Biliary innate immunity -function and modulation -

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ABSTRACT

Biliary innate immunity is involved in the pathogenesis of cholangiopathies in patients with primary biliary cirrhosis (PBC) and biliary atresia. Biliary epithelial cells possess an innate immune system consisting of the Toll-like receptor (TLR) family and recognize pathogen-associated molecular patterns (PAMPs). Tolerance to bacterial PAMPs such as lipopolysaccharides is also important to maintain homeostasis in the biliary tree, but tolerance to double-stranded RNA (dsRNA) is not found. In PBC, CD4-positive Th17 cells characterized by the secretion of IL-17 are implicated in the chronic inflammation of bile ducts and the presence of Th17 cells around bile ducts is causally associated with the biliary innate immune responses to PAMPs. Moreover, a negative regulator of intracellular TLR signaling, peroxisome proliferator-activated receptor-\(\gamma\) (PPAR\(\gamma\)), with a Th1-predominant cytokine milieu is involved in the pathogenesis of cholangitis. Immunosuppression using PPAR\(\gamma\) ligands may help to attenuate the bile duct damage in PBC patients. In biliary atresia characterized by a progressive, inflammatory, and sclerosing cholangiopathy, dsRNA viruses are speculated to be an etiological agent and to directly induce enhanced biliary apoptosis via the expression of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). Moreover, the epithelial-mesenchymal transition (EMT) of biliary epithelial cells is also evoked by the biliary innate immune response to dsRNA.
**Introduction**

Clarification of the molecular mechanisms of innate immunity and significance of innate immune responses to the pathogenesis of immune-mediated diseases as well as to defense against infections has progressed steadily since the cloning of Tolls in drosophila and Toll-like receptors (TLRs) in mammals including humans [1,2]. Innate immunity was initially thought to be limited to immunocompetent cells such as dendritic cells and macrophages, but epithelial cells also possess TLRs and proper innate immune systems. Liver and extrahepatic bile ducts consisting of hepatocytes and biliary epithelial cells (BECs) are also exposed to microorganisms and their components originating from the intestines via portal blood and duodenum. In the gastrointestinal tract, TLRs expressed in intestinal epithelial cells may be involved in innate immunity to maintain mucosal homeostasis and also the development of enterocolitis by producing inflammatory molecules [3]. Similar processes using TLRs may operate in the biliary tree. Human bile is sterile under normal conditions, but bacterial components such as lipopolysaccharide (LPS), lipoteichoic acid, and bacterial DNA fragments, known as pathogen-associated molecular patterns (PAMPs), are detectable in normal and pathologic bile [4-7] and cultivable bacteria are also detectable in bile of patients with inflammatory biliary
diseases [8-11], indicating that BECs are exposed to bacterial components under physiological as well as pathological conditions (Table 1). Although hepatocytes are usually infected by the hepatitis virus, no microorganisms showing BEC-specific tropism have been identified. The participation of microorganisms, however, in the etiology or pathogenesis of various cholangiopathies and biliary diseases has been reported or speculated. In this review, we describe the biliary innate immune system, its association with the pathogenesis of cholangiopathy and biliary diseases, and finally a strategy for the attenuation of cholangiopathy, particularly cholangitis, by the regulation of innate immune responses.

**Association with biliary innate immunity in biliary diseases**

Infectious agents have been implicated in the etiology or progression of cholangiopathies including cholangitis, bile duct loss, and lithiasis as a trigger or aggravating factor. Notably, several enterobacteria and viruses are speculated to be primary or secondary factor for primary biliary cirrhosis (PBC), primary sclerosing cholangitis, biliary atresia, hepatolithiasis, and chronic cholecystitis [4,5,12-16] (Table 1).

**Primary biliary cirrhosis (PBC):** PBC patients have an increased incidence
of recurrent urinary tract infections compared to patients with other chronic liver
diseases [17-19]. Recent findings also support an association between vaginal or
urinary tract infections and PBC [20]. Furthermore, endotoxin and lipoteichoic acids
abnormally accumulate in or around the intrahepatic bile ducts [7,16,21] and DNA of
*Propionibacterium acnes* (*P. acnes*) was detected as a major clone in the granulomas
of PBC patients (Table 1) [22]. Because these bacterial components, whether proteins
or nucleic acids, act as PAMPs, the presence of PAMPs in bile or around bile ducts is
known to induce a variety of inflammatory reactions and speculated to underlie the
etiopathogenesis of the cholangiopathy in cases of PBC.

The major autoantigens against antimitochondrial antibodies (AMAs) in PBC
are members of the 2-oxo-acid dehydrogenase complex (2-OADC), which includes
the E2 subunit of the pyruvate dehydrogenase complex (PDC-E2) [23]. An immune
response to intrahepatic BECs through 2-OADC-specific CD4⁺ helper T cells and
CD8⁺ cytotoxic T cells is thought to be the major mechanism responsible for the
immunological destruction of BECs in PBC and these T cells show molecular mimicry
between human and bacterial PDC-E2 [24,25]. Therefore, environmental factors such
as microorganisms and xenobiotics are speculated to disrupt the self-tolerance to
autoantigens as a specific intrahepatic BEC malfunction, supporting the forementioned
role of PAMPs in the etiopathogenesis of PBC.

**Biliary atresia:** Biliary atresia consists of a fetal type affecting 10-25% of patients, and the more common perinatal type. The perinatal type is characterized by a progressive, inflammatory, and sclerosing cholangiopathy. The presence of several viruses including Reoviridae (type 3 reovirus and type C rotavirus) (Table 1) in liver tissue or affected bile duct specimens obtained from patients with biliary atresia during the Kasai procedure or a liver transplantation, has been demonstrated [13,26-31], though conflicting results have been reported [13,26,28-30,32]. Immunostaining for Mx proteins, which mediate an early innate immune response and are highly sensitive markers for type I interferon (IFN) activity, revealed that hepatocytes and intrahepatic bile ducts in biliary atresia are positive for Mx, suggesting the presence of viruses in hepatocytes and biliary epithelial cells of patients with biliary atresia [33]. Among these viruses, Reoviridae having a double-stranded RNA (dsRNA) genome, in particular, are characterized by epithelial tropism, and rotavirus type A is the most frequent etiological agent in cases of acute infantile diarrhea in young children. Moreover, the infection of newborn Balb/c-mice with Reoviridae including type A rhesus rotavirus (RRV) and type 3 reovirus (Abney) leads to a cholestasis and biliary obstruction resembling human biliary atresia [34,35].
Therefore, it is likely that BECs are a target of these viruses which directly cause the cholangiopathy in cases of biliary atresia.

