

# The Role of Inflammation in Cholestatic Liver Injury

Jie Chen, Shujun Zhang 

Chongqing Key Laboratory of Infectious Diseases and Parasitic Diseases, Department of Infectious Diseases, the First Affiliated Hospital of Chongqing Medical University, Chongqing, People's Republic of China

Correspondence: Shujun Zhang, Tel/Fax +86-23-89012427, Email sjzhang@hospital.cqmu.edu.cn

**Abstract:** Cholestasis is a common clinical event in which bile formation and excretion are blocked, leading to retention of bile acids or bile salts; whether it occurs intra- or extrahepatically, primary or secondary, its pathogenesis is still unclear and is influenced by a combination of factors. In a variety of inflammatory and immune cells such as neutrophils, macrophages (intrahepatic macrophages are also known as Kupffer cells), mast cells, NK cells, and even T cells in humoral immunity and B cells in cellular immunity, inflammation can be a “second strike” against cholestatic liver injury. These cells, stimulated by a variety of factors such as bile acids, inflammatory chemokines, and complement, can be activated and accumulate in the cholestatic liver, and with the involvement of inflammatory mediators and modulation by cytokines, can lead to destruction of hepatocytes and bile duct epithelial cells and exacerbate (and occasionally retard) the progression of cholestatic liver disease. In this paper, we summarized the new research advances proposed so far regarding the relationship between inflammation and cholestasis, aiming to provide reference for researchers and clinicians in the field of cholestatic liver injury research.

**Keywords:** inflammation, immune cells, cholestasis, cholestatic liver injury

## Introduction

Cholestasis is a pathological condition in which the formation, secretion, and excretion of bile flow are inhibited by various factors, resulting in the inability of bile to drain smoothly into the duodenum and thus return to the blood.<sup>1</sup> In the early stage, there is no specific clinical manifestation, only an increase in serum alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) levels; some patients may experience weakness, nausea, poor appetite, and epigastric discomfort. After gradual progression, jaundice, skin itching, fatigue, steatorrhea, yellow tumor, hepatic osteodystrophy, and fat-soluble vitamin deficiency may occur. In severe cases, it may even lead to liver failure and death.<sup>2</sup> Hepatobiliary disorders characterized by cholestasis are called cholestatic liver diseases, including primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), intrahepatic cholestasis in pregnancy syndrome (ICP), IgG4-related sclerosing cholangitis, and progressive familial intrahepatic cholestasis (PFIC). Cholestasis can also occur in alcoholic liver disease, viral hepatitis, drug-induced liver injury, and non-alcoholic fatty liver disease.<sup>3</sup>

The occurrence of cholestasis is associated with abnormal bile duct cell proliferation, peribiliary fibrosis, myofibroblast activation, toxic bile acid accumulation, and bile duct reactions, all of which can drive disease progression.<sup>4</sup> The processes of cholestasis and cholestatic liver injury are extremely complex, and the etiology of cholestatic liver injury varies greatly. The invasion of inflammatory cells, which facilitates bile duct cell death, results in blockage of bile ducts and abnormal bile acid excretion in PBC and PSC, etc. And the accumulating bile acids might act as a second hit, causing inflammation and fibrosis.<sup>5</sup> In cholestatic liver diseases such as ICP and PFIC, which are due to abnormalities of the bile salt transporter pump, the first strike is bile acid accumulation due to inadequate bile acid excretion, which causes a second hit of immunological imbalance, inflammation, and fibrosis, leading to aggravation of cholestatic liver injury.<sup>6,7</sup> Inflammation might be the initial cause of cholestasis and the following hit to the liver. As a result, many scientists regard regulation of the inflammatory response as a viable therapeutic target for cholestasis and its accompanying liver harm.<sup>8</sup>

Therefore, this article provides a review of the mechanisms of inflammation and cholestasis from the aspects of inflammatory cells, humoral immunity, inflammatory factors, and Inflammatory vesicles, etc.

## Discussion

### Inflammatory Cells in Cholestasis

#### Neutrophils

Neutrophils are usually the first responders to the inflammatory response. During bacterial infections, neutrophils are able to transform from a resting state to an activated state stimulated by C5a, lipopolysaccharides (LPS), and cytokines, and thus have a direct role in clearing pathogens.<sup>9</sup> Usually, neutrophils execute their cytotoxicity through the production of reactive oxygen species (ROS) and hypochlorous acid, both of which have strong toxic effects on liver cells.<sup>10</sup> In addition, neutrophils can produce a substance called serine protease, which not only has a direct cytotoxic effect but also participates in the activation of inflammatory cytokines, and thus exacerbate liver damage.<sup>11</sup>

