Advances in cholangiocyte immunobiology

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Syal G, Fausther M, Dranoff JA. Advances in cholangiocyte immunobiology. Am J Physiol Gastrointest Liver Physiol 303: G1077–G1086, 2012. First published September 6, 2012; doi:10.1152/ajpgi.00227.2012.—Cholangiocytes, or bile duct epithelia, were once thought to be the simple lining of the conduit system comprising the intra- and extrahepatic bile ducts. Growing experimental evidence demonstrated that cholangiocytes are in fact the first line of defense of the biliary system against foreign substances. Experimental advances in recent years have unveiled previously unknown roles of cholangiocytes in both innate and adaptive immune responses. Cholangiocytes can release inflammatory modulators in a regulated fashion. Moreover, they express specialized pattern-recognizing molecules that identify microbial components and activate intracellular signaling cascades leading to a variety of downstream responses. The cytokines secreted by cholangiocytes, in conjunction with the adhesion molecules expressed on their surface, play a role in recruitment, localization, and modulation of immune responses in the liver and biliary tract. Cholangiocyte survival and function is further modulated by cytokines and inflammatory mediators secreted by immune cells and cholangiocytes themselves. Because cholangiocytes act as professional APCs via expression of major histocompatibility complex antigens and secrete antimicrobial peptides in bile, their role in response to biliary infection is critical. Finally, because cholangiocytes release mediators critical to myofibroblastic differentiation of portal fibroblasts and hepatic stellate cells, cholangiocytes may be essential in the pathogenesis of biliary cirrhosis.

toll-like receptor; interleukin-6; interleukin-8; tumor necrosis factor-α; monocyte chemoattractant protein-1; interferon-γ; defensins; Mx proteins; trefoil factors

THE MAMMalian BILIARY TRACT is composed of a network of small to large ducts lined by epithelial cells called cholangiocytes, also known as bile duct epithelia (BDE). The biliary tract begins within the hepatic lobule and branches successively into larger ducts, ultimately culminating in the extrahepatic bile ducts (5). Cholangiocytes were once thought to be simple columnar epithelia with a passive role in liver function, but an explosion of experimental evidence in the last 20–30 years has shown that these cells are dynamic and critical to liver (patho)physiology (10, 13). Cholangiocytes constitute only 4–5% of total liver cell mass but contribute to around 40% of total daily bile secretion, depending on mammalian species (6, 54). Bile is not only essential in digestion but is also a means of excretion of endogenous byproducts of body metabolism and exogenous substances like drug metabolites. The distal portion of the biliary tract, the common bile duct, drains the bile into the duodenum and is in open communication with the gastrointestinal tract. Indeed, microorganisms present in the duodenum are the major source of ascending hepatobiliary infections. Like other epithelial cells, cholangiocytes are equipped with a variety of defense mechanisms against such infections.

The spectrum of cholangiocytes participation in the immune response was once thought to be limited to secretion of immunoglobulin (Ig) A into the bile (54), but recent experimental advances have unveiled many previously unknown mechanisms of biliary epithelial defense. Cholangiocytes are now known to be active players in the immune pathogenesis of both infectious and noninfectious hepatobiliary diseases, since they are now known to be immunologically active cells with an important role in both innate and adaptive immunity. Recognition of pathogen-associated molecular patterns (PAMPs) by toll-like receptors (TLRs) expressed by cholangiocytes leads to activation of complex downstream effects eventually leading to secretion of antimicrobial peptides, expression of adhesion molecules, and secretion of inflammatory cytokines. This complex interplay of innate and adaptive immune responses not only promotes biliary defense but also leads to immune damage to the tissue. Various immune regulatory mechanisms exist to minimize this self-inflicted damage; however, the tradeoff for this adaptive mechanism may be a predisposition to immune-mediated liver diseases. In this review, we will focus on the immunological functions of cholangiocytes and their potential implications in the pathogenesis of cholangiopathic diseases.