**Hepatolithiasis:** Hepatolithiasis is not a rare disease in East Asian countries including Japan and is characterized by the formation of stones and histologically "chronic proliferative cholangitis". Bacterial infections in the biliary tree and cholestasis have been implicated as the major etiopathogenic factor for lithogenesis in patients with calcium-bilirubinate stones. *Escherichia coli* (*E.*coli) is the bacterium most frequently isolated, followed by several species shown to have $\beta$-glucuronidase (Table 1). Moreover, the presence of *Campylobacter* species-specific DNA has been demonstrated in bile samples and biliary mucosa specimens in cases of hepatolithiasis, by PCR (Table 1) [9]. These bacteria in the biliary epithelium are speculated to influence the occurrence and development of cholangitis and lithogenesis, though the mechanism of such an effect is still unclear.

**Basic mechanisms of biliary innate immunity**

BECs are immunologically potent cells. The BECs of inflamed bile ducts actively participate in the inflammation by secreting cytokines and expressing immune receptors. In addition to immunocompetent cells, epithelial cells including BECs
recognize microbes and their constituents via a set of receptors, referred to as pattern-recognition receptors (PRRs). TLRs are the major epithelial PRRs recognizing PAMPs. Ten TLRs (TLR1 to TLR10) have been identified in humans, with TLR4 known to mediate inflammatory responses to LPS. In immunocompetent cells, the response to LPS is mediated by interaction with the TLR4 in conjunction with TLR4 accessory proteins MD-2 and CD14, triggering transduction of intracellular signals followed by the activation of TLR-associated adapter proteins, myeloid differentiation factor 88 (MyD88) and IL-1 receptor-associated kinase (IRAK)-1, leading to the activation of nuclear factor-κB (NF-κB) and then to the synthesis of antibiotics and proinflammatory cytokines. In contrast to bacterial PAMPs, dsRNA viruses such as Reoviridae (reovirus and rotavirus) are recognized by TLR3, IFN-inducible helicase retinoic acid-induced protein I (RIG-I), and melanoma differentiation-associated gene-5 (MDA-5). The stimulation of these receptors by dsRNA transduces signals to activate the transcription factor interferon regulatory factor 3 (IRF3) as well as NF-κB. Human and murine BECs possess at least TLR1-TLR5, related molecules (MD-2, MyD88, and IRAK-1), RIG-I, and MDA-5 [4,36-38]. Moreover, immunohistochemistry has confirmed that TLR1-TLR5, MyD88 and IRAK-1 are distributed diffusely in the intrahepatic biliary tree of normal human liver, irrespective
of anatomical levels [38] (Fig.1). In addition to the expression of these receptors, the responsiveness of BECs to the corresponding PAMPs is also found. For example, LPS binds to the surface of cultured BECs, and induces the production of TNF-α mRNA in an NF-κB-dependent manner [4]. Moreover, stimulation with polyinosinic-polycytidylic acid (poly(I:C), a synthetic analog of viral dsRNA) induces the activation of NF-κB and IRF3 and the production of IFN-β1 and MxA as potent antiviral responses [37]. Therefore, BECs possess functional TLR signaling systems and participate in innate immunity.

Chemical mediators produced by a biliary innate immune response

Innate immunity provides defense against bacterial and viral infections. Therefore, as part of an innate immune response, several antibiotics are produced. Cytokines and chemokines are also produced in immunocompetent cells and play an important role in subsequent acquired immunity. Moreover, BECs have been shown to secrete polymeric immunoglobulin A, several antibiotics against bacteria (lactoferrin, lysozyme, and defensins) and viruses (IFN-β1 and MxA), cytokines, and chemokines on treatment with PAMPs, thereby contributing to biliary mucosal defense and subsequent acquired immunity [39-41].
**Defensin:** Defensins are antimicrobial peptides identified as key elements in innate immunity. Structurally, they are divided into α- and β-defensins. The β-defensin family is distributed in the epithelium of several organs, constituting an important barrier at mucosal surfaces. So far, human β-defensins (hBD-1 to -6) have been identified. hBD-1 is constitutively expressed in cultured BECs and diffusely distributed in the cytoplasm of intrahepatic bile ducts irrespective of anatomical levels [42] (Fig.2). Moreover, because hBD-1 is constantly detectable in bile samples, hBD-1 is believed to play a role in the constitutive antimicrobial defense of the hepatobiliary system [42]. This may be why biliary tract infections are rare and bile is sterile under physiological conditions, though the biliary tree is potentially exposed to enteric bacteria. In contrast, hBD-2 is not detected in BECs cultured without a stimulant, but *de novo* expression is found in LPS- or *E.coli*-treated BECs. *In vivo*, hBD-2 expression is restricted to the intrahepatic large bile ducts and peribiliary glands, in particular, showing cholangitis in extrahepatic biliary obstruction and hepatolithiasis (Fig.2) [42]. Because in these diseased livers, enteric bacteria are mostly cultivable in bile, the participation of bacteria-related cholangitis is closely associated with the hBD-2 expression in BECs. hBD-1 plays a constitutive role in biliary antimicrobial defense, while hBD-2 expression is induced in response to local
infections and may play a role in additional antimicrobial defenses.

