Innate immunity in the pathogenesis of cholangiopathy: A recent update

Review Article

Innate immunity in the pathogenesis of cholangiopathy, A recent update

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ABSTRACT

Biliary innate immunity is involved in the pathogenesis of cholangiopathies in patients with various biliary diseases. Biliary epithelial cells possess an innate immune system consisting of the Toll-like receptor (TLR) family and recognize pathogen-associated molecular patterns (PAMPs). Recently, regulatory mechanisms by intracellular negative regulators including peroxisome proliferator-activated receptor-γ and micro-RNA have been clarified. In primary biliary cirrhosis (PBC) and primary sclerosing cholangitis, dysregulated biliary innate immunity, namely hyper-responsiveness to PAMPs, is associated with the histopathogenesis of cholangiopathy. Moreover, biliary epithelial cells produce monocyte chemotactic protein-1 (MCP-1/CCL2) as a result of the innate immune response and bile ductules play a role in hepatic fibrosis caused by hepatic stellate cells (HSCs). Also, biliary innate immune responses induce the production of two chemokines, fractalkine and macrophage inflammatory protein-3α (MIP-3α), causing the migration of inflammatory cells and a population of antigen-presenting cell found in epithelium, Langerhans cell, and involve chronic cholangitis associated with biliary epithelium-specific innate and acquired immunity in PBC.

KEY WORDS : biliary epithelial cell, cholangiopathy, innate immunity, Toll-like receptor, chemokine, primary biliary cirrhosis

Introduction

Infectious agents are involved in various cholangiopathies including cholangitis, bile duct loss, and lithiasis as either etiopathological or aggravating factors. Several enterobacteria and viruses are speculated to be primary or secondary factors for primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), biliary atresia, and biliary lithiasis. Such cholangiopathy is closely associated with biliary innate immunity and the periductal cytokine network [1-7]. Epithelial cells as well as immunocompetent cells such as dendritic cells (DCs) possess pathogen-recognition receptors such as Toll-like receptors (TLRs) reflecting the proper micro-environment and function of each epithelial cell type. Biliary epithelial cells (BECs, cholangiocytes) also possess TLR-related innate immune systems [6, 8-10]. Because no microorganisms showing BEC-specific tropism have been identified, an innate immune response specific to BEC rather than pathogen-associated molecular patterns (PAMPs) is important in the pathogenesis of cholangiopathy. We have previously reviewed the basic molecular mechanism of biliary innate immunity in this journal [11]. In this review, we attempted to summarize recent progress in the study of the pathophysiological role of biliary innate immunity in the mechanisms of cholangiopathy, mainly based on our own data (Table 1).

Regulatory system of biliary innate immunity: update

Human BECs possess functional PAMP-recognizing receptors including at least TLR1-5 (Table 1). These TLRs are distributed throughout the intrahepatic biliary tree in normal and diseased human livers, irrespective of anatomical level, implying that BECs participate directly in innate immunity and show a prompt response to pathogens

without any help from immunocompetent cells [6, 9, 10, 13, 18, 28, 29].

Although the luminal surface of the bile duct is continually exposed to PAMPs in bile, mainly originating from the intestines, BECs physiologically do not elicit an inflammatory response. Tight control of TLR signaling provides tolerance to physiological amounts of intestinal endotoxin in bile to avoid constant innate immune activation in BECs. This failure to respond to PAMPs, especially LPS, could be due to "endotoxin tolerance." In human BECs, IRAK-M and peroxisome proliferator-activated receptor γ (PPARγ) are closely associated with the maintenance of biliary homeostasis as a regulator of tolerance by attenuating inflammatory signals to commensal PAMPs in bile, as summarized in our previous review of this journal [11].