Biliary Innate Immunity

Toll-like receptors. TLR EXPRESSION AND FUNCTION. TLRs are a group of membrane-spanning receptors that are important mediators of the innate immune system. TLRs recognize structurally conserved microbial molecules PAMPs to produce specific intracellular downstream effects and eventual secretion of proinflammatory cytokines and chemokines. Human bile is sterile under physiological conditions; bacteria can be cultured from bile only in inflammatory biliary diseases (35, 83, 105).
Nevertheless, bacterial components like lipopolysaccharide (LPS), lipoteichoic acid (LTA), and bacterial DNA fragments can be detected in the bile or accumulated in cholangiocytes in normal and pathological conditions (43, 89, 101). The human (SV40-transformed) cholangiocyte H69 cell line expresses mRNAs of all known human TLRs (TLR1–10) (11). Studies have also confirmed the expression of multiple TLRs and related proteins, including myeloid differentiation primary response protein 88 (MyD88), myeloid differentiation protein-2 (MD-2), and cluster of differentiation 14 (CD14), on immortalized and primary human cholangiocytes (31, 34, 121). Activation of TLRs in cholangiocytes has been shown to occur in the setting of bacterial, viral, and parasitic infections in vitro and in vivo (11, 46). The inflammatory cytokines and other antimicrobial molecules secreted by cholangiocytes subsequent to TLR stimulation mediate various immune responses thought to be important to maintain mucosal homeostasis. LPS interaction with TLR4, in conjunction with accessory proteins MD-2 and CD14, triggers activation of the adaptor proteins MyD88 and interleukin (IL)-1 receptor-associated kinase-1 (IRAK-1). This eventually leads to activation of nuclear factor (NF)-κB and induces cholangiocyte release of a variety of cytokines, including IL-1β, IL-8, IL-6, monocyte chemotactic protein-1 (MCP-1), tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), and transforming growth factor-β (TGF-β). Interestingly, this pathway is thought to be important not only in the protection against biliary infection, but also in the pathogenesis of noninfectious biliary diseases, such as primary sclerosing cholangitis (PSC) (34, 47, 57, 121). Similar effects have been observed in the cholangiocyte LTA/TLR2 axis (121). TLR2 and TLR4 stimulation with subsequent activation of the NF-κB pathway regulates the cholangiocyte response to Cryptosporidium parvum infection (11), and TLR4 is important for the eradication of biliary C. parvum infection in vivo (86).

The roles of other TLR pathways in noninfectious cholangiopathies are more poorly understood. TLR3 expression has also been found to be upregulated at sites of ductular reaction in primary biliary cirrhosis (PBC), autoimmune hepatitis (AIH), and chronic viral hepatitis (82). TLR3 and TLR7 have been linked to the pathogenesis of biliary atresia (BA); because TLR3 and TLR7 are activated by viral RNA, viral infection has been strongly considered in BA pathogenesis (37, 46). In addition to microbial components, several endogenous molecules have also been reported to activate TLRs (88, 102, 114). Some of the endogenous TLR activators, including heat shock proteins, fibronectin, and hyaluronic acid, have been detected in human bile and may play a role in perpetuation of inflammatory damage in cholangiopathies. Heat shock proteins are expressed by cholangiocytes of patients with PBC, obstructive jaundice, and PSC (8, 73). Biliary fibronectin levels are elevated in both benign and malignant biliary tract disease (59). Similarly, hyaluronic acid levels are elevated in bile of patients with biliary stone disease (69). These data support the concept that TLR function may be important in cholangiopathy pathogenesis in the setting of liver fibrosis (Table 1). Regulation of TLR expression and signaling. The expression of TLRs on epithelial cells is a highly regulated process (10, 13). This serves to promote appropriate activation of the innate immune system against exogenous invading pathogens and prevent immune responses against endogenous ligands and commensal microorganisms. As described previously, selective upregulation of TLR2 and TLR4 expression has been demonstrated upon infection of cultured human cholangiocytes with C. parvum (11). IFN-γ and TNF-α upregulate expression of TLRs in cultured human intrahepatic cholangiocarcinoma cell lines and human intrahepatic biliary epithelial cell (HIBE C) lines (31). TLRs have been found to be upregulated in cholangiopathies, including PBC, PSC, and obstructive jaundice (57, 78, 82, 116), but it is not clear if such TLR upregulation is primary to the pathogenesis of these conditions or is instead a reactive phenomenon.