**Interleukin 8:** IL-8 is a major cytokine of neutrophils, and functions not only as a chemoattractant of neutrophils, basophiles, and some populations of T cells, but also as an activator of neutrophils for releasing leukotrienes, activated oxygen, and neutrophil defensins. Bacteria or their products have been reported to induce the secretion of IL-8 from intestinal or gingival epithelial cells, and such cytokines and chemokines are speculated to be involved in epithelial cell damage during bacterial or fungal infections. Cultured human BECs express and release IL-8 in response to bacterial PAMPs including LPS [43]. IL-8 expression is found in proliferating bile ductules in various diseased livers and closely associated with neutrophilic infiltration [43]. Particularly cholangitis lenta defined as bile ductular proliferation, ductular cholestasis, and ductular epithelial damage, is also accompanied by a prominent neutrophilic infiltration [44]. Cholangitis lenta is usually encountered in septic conditions, so circulating infectious reagents such as bacterial toxins or products, cytokines, or chemokines are speculated to be involved in its pathogenesis. In a state of sepsis, particularly endotoxemia, BECs may secrete IL-8 and attract neutrophils to reactive bile ductules. IL-8 produced in bile ductular biliary epithelia is a potential target in the prevention of liver and biliary damage in diseased livers such as septic
Fractalkine: Fractalkine (CX3CL1) is a chemokine with both chemoattractant and cell-adhesive functions and plays an important role in the migration of leukocytes to target sites under physiological as well as pathological conditions. Fractalkine is expressed in several epithelial cells under normal conditions and involved in the chemoattraction to the epithelial layer and adhesion of mononuclear cells expressing its receptor, CX3CR1. The fractalkine level elevated in serum concurrent with increased expression of CX3CR1 in liver-infiltrating mononuclear cells in patients with PBC [45], suggesting fractalkine to be critical for the generation and persistence of the portal lymphocytic infiltration in PBC. In fact, fractalkine is detectable in BECs of small bile ducts in normal and diseased livers, but it is increased in injured bile ducts of PBC [45]. Moreover, many CX3CR1-positive mononuclear cells infiltrate into portal tracts and most biliary intraepithelial lymphocytes in injured bile ducts are also positive for CX3CR1. Production of fractalkine in BECs is responsible for the chemoattraction of CX3CR1-positive lymphocytes into portal tracts and into biliary epithelia [45]. Recently, Shimoda et al [46] demonstrated the significance of fractalkine and the precise mechanism of its production using populations of multiple intrahepatic cell types, including endothelial
cells, liver sinusoidal endothelial cells, and BECs, to directly study the interaction of
fractalkine-producing cells with liver-infiltrating mononuclear cells. Endothelial cells
produced large amounts of fractalkine upon stimulation by PAMPs, though liver
sinusoidal endothelial cells produced no fractalkine. Moreover, BECs also produced
fractalkine; TLR3-stimulated BECs produced fractalkine after direct contact with
TLR4-stimulated autologous monocytes. Innate immunity may lead to increased
expression of fractalkine in the liver and contribute to the development of
cholangiopathy in cases of PBC.

Tolerance

The luminal surface of the biliary tree is continually exposed to PAMPs via
bile and/or portal blood, but no inflammatory response is elicited in BECs. This lack
of response to PAMPs, especially LPS, could be due to "endotoxin tolerance," an
important mechanism of maintaining the homeostasis of organs such as the intestines
which have commensal bacterial flora and to avoid excessive tissue damage [47]. In
addition to intestinal epithelial cells, BECs possess similar tolerance; treatment with
LPS for 24hrs significantly induced tolerance to a subsequent exposure to LPS as
assessed by measuring levels of NF-κB activity and TNF-α mRNA production in
cultured BEC cells (Fig.3) [48]. Moreover, pretreatment with Pam\textsubscript{3}CSK\textsubscript{4} (TLR1/2 ligand) effectively induced tolerance to subsequent stimulation with LPS (TLR4 ligand) (Fig.3) [48]. This cross-tolerance has been demonstrated in monocytes and intestinal epithelial cells [47]. However, treatment with poly(I:C) (TLR3 ligand) significantly enhanced NF-κB activity in fresh cultured BECs and pretreatment did not lead to tolerance to poly(I:C) (Fig.3) [49]. Levels of production of MxA and TRAIL were also preserved. Therefore, tolerance to a TLR3 ligand (poly(I:C)) is not found in BECs.

In response to LPS, the structural complex formed by myeloid differentiation factor 88 (MyD88), IL-1 receptor-associated kinase (IRAK)-1, IRAK-4, and TNF receptor-associated factor 6 (TRAF6) induces a series of phosphorylation events, leading to the activation of nuclear transcription factors. IRAK-M plays a critical negative regulatory role in the signaling between MyD88 and IRAK-1 [50]. In LPS-tolerant cultured BECs, levels of the IRAK-M mRNA and protein were increased, implying that the expression of IRAK-M interferes with the association between IRAK-1 and MyD88 and is crucial to the LPS-induced tolerance of endotoxin in BECs [48]. Moreover, Pam\textsubscript{3}CSK\textsubscript{4} as well as LPS induced IRAK-M expression in BECs [48], suggesting that the tolerance caused by the up-regulation of IRAK-M expression is
also associated with cross-tolerance to LPS induced by Pam\textsubscript{3}CSK\textsubscript{4}. Immunohistochemically, IRAK-M was constitutively expressed in the cytoplasm of BECs, irrespective of intrahepatic biliary levels (Fig.4). This finding suggests that the expression of IRAK-M is associated with hypo- or un-responsiveness to bacterial PAMPs in bile and/or portal flow. In contrast, although IRAK-M mRNA expression was upregulated by stimulation with dsRNA (TLR3 ligand), no tolerance to the dsRNA was found in cultured BECs. This is reasonable because the intracellular signaling of dsRNA-related receptors is a MyD88-independent pathway, that is, the dsRNA-related response is not affected by IRAK-M [51]. Moreover, the upregulation of IRAK-M expression on treatment with poly(I:C) is speculated to cause dsRNA-stimulated BECs to become tolerant to TLR2- and TLR3-related PAMPs including LPS. However, BECs are speculated to be in an entirely virus-free state and at the time of infection of a dsRNA virus, an innate response to dsRNA develops and continues in the presence of virus in the biliary tree, suggesting that the infection likely causes progressive destruction in the biliary epithelium without inducing tolerance.

