Taurocholic Acid Feeding Prevents Tumor Necrosis Factor-α–Induced Damage of Cholangiocytes by a PI3K–Mediated Pathway

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Cholangiopathies, such as primary biliary cirrhosis and primary sclerosis cholangitis, are characterized at the end stage by ductopenia due to increased cholangiocyte apoptosis and decreased cholangiocyte proliferation. Although cholangiocyte proliferation is associated with an increased number of intrahepatic bile ducts and secretin-stimulated ductal secretion, ductopenia is coupled with decreased ductal mass and secretin-induced ductal secretory activity. We have shown that a single injection of actinomycin D + tumor necrosis factor-α (TNF-α) to bile duct–ligated (BDL) rats induces cholangiocyte injury, which is characterized by loss of cholangiocyte proliferation, and secretory activity and by an increase in cholangiocyte apoptosis. We also have shown that taurocholic acid both *in vivo* and *in vitro* stimulates cholangiocyte proliferation. We hypothesize that taurocholic acid feeding protects cholangiocytes against TNF-α–induced apoptosis through a phosphatidylinositol-3-kinase (PI3K)–dependent pathway. Immediately after BDL, rats were fed taurocholic acid or control diet in the absence/presence of daily injections of wortmannin for 1 week. Seven days later, control-fed or taurocholic acid–fed rats were treated with a single intraperitoneal injection of actinomycin D + TNF-α. Twenty-four hours later we evaluated: (i) cholangiocyte apoptosis and proliferation in liver sections and (ii) basal and secretin-stimulated bile and bicarbonate secretion in bile fistula rats. Taurocholic acid feeding prevented TNF-α–induced increases in cholangiocyte apoptosis and decreases in growth and secretin-stimulated bile and bicarbonate secretion, changes that were blocked by PI3K inhibition. The PI3K survival pathway is important in bile acid protection against immune-mediated cholangiocyte injury in cholestatic liver diseases. Exp Biol Med 232:942–949, 2007

Key words: apoptosis; bile flow; intrahepatic biliary epithelium; proliferation; secretin

Introduction

Bile is first secreted at the bile canaliculus of hepatocytes (1), and before it reaches the small intestine it is modified by cholangiocytes through a series of reabsorptive and secretory events that are modulated by several factors, including gastrointestinal hormones (e.g., secretin, somatostatin, and gastrin), neurotransmitters, and bile salts (2–8). Secretin is of particular importance, because its effects on bile secretion (4, 7, 9) and the expression of its receptor (present only in cholangiocytes in the rat liver; Ref. 10) is altered based on changes in

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cholangiocyte proliferation and bile duct mass observed in cholestatic liver diseases (3, 4, 10–12). For instance, in pathologic conditions associated with enhanced cholangiocyte proliferation (e.g., following bile duct ligation [BDL], partial hepatectomy or chronic bile acid feeding; Refs. 3, 4, 11) there is enhanced secretin receptor gene expression and increased secretin-stimulated ductal secretion (3, 4, 10, 11, 13). Conversely, reduced cholangiocyte growth (associated with bile duct damage, for example, after acute carbon tetrachloride [CCl_4] administration) is coupled with reduced secretin receptor expression and decreased secretin-stimulated, bicarbonate-rich choleresis (12). The stimulatory effect of secretin on ductal secretion is mediated by its interaction with basolateral receptors and an increase in intracellular cAMP levels (13, 14). Increased cAMP levels (13, 14) leads to opening of the Cl^- channel, cystic fibrosis transmembrane regulator (15), and activation of the Cl^-/HCO_3^- exchanger (16), with subsequent secretion of bicarbonate into bile (4).

Impaired bile secretion (during cholestasis) is due to functional damage of either hepatocytes or cholangiocytes (9, 17). Chronic cholestasis is a characteristic of several chronic liver diseases, including biliary strictures (18), primary sclerosing cholangitis (PSC; Ref. 19), and cholangiocarcinoma (20). Studies have demonstrated the role of impaired canalicular membrane transport function as well as the effects of impaired bile flow and bile salt accumulation on hepatocyte damage (21–24). Although there is growing information on the role of cholangiocytes in the development of cholestasis (12, 25, 26), the mechanisms of cholangiocyte injury during chronic cholestasis remain unclear.

Tumor necrosis factor-α (TNF-α) regulates epithelial cell injury as well as immune-mediated cholangiocyte damage (27). Immune-mediated cholangiocyte injury has been suggested to play a role in the pathogenesis of cholangiopathies, including primary biliary cirrhosis (PBC) and PSC (9, 28). Furthermore, systemic levels of TNF-α are enhanced following biliary obstruction in mice (29). *In vitro* studies have shown that TNF-α, in combination with interleukin-1 (IL-1), IL-6, and interferon-γ, inhibits cAMP-dependent ductal secretion (30). Although we have shown (26) that TNF-α, when administered in combination with actinomycin D, induces cholangiocyte damage and loss of ductal secretion in BDL rats, information on the role of TNF-α and death receptors in the modulation of cholangiocyte function during chronic cholestasis is limited.

Bile acids regulate the apoptotic, proliferative, and secretory activities of the intrahepatic biliary epithelium (2, 3, 9, 31–34) by entering cholangiocytes by a specific transporter, the Na^+-dependent apical bile acid transporter, ASBT (35). Studies have shown that bile acids (taurocholic acid and ursodeoxycholic acid) protect intrahepatic bile ducts against injury induced by parasympathetic (32, 34) or sympathetic (36) denervation or CCl_4 administration (33) by a phosphatidylinositol-3-kinase (PI3K)–dependent pathway (37), a major determinant of cell growth and survival (33, 34). No information exists regarding either (i) the protective effect of taurocholic acid against TNF-α–induced changes in cholangiocyte apoptosis and proliferation and secretin-stimulated ductal secretion, and (ii) role of the PI3K system in taurocholic acid prevention of TNF-α–induced changes in cholangiocyte functions. Thus, we posed the following questions: (i) Does taurocholic acid feeding protect the intrahepatic biliary epithelium from the damage induced by the administration of actinomycin + TNF-α administration to BDL rats (26)? and (ii) Is taurocholic acid prevention of TNF-α–induced changes in cholangiocyte apoptosis and proliferation blocked by the *in vivo* administration of the PI3K inhibitor, wortmannin (34)?