Recently, novel regulatory mechanisms of biliary innate immunity associated with the TLR system have been clarified. Micro-RNAs play important roles in a wide range of biological events through post-transcriptional suppression of target mRNAs. Recent studies indicate Micro-RNA-mediated post-transcriptional pathways to be critical to host-cell regulatory responses to microbial infections. Cultured human BECs express let-7 family members which post-transcriptionally down-regulate TLR4 expression and infections of *C. parvum* decrease the expression of let-7 resulting in the up-regulation of TLR4 [16]. Moreover, microRNA-98 and let-7, suppressing cytokine-inducible Src homolog 2-containing protein (CIS, a suppressor of cytokine signaling family) at the translational level, are expressed in BECs, and LPS and *C. parvum* infections downregulate these micro-RNAs, suggesting the regulation of the TLR-mediated biliary innate immune response [17].

In PBC, compared with Th2, a Th1-dominant cytokine milieu is associated with the pathogenesis including bile duct injury [30]. BECs possess the receptor for IFN-γ

(Th1 cytokine) and IFN-γ up-regulates the expression of TLRs and susceptibility to PAMPs in BECs, as mentioned above, impairing the regulation of biliary innate immunity. The upregulation of TLR4 and TLR9 in BECs and of TLR3 and type I IFN signaling pathways in portal tracts and parenchyma are found in PBC [28, 29, 31]. Moreover, IL-4 (Th2 cytokine) and IFN-γ up- and down-regulate the expression of PPARγ in cultured human BECs [15, 32]. In PBC liver, PPARγ expression is significantly down-regulated in the affected bile ducts as a Th1-dominant periductal cytokine milieu [15]. These findings indicate an increased susceptibility to PAMPs, suggesting an association with the pathogenesis of cholangiopathy in PBC.

Moreover, in PSC, inappropriate biliary innate immune responses to intestinal endotoxin and subsequent endotoxin intolerance have been reported to contribute to the development and perpetuation of chronic cholangitis [33]. BECs from PSC livers show reversibly increased TLR expression and activation of TLR-related intracellular signaling. Consequently, PSC BECs exhibit inappropriate innate immune responses to endotoxin and do not develop tolerance after repeated exposure to endotoxin. This hyper-responsiveness is probably because of the stimulatory effect of abundantly expressed IFN-γ and TNF-α in PSC livers, which stimulate TLR4-mediated endotoxin signaling in BECs, leading to increased TLR4-mediated endotoxin incorporation and impaired inactivation of the TLR4 signaling cascade.

Hepatic fibrosis and biliary innate immunity

A major feature of chronic liver diseases, hepatic fibrosis, spreads within portal tracts and also periportal areas in patients with hepatitic and cholestatic liver diseases, irrespective of etiology. In particular, a fibrogenic cytokine-rich environment caused by

inflammation and infection is closely associated with hepatic fibrosis.

Periportal fibrosis is thought to be associated with the accumulation and activation of hepatic stellate cells (HSCs). In damaged liver, HSCs are activated, proliferate, and migrate into the injured area in response to the chemoattractive effects of chemokines and are considered the most important effecter cells associated with fibrogenesis in hepatic parenchyma including the interface between portal tracts and periportal hepatocytes, and the fibrous enlargement of portal tracts and fibrous extension from portal areas are closely associated with activated HSCs and their transformed version, myofibroblasts. Then, HSCs, particularly activated HSCs, migrate into damaged areas in response to a chemokine, secreting monocyte chemotactic protein-1 (MCP-1/CCL2) which attracts monocyte/macrophages [34].

BECs directly promote fibrogenesis by a number of mechanisms including the synthesis of matrix constituents and the release of fibrogenic cytokines such as MCP-1, platelet-derived growth factor, TGF-β, connective tissue growth factor (CTGF), and endothelin-1 [35]. Moreover, only the expression of MCP-1 positively regulated by TLR ligands (PAMPs) occurs in an NF-κB-dependent manner, suggesting that the biliary innate immune response is a critical trigger of MCP-1 production.