**Cholangiopathy associated with biliary innate immunity**
**Cell death:** BECs lining the biliary tree are tolerant of non-pathogenic commensal bacterial PAMPs so as to maintain the homeostasis of biliary innate immunity under physiological conditions. Even though biliary innate immunity is activated by pathological PAMPs and provides ‘danger signals’ to the biliary tree, more effective negative mechanisms occur to avoid tissue damage.

However, because innate immune tolerance of dsRNA is lacking in BECs, cell and tissue damage is found in the biliary innate immune response via TLR3 [49]. Stimulation with poly(I:C) induced the activation of NF-κB and IRF-3, followed by the production of antiviral IFN-β1 [37] and also enhanced apoptosis via production of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL).[37] Moreover, in biliary atresia in which Reoviridae are speculated to be an etiological agent, BECs lining extrahepatic bile ducts diffusely and constantly expressed TLR3 and showed an enhancement of TRAIL and single-stranded DNA (ssDNA)-positive apoptosis as well as the activation of NF-κB and IRF-3 and increased expression of an anti-viral product, MxA [33,37,38]. Therefore, BECs not only directly participate in the antiviral innate immune response through the production of antiviral effectors to prevent viral replication by secreting antibiotics in response to dsRNA, but also play a role in the generation of apoptotic responses to infected cells. This additional mechanism
concerning cell death in biliary innate immunity is directly associated with the pathogenesis of cholangiopathies in biliary atresia.

**Epithelial-mesenchymal transition (EMT):** Fundamental to EMT is a loss of normal epithelial features such as cell-to-cell adhesion molecules and the gain of a mesenchymal phenotype [52]. Recently, the EMT of BECs has been speculated to be associated with periductal fibrosis and portal fibrosis in several chronic hepatobiliary diseases [53-56]. In biliary atresia, in particular, the mesenchymal markers vimentin and S100A4 (also known as fibroblast-specific protein 1), and an essential transcription factor for EMT, Snail, are expressed but the biliary-type cytokeratin CK19 and the common epithelial marker E-cadherin are not, in BECs of extrahepatic bile ducts and peribiliary glands. The occurrence of EMT in the BECs of these anatomical biliary components is closely associated with the pathogenesis of sclerosing cholangiopathy in biliary atresia [56,57]. As mentioned, although the biliary innate immune response to dsRNA reduces the viability of cultured human BECs via TRAIL-mediated apoptosis, the rate of cell death is approximately 30% [37]. The cells that evade apoptosis show a gradual loss of CK19 and E-cadherin, and increased expression of S100A4 and Snail, demonstrating the occurrence of biliary EMT. Because EMT confers resistance to apoptotic effects in fetal rat hepatocytes [58],
biliary EMT is thought to be a survival mechanism and associated with an incomplete induction of apoptosis caused by the biliary innate immune response.

TGF-β1 and basic fibroblast growth factor (bFGF) are the major inducers of EMT and TGF-β1 plays an important role in the initiation and progression of liver fibrosis [59,60]. Moreover, because expression of the TGF-β1 pseudoreceptor, bone morphogenic protein and activin membrane-bound inhibitor (Bambi), is decreased by an innate immune response and consequently, susceptibility to TGF-β1 is increased, loss of Bambi and upregulation of the TGF-β receptor are also speculated to be inducers of EMT [61]. Cultured human BECs constantly express TGF-β1, its receptor TGFβR1, and the bFGF receptor (FGFR1) [54,57]. However, because Bambi is also expressed in BECs, the induction of EMT is likely inhibited by the effect of Bambi. Treatment with poly(I:C) gradually decreases and increases the expression of Bambi and bFGF, respectively, and stimulation with bFGF quickly induces a reduction in the level of Bambi. Therefore, the biliary innate immune response to dsRNA could increase susceptibility to TGF-β1, and both TGF-β and bFGF play important roles in the biliary EMT induced by a dsRNA-related innate immune response.

**Chronic inflammation:** Recently, in addition to Th1 and Th2 cells, a third type of pathogenic helper T cell, the Th17 cell, and its association with the chronic
inflammation of autoimmune diseases, has been noted [62]. Human Th17 cells are characterized by the production of interleukin (IL)-17 (IL-17A and IL-17F) and differentiate from naïve T cells (Th0). Th17 cells are part of the mucosal host defense system and have a major role in protection against bacterial infections. Moreover, with the discovery that Th17 cells are also involved in the pathogenesis of chronic inflammatory disorders including models of some autoimmune diseases, there has been intense interest in the relative contributions of Th17 and Th1/Th2 cells to the pathogenesis of these diseases. In liver, IL-17-positive cells identified as Th17 cells are mainly present at the interface of inflammed portal tracts in cases of PBC and CVH-C, and also, in PBC, accumulated around damaged interlobular bile ducts [63]. Th17 cells are associated with interface hepatitis in chronic liver diseases. Moreover, the Th17-related peribiliary cytokine milieu is enhanced in PBC and implicated in the histogenesis of the sustained cholangitis of PBC.

In human Th17 cells, IL-6 and IL-1β are required for differentiation [64], while IL-23 is necessary for maintaining or stabilizing cellular functions and survival, but not differentiation [62]. Bacterial PAMPs including LPS cause the production of Th17-inducible cytokines (IL-6 and IL-1β) and a Th17-maintaining cytokine (IL-23) in cultured BECs [63]. Moreover, BECs lining damaged bile ducts in PBC, express
IL-6, IL-1β, and IL-23 p19 [63]. The biliary innate immune response to bacterial components involves the production of Th17-inducible and -maintaining cytokines in BECs and also the differentiation into Th17 cells of periductal dendritic cells and macrophages. Biliary innate immunity plays a role in the induction and maintenance of Th17 cells in the periductal area in cases of PBC.