Materials and Methods

Materials. Reagents were purchased from Sigma Chemical (St. Louis, MO) unless otherwise indicated. Rat chow (containing AIN 76, control diet, or 1% taurocholic acid in AIN 76) was prepared by Dyets Inc. (Bethlehem, PA). Porcine secretin was purchased from Peninsula Laboratories (Belmont, CA). The monoclonal mouse antibody against proliferating cellular nuclear antigen (PCNA) was purchased from DAKO (Kyoto, Japan). The mouse anti–cytokeratin-19 (anti–CK-19) antibody was purchased from Amersham (Arlington Heights, IL). Recombinant TNF-α was purchased from R&D Systems (Minneapolis, MN).

Animal Models. Male Fisher 344 rats (175–200 g) were purchased from Charles River (Wilmington, MA), maintained in a temperature-controlled environment (20°C–22°C) with a 12:12-hr light:dark cycle, and fed *ad libitum* standard rat chow. Rats had free access to drinking water. The studies were performed in two models. The first included BDL (for liver block collection) or bile duct–incannulated (BDI, for bile collection) rats that, immediately after BDL (4) or BDI (4), were fed control diet (AIN 76) for 1 week, followed by a single intraperitoneal injection of 0.9% NaCl or actinomycin D (100 μg/kg body wt) + TNF-α (50 ng/kg body wt; Ref. 26). Twenty-four hours later, the animals were sacrificed for the selected experiments. The second model included BDL or BDI rats that, immediately after BDL or BDI (4), were fed 1% taurocholic acid (which represents an approximate dose of 275 μmol/day; Ref. 3) for 1 week in the presence of daily injections of 0.9% NaCl or wortmannin (0.7 mg/kg body wt; Ref. 34) dissolved in dimethyl sulfoxide (DMSO), followed by a single intraperitoneal injection of actinomycin D (100 μg/kg body wt) + TNF-α (50 ng/kg body wt; Ref. 26). Twenty-four hours later, the animals were sacrificed for the selected experiments. BDL and BDI were performed as described (4). The groups of animals used in the study are summarized in Table 1. Since we have previously shown that daily injections of DMSO or wortmannin to BDL rats do not affect

Table 1. Animal Models[a]

Treatment	Surgery	Treatment
BDL + BA control feeding + NaCl	BDL or BDI	Immediately after surgery, rats were fed BA control diet for 1 week. After 1 week, rats were treated with a single injection of NaCl. After injection, rats were used 24 hrs later
BDL + BA control feeding + AT	BDL or BDI	Immediately after surgery, rats were fed BA control diet for 1 week. After 1 week, rats were treated with a single injection of AT. After injection, rats were used 24 hrs later
BDL + TC feeding + AT	BDL or BDI	Immediately after surgery, rats were fed TC for 1 week. After 1 week, rats were treated with a single injection of AT. After injection, rats were used 24 hrs later
BDL + TC feeding + wortmannin + AT	BDL or BDI	Immediately after surgery, rats were fed TC for 1 week and simultaneously treated by intraperitoneal injections of wortmannin. After 1 week, rats were treated with a single injection of AT After injection, rats were used 24 hrs later

[a] BA, bile acid; AT, actinomycin D + TNF-α; TC, taurocholic acid.

cholangiocyte survival, proliferation, and functional activity (36, 38), and that taurocholic acid feeding to BDL rats does not alter cholangiocyte apoptosis and increase cholangiocyte growth (32), these groups of animals were not included in the study. Before each experimental procedure, the animals were anesthetized intraperitoneally with pentobarbital sodium (50 mg/kg body wt) according to the regulations of the panel on euthanasia of the American Veterinarian Medical Association.

Measurement of Cholangiocyte Apoptosis, Proliferation, and Secretion. We performed studies to evaluate whether: (i) *in vivo* administration of actinomycin D + TNF-α to BDL rats induces damage of intrahepatic bile ducts, leading to enhanced apoptosis and loss of proliferative and secretory activity of cholangiocytes; (ii) taurocholic acid feeding prevents actinomycin D + TNF-α–induced duct damage and loss of cholangiocyte proliferation and secretion (26); and (iii) consistent with the concept that PI3K plays a role in taurocholic acid prevention of actinomycin D + TNF-α duct damage, chronic daily intraperitoneal injections of wortmannin block taurocholic acid prevention of actinomycin D + TNF-α–induced changes in cholangiocyte apoptosis, proliferation, and secretion.

Cholangiocyte apoptosis was evaluated by TUNEL analysis in liver sections using a commercially available kit (Wako Chemicals, Tokyo, Japan). Following counterstaining with hematoxylin solution, sections (4 μm thick; three slides evaluated per group of animals) were examined by light microscopy with an Olympus BX-40 (Melville, NY) microscope equipped with a CCD camera. Approximately 200 cells per slide were counted in a coded fashion in seven nonoverlapping fields.

Cholangiocyte proliferation was measured by quantitative evaluation of the number of PCNA- and CK-19–positive cholangiocytes in liver sections (5 μm thick; three slides evaluated per group of animals) from the selected group of animals. Immunohistochemistry for PCNA or CK-19 was performed in paraffin-embedded sections (5 μm; three slides analyzed for each group) as described (11, 25). Sections were counterstained with hematoxylin and examined with a microscope (U-PMTVC; Olympus Optical Co., Tokyo, Japan). More than 100 cholangiocytes were counted in a random, blinded fashion in three different fields for each group of animals. Data were expressed as number of PCNA- or CK-19–positive cholangiocytes per 100 cholangiocytes.

Following anesthesia with sodium pentobarbital, the selected rats were surgically prepared for bile collection as described (4). When steady-state bile flow was reached (60–70 mins from the infusion of Krebs Ringer Henseleit [KRH]), the selected animal was infused with secretin (100 n*M*) for 30 mins and a final infusion of KRH for 30 mins. Bile was collected every 10 mins in preweighed tubes and immediately stored at –70°C before determination of bicarbonate concentration. Bicarbonate concentration (measured as total CO_2) in the selected bile samples was determined by an ABL 520 Blood Gas System (Radiometer Medical A/S, Copenhagen, Denmark).