A recent study demonstrated that antibodies to cholangiocytes in patients with PSC may activate innate immune responses. Specifically, IgG antibodies to cholangiocytes induced expression of TLR4 and NF-κB (57). Additionally, bile ducts stained positively for TLR4 and TLR9 in 58% of liver specimens from patients with PSC who expressed such antibodies compared with 14% positive staining in patients with PSC without IgG anti-cholangiocyte expression (57). The role of cholangiocyte antibodies in production of post liver transplantation cholangitis and acute rejection was also suggested in a study in liver transplant patients, in whom cholangiocytes antibodies induced cholangiocyte expression of TLR2 and TLR3 and production of proinflammatory cytokines and chemokines (24).

Several mechanisms negatively regulating the expression of TLRs by cholangiocytes have been elucidated. Cultured H69 cells express the lethal-7 (let-7) family of microRNAs (miRNAs), and let-7 downregulates cholangiocyte TLR4 expression via posttranscriptional suppression (12). C. parvum infection of H69 cells downregulates expression of let-7i, resulting in a secondary increase in expression of TLR4 (12). Conversely, HIV-1 tat protein suppresses TLR4 protein expression on H69 cells through translational inhibition, contributing to unusual susceptibility of HIV-infected patients to developing biliary cryptosporidiosis (87).

LPS is found in human bile in both pathological and physiological circumstances but does not elicit inflammatory responses under physiological conditions. A physiological mechanism for “endotoxin tolerance” has been thought to play a role in limiting the activation of TLRs in response to bacterial PAMPs. This was first described in the intestines, which are continuously exposed to commensal bacterial flora without causing inflammatory tissue damage due to TLR activation (90). Harada et al. (32) demonstrated that pretreatment of HIBE C lines with the TLR4 ligand LPS rendered them tolerant to further exposure to LPS as measured by cell NF-κB activity and TNF-α mRNA production. The LPS-tolerant cells had an increased level of IL-1 receptor-associated kinase-M (IRAK-M) mRNA, which is a negative regulator of TLR signaling, implying the role of IRAK-M in inducing endotoxin tolerance (32). Subsequent studies have failed to show the existence of such tolerance to TLR3 receptor ligands (36). Recently, loss of function of cystic fibrosis transmembrane conductance regulator (CFTR) was observed to cause loss of endotoxin tolerance and increased production of cytokines mediated by TLR4 and NF-κB (22). This finding implies that cystic fibrosis cholangiopathy pathogenesis is due not only to altered physical and chemical characteristics of bile but also to dysregulated activation of the biliary innate immune system in the absence of CFTR. Taken together, these findings suggest that TLR4
may be of particular importance in the pathogenesis of cholangiopathies and certainly merits further investigation in specific disease states.

Cholangiocyte/T helper 17 cell interactions. A novel development that merits special mention is the potential interaction between cholangiocytes and T helper (Th) 17 cells. The role of Th17 cells in the pathogenesis of chronic inflammatory diseases is increasingly appreciated. Th17 cells are derived from naive Th0 cells (108) but are specifically linked to the cytokine IL-23. TLR signaling in response to bacterial PAMPs has been shown to induce the production of cytokines IL-6 and IL-1β that are necessary for Th17 differentiation (2) and IL-23 for phenotypic maintenance (63) in the cultured human cholangiocyte cell line BEC1–3 (39). In biopsy specimens from patients with PBC, Th17 cells have been found to be distributed around inflamed portal tracts and damaged interlobular bile ducts (39).
However, the specific roles of Th17 cells in PBC and other cholangiopathies are currently unknown.

**Secretion of antimicrobial molecules.** IMMUNOGLOBULIN A. IgA provides mucosal immunity in various organ systems and aids in local pathogen clearance (117). The predominant immunoglobulin present in bile is secretory IgA (sIgA), whereas IgM and IgG are present in much lower concentration (94). The secretion of IgA in the bile involves binding of serum IgA to the secretory component on the basolateral membrane of the cell, endocytosis of the antibody, and transcellular passage through a network of tubulovascular pathways to the apical membrane. At the apical membrane, proteolytic cleavage of the secretory component takes place with secretion of the extracellular fragment and the antibody (81). In some experimental animals (rat, mice, and rabbits), transcytosis and secretion of IgA into the bile occurs in hepatocytes (26, 55). However, in human liver, because the secretory component is predominately expressed by cholangiocytes, the secretion of IgA in bile is a function of epithelial cells of small biliary ductules (16, 98, 112).