Modulation of biliary innate immunity as a therapeutic strategy

Therapeutic strategies have been proposed for the modulation of hepatic innate immunity, but rarely for biliary innate immunity. Peroxisome proliferator-activated receptor γ (PPARγ) is a nuclear receptor involved in regulating adipocyte differentiation and also anti-inflammatory activities [65]. The activation of PPARγ by its ligands is shown to inhibit the expression of pro-inflammatory cytokines such as TNF-α, the induction of which is mediated via NF-κB [66]. In liver, PPARγ is constitutively expressed in intrahepatic bile ducts, irrespective of the anatomical level (Fig.5), and may relate to the maintenance of biliary homeostasis and absence of inflammatory reactions by attenuating inflammatory signals in BECs to PAMPs [67]. In PBC liver, PPARγ expression is significantly down-regulated in the affected bile ducts (Fig.5), indicating an increased susceptibility to PAMPs. IL-4 (Th2 cytokine)
and IFN-γ (Th1 cytokine) up- and down-regulate PPARγ expression, respectively, in cultured human BECs. An unique cytokine milieu is associated with the reduction in PPARγ expression in the affected bile ducts of PBC liver [67].

Several PPARγ ligands have been identified, including the prostaglandin D metabolite 15-deoxy-Δ^{12,14}-prostaglandin J2 (15d-PGJ2) and thiazolidinedione derivatives. 15d-PGJ2 functions as an endogenous ligand for PPARγ and attenuates the activation of NF-κB by preventing the phosphorylation of its inhibitor protein (I-κB). In cultured human BECs, 15d-PGJ2 treatment attenuates PAMP (LPS or peptidoglycan)-induced NF-κB activation and also TNF-α production (Fig.6). PPARγ ligands provide protection against biliary inflammation in PBC, but 15d-PGJ2 inhibited NF-κB’s activation independent of PPARγ [67,68]. In fact, a PPARγ antagonist (GW9662) partially blocked the inhibitory effects of 15d-PGJ2 on NF-κB activity. Therefore, 15d-PGJ2 is expected to be effective in the anti-inflammatory treatment of bile ducts with reduced as well as preserved PPARγ expression in PBC. Because PPARγ is a key immunomodulatory molecule, a reduction in its expression in the bile ducts of PBC liver may be important to the immunopathogenesis of chronic cholangitis. Therefore, immunosuppression using PPARγ ligands may help to reduce bile duct damage in PBC.
Conclusion

Biliary innate immunity consisting of an organ-specific system is important for the mucosal immunity in intrahepatic and extrahepatic bile ducts. Biliary innate immunity is surely associated with the pathogenesis of biliary diseases as well as defense against microbial infections. The molecular mechanisms involved have recently been clarified. Targeting of NF-κB is thought to be a potential therapeutic strategy in various cells, but TLR signaling-specific molecules are also likely to be suitable molecular targets. Further translational research concerning the regulation of biliary innate immunity is needed.
Table 1  Bacteria and viruses possible etiological of biliary diseases

Primary biliary cirrhosis
• Detection of microorganisms
  - lipopolysaccharide (LPS)
  - lipoteichoic acid
  - Helicobacter
  - β-retrovirus
  - P.acnes

• Molecular mimicry between human and microbial PDC-E2
  - E.coli
  - Mycobacterium
  - Novosphingobium
  - Lactobacillus
  - Chlamydia

Biliary atresia
- Reovirus
- Rotavirus
- cytomegalovirus (CMV)
- adenovirus
- enterovirus
- Ebstein-Barr virus (EBV)

Primary sclerosing cholangitis
- Helicobacter
- a-hemolytic streptococcus

Hepatolithiasis
- Escherichia coli (E.coli)
- Klebsiella
- Streptococcus
- Pseudomonas
- Bacteroides
- Clostridium
- Campylobacter
REFERENCES

1. Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffmann JA. The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in Drosophila adults. Cell 1996; 86: 973-983.

2. Akira S, Takeda K. Toll-like receptor signalling. Nat Rev Immunol 2004; 4: 499-511.

3. Hugot JP, Chamaillard M, Zouali H, Lesage S, Cezard JP, Belaiche J, Almer S, et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn’s disease. Nature 2001; 411: 599-603.

4. Harada K, Ohira S, Isse K, Ozaki S, Zen Y, Sato Y, Nakanuma Y. Lipopolysaccharide activates nuclear factor-kappaB through toll-like receptors and related molecules in cultured biliary epithelial cells. Lab Invest 2003; 83: 1657-1667.

5. Hiramatsu K, Harada K, Tsuneyama K, Sasaki M, Fujita S, Hashimoto T, Kaneko S, et al. Amplification and sequence analysis of partial bacterial 16S ribosomal RNA gene in gallbladder bile from patients with primary biliary cirrhosis. J Hepatol 2000; 33: 9-18.

6. Osnes T, Sandstad O, Skar V, Osnes M. Lipopolysaccharides and beta-glucuronidase activity in choledochal bile in relation to choledocholithiasis. Digestion 1997; 58: 437-443.

7. Sasatomi K, Noguchi K, Sakisaka S, Sata M, Tanikawa K. Abnormal accumulation of endotoxin in biliary epithelial cells in primary biliary cirrhosis and primary sclerosing cholangitis. J Hepatol 1998; 29: 409-416.

8. Sheen-Chen S, Chen W, Eng H, Sheen C, Chou F, Cheng Y, Lee T. Bacteriology and antimicrobial choice in hepatolithiasis. Am J Infect Control 2000; 28: 298-301.

9. Harada K, Ozaki S, Kono N, Tsuneyama K, Katayanagi K, Hiramatsu K, Nakanuma Y. Frequent molecular identification of Campylobacter but not Helicobacter genus in bile and biliary epithelium in hepatolithiasis. J Pathol 2001; 193: 218-223.