Proliferating bile ductules are thought to be part of a non-specific reaction in various hepatobiliary diseases, and bile ductules located between interlobular bile ducts in portal tracts and bile canaliculi in hepatocytes are frequently increased in number under a variety of pathologic conditions of the liver and take part in hepatic fibrosis and the progression of hepatobiliary diseases [26, 36, 37]. MCP-1 is expressed in bile ductules in areas of interface hepatitis (Fig.1), whereas normal livers lacked these findings. Moreover, α-smooth muscle actin (α-SMA)-positive activated HSCs

(myofibroblasts) are accumulated around MCP-1-expressing bile ductules (Fig.1), suggesting that MCP-1 derived from BECs consisting of bile ductules plays a role in the chemoattraction of HSCs. Because the interface areas are rich in several cytokines caused by immune-mediated (necro)inflammatory reactions against virus-infected hepatocytes and bile-derived PAMPs, BECs in bile ductules could produce MCP-1, particularly, via biliary innate immunity, suggesting that biliary innate immunity directly plays an important role in the recruitment of HSCs to interface areas and the activation of HSCs resulting in the progression of periportal fibrosis.

Fractalkine and innate immunity in PBC

PBC mainly affects middle-aged females, and histologically, the interlobular bile ducts are primarily damaged and show characteristic findings such as chronic nonsuppurative destructive cholangitis (CNSDC) followed by progressive bile duct loss [38]. There is considerable evidence that the damage of bile ducts is mediated by autoreactive or cytotoxic T cells [39], and molecular mechanisms of the migration of pathogenic T cells around or within bile ducts have gradually been clarified.

There have been several reports that bacterial components such as lipopolysaccharide (LPS) and DNA fragments are detectable in pathologic bile of patients with PBC [5, 6], and also endotoxin and lipoteichoic acids abnormally accumulate in or around the intrahepatic bile ducts of PBC [5-7, 40, 41]. Unusual immune responses to these infectious agents or their components are now suspected to underlie the etiopathogenesis of PBC [42-45].

The chemokine-adhesion molecule, fractalkine (CX3CL1), plays an important role in the migration of leukocytes to target sites under physiological as well as

pathological conditions. Unlike other chemokines, fractalkine is expressed as a membrane-bound form on cells and also can be shed by ADAM10 or ADAM17 as a soluble chemotactic form. Vascular endothelial cells express fractalkine and its soluble form is a potent chemoattractant for CD16+NK cells, CD8+ cytotoxic T cells, CD4+T cells, γδT cells, monocytes, mature macrophages, and mucosal DC cells, expressing its receptor (CX3CR1). The level of soluble fractalkine is significantly elevated in the sera of PBC patients and also *in situ* expression of fractalkine in small bile ducts, particularly damaged bile ducts, is upregulated in PBC [24] (Fig.2), suggesting that fractalkine is an important mediator associated with the continuous portal, particularly periductal, inflammation of PBC.

Upregulation of fractalkine expresion in BECs is induced by PAMPs including LPS, suggesting that the dysregulated biliary innate immunity against commensal or pathological PAMPs is closely associated with the production of fractalkine in BECs and that secreted soluble fractalkine may be involved in the chemoattraction of infiltrating cells expressing CX3CR1 around these pathological bile ducts and in the recruitment of biliary intraepithelial leukocytes. Moreover, the up-regulation of fractalkine expression in BECs is promoted by TLR3 ligand (poly(I:C)), via activated monocytes through direct contact and the secreation of TNF-α by monocytes [25]. Intraepithelial lymphocytes in damaged bile ducts of PBC patients contain a significantly higher percentage of CD8+ T cells and show strongly increased cytolytic activity against epithelial-derived target cells [46]. Then, CX3CR1-positive leukocytes invade into bile ducts by engaging fractalkine, thereby causing cholangitis including CNSDC [25].

In addition to PBC, fractalkine is associated with the chronic inflammation in

other diseases including rhematoid arthritis and inflammatory bowel disease. Recently, an anti-fractalkine monoclonal antibody has been reported to almost completely suppress the clinicopathological activities in inflammatory bowel disease models [47]. Therefore, fractalkine-CX3CR1 signaling might be a molecular target for the treatment of PBC.