Secretion of IgA in bile has important physiological functions. It provides a barrier of defense against infective pathogens in the biliary tract by aggregating bacteria and preventing binding to the mucosal surface (109). IgA antibodies to intestinal bacteria are naturally present in bile, and studies have shown that inoculation of bacterial antigen in the intestinal lymphoid tissue leads to secretion of antigen-specific IgA antibodies in the bile (1). Various in vitro and in vivo studies in animals have confirmed secretion of circulating serum antigen in the bile complexed with naturally induced or passively administered IgA antibody (40, 91). It is postulated that this mechanism applies to the excretion of both systemic and local antigens thus reducing the inflammatory response elicited by the immune complexes (immune complex disease). Because IgA is less immunogenic than IgG (96), one role of biliary sIgA may be reduction of the net inflammatory response. Finally, transcellular transport of IgA from the basolateral to apical cell membrane can also serve as a host defense mechanism against intracellular pathogens, since IgA antibodies can bind to intracellular microbial pathogens during cellular transcytosis (although this has not been shown in cholangiocytes) (75).

**Human β-defensins.** Defensins are small, cationic cysteine-rich proteins that possess antimicrobial activity against bacteria, fungi, and viruses (41). Defensins act by binding to the microbial membrane and forming pore-like membrane defects, thus allowing efflux of essential intracellular ions, ultimately resulting in cell death. While α-defensins are typically limited to immune cells, β-defensins are widely distributed and are secreted by leukocytes and a variety of epithelia. Cholangiocytes from healthy and diseased patients have been shown to release the human β-defensin (HBD) 1, and bile from such patients expresses this molecule (33). In contrast, HBD-2 is expressed only in diseased livers, specifically in the bile ducts exhibiting active inflammation (33). This finding suggests that HBD-1 might play a role in constitutive biliary innate immunity while HBD-2 expression is induced in the presence of local infection or tissue inflammation. In another study, constitutive expression of HBD-1 and HBD-3 was found in H69 cells (11). Expression of HBD-2 was found to be low but underwent significant upregulation upon infection of the cells with *C. parvum* in a TLR2- and TLR4-sensitive fashion (11). This reinforces the hypothesis that, while HBD-1 and HBD-3 play a role in nonspecific antimicrobial immunity in the biliary epithelium, HBD-2 is expressed in response to TLR activation and provides immunity against specific microbial infections.

**MX proteins.** Mx proteins belong to the dynamin superfamily of large guanosine triphosphatases induced by interferons (29). Mx proteins exhibit antiviral activity against RNA viruses via recognition of the viral nucleocapsid and interference with viral genomic replication (29).

The finding of Mx protein expression in cholangiocytes in the recent years has led to the possible hypothesis of involvement of these intracellular proteins in the immunopathogenesis of certain biliary diseases. In a normal liver tissue, only a small number of Kupffer cells express Mx proteins. In chronic liver diseases and fulminant hepatic failure, an increased number of Kupffer cells, lymphocytes, hepatocytes, and cholangiocytes has been found to express Mx proteins (64). This increase in Mx protein expression correlates with increased expression of IFN-α. A significant increase in expression of Mx proteins has been reported in patients with early stages of BA, supporting the viral hypothesis of that disease (3, 46). However, at present, the net contribution of Mx proteins to cholangiocyte immune function is unknown.

**TNF superfamily proteins.** As discussed above, the mechanism of endotoxin tolerance prevents tissue damage by regulating innate immune responses in response to exposure to nonpathogenic commensal bacterial PAMPs in the biliary tree. Because cholangiocytes lack tolerance to TLR3 activation, tissue damage is caused on exposure of cholangiocytes to the TLR3 ligand double-stranded RNA or synthetic agonists. In cholangiocytes, stimulation of TLR3 by synthetic ligand poly(I:C) causes production of IFN-β via NF-κB activation and enhancement of apoptosis by production of TNF-related apoptosis-inducing ligand (TRAIL) (37). In support of the RNA viral hypothesis of BA, cholangiocytes from extrahepatic bile ducts exhibited increased expression of TLR3, activation of NF-κB and interferon regulatory factor-3, increased expression of IFN-γ and Mx proteins, and enhancement of TRAIL-induced apoptosis (46). This discovery supports the hypothesis that cholangiocytes not only play a role in biliary innate immunity by production of antimicrobial proteins but also by inducing apoptosis of the infected cells. Fas ligand (FasL) is a transmembrane protein of the TNF family that induces apoptosis after binding to its receptor FasR or CD95 (14). Increased expression of FasL has also been found in the cell membrane and endoplasmic reticulum of cholangiocytes of patients with chronic nonsuppurative destructive cholangitis in PBC, suggesting the role of increased apoptotic cell loss by the Fas-mediated pathway in this disease (62).