Statistical Analysis. All data are expressed as mean ± SEM. Differences between groups were analyzed by the Student's unpaired *t* test when two groups were analyzed and analysis of variance (ANOVA) when more than two groups were analyzed.

Results

Measurement of Cholangiocyte Apoptosis, Proliferation, and Secretion. In agreement with previous studies (26), a single injection of actinomycin D + TNF-α to 1-week BDL rats significantly increased the number of apoptotic cholangiocytes compared with liver sections from BDL rats treated with a single injection of NaCl (Fig. 1). Taurocholic acid feeding partially prevented the actinomycin D + TNF-α–induced increase in cholangiocyte apoptosis (Fig. 1). Consistent with the concept that the PI3K system plays a role in taurocholic acid protective effects on bile duct injury, wortmannin intraperitoneal

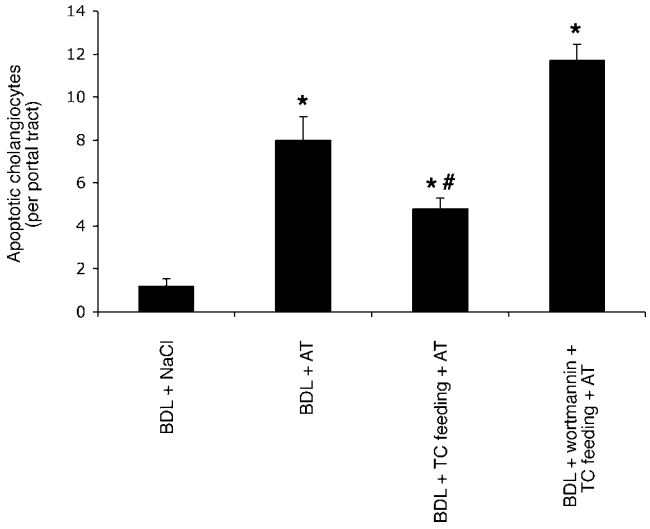

Figure 1. Measurement of cholangiocyte apoptosis by TUNEL analysis in liver sections from the selected group of animals. A single injection of actinomycin D + TNF-α to BDL rats significantly increased the number of apoptotic cholangiocytes compared with liver sections from BDL rats treated with a single injection of NaCl. Taurocholic acid feeding prevented actinomycin D + TNF-α–induced increase in cholangiocyte apoptosis. Wortmannin blocked taurocholic acid prevention of actinomycin D + TNF-α–induced increase in cholangiocyte apoptosis. Data are mean ± SEM of seven cumulative values from 10 randomly selected portal areas. *$P < 0.05$ *versus* cholangiocyte apoptosis from BDL rats treated with a single injection of NaCl. #$P < 0.05$ *versus* cholangiocyte apoptosis from: (i) 1-week BDL rats treated with a single injection of actinomycin D + TNF-α and (ii) rats that immediately after BDL were fed taurocholic acid for 1 week and simultaneously treated by intraperitoneal injections of wortmannin before being treated with a single injection of actinomycin D + TNF-α.

injections blocked taurocholic acid prevention of actinomycin D + TNF-α–induced increase in cholangiocyte apoptosis (Fig. 1). A single injection of actinomycin D + TNF-α decreased the number of PCNA- and CK-19–positive cholangiocytes compared with liver sections from BDL rats treated with a single injection of NaCl (Figs. 2 and 3). Taurocholic acid prevention of actinomycin D + TNF-α changes in cholangiocyte proliferation was blocked by wortmannin (Figs. 2 and 3).

Basal and secretin-stimulated bile flow, bicarbonate concentration, and secretion of BDI rats treated with a single injection of NaCl were similar to those of previous studies (Table 2; Refs. 4, 26). In agreement with our previous findings (26), in BDI rats treated with a single injection of actinomycin D + TNF-α, secretin did not increase bile flow and bicarbonate concentration and secretion compared with BDI rats treated with a single injection of NaCl (Table 2). Taurocholic acid feeding to BDI rats (treated with a single injection of actinomycin D + TNF-α) prevented TNF-α inhibition of secretin-stimulated bicarbonate-rich choleresis, which was similar to that of BDI rats treated with NaCl (Table 2). Consistent with the concept that the PI3K system regulates bile acid effects on cholangiocyte functions, chronic administration of wortmannin to taurocholic acid–

Table 2. Measurement of Basal and Secretin-Stimulated Bile Flow and Bicarbonate Concentration and Secretion[a]

Treatment	Basal bile flow (μl/min/kg body weight)	Secretin bile flow (μl/min/kg body weight)	Basal bicarbonate concentration (mEq/l)	Secretin bicarbonate concentration (mEq/l)	Basal bicarbonate secretion (μEq/min/kg body weight)	Secretin bicarbonate secretion (μEq/min/kg body weight)
BDI + BA control feeding + NaCl ($n = 9$)	93.8 ± 8.0	125.0 ± 10.7*	35.0 ± 2.4	46.5 ± 4.3**	3.2 ± 0.2	5.6 ± 0.5***
BDI + BA control feeding + AT ($n = 14$)	101.7 ± 4.8	113.2 ± 7.2****	45.5 ± 0.5	46.5 ± 2.7****	4.6 ± 0.3	5.2 ± 0.1****
BDI + TC feeding + AT ($n = 6$)	90.4 ± 7.1	135.1 ± 12.1*	34.2 ± 1.4	39.9 ± 1.6**	3.1 ± 0.3	5.2 ± 0.3***
BDI + TC feeding + wortmannin + AT ($n = 6$)	76.1 ± 9.1	85.8 ± 7.5****	43.6 ± 1.1	46.6 ± 2.0****	3.3 ± 0.4	4.1 ± 0.6****

[a] Data are means ± SE. BA, bile acid; AT, actinomycin D + TNF-α; TC, sodium taurocholate.
* $P < 0.05$ *versus* its corresponding value of basal bile flow; ** $P < 0.05$ *versus* its corresponding value of basal bicarbonate concentration; *** $P < 0.05$ *versus* its corresponding value of basal bicarbonate secretion; **** Not significant *versus* corresponding basal value of bile flow, bicarbonate concentration, or secretion. The differences between groups were analyzed by Student's *t* test when two groups were analyzed or analysis of variance (ANOVA) when more than two groups were analyzed.

