [73]. Both the decrease in canalicular P-gp content described in EE-induced cholestasis [67] and the marked increase in P-gp expression after the first days of CBDL [51, 66, 67] would provide a potential molecular mechanism for these functional alterations, although it was not demonstrated that these changes in P-gp expression involve the *mdr2* isoform (phospholipid export pump).

The results in *mdr2* (-/-) knockout mice have shown that a defect in biliary phospholipid secretion at the hepatocellular level can lead to cholangiopathy and severe liver disease [20, 75]. Therefore, the pathophysiology of a number of human neonatal and adult choletastatic syndromes deserves re-evaluation in the light of a possible *MDR3* (human *mdr2* homologue) defect. So far, *MDR3* mRNA was found to be absent in one single patient with a subtype of progressive familial intrahepatic cholestasis (type 3) characterized by elevated serum gamma-glutamyl transpeptidase activity, as well as bile duct proliferation and inflammatory infiltrate in portal areas, thereby resembling the hepatic injury observed in *mdr2* (-/-) mice, and the biliary phospholipid levels were substantially decreased in another patient with this disease [76]. However, no evidence for deficient or reduced intrahepatic *MDR3* mRNA levels in liver biopsy samples from patients with primary biliary cirrhosis was found by reverse transcription/competitive polymerase chain reaction [77], suggesting that decreased *MDR3* gene expression does not play a role in the pathogenesis of primary biliary cirrhosis.

Cholesterol

Biliary cholesterol excretion is impaired in various experimental models of cholestasis such as EE-induced cholestasis [72], CSA-induced cholestasis [73] and CBDL [74]. The detailed molecular mechanism for these findings remains currently unclear although impaired bile salt excretion observed in these models probably plays a major role [16, 78].

Interestingly, a putative hepatic cholesterol carrier has been identified recently and awaits further characterization in experimental models of cholestasis [79].

Glutathione

Biliary glutathione (GSH) excretion is markedly impaired in LPS-induced cholestasis [50] and CBDL [46, 80] and results in increased hepatocellular GSH levels in these models of cholestasis [48, 50, 80]. In contrast to the decrease of biliary GSH excretion in livers from LPS-treated rats, GSH transport is normal *in vitro* in canalicular membrane vesicles isolated from these animals [81]. This finding of an apparent discrepancy between the intact liver and vesicle transport of GSH is reminiscent of what has been described in EHBR rats [82] with congenital absence of *mrp2* (see above) where defective biliary GSH excretion is thought to be either a direct [24] or indirect effect [82, 83] of the *mrp2* mutation. Impaired biliary excretion of GSH in mutant rats and these models of cholestasis could be best explained as a secondary defect due to cis-inhibition of canalicular GSH transport from retained substrates for the defective *mrp2* and/or loss of trans-stimulation by these same substrates which are normally excreted into the bile canaliculus [82]. A recent report raised the possibility that MRP may transport GSH in cell lines transfected with *MRP* [84], and it was, therefore, suggested that its canalicular isoform *MRP2/mrp2* might also directly participate in canalicular GSH transport [17, 24]. Although this would provide a "unifying hypothesis" for both impaired biliary GSH excretion and impaired excretion of amphiphilic anionic conjugates under conditions with a congenital (e.g., mutant rats) or acquired (e.g., LPS, CBDL) *mrp2* defect, a major role for *MRP2/mrp2* in canalicular GSH transport appears unlikely, considering the fact that canalicular GSH transport is electrogenic (but not ATP-dependent) and bidirectional (but not unidirectional) as opposed to transport mediated by *MRP2/mrp2* [83].