**Adhesion molecules.** Cholangiocytes express several different classes of adhesion molecules on their surfaces, which may mediate interaction with immune cells, thus contributing to localization and regulation of immune responses. Cholangiocytes constitutively express human leukocyte antigen (HLA) class I, HLA class II, intercellular adhesion molecule-1 (ICAM-1), and lymphocyte function-associated antigen-3 (LFA-3) at the plasma membrane (4, 15, 79). Other adhesion molecules expressed by cholangiocytes include epidermal growth factor receptor, Fas receptor (CD95), CD40, and CD44 (15). Importantly, inflammatory cytokines regulate the expression of adhesion molecules on cholangiocytes. IFN-γ, TNF-α and IL-1 have all been shown to increase expression of major
histocompatibility complex-1 (MHC-I), MHC-II, and ICAM-1 (4, 15, 79). On the other hand, TGF-β downregulates cholangiocyte expression of these molecules (4, 15, 79). Several lines of evidence suggest that cholangiocytes upregulate plasma membrane adhesion molecules in infectious and noninfectious cholangiopathies. MHC I is upregulated in cultured human cholangiocytes infected with cytomegalovirus (CMV) (104). In murine cholangiocytes, expression of MHC I and MHC II antigens was amplified after stimulation with IFN-γ and CMV infection (44).

The interaction between T lymphocytes and cholangiocytes mediated by cholangiocyte cell surface adhesion molecules regulates T cell-mediated immunity. Interactions between cholangiocyte ICAM-1 and cytotoxic T lymphocyte LFA-1 causes cholangiocyte injury (65). In addition to activation of cytotoxic T cells by MHC-I/T cell receptor (TCR) interaction, cytotoxic T lymphocytes can alternately be activated by a mechanism involving CD40-CD40L interaction (42, 103). CD40 is constitutively expressed on cholangiocytes, and its expression is upregulated by IFN-γ stimulation (15).

Fractalkine or chemokine (C-X3-C motif) ligand 1 (CX3CL1) is a cytokine of the CX3C family, which serves as a chemoattractant and cell adhesion molecule (48). In its soluble form, fractalkine is a chemokine receptor for T lymphocytes and monocytes, and in its cell-bound form, fractalkine causes adhesion of leukocytes to the cells expressing CX3CR1 receptor (48). Fractalkine is expressed by cholangiocytes of normal and diseased livers (50). Because soluble fractalkine is increased in the serum of patients with PBC, and these patients exhibit increased liver infiltration with CX3CR1-positive mononuclear and T cells, fractalkine may be important in PBC pathogenesis (50). Injured bile ducts upregulate fractalkine expression in response to LPS and Th1-cytokines, leading to chemotraction of mononuclear and T cells and increased adhesion of these cells to cholangiocytes (50). TLR3 and IFN-α upregulate cholangiocyte fractalkine fractalkine expression in PBC, suggesting a possible pathogenic role in PBC pathogenesis.

Cell surface antigen recognition molecules. Antigen presentation is one of the most important initial steps in stimulation of cell-mediated immune responses. MHC II antigen expression is the hallmark of antigen-presenting cells (APCs). Cholangiocytes constitutively express MHC II antigen at the cell surface; thus, they are potential APCs (4, 15, 97). MHC II expression on cholangiocytes is enhanced after stimulation with the inflammatory cytokines IL-1, TNF-α and IFN-γ in vitro. Moreover, cholangiocyte MHC II overexpression has been observed in injured bile ducts from livers with allograft rejection, graft vs. host disease, PBC, and PSC (4, 15, 44, 76, 115). More indirect evidence supporting the fact that cholangiocytes can act as APCs comes from the fact that, in liver transplant rejection, HLA class II-specific lymphocytes infiltrate intrahepatic bile ducts and are thought to induce cholangiocyte destruction (71).