10. Nilsson HO, Taneera J, Castedal M, Glatz E, Olsson R, Wadstrom T. Identification of Helicobacter pylori and other Helicobacter species by PCR, hybridization, and partial DNA sequencing in human liver samples from patients with primary sclerosing cholangitis or primary biliary cirrhosis. J Clin Microbiol 2000; 38: 1072-1076.
11. Sung JY, Costerton JW, Shaffer EA. Defense system in the biliary tract against bacterial infection. Dig Dis Sci 1992; 37: 689-696.
12. Xu L, Shen Z, Guo L, Fodera B, Keogh A, Joplin R, O'Donnell B, et al. Does a betaretrovirus infection trigger primary biliary cirrhosis? Proc Natl Acad Sci U S A 2003; 100: 8454-8459.
13. Tyler KL, Sokol RJ, Oberhaus SM, Le M, Karrer FM, Narkewicz MR, Tyson RW, et al. Detection of reovirus RNA in hepatobiliary tissues from patients with extrahepatic biliary atresia and choledochal cysts. Hepatology 1998; 27: 1475-1482.
14. Nilsson I, Kornilovs'ka I, Lindgren S, Ljungh A, Wadstrom T. Increased prevalence of seropositivity for non-gastric Helicobacter species in patients with autoimmune liver disease. J Med Microbiol 2003; 52: 949-953.
15. Fox JG, Dewhirst FE, Shen Z, Feng Y, Taylor NS, Paster BJ, Ericson RL, et al. Hepatic Helicobacter species identified in bile and gallbladder tissue from Chileans with chronic cholecystitis. Gastroenterology 1998; 114: 755-763.
16. Tsuneyama K, Harada K, Kono N, Hiramatsu K, Zen Y, Sudo Y, Gershwin ME, et al. Scavenger cells with gram-positive bacterial lipoteichoic acid infiltrate around the damaged interlobular bile ducts of primary biliary cirrhosis. J Hepatol 2001; 35: 156-163.
17. Burroughs AK, Rosenstein IJ, Epstein O, Hamilton-Miller JM, Brumfitt W, Sherlock S. Bacteriuria and primary biliary cirrhosis. Gut 1984; 25: 133-137.
18. Butler P, Valle F, Hamilton-Miller JM, Brumfitt W, Baum H, Burroughs AK. M2 mitochondrial antibodies and urinary rough mutant bacteria in patients with primary biliary cirrhosis and in patients with recurrent bacteriuria. J Hepatol 1993; 17: 408-414.
19. Butler P, Hamilton-Miller JM, McIntyre N, Burroughs AK. Natural history of bacteriuria in women with primary biliary cirrhosis and the effect of antimicrobial therapy in symptomatic and asymptomatic groups. Gut 1995; 36: 931-934.
20. Parikh-Patel A, Gold EB, Worman H, Krivy KE, Gershwin ME. Risk factors for primary biliary cirrhosis in a cohort of patients from the united states. Hepatology 2001; 33: 16-21.
21. Haruta I, Hashimoto E, Kato Y, Kikuchi K, Kato H, Yagi J, Uchiyama T,
et al. Lipoteichoic acid may affect the pathogenesis of bile duct damage in primary biliary cirrhosis. Autoimmunity 2006; 39: 129-135.

22. Harada K, Tsuneyama K, Sudo Y, Masuda S, Nakanuma Y. Molecular identification of bacterial 16S ribosomal RNA gene in liver tissue of primary biliary cirrhosis: is Propionibacterium acnes involved in granuloma formation? Hepatology 2001; 33: 530-536.

23. Gershwin ME, Mackay IR, Sturgess A, Coppel RL. Identification and specificity of a cDNA encoding the 70 kd mitochondrial antigen recognized in primary biliary cirrhosis. J Immunol 1987; 138: 3525-3531.

24. Shimoda S, Nakamura M, Ishibashi H, Hayashida K, Niho Y. HLA DRB4 0101-restricted immunodominant T cell autoepitope of pyruvate dehydrogenase complex in primary biliary cirrhosis: evidence of molecular mimicry in human autoimmune diseases. J Exp Med 1995; 181: 1835-1845.

25. Kita H, Lian ZX, Van de Water J, He XS, Matsumura S, Kaplan M, Luketic V, et al. Identification of HLA-A2-restricted CD8(+) cytotoxic T cell responses in primary biliary cirrhosis: T cell activation is augmented by immune complexes cross-presented by dendritic cells. J Exp Med 2002; 195: 113-123.

26. Morecki R, Glaser JH, Cho S, Balistreri WF, Horwitz MS. Biliary atresia and reovirus type 3 infection. N Engl J Med 1982; 307: 481-484.

27. Morecki R, Glaser JH, Johnson AB, Kress Y. Detection of reovirus type 3 in the porta hepatis of an infant with extrahepatic biliary atresia: ultrastructural and immunocytochemical study. Hepatology 1984; 4: 1137-1142.

28. Riepenhoff-Talty M, Gouvea V, Evans MJ, Svensson L, Hoffenberg E, Sokol RJ, Uhnoo I, et al. Detection of group C rotavirus in infants with extrahepatic biliary atresia. J Infect Dis 1996; 174: 8-15.

29. Brown WR, Sokol RJ, Levin MJ, Silverman A, Tamaru T, Lilly JR, Hall RJ, et al. Lack of correlation between infection with reovirus 3 and extrahepatic biliary atresia or neonatal hepatitis. J Pediatr 1988; 113: 670-676.

30. Bobo L, Ojeh C, Chiu D, Machado A, Colombani P, Schwarz K. Lack of evidence for rotavirus by polymerase chain reaction/enzyme immunoassay of hepatobiliary samples from children with biliary atresia. Pediatr Res 1997; 41: 229-234.
31. Saito T, Shinozaki K, Matsunaga T, Ogawa T, Etoh T, Muramatsu T, Kawamura K, et al. Lack of evidence for reovirus infection in tissues from patients with biliary atresia and congenital dilatation of the bile duct. J Hepatol 2004; 40: 203-211.