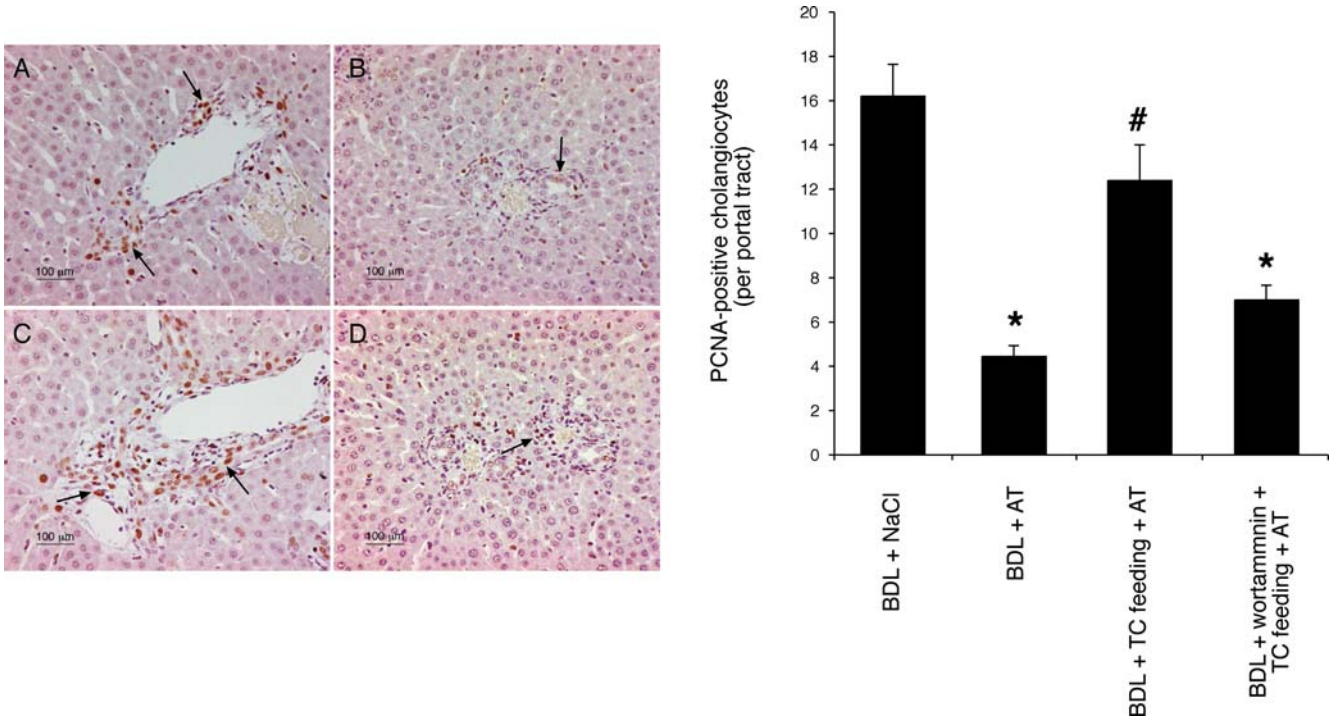

Figure 2 Measurement of PCNA-positive cholangiocytes in liver sections from the selected group of animals. A single injection of actinomycin D + TNF-α decreased the number of PCNA-positive cholangiocytes compared with liver sections from BDL rats treated with a single injection of NaCl. Taurocholic acid prevention of actinomycin D + TNF-α decrease in cholangiocyte proliferation was blocked by wortmannin. *$P < 0.05$ *versus* cholangiocyte apoptosis from BDL rats treated with a single injection of NaCl. Data are mean ± SEM of seven cumulative values from 10 randomly selected portal areas. #$P < 0.05$ *versus* cholangiocyte apoptosis from: (i) BDL rats treated with a single injection of actinomycin D + TNF-α and (ii) rats that immediately after BDL were fed taurocholic acid for 1 week and simultaneously treated by intraperitoneal injections of wortmannin before being treated with a single injection of actinomycin D + TNF-α. Bar = 100 μm.

fed BDI rats (treated with a single injection of actinomycin D + TNF-α) blocked the protective effect of taurocholic acid on secretin-stimulated, bicarbonate-rich choleresis (Table 2). In this group of animals, secretin did not increase bile flow and bicarbonate concentration and secretion (Table 2).

Discussion

The findings of this study relate to the protective role of taurocholic acid feeding *versus* TNF-α–induced changes in cholangiocyte apoptosis and proliferation and secretin-stimulated ductal secretion. We have shown that taurocholic acid feeding prevented TNF-α–induced increases in cholangiocyte apoptosis and decreases in biliary growth and secretin-stimulated ductal secretion, changes that were blocked by the simultaneous administration of the PI3K inhibitor, wortmannin (34).

Although cholangiopathies have different etiologies, they are characterized by chronic damage of different portions of the biliary tree, with consequent dysregulation of the balance between survival and death of cholangiocytes (9, 28, 39), a pathologic condition leading to vanishing of bile ducts and liver failure (9, 28, 39). This sequence of pathologic events seems to be paced by the progressive loss of bile ducts to adequately provide a compensatory proliferative response to chronic liver injury (28). Unfortunately, the knowledge of the factors modulating cholangiocyte proliferative response to liver injury is limited. Our study demonstrates that the balance between apoptosis and proliferation of cholangiocytes and their functional activity in the course of cholestasis (following extrahepatic bile duct obstruction) are likely modulated by the PI3K system (sensitivity to wortmannin) by the cooperative action of bile acids and TNF-α.