Activation of cell-mediated immunity requires interaction of costimulatory molecules in addition to MHC-II/TCR interaction. (Patho)physiologically important costimulatory molecules include B7-1 (CD80) and B7-2 (CD86) (61). Studies to test the expression of B7-1 and B7-2 on cholangiocytes have produced mixed results. Leon et al. (67) showed that these costimulatory molecules are not expressed by cholangiocytes, either constitutively or after stimulation with IFN-γ and TNF-α (106). Functional studies performed by this group showed that HIBECs lacked the ability to cause CD4+ T cell activation measured as lymphoproliferation and production of IL-2, and the authors concluded that this was due to lack of costimulatory molecule expression (66). On the other hand, B7 expression was demonstrated in cholangiocytes from patients with PBC diagnosed in early stages (stage I or II) (113). B7-1 (CD80), B7-2 (CD86), MHC I, and MHC II were also demonstrated in purified cholangiocytes isolated from cholestatic mice (45). In the presence of these conflicting data, further studies are warranted to conclude definitively whether cholangiocytes can function as APCs.

There are potential mechanisms by which cholangiocytes may inhibit T cell activation in the setting of cholangiocyte/T cell interaction. Programmed cell death ligand 1 (PD-L1) and programmed cell death ligand 2 (PD-L2) belong to the B7 family of transmembrane proteins, which interact with the PD-1 receptor (CD279) and transmit inhibitory signals to suppress proliferation of T cells (9). In the normal state, H69 cells and isolated primary HIBECs express PD-L1 (B7-H1) mRNA but not PD-L1 protein due to inhibition of translation by miRNAs; however, stimulation of cholangiocytes by IFN-γ blocks this downregulated protein expression, leading to net upregulation of PD-L1 (27). IFN-γ has also been shown to upregulate H69 cells and primary HIBEC PD-L1 and PD-L2 expression. Antigen-stimulated cholangiocytes in which PD-L1 and PD-L2 function was inhibited by blocking antibodies have been shown to activate T cells, but only to a modest level (15). Release of prostaglandin E 2 by cholangiocytes treated with IFN-α or IL-1β by cholangiocytes inhibited T cell stimulation in a cell contact-independent manner (56). Taken together, these data provide provocative evidence that cholangiocytes themselves can directly regulate T cell function; thus, cholangiocyte/T cell interactions may be important initiating or perpetuating events in the pathogenesis of cholangiopathies.

Prolinflammatory cytokines. Chemoattraction and transendothelial migration are fundamental steps in the pathogenesis of inflammation that lead to homing of immunologically active cells to the site of inflammatory process (95). Cholangiocytes secrete a variety of cytokines and chemokines that regulate recruitment of circulating immune cells. Cultured primary human intrahepatic cholangiocytes express and secrete IL-8, which induces neutrophil chemotaxis, and MCP-1, which induces monocyte chemotaxis and differentiation (80). As will be discussed below, cholangiocyte release of MCP-1 may also be important in regulation of liver myofibroblast function (80). Cholangiocyte MCP-1 expression is upregulated in regenerating bile ducts and correlates with macrophage/macrophagocytic infiltration in chronic viral hepatitis and advanced cirrhosis (72). Prolinflammatory cytokines themselves modulate the cholangiocyte IL-8 and MCP-1 release. IL-1 and TNF-α upregulate secretion of both IL-8 and MCP-1, whereas IFN-γ upregulates MCP-1 and downregulates IL-8 release (80). Ductular reaction in liver diseases, including cholestasis of sepsis, extrahepatic biliary obstruction, and fulminant hepatitis, correlates with cholangiocyte IL-8 expression (49). Cholangiocytes release macrophage inflammatory protein-α in response to IL-1β, TNF-α, and IL-17 or activation of cholangiocyte TLRs; this in turn induces chemotaxis of antigen-presenting dendritic cells (38). Stromal-derived growth factor-1 (SDF-1) has also been shown to be upregulated in BECs in inflammatory liver dis-
Cholangiocytes also play an important role in regulation of immune responses of the recruited immune cells via regulated release of inflammatory cytokines and chemokines. Multiple studies have demonstrated that cholangiocytes release IL-6 in a regulated fashion. IL-1 induces IL-6 release from primary cultured HIBECS (74), and cholangiocytes from damaged intrahepatic bile ducts in PBC release IL-6 and TNF-α (118). Because cholangiocytes have been shown to express receptors for IL-6 and TNF-α, one function of these cytokines may be to alter cholangiocyte function in an autocrine fashion (118). One autocrine function of IL-6 that may be of critical importance is maintenance of cholangiocyte cell populations (to be discussed below). TNF-α modulates cholangiocyte immune responses by increasing expression of MHC II, secretion of proinflammatory cytokines IL-8 and MCP-1, and induction of cytotoxic T lymphocyte function (4, 80). TNF-α may also promote apoptotic cell death in cholangiocytes as has been demonstrated in hepatocytes (7), thus contributing to bile duct damage. In addition, cholangiocytes secrete TGF-β in a regulated fashion (77). TGF-β has a variety of downstream effects, including stimulation of cholangiocyte endothelin-1 secretion thought to play a central role in pathogenesis of hepatopulmonary syndrome after common bile duct ligation, promotion of myofibroblastic differentiation of portal fibroblasts (PF), and inhibition of mitogenesis in primary human cholangiocytes (68, 70, 120). TGF-β may play a role in paracrine regulation of extracellular matrix secretion in the adjacent mesenchymal cells (77).