32. Rauschenfels S, Krassmann M, Al-Masri AN, Verhagen W, Leonhardt J, Kuebler JF, Petersen C. Incidence of hepatotropic viruses in biliary atresia. Eur J Pediatr 2009; 168: 469-476.

33. Al-Masri AN, Flemming P, Rodeck B, Melter M, Leonhardt J, Petersen C. Expression of the interferon-induced Mx proteins in biliary atresia. J Pediatr Surg 2006; 41: 1139-1143.

34. Riepenhoff-Talty M, Schaekel K, Clark HF, Mueller W, Uhnoo I, Rossi T, Fisher J, et al. Group A rotaviruses produce extrahepatic biliary obstruction in orally inoculated newborn mice. Pediatr Res 1993; 33: 394-399.

35. Szavay PO, Leonhardt J, Czech-Schmidt G, Petersen C. The role of reovirus type 3 infection in an established murine model for biliary atresia. Eur J Pediatr Surg 2002; 12: 248-250.

36. Takii Y, Nakamura M, Ito M, Yokoyama T, Komori A, Shimizu-Yoshida Y, Nakao R, et al. Enhanced expression of type I interferon and toll-like receptor-3 in primary biliary cirrhosis. Lab Invest 2005; 85: 908-920.

37. Harada K, Sato Y, Itatsu K, Isse K, Ikeda H, Yasoshima M, Zen Y, et al. Innate immune response to double-stranded RNA in biliary epithelial cells is associated with the pathogenesis of biliary atresia. Hepatology 2007; 46: 1146-1154.

38. Harada K, Isse K, Nakanuma Y. Interferon gamma accelerates NF-kappaB activation of biliary epithelial cells induced by Toll-like receptor and ligand interaction. J Clin Pathol 2006; 59: 184-190.

39. Saito K, Nakanuma Y. Lactoferrin and lysozyme in the intrahepatic bile duct of normal livers and hepatolithiasis. An immunohistochemical study. J Hepatol 1992; 15: 147-153.

40. Bals R, Wang X, Wu Z, Freeman T, Bafna V, Zasloff M, Wilson JM. Human beta-defensin 2 is a salt-sensitive peptide antibiotic expressed in human lung. J Clin Invest 1998; 102: 874-880.

41. Sugiura H, Nakanuma Y. Secretory component and immunoglobulins in the intrahepatic biliary tree and peribiliary gland in normal livers and hepatolithiasis. Gastroenterol Jpn 1989; 24: 308-314.
42. Harada K, Ohba K, Ozaki S, Isse K, Hirayama T, Wada A, Nakanuma Y. Peptide antibiotic human beta-defensin-1 and -2 contribute to antimicrobial defense of the intrahepatic biliary tree. Hepatology 2004; 40: 925-932.

43. Isse K, Harada K, Nakanuma Y. IL-8 expression by biliary epithelial cells is associated with neutrophilic infiltration and reactive bile ductules. Liver Int 2007; 27: 672-680.

44. Lefkowitch JH. Bile ductular cholestasis: an ominous histopathologic sign related to sepsis and "cholangitis lenta". Hum Pathol 1982; 13: 19-24.

45. Isse K, Harada K, Zen Y, Kamihira T, Shimoda S, Harada M, Nakanuma Y. Fractalkine and CX3CR1 are involved in the recruitment of intraepithelial lymphocytes of intrahepatic bile ducts. Hepatology 2005; 41: 506-516.

46. Shimoda S, Harada K, Niiro H, Taketomi A, Maehara Y, Tsuneyama K, Kikuchi K, et al. CX3CL1 (fractalkine): A signpost for biliary inflammation in primary biliary cirrhosis. Hepatology 2009.

47. Otte JM, Cario E, Podolsky DK. Mechanisms of cross hyporesponsiveness to Toll-like receptor bacterial ligands in intestinal epithelial cells. Gastroenterology 2004; 126: 1054-1070.

48. Harada K, Isse K, Sato Y, Ozaki S, Nakanuma Y. Endotoxin tolerance in human intrahepatic biliary epithelial cells is induced by upregulation of IRAK-M. Liver Int 2006; 26: 935-942.

49. Harada K, Sato Y, Isse K, Ikeda H, Nakanuma Y. Induction of innate immune response and absence of subsequent tolerance to dsRNA in biliary epithelial cells relate to the pathogenesis of biliary atresia. Liver Int 2008; 28: 614-621.

50. Kobayashi K, Hernandez LD, Galan JE, Janeway CA, Jr., Medzhitov R, Flavell RA. IRAK-M is a negative regulator of Toll-like receptor signaling. Cell 2002; 110: 191-202.