Studies suggest that TNF-α (a key mediator in inducing and processing inflammatory events; Refs. 40, 41) may play a role in the development of immune-mediated liver diseases (27). For example, during intrahepatic cholestasis there is enhanced formation of basal and endotoxin-induced TNF-α, which has been linked with the severity of liver damage (41). In experimental models of cholestasis (41) as well as in patients with PBC (42), there is increased expression of TNF-α and related receptors in cholangiocytes (41, 42). Moreover, TNF-α (when administered in combination with actinomycin D) induces cholangiocyte damage, decreased cholangiocyte proliferation, and loss of ductal secretion in BDL rats (26). Although oral bile acid replacement inhibits endotoxin-induced TNF-α production in cholestatic rats (41), no information exists regarding the coordinated role of bile acids and TNF-α in the regulation of cholangiocyte functions.

We first evaluated the effect of taurocholic acid feeding on TNF-α–induced changes in cholangiocyte functions and

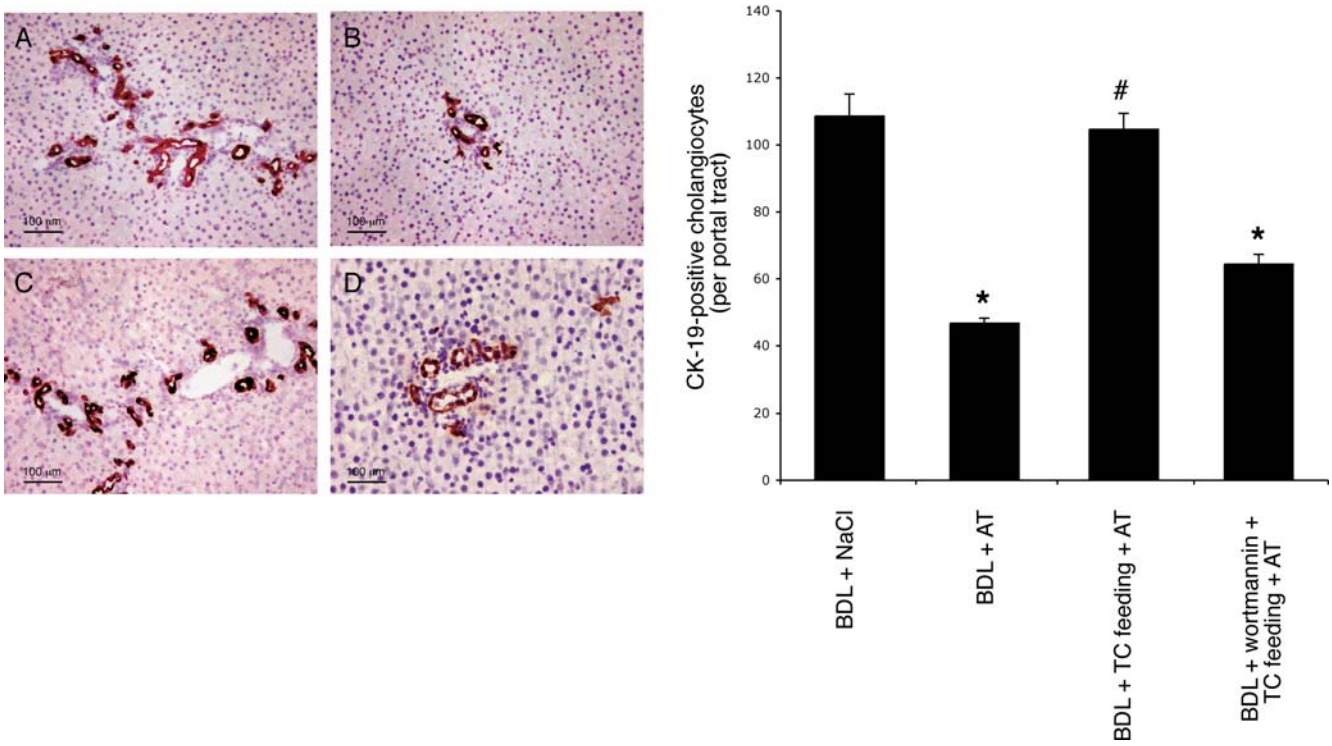

Figure 3. Measurement of CK-19–positive cholangiocytes in liver sections from the selected group of animals. A single injection of actinomycin D + TNF-α decreased the number of CK-19–positive cholangiocytes compared with liver sections from BDL rats treated with a single injection of NaCl. Taurocholic acid prevention of actinomycin D + TNF-α decrease in the number of CK-19–positive cholangiocytes was blocked by wortmannin. Data are mean ± SEM of seven cumulative values from 10 randomly selected portal areas. *$P < 0.05$ *versus* cholangiocyte apoptosis from (i) BDL rats treated with a single injection of NaCl; and (ii) rats that immediately after BDL were fed taurocholic acid for 1 week and simultaneously treated by intraperitoneal injections of wortmannin before being treated with a single injection of actinomycin D + TNF-α. Bar = 100 μm.

showed that taurocholic acid feeding prevents both the increase in cholangiocyte apoptosis and the decrease in cholangiocyte growth and secretin-stimulated, bicarbonate-rich choleresis induced by TNF-α. Our data support the concept that bile acids modulate the balance between cholangiocyte damage and proliferation. In support of this notion, although some bile acids (e.g., chenodeoxycholic and deoxycholic acids) are cytotoxic (27, 43, 44), other bile salts (e.g., taurochenodeoxycholic, ursodeoxycholic, tauroursodeoxycholic, and taurocholic acids) are cytoprotective in a number of different cells, including cholangiocytes (27, 32–34, 43, 45–48). Taurocholic acid, the most predominant "tauro-conjugated" bile acid in human bile (49), plays a key role in the regulation of the balance between cholangiocyte apoptosis and growth (3, 32, 33, 50). In support of this concept, prolonged bile depletion reduced the endogenous bile acid pool, cholangiocyte growth, and secretin-stimulated ductal secretion of BDL rats (50). In addition, taurocholic acid both *in vivo* and *in vitro* protects BDL cholangiocytes from a CCl_4-induced increase in apoptosis and loss of proliferative and secretory activity (33).