Antigen-presenting cells and biliary innate immunity in PBC

The presence and response to mitochondrial autoantigen(s) of autoreactive T cells [39] is believed to require interaction with professional antigen-presenting cells (APCs) such as DCs. DCs are generally classified into myeloid DCs (mDCs) and plasmacytoid DCs (pDCs) and reside in tissues of most organs under normal conditions. In the periphery, immature DCs such as Langerhans cells (LCs, a population of DCs only found in epithelia) capture antigens and, under the influence of inflammatory stimuli, subsequently migrate. The presence of APCs in biliary trees is likely important to the bile duct-specific autoimmunity in PBC. In the inflamed bile ducts, several DCs including immature DCs [48], S-100[+] DCs,[49] and LCs[50, 51] are found, indicating a role in the initiation of inflammatory responses in cases of PBC. Moreover, periductal DCs are mostly mDCs and the major representative type of mDCs, Langerin[+] LCs, are attached to or embedded in the damaged bile ducts of PBC patients (Fig.3) [23], suggesting that LCs existing around and within bile ducts play an important role as APCs presenting bile duct-derived autoantigens.

Because LCs possess chemokine receptors (CCR4 and CCR6), ligands of these receptors including CCL17, CCL22, and MIP-3α (CCL20) could cause LCs to migrate. Among them, MIP-3α plays a central role in recruiting LC precursors into the

epithelium during inflammation and also an important role in the pathogenesis of ulcerative colitis [52, 53]. In PBC, MIP-3α-expressing BECs are frequently found in the damaged bile ducts accompanying Langerin[+] LCs, suggesting that the BECs producing MIP-3α directly play an important role in the migration of LCs in biliary layers. Moreover, the expression of MIP-3α is selectively restricted at sites of inflammation including cholangitis in PBC (Fig.3) and bile duct-related acquired immunity is speculated to be closely associated with the periductal cytokine milieu and biliary innate immunity [6, 9, 15, 24, 30]. Because human BECs possess receptors for some cytokines (IL-1β, IL-6, IL-4, IFN-γ, TNF-α, and IL-17) as well as TLRs [9, 21], they could show a response to these cytokines as well as PAMPs and induce various immunological reactions. IL-17 and two inflammatory cytokines (IL-1β and TNF-α) significantly upregulate the expression of MIP-3α in human BECs. IL-17-positive cells (namely Th17 cells) are accumulated around the damaged bile ducts in PBC and the expression of IL-1β and TNF-α was increased in damaged bile ducts including CNSDC of PBC patients [20, 21]. Therefore, the production of MIP-3α in PBC is closely associated with periductal cytokines including IL-1β, TNF-α, and IL-17. In addition to these cytokine milieus, the damaged bile ducts and/or portal tracts show an increased susceptibility to PAMPs and enhanced production and secretion of innate inflammatory mediators in PBC [15, 29]. PAMPs directly induce the production of MIP-3α in human BECs [23]. Therefore, LCs existing around or within biliary epithelial layers are induced to migrate via BEC-derived MIP-3α expression evoked via a PBC-specific periductal cytokine milieu and biliary innate immunity and are important as biliary epithelium-specific APCs in the development of acquired immunity and the aggravating and continuous cholangitis in PBC.

Conclusion and perspectives

Biliary innate immunity consisting of an bile duct-specific system is important for the mucosal immunity in intrahepatic and extrahepatic bile ducts and also associated with the pathogenesis of several cholangiopathies in biliary diseases as well as defense against microbial infections. We speculate that biliary innate immunity is solely associated with the etiology of biliary diseases as the initial event and that the presence of causative microorganisms is not necessary in the pathogenesis of cholangiopathy caused by a subsequent acquired immunity. It is mandatory to understand the molecular basis underlying the immunophysiology and immunopathology of cholangiopathy in terms of innate as well as acquired immunity.

DISCLOSURE

This manuscript is an updated version of my previously published manuscript.