Bicarbonate

Biliary HCO_3^- excretion is markedly impaired in EE-induced cholestasis [85] and to a lesser degree in LPS-induced cholestasis [50]. However, in EE-treated animals, canalicular $\text{Cl}^-/\text{HCO}_3^-$ anion exchanger activity and its hormonal regulation are normal, and impaired biliary HCO_3^- excretion is apparently caused by regurgitation of biliary HCO_3^- via leaky tight junctions from the bile canaliculus into the liver sinusoids [85]. In contrast, CBDL leads to increased biliary HCO_3^- excretion from proliferating bile ductules, particularly when stimulated with secretin [74]. No molecular data are so far available regarding the expression of $\text{Cl}^-/\text{HCO}_3^-$ anion exchanger (*AE2*) genes in these experimental models of cholestasis in rodents. However, recent studies in humans have revealed abnormal expression of *AE2* in liver from patients with primary biliary cirrhosis [86-88]. *AE2* immunoreactivity at canaliculi and bile ducts was decreased in such livers, and *AE2* mRNA levels in liver tissue from these patients were also reduced. Since $\text{Cl}^-/\text{HCO}_3^-$ -exchange activity is believed to be essential to secretion of both canalicular and ductular bile, decreased expression of *AE2* in the liver may lead to impaired bile secretion. Diminished *AE2* expression has also been reported in salivary glands from patients with primary biliary cirrhosis and Sicca syndrome [87], which may indicate a more generalized epithelial " HCO_3^- secretory failure" in PBC.

SUMMARY AND CONCLUSIONS

The canalicular excretion of bile acids and various non-bile acid organic anions is impaired in several experimental models of cholestasis, such as endotoxin (LPS)-induced cholestasis, estrogen (EE)-induced cholestasis and bile duct ligation (CBDL). Recent advances in molecular biology have permitted studies on the expression of canalicular

transport proteins in these models of cholestasis, and have provided new insights in the molecular pathogenesis of cholestasis. LPS, EE, and CBDL all down-regulate the expression of the canalicular conjugate export pump (*mrp2*) which may explain impaired excretion of amphiphilic anionic conjugates (e.g., conjugated bilirubin) in these forms of cholestasis. Down-regulation of transporter genes such as *mrp2* may represent an important general mechanism of cholestasis in these experimental models. The expression of P-glycoproteins remains essentially unchanged in LPS- and EE-induced cholestasis, but increases dramatically following CBDL due to the *mdr1* isoform, which may reflect an attempt of the hepatocyte to facilitate the biliary excretion of lipophilic cholephiles retained in the liver in cholestasis. Although canalicular bile salt transport is also impaired in cholestasis, conclusive molecular studies have to await the cloning of the "definitive" canalicular bile acid transporter (cBAT/cbat). With the increasing availability of molecular probes for these transport systems in humans, new information on the molecular regulation of canalicular transport proteins in human cholestatic liver diseases should bring new insights into their pathophysiology and treatment.

Added in proof: Since submission of this manuscript the sister of P-glycoprotein (*spgp*) has been identified as the canalicular bile acid transporter (cBAT/cbat) of mammalian liver (Gerloff et al., *Hepatology* 26:358A, 1997 and *J. Biol. Chem.* in press, 1998). Mutations of the human *SPGP* gene could be responsible for progressive familial intrahepatic cholestasis type 2 (Byler's Syndrome) (Strautnieks et al., *Am. J. Hum. Genet.* 61:630-633, 1997). Another subtype, progressive familial intrahepatic cholestasis type 3 is caused by a mutation of the human *MDR3* gene resulting in the absence of MDR3 P-glycoprotein in the liver and reduced/absent biliary phospholipid excretion in these patients (deVree et al., *Proc. Natl. Acad. Sci. U.S.A.* 95:282-287, 1998). Progressive familial intrahepatic cholestasis type I (Byler's Disease) and benign recurrent intrahepatic cholestasis appear to be due to a mutation of the same gene, despite their entirely different phenotypes and prognosis. This gene (called *FIC1*) normally encodes a P-type ATPase which is expressed more in the small intestine than in liver and likely plays a role in the enterohepatic circulation of bile acids (Bull et al., *Nature Genetics* 18:219-224, 1998).

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