We next performed experiments to demonstrate that the protective effects of taurocholic acid on TNF-α–induced changes in cholangiocyte apoptosis, proliferation, and secretin-stimulated ductal secretion occur through a PI3K-dependent pathway (sensitivity to wortmannin) that plays a role in the modulation of cell growth and survival (33, 34). Consistent with this concept, taurocholic acid prevention of TNF-α effects on cholangiocyte apoptosis, proliferation, and secretin-stimulated ductal secretion were ablated by wortmannin. Our studies are consistent with the concept that PI3K plays an important role in bile acid modulation of cholangiocyte functions. The regulatory protein PI3K is involved in many different signaling pathways and regulates key functions of a number of cells, including cholangiocytes (32–34, 50–52). PI3K is considered a key intracellular factor that is responsible for the regulation of antiapoptotic signals and modulation of the balance between cell loss/growth (32–34, 53, 54). Overexpression of PI3K in cells is accompanied by a marked antiapoptotic effect and a significant increase in cell survival following radiation (19, 24). In support of our findings, the hydrophobic bile salt, taurochenodeoxycholate, activates PI3K-dependent survival mechanisms in hepatocytes, an effect preventing the cytotoxicity of this bile acid (43). In addition, cytoprotection against bile acid–induced apoptosis in rat hepatocytes is dependent on the PI3K pathway (55, 56).

We have shown recently that both ursodeoxycholic and taurocholic acids exert similar prosurvival effects on cholangiocytes (32–34). Specifically, in models where

cholestasis and induction of cholangiocyte death are simultaneously recreated, the administration of ursodeoxycholic and taurocholic acids prevents cholangiocyte apoptosis and maintains bile duct mass and functional activity to a similar extent. Moreover, we treated tauroursodeoxycholic acid (TUDCA)–fed BDL rats with a single intraperitoneal injection of actinomycin D + TNF-α, and we observed[1] that, similar to taurocholic acid, the treatment with TUDCA resulted in the prevention of the reduction of cholangiocyte growth and of the increase of cholangiocyte apoptosis.

In summary, our study demonstrates that taurocholic acid feeding prevents (through a PI3K-dependent pathway) cholangiocyte loss and the lack of proliferative and functional response to extrahepatic bile duct obstruction due to TNF-α. The present studies allow a better understanding of the mechanisms by which bile ducts are damaged and how certain bile acids protect against immune-mediated cholangiocyte injury in cholestatic liver diseases, including PBC and PSC (9, 28). Failure of bile acids to activate specific cytoprotective mechanisms in cholangiocytes may promote apoptosis and loss of cholangiocytes in these diseases.

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1. Nathanson MH, Boyer JL. Mechanisms and regulation of bile secretion. Hepatology 14:551–566, 1991.
2. Xia X, Francis H, Glaser S, Alpini G, LeSage G. Bile acid interactions with cholangiocytes. World J Gastroenterol 12:3553–3563, 2006.
3. Alpini G, Glaser S, Ueno Y, Rodgers R, Phinizy JL, Francis H, Baiocchi L, Holcomb LA, Caligiuri A, LeSage G. Bile acid feeding induces cholangiocyte proliferation and secretion: evidence for bile acid-regulated ductal secretion. Gastroenterology 116:179–186, 1999.
4. Alpini G, Lenzi R, Sarkozi L, Tavoloni N. Biliary physiology in rats with bile ductular cell hyperplasia. Evidence for a secretory function of proliferated bile ductules. J Clin Invest 81:569–578, 1988.
5. Barbaro B, Glaser S, Francis H, Taffetani S, Marzioni M, LeSage G, Alpini G. Nerve regulation of cholangiocyte functions. In: Alpini G, Alvaro D, LeSage G, Marzioni M, LaRusso NF, Eds. Pathophysiology of the Bile Duct System. Georgetown, TX: Landes Biosciences, pp199–209, 2004.
6. LeSage G, Alvaro D, Glaser S, Francis H, Marucci L, Roskams T, Phinizy JL, Marzioni M, Benedetti A, Taffetani S, Barbaro B, Fava G, Ueno Y, Alpini G. Alpha-1 adrenergic receptor agonists modulate ductal secretion of BDL rats via $Ca^{(2+)}$- and PKC-dependent stimulation of cAMP. Hepatology 40:1116–1127, 2004.
7. Kanno N, LeSage G, Glaser S, Alpini G. Regulation of cholangiocyte bicarbonate secretion. Am J Physiol Gastrointest Liver Physiol 281: G612–G625, 2001.