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Table 1

Summary of biliary innate immunity

  Receptors recognizing PAMPs
      TLRs
         TLR1 [12, 13]
         TLR2 [9, 12, 13]
         TLR3 [9, 12-14]
         TLR4 [6, 9, 12, 13]
         TLR5 [9, 12, 13]
         TLR6 [9, 12, 13]
         TLR7 [12]
         TLR8 [12]
         TLR9 [12, 13]
      RIG-I [10]
      MDA-5 [10]

  Negative regulators of TLR signalings
      IRAK-M [8]
      PPAR-γ [15]
      micro-RNA [16, 17]

  Antibiotics, cytokine, and chemokines produced via biliary innate immunity
      defensin [18]
      IFN-β1 [10]
      TNF-α [19, 20]
      IL-6 [13, 20, 21]
      IL-1β [21]
      IL-23 p19 [21]
      MCP-1[13, 22]
      MIP-3α [23]
      Fractalkine (CX3CL1) [24, 25]
      IL-8 [13, 26]
      CXCL16 [27]

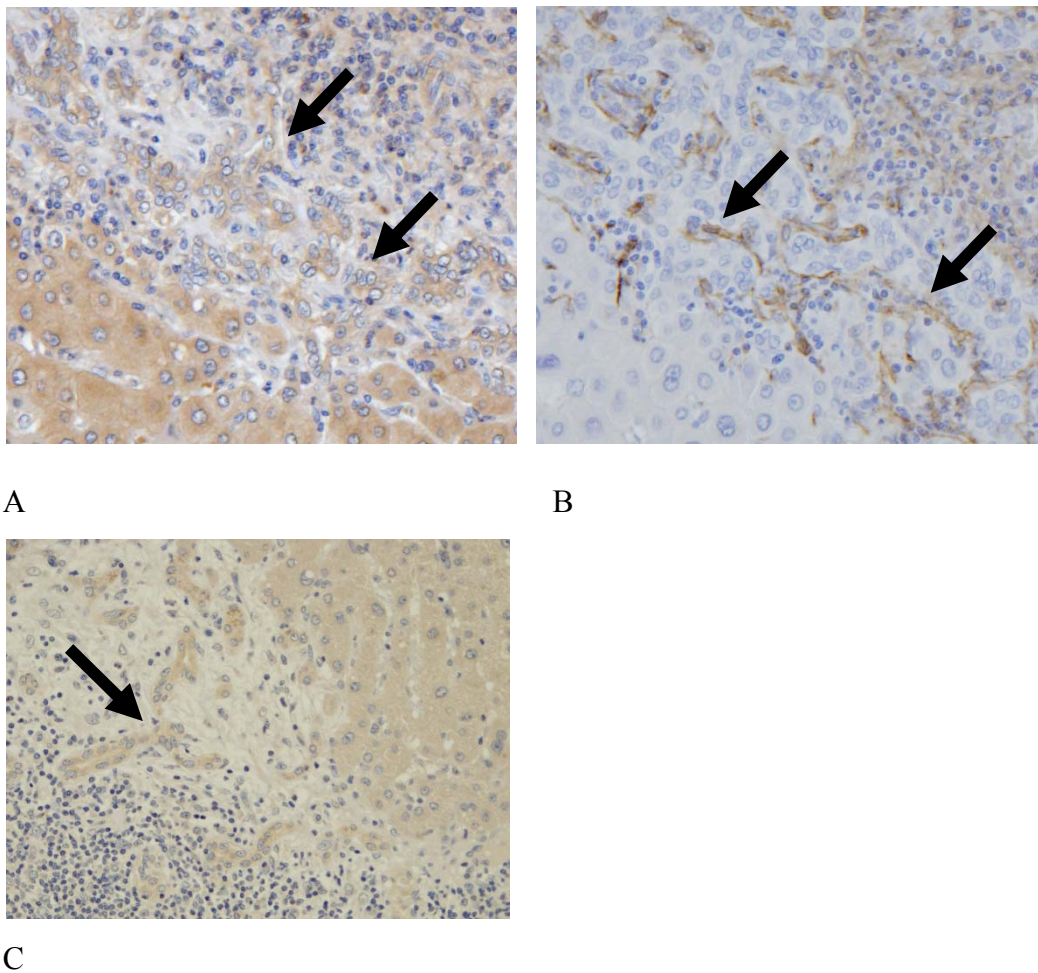Parentheses denote reference numbers.