The potential importance of adaptive immune responses in cholangiopathy pathogenesis cannot be understated. Cholangiocytes are active participants in pathophysiological responses that themselves induce immune-mediated ductular injury. Furthermore, cholangiocytes release mediators to recruit immunologically active cells to the biliary milieu and enhance cell-mediated immune responses by expressing cell adhesion molecules. Thus, the innate and adaptive immune responses may orchestrate the progression of chronic cholangiopathies, ductular injury, and subsequent development of liver fibrosis.

**Immunologic Roles of Cholangiocytes in Liver Fibrosis**

Cirrhosis is a dreaded complication of chronic liver disease, resulting from dysregulated scar formation by liver myofibroblasts (95). Two major cell populations form the sources of liver myofibroblasts: PF and hepatic stellate cells (HSC). PF are located adjacent to cholangiocytes throughout the biliary tree and are thought to be particularly important in the pathogenesis of biliary fibrosis, whereas HSC are sinusoidal pericytes thought to be of critical importance in both biliary and nonbiliary liver fibrosis (20, 23, 28, 92). Release of inflammatory cytokines and chemokines by cholangiocytes appears to be critical in the pathogenesis of liver fibrosis.

The regulated release of IL-6 by cholangiocytes in biliary cirrhosis is well characterized. Cholangiocytes express a baso-
lateral P2Y nucleotide receptor linked to increases in intracellular Ca\(^{2+}\) and cAMP, both of which act on Ca\(^{2+}\)/cAMP response elements on the IL-6 promoter (19). Stimulation of this receptor with ATP induces cholangiocyte IL-6 release. IL-6 then acts on the PF IL-6 receptor, resulting in downregulation of the ATP-cleaving ectoenzyme ectonucleoside triphosphate diphosphohydrolase-2 (NTPD2), resulting in profound transcriptional downregulation of NTPD2 expression by PF (18, 52). Because E-NTPD2 is the primary mechanism by which cholangiocyte P2Y receptor function is regulated (52), this process sets up a self-perpetuating loop leading to further increases in cholangiocyte IL-6 release. IL-6 works in two ways to maintain cholangiocyte mass (in fact to increase it) in biliary cirrhosis. First, IL-6 itself induces cholangiocyte proliferation in an autocrine fashion (119). Second, because extracellular ATP (but no other nucleotide) stimulates cholangiocyte proliferation, IL-6-sensitive downregulation of PF E-NTPD2 levels leads to net upregulation of cholangiocyte proliferation (122). Note that the effect of IL-6 on maintenance of biliary mass is of critical homeostatic importance; mice lacking IL-6 signaling have a marked increase in mortality in biliary cirrhosis (21).