8. Glaser S, Rodgers RE, Phinizy JL, Robertson WE, Lasater J, Caligiuri A, Tretjak Z, LeSage G, Alpini G. Gastrin inhibits secretin-induced ductal secretion by interaction with specific receptors on rat cholangiocytes. Am J Physiol Gastrointest Liver Physiol 273:G1061–G1070, 1997.
9. Alpini G, Prall RT, LaRusso NF. The pathobiology of biliary epithelia. In: Arias IM, Boyer JL, Chisari FV, Fausto N, Jakoby W, Schachter D, Shafritz D, Eds. The Liver: Biology & Pathobiology, 4th Ed. Philadelphia, PA: Lippincott Williams & Wilkins, pp421–435, 2001.
10. Alpini G, Ulrich CD 2nd, Phillips JO, Pham LD, Miller LJ, LaRusso NF. Upregulation of secretin receptor gene expression in rat cholangiocytes after bile duct ligation. Am J Physiol Gastrointest Liver Physiol 266:G922–G928, 1994.
11. LeSage G, Glaser S, Gubba S, Robertson WE, Phinizy JL, Lasater J, Rodgers RE, Alpini G. Regrowth of the rat biliary tree after 70% partial hepatectomy is coupled to increased secretin-induced ductal secretion. Gastroenterology 111:1633–1644, 1996.
12. LeSage G, Glaser S, Marucci L, Benedetti A, Phinizy JL, Rodgers R, Caligiuri A, Papa E, Tretjak Z, Jezequel AM, Holcomb LA, Alpini G. Acute carbon tetrachloride feeding induces damage of large but not small cholangiocytes from BDL rat liver. Am J Physiol Gastrointest Liver Physiol 276:G1289–G1301, 1999.
13. Alpini G, Glaser S, Ueno Y, Pham L, Podila PV, Caligiuri A, LeSage G, LaRusso NF. Heterogeneity of the proliferative capacity of rat cholangiocytes after bile duct ligation. Am J Physiol Gastrointest Liver Physiol 274:G767–G775, 1998.
14. Alpini G, Roberts S, Kuntz SM, Ueno Y, Gubba S, Podila PV, LeSage G, LaRusso NF. Morphological, molecular, and functional heterogeneity of cholangiocytes from normal rat liver. Gastroenterology 110: 1636–1643, 1996.
15. Fitz JG, Basavappa S, McGill J, Melhus O, Cohn JA. Regulation of membrane chloride currents in rat bile duct epithelial cells. J Clin Invest 91:319–328, 1993.
16. Alvaro D, Cho WKC, Mennone A, Boyer JL. Effect of secretin on intracellular pH regulation in isolated rat bile duct epithelial cells. J Clin Invest 92:1314–1325, 1993.
17. Beuers U, Nathanson MH, Isales CM, Boyer JL. Tauroursodeoxycholic acid stimulates hepatocellular exocytosis and mobilizes extracellular Ca^{++} mechanisms defective in cholestasis. J Clin Invest 92:2984–2993, 1993.
18. Theilmann L, Kuppers B, Kadmon M, Roeren T, Notheisen H, Stiehl A, Otto G. Biliary tract strictures after orthotopic liver transplantation: diagnosis and management. Endoscopy 26:517–522, 1994.
19. Kowdley KV. Ursodeoxycholic acid therapy in hepatobiliary disease. Am J Med 108:481–486, 2000.
20. Suzuki S, Kurachi K, Yokoi Y, Tsuchiya Y, Okamoto K, Okumura T, Inaba K, Konno H, Nakamura S. Intrahepatic cholangiojejunostomy for unresectable malignant biliary tumors with obstructive jaundice. J Hepatobiliary Pancreat Surg 8:124–129, 2001.
21. Green RM, Gollan JL, Hagenbuch B, Meier PJ, Beier DR. Regulation of hepatocyte bile salt transporters during hepatic regeneration. Am J Physiol Gastrointest Liver Physiol 273:G621–G627, 1997.
22. Kullak-Ublick GA, Stieger B, Hagenbuch B, Meier PJ. Hepatic transport of bile salts. Semin Liver Dis 20:273–292, 2000.
23. Lee J, Azzaroli F, Wang L, Soroka CJ, Gigliozzi A, Setchell KD, Kramer W, Boyer JL. Adaptive regulation of bile salt transporters in kidney and liver in obstructive cholestasis in the rat. Gastroenterology 121:1473–1484, 2001.
24. Xu Y, Jones BE, Neufeld DS, Czaja MJ. Glutathione modulates rat and mouse hepatocyte sensitivity to tumor necrosis factor toxicity. Gastroenterology 115:1229–1237, 1998.
25. LeSage G, Glaser S, Ueno Y, Alvaro D, Baiocchi L, Kanno N, Phinizy JL, Francis H, Alpini G. Regression of cholangiocyte proliferation after cessation of ANIT feeding is coupled with increased apoptosis. Am J Physiol Gastrointest Liver Physiol 281:G182–G190, 2001.
26. Alpini G, Ueno Y, Tadlock L, Glaser S, LeSage G, Francis H, Taffetani S, Marzioni M, Alvaro D, Patel T. Increased susceptibility of cholangiocytes to tumor necrosis factor-alpha cytotoxicity after bile duct ligation. Am J Physiol Cell Physiol 285:C183–C194, 2003.
27. Patel T, Roberts LR, Jones BA, Gores GJ. Dysregulation of apoptosis