Figures and legends

A

B

C

Fig.1   Immunohistochemistry for MCP-1 and αSMA. A and B; HCV-related chronic viral hepatitis. Proliferating bile ductules at the interface of periportal areas express MCP-1(A, arrows) and αSMA-positive cells showing hepatic stellate cell (HSC) morphology are found in the same area (B, arrows). C; primary biliary cirrhosis. MCP-1-positive proliferating bile ductules are found (arrow).
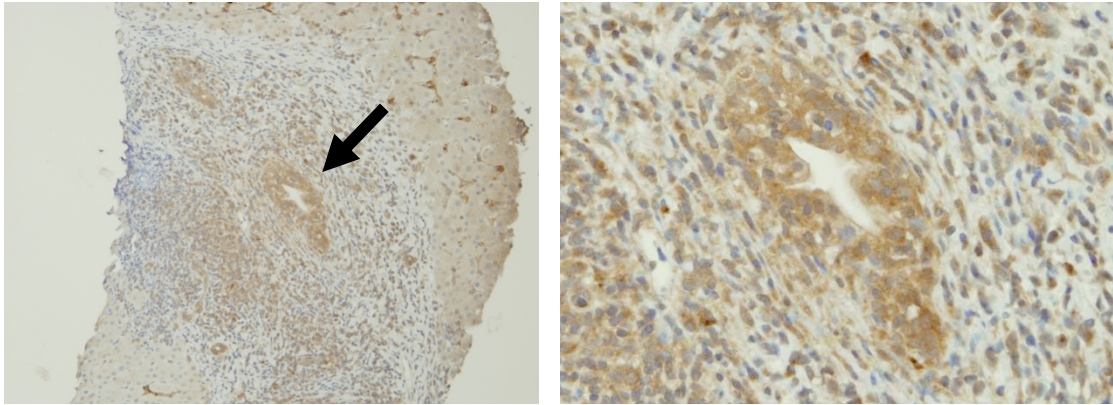
A   B

Fig.2   Immunohistochemistry for fractalkine. Primary biliary cirrhosis. Fractalkine is
expressed in damaged bile duct (arrow in A) and periductal inflammatory cells
in portal tracts and intrasinusoidal cells, probably Kupffer cells, in parenchyma.
B is a higher magnification of A.
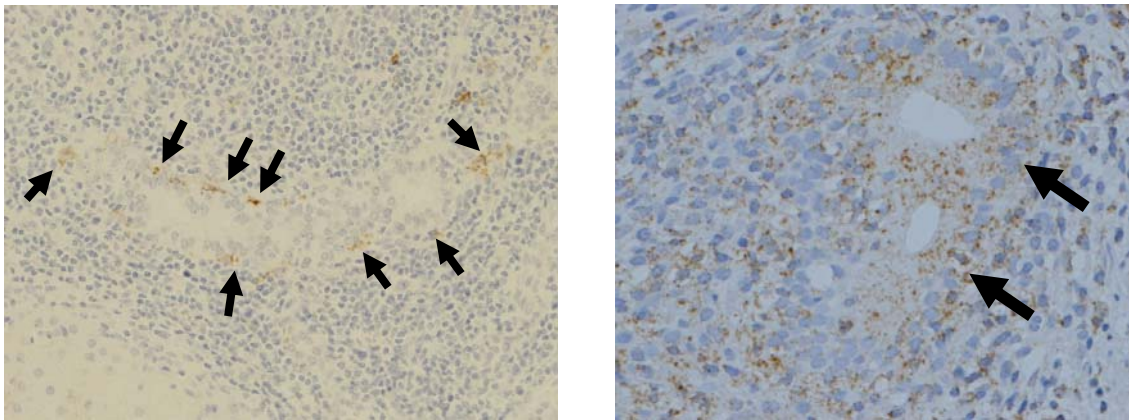
A

B

Fig.3. Detection of Langerhans cells (LCs) and macrophage inflammatory protein-3α (MIP-3α) in liver sections. (A) Langerin[+] LCs are found around and within bile ducts showing cholangitis in primary biliary cirrhosis (PBC). (B) MIP-3α is expressed in infiltrating mononuclear cells and the damaged bile ducts (arrows) in PBC.