51. Takeda K, Akira S. Toll-like receptors in innate immunity. Int Immunol 2005; 17: 1-14.

52. Thiery JP. Epithelial-mesenchymal transitions in development and pathologies. Curr Opin Cell Biol 2003; 15: 740-746.

53. Nakanuma Y, Kono N. Expression of vimentin in proliferating and damaged bile ductules and interlobular bile ducts in nonneoplastic
hepatobiliary diseases. Mod Pathol 1992; 5: 550-554.
54. Rygiel KA, Robertson H, Marshall HL, Pekalski M, Zhao L, Booth TA, Jones DE, et al. Epithelial-mesenchymal transition contributes to portal tract fibrogenesis during human chronic liver disease. Lab Invest 2008; 88: 112-123.
55. Sato Y, Harada K, Ozaki S, Furubo S, Kizawa K, Sanzen T, Yasoshima M, et al. Cholangiocytes with mesenchymal features contribute to progressive hepatic fibrosis of the polycystic kidney rat. Am J Pathol 2007; 171: 1859-1871.
56. Diaz R, Kim JW, Hui JJ, Li Z, Swain GP, Fong KS, Csiszar K, et al. Evidence for the epithelial to mesenchymal transition in biliary atresia fibrosis. Hum Pathol 2008; 39: 102-115.
57. Harada K, Sato Y, Ikeda H, Isse K, Ozaki S, Enomae M, Ohama K, et al. Epithelial-mesenchymal transition induced by biliary innate immunity contributes to the sclerosing cholangiopathy of biliary atresia. J Pathol 2009; 217: 654-664.
58. Valdes F, Alvarez AM, Locascio A, Vega S, Herrera B, Fernandez M, Benito M, et al. The epithelial mesenchymal transition confers resistance to the apoptotic effects of transforming growth factor Beta in fetal rat hepatocytes. Mol Cancer Res 2002; 1: 68-78.
59. Zavadil J, Bottinger EP. TGF-beta and epithelial-to-mesenchymal transitions. Oncogene 2005; 24: 5764-5774.
60. Dooley S, Streckert M, Delvoux B, Gressner AM. Expression of Smads during in vitro transdifferentiation of hepatic stellate cells to myofibroblasts. Biochem Biophys Res Commun 2001; 283: 554-562.
61. Seki E, De Minicis S, Osterreicher CH, Kluwe J, Osawa Y, Brenner DA, Schwabe RF. TLR4 enhances TGF-beta signaling and hepatic fibrosis. Nat Med 2007; 13: 1324-1332.
62. Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, McClanahan T, et al. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J Exp Med 2005; 201: 233-240.
63. Harada K, Shimoda S, Sato Y, Isse K, Ikeda H, Nakanuma Y. Periductal interleukin-17 production in association with biliary innate immunity contributes to the pathogenesis of cholangiopathy in primary biliary cirrhosis. Clin Exp Immunol 2009; 157: 261-270.
64. Acosta-Rodriguez EV, Napolitani G, Lanzavecchia A, Sallusto F. Interleukins 1beta and 6 but not transforming growth factor-beta are essential for the differentiation of interleukin 17-producing human T helper cells. Nat Immunol 2007; 8: 942-949.

65. Nakajima T, Kamijo Y, Tanaka N, Sugiyama E, Tanaka E, Kiyosawa K, Fukushima Y, et al. Peroxisome proliferator-activated receptor alpha protects against alcohol-induced liver damage. Hepatology 2004; 40: 972-980.

66. Nakajima A, Wada K, Miki H, Kubota N, Nakajima N, Terauchi Y, Ohnishi S, et al. Endogenous PPAR gamma mediates anti-inflammatory activity in murine ischemia-reperfusion injury. Gastroenterology 2001; 120: 460-469.

67. Harada K, Isse K, Kamihira T, Shimoda S, Nakanuma Y. Th1 cytokine-induced downregulation of PPARgamma in human biliary cells relates to cholangitis in primary biliary cirrhosis. Hepatology 2005; 41: 1329-1338.

68. Okano H, Shiraki K, Inoue H, Yamanaka Y, Kawakita T, Saitou Y, Yamaguchi Y, et al. 15-deoxy-delta-12-14-PGJ2 regulates apoptosis induction and nuclear factor-kappaB activation via a peroxisome proliferator-activated receptor-gamma-independent mechanism in hepatocellular carcinoma. Lab Invest 2003; 83: 1529-1539.
Fig. 1

Representative expression pattern of TLR4 in interlobular bile ducts. Membranous (mainly lateral) in addition to weakly cytoplasmic expression is found in Primary biliary cirrhosis. Immunohistochemistry for TLR4.
Immunohistochemical staining for human beta defensin (hBD)-1 (A) and hBD-2 (B). (A) Normal liver. Septal bile ducts are positive for hBD-1. (B) Extrahepatic biliary obstruction. Biliary epithelium of the intrahepatic large bile duct showing cholangitis strongly expresses hBD-2.
Induction of tolerance in cultured human biliary epithelial cells (BECs). BECs are pretreated with lipopolysaccharide (LPS, TLR4 ligand) or Pam3CSK4 (Pam3, TLR1/2 ligand) for 24h and subjected to another LPS challenge. Pretreatment with LPS and Pam3CSK4 significantly decreases NF-κB activity in response to a subsequent LPS challenge. In contrast, the pretreatment with poly(I:C) (TLR3 ligand) or LPS does not inhibit NF-κB’s activation in response to a subsequent poly(I:C) challenge (*<0.05).
Fig. 4

IRAK-M is constitutively expressed in the cytoplasm of septal bile ducts in normal liver.

Immunohistochemistry for IRAK-M.
Immunohistochemistry for peroxisome proliferator-activated receptor γ (PPARγ) A: Normal liver. PPARγ is expressed in the cytoplasm of bile ducts (arrow). B: Primary biliary cirrhosis (PBC). Damaged bile ducts (arrowhead) show reduced expression of PPARγ, though evidently positive biliary cells (arrow) also remain.
Effect of the peroxisome proliferator-activated receptor γ (PPARγ) ligand, 15d-PGJ2, on lipopolysaccharide (LPS, TLR4 ligand)- and peptidoglycan (TLR2 ligand)-induced NF-κB activation in cultured human biliary epithelial cells (BECs). BECs are pretreated in the presence or absence of 15d-PGJ2 (20μM) before stimulation with LPS or peptidoglycan. Pretreatment with 15d-PGJ2 significantly prevents PAMP-induced NF-κB activation (*<0.05).
Fig. 1
Fig. 3

Graph showing NF-κB activation levels with different stimulants. The graph compares non-stimulated (Non) with LPS, Pam3, and PolyIC treatments. The 1st and 2nd stimulants are indicated with Non, LPS, Pam3, and PolyIC. The 1st stimulant groups are Non, LPS, Pam3, and Non, while the 2nd stimulant groups are LPS, LPS, LPS, and PolyIC. Asterisks indicate statistical significance (*) between treatment groups.
Fig. 5A