[1] Alpini, unpublished data, 2007

as a mechanism of liver disease: an overview. Semin Liver Dis 18:105–114, 1998.
28. Lazaridis KN, Strazzabosco M, LaRusso NF. The cholangiopathies: disorders of biliary epithelia. Gastroenterology 127:1565–1577, 2004.
29. Bemelmans MH, Gouma DJ, Greve JW, Buurman WA. Cytokines tumor necrosis factor and interleukin-6 in experimental biliary obstruction in mice. Hepatology 15:1132–1136, 1992.
30. Spirli C, Nathanson MH, Fiorotto R, Duner E, Denson LA, Sanz JM, Di Virgilio F, Okolicsanyi L, Casagrande F, Strazzabosco M. Proinflammatory cytokines inhibit secretion in rat bile duct epithelium. Gastroenterology 121:156–169, 2001.
31. Palmer RH, Ruban Z. Production of bile duct hyperplasia and gallstones by lithocholic acid. J Clin Invest 45:1255–1267, 1966.
32. Marzioni M, LeSage G, Glaser S, Patel T, Marienfeld C, Ueno Y, Francis H, Alvaro D, Tadlock L, Benedetti A, Marucci L, Baiocchi L, Phinizy JL, Alpini G. Taurocholate prevents the loss of intrahepatic bile ducts due to vagotomy in bile duct-ligated rats. Am J Physiol Gastrointest Liver Physiol 284:G837–G852, 2003.
33. Marucci L, Alpini G, Glaser S, Alvaro D, Benedetti A, Francis H, Phinizy JL, Marzioni M, Mauldin J, Venter J, Baumann B, Ugili L, LeSage G. Taurocholate feeding prevents CCl_4-induced damage of large cholangiocytes through PI3-kinase-dependent mechanism. Am J Physiol Gastrointest Liver Physiol 284:G290–G301, 2003.
34. Marzioni M, Francis H, Benedetti A, Ueno Y, Fava G, Venter J, Reichenbach R, Mancino MG, Summers R, Alpini G, Glaser S. Ca^{2+}-dependent cytoprotective effects of ursodeoxycholic and tauroursodeoxycholic acid on the biliary epithelium in a rat model of cholestasis and loss of bile ducts. Am J Pathol 168:398–409, 2006.
35. Alpini G, Glaser S, Rodgers R, Phinizy JL, Robertson WE, Lasater J, Caligiuri A, Tretjak Z, LeSage G. Functional expression of the apical Na^+-dependent bile acid transporter in large but not small rat cholangiocytes. Gastroenterology 113:1734–1740, 1997.
36. Glaser S, Alvaro D, Francis H, Ueno Y, Marucci L, Benedetti A, De Morrow S, Marzioni M, Mancino MG, Phinizy JL, Reichenbach R, Fava G, Summers R, Venter J, Alpini G. Adrenergic receptor agonists prevent bile duct injury induced by adrenergic denervation by increased cAMP levels and activation of Akt. Am J Physiol Gastrointest Liver Physiol 290:G813–G826, 2006.
37. Pugazhenthi S, Nesterova A, Sable C, Heidenreich KA, Boxer LM, Heasley LE, Reusch JE. Akt/protein kinase B up-regulates Bcl-2 expression through cAMP-response element-binding protein. J Biol Chem 275:10761–10766, 2000.
38. LeSage G, Alvaro D, Benedetti A, Glaser S, Marucci L, Baiocchi L, Eisel W, Caligiuri A, Phinizy JL, Rodgers R, Francis H, Alpini G. Cholinergic system modulates growth, apoptosis, and secretion of cholangiocytes from bile duct-ligated rats. Gastroenterology 117:191–199, 1999.
39. Desmet VJ. Vanishing bile duct disorders. Prog Liver Dis 10:89–121, 1992.
40. Neuman M, Angulo P, Malkiewicz I, Jorgensen R, Shear N, Dickson ER, Haber J, Katz G, Lindor K. Tumor necrosis factor-alpha and transforming growth factor-beta reflect severity of liver damage in primary biliary cirrhosis. J Gastroenterol Hepatol 17:196–202, 2002.
41. Sheen-Chen SM, Chen HS, Ho HT, Chen WJ, Sheen CC, Eng HL. Effect of bile acid replacement on endotoxin-induced tumor necrosis factor-alpha production in obstructive jaundice. World J Surg 26:448–450, 2002.
42. Floreani A, Guido M, Bortolami M, Della Zentil G, Venturi C, Pennelli N, Naccarato R. Relationship between apoptosis, tumour necrosis factor, and cell proliferation in chronic cholestasis. Dig Liver Dis 33: 570–575, 2001.
43. Rust C, Karnitz LM, Paya CV, Moscat J, Simari RD, Gores GJ. The bile acid taurochenodeoxycholate activates a phosphatidylinositol 3-kinase-dependent survival signaling cascade. J Biol Chem 275:20210–20216, 2000.
44. Ricchi M, Bertolotti M, Anzivino C, Carulli L, Canedi I, Bormioli ML, Tiozzo R, Croce MA, Lonardo A, Carulli N, Loria P. 17Beta-estradiol prevents cytotoxicity from hydrophobic bile acids in HepG2 and WRL-68 cell cultures. J Gastroenterol Hepatol 21:894–901, 2006.
45. Takikawa Y, Miyoshi H, Rust C, Roberts P, Siegel R, Mandal PK, Millikan RE, Gores GJ. The bile acid-activated phosphatidylinositol 3-kinase pathway inhibits Fas apoptosis upstream of bid in rodent hepatocytes. Gastroenterology 120:1810–1817, 2001.
46. Rajesh KG, Suzuki R, Maeda H, Yamamoto M, Yutong X, Sasaguri S. Hydrophilic bile salt ursodeoxycholic acid protects myocardium against reperfusion injury in a PI3K/Akt dependent pathway. J Mol Cell Cardiol 39:766–776, 2005.
47. LeSage G, Glaser S, Alpini G. Regulation of cholangiocyte proliferation. Liver 21:73–80, 2001.
48. Wang L, Piguet AC, Schmidt K, Tordjmann T, Dufour JF. Activation of CREB by tauroursodeoxycholic acid protects cholangiocytes from apoptosis induced by mTOR inhibition. Hepatology 41:1241–1251, 2005.
49. Hofmann AF. Bile acids. In: Arias IM, Boyer J, Fausto N, Jakoby W, Schachter D, Shafritz DA, Eds. The Liver: Biology & Pathobiology, 3rd Ed. New York, NY: Raven Press, pp667–718, 1994.
50. Alpini G, Glaser S, Alvaro D, Ueno Y, Marzioni M, Francis H, Baiocchi L, Stati T, Barbaro B, Phinizy JL, Mauldin J, LeSage G. Bile acid depletion and repletion regulate cholangiocyte growth and secretion by a phosphatidylinositol 3-kinase-dependent pathway in rats. Gastroenterology 123:1226–1237, 2002.
51. Davy DA, Campbell HD, Fountain S, de Jong D, Crouch MF. The flightless I protein colocalizes with actin- and microtubule-based structures in motile Swiss 3T3 fibroblasts: evidence for the involvement of PI 3-kinase and Ras-related small GTPases. J Cell Sci 114:549–562, 2001.
52. Misra S, Ujhazy P, Gatmaitan Z, Varticovski L, Arias IM. The role of phosphoinositide 3-kinase in taurocholate-induced trafficking of ATP-dependent canalicular transporters in rat liver. J Biol Chem 273:26638–26644, 1998.
53. Fan S, Ma YX, Wang JA, Yuan RQ, Meng Q, Cao Y, Laterra JJ, Goldberg ID, Rosen EM. The cytokine hepatocyte growth factor/scatter factor inhibits apoptosis and enhances DNA repair by a common mechanism involving signaling through phosphatidyl inositol 3 kinase. Oncogene 19:2212–2223, 2000.
54. Ibuki Y, Goto R. Suppression of apoptosis by UVB irradiation: survival signaling via PI3-kinase/Akt pathway. Biochem Biophys Res Commun 279:872–878, 2002.
55. Webster CR, Anwer MS. Cyclic adenosine monophosphate-mediated protection against bile acid-induced apoptosis in cultured rat hepatocytes. Hepatology 27:1324–1331, 1998.
56. Webster CR, Anwer MS. Phosphoinositide 3-kinase, but not mitogen-activated protein kinase, pathway is involved in hepatocyte growth factor-mediated protection against bile acid-induced apoptosis in cultured rat hepatocytes. Hepatology 33:608–615, 2001.