As discussed above, there is strong evidence of cholangiocyte MCP-1 secretion. Although MCP-1 was originally identified as a monocytic and lymphocytic chemoattractant, new evidence suggests that MCP-1 release by cholangiocytes may be of particular importance in the pathogenesis of biliary cirrhosis (30, 60). MCP-1 expression is upregulated in regenerating bile ducts in pediatric liver diseases (including BA), and the degree of upregulation correlates with disease severity (72). Furthermore, in the rhesus rotavirus model of BA, cholangiocytes upregulate MCP-1 release (51). Cholangiocyte-secreted MCP-1 is fibrogenic. Specifically, MCP-1 induces myofibroblastic differentiation, proliferation, and collagen-1 release by PF (60). Because MCP-1 induces homing of HSC (30, 93), it is likely that cholangiocyte release of MCP-1 perpetuates scar formation in cholangiopathic injury. Hence, cholangiocytes themselves promote worsening of liver injury via mediation of the hallmarks of the ductular reaction: duct injury and repair, inflammatory infiltration, and myofibroblastic differentiation of PF. Defining specific targets contributing to each of these aspects of the ductular reaction should lead to identification of novel, useful targets for the prevention and/or treatment of biliary (and perhaps nonbiliary) cirrhosis.

Maintenance of Biliary Epithelial Integrity

The trefoil factor family (TFF) peptides consist of small protease-resistant proteins that are secreted by mucus-secreting cells of the gastrointestinal tract (111). The members of this peptide family, TFF-1, TFF-2, and TFF-3, have a highly conserved motif of six cysteine residues called the “trefoil” domain (111). TFF peptides increase the viscosity of intestinal mucus, thereby protecting the intestinal epithelium from injury, and stimulate the restitution phase of wound healing by enhancing intestinal epithelial spreading and migration. TFF-1 and TFF-3 are constitutively expressed in large bile ducts of normal liver tissue, and their expression is increased after injury to large bile ducts (58, 84, 99, 100, 107). In contrast, TFF-2 expression is absent from the normal liver tissue; however, TTF-2 expression is upregulated after injury to small intrahepatic bile ducts (100). In PBC, there is differential expression of TFF-3 in the large bile ducts in response to damage compared with small bile ducts (58). Because of the absence of mucoprotective TTF-3, small bile ducts are more vulnerable to pathological damage, which could explain the selective injury small bile ducts observed in PBC. Cholangiocyte TFF-3 expression is regulated by the IL-6/gp130/STAT3 pathway (53, 84). Mucin-secreting cholangiocytes of large intrahepatic and extrahepatic bile ducts constitutively express phosphorylated STAT3 and TFF-3, whereas small- and medium-sized intrahepatic bile ducts are phospho-STAT3- and TFF3-negative (84).

Small proline-rich proteins (SPRR) are encoded as a part of the epidermal differentiating complex (EDC) region on chromosome 1 (25). Genes located in EDC are involved in terminal differentiation of the epidermis (25). Specifically, SPRRs function as cross-linking proteins that form bridges between other proteins (encoded in EDC) comprising cornified cell envelope, which is an effective barrier against the external environment. In normal mouse liver, SPRR2A are not expressed by cholangiocytes; however, SPRR2A is expressed by cholangiocytes after BDL (85). The IL-6/gp130/STAT3 pathway upregulates cholangiocyte SPRR2 expression (85). Moreover, IL-6/- mice lack cholangiocyte SPRR2 function after BDL, and this is reversed by external IL-6 replacement (85). The proposed functions of cholangiocyte SPRR2 expression include maintenance of barrier function, resistance to oxidative injury, and wound healing (17).

Conclusion

Cholangiocytes are not merely epithelia passively lining bile ducts. Rather, they are dynamic cells, with specific immune systems allowing the detection of foreign materials (Fig. 1) and release of substances regulating immune function (Fig. 2). Cholangiocyte immunobiology is emerging as a pathophysiological field, and it appears to be particularly relevant to the pathogenesis of a variety of cholangiopathic disease. Although an integrated view of cholangiocyte immunobiology is still lacking, this field has advanced markedly in recent years. Because it is likely that disease-specific differences in cholangiocyte immune function mediate observed differences in such diseases, further research should focus on disease-specific alterations in the immune functions of cholangiocytes.

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DISCLOSURES

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