Neutrophil recruitment in the hepatic microvascular system can be found in bile duct ligation (BDL) or bile-fed mice and has been demonstrated in several previous studies.<sup>12,13</sup> This recruitment process begins 6 hours after BDL<sup>14</sup> and the level of neutrophils will peak after two to three days, becoming the main inflammatory cell in the cholestasis model.<sup>15</sup> This was also found in the livers of ICP patients, accompanied by the decrease in serum neutrophil counts.<sup>16</sup> After recruitment, neutrophils exocytose into the surrounding parenchymal tissue and portal bundles in an intercellular adhesion molecule-1 (ICAM-1)-dependent manner on the endothelium, at which point the Na(+)/H(+) exchange factor 1 (NHERF-1) and the cytoskeletal protein ezrin-radixin-moesin (ERM) act as scaffolding proteins for the neutrophils. The process involves a variety of inflammatory mediators, which are involved in the migration of neutrophils across the endothelial and epithelial cells.<sup>17,18</sup> The process is mediated by a variety of inflammatory mediators, which are involved in the migration of neutrophils across the endothelial and epithelial cells. In addition, in a mouse model of obstructive cholestasis, the bone bridge protein (OPN) released from the bile duct cells acted as a pro-inflammatory mediator in initiating the early neutrophil-mediated phase of injury after cleavage to a pro-inflammatory form by the matrix metalloproteinases (MMP).<sup>19</sup> We are, therefore, expected to reduce liver damage by exploring multiple receptor blockers of inflammatory mediators and blocking neutrophil chemotaxis.<sup>20</sup>

Earlier studies have found that neutrophils' bactericidal ability, phagocytosis and intracellular killing ability are significantly reduced in the early and advanced stages of PBC.<sup>21</sup> In the rat model of obstructive cholestasis, the decrease in phagocytic function of polymorphonuclear leukocytes can also be found.<sup>22</sup> On the contrary, it was found that in the rat model of common bile duct obstruction, neutrophils have enhanced phagocytosis, and can produce superoxide.<sup>23</sup> Therefore, we speculate that in the early stages of cholestasis, neutrophils will recruit locally to promote the occurrence and progression of inflammation, and subsequently its function will be weakened, resulting in a series of complications, such as the occurrence of bacterial peritonitis. Therefore, neutrophils may have a contradictory role in the development of cholestatic liver diseases. On the one hand, they may produce an inflammatory protective response, on the other hand, also can promote disease progression.

#### Macrophages

The liver possesses the largest population of macrophages in the body, including two main cell subpopulations, Kupffer cells (KCs) and infiltrating macrophages (ie monocyte-derived macrophages, MoMFs). KCs express danger recognition receptors and clearance of receptors, and they are the main natural immune cells that eliminate a large number of bacteria, fungi and viruses.<sup>24,25</sup> The use of gadolinium chloride (an inhibitor of KCs) attenuated liver injury and fibrosis in a BDL model, suggesting that KCs can promote BDL-induced liver injury.<sup>26</sup> Studies have confirmed that KCs can be activated after BDL, leading to the release of reactive oxygen species (ROS)<sup>27</sup> and the production of pro-inflammatory cytokines induced by LPS; this further activates the signaling pathways, such as nuclear factor kappa B (NF- $\kappa$ B) and mitogen-activated protein kinase (MAPK) in the hepatocytes and bile duct cells, affecting the function of the bile acid transport proteins and causing bile acid accumulation.<sup>28,29</sup> In recent years, it has been found in mouse models of PBC that hepatic macrophages not only secrete cytokines, but also regulate natural killer (NK) cells to jointly participate in the inflammatory process through a signaling pathway mediated by natural killer group 2, member D (NKG2D) and its ligand RAE-1.<sup>30</sup> Interestingly, KCs can also reduce cholestatic liver injury by expressing TGR5 (a bile acid-activated G protein-coupled receptor), inhibiting NF- $\kappa$ B and c-Jun N-terminal kinase (JNK) signaling pathways, and inhibiting NLRP3 inflammatory vesicle activation so they may play different roles in the different stages of BDL-induced liver injury.<sup>31,32</sup> However, further studies are needed to elucidate whether inhibiting TGR5 activation in Kupffer cells is beneficial for the progression of cholestatic diseases.

In patients with PSC, not only macrophage activation was found, but also bile acids were found to prompt macrophages to produce more histamine through paracrine.<sup>33</sup> In patients with biliary atresia and Alagille syndrome, Taylor et al defined three types of macrophages through single-cell RNA sequencing analysis and immunofluorescence isolation: lipid-associated macrophages, monocytoïd macrophages and adaptive macrophages, all of which were pathogenic, and can express immunomodulatory gene RORA and TREM2, which may serve as a new therapeutic target for alleviating inflammatory damage in cholestatic liver disease.<sup>34</sup> Tian et al also proposed a new potential therapeutic strategy: selectively depletion of long chain non-coding RNA-H19 (lncRNA-H19) of macrophages, which involved in the proliferation of cholangiocytes in cholestatic livers and promotes hepatic fibrosis, helps to inhibit cholestatic liver damage and fibrosis.<sup>35</sup> However, macrophages in cholestasis models do not always have significant pathogenicity, such as the reduction in macrophage clearance found in infants with biliary atresia,<sup>36</sup> which may contribute to the accumulation of autoimmune complexes in the liver, and indirectly leads to liver damage.

## Mast Cells

Mast cells (MC) are immune cells of the bone marrow spectrum with pro/anti-inflammatory, pro-fibrotic, and immunomodulatory effects,<sup>37,38</sup> which were found to be increased in PBC, PSC, bile duct obstruction, hepatitis, fatty liver, liver fibrosis, hepatocellular carcinoma, cholangiocarcinoma, and liver failure.<sup>39</sup> Mast cells infiltrating the liver can secrete a large number of pro-inflammatory mediators, such as histamine, heparin, trypsin, chymotrypsin, carboxypeptidase A3, granzyme B, and cytokines including TNF- $\alpha$ , IL-1 $\beta$  and IL-3, etc. Among them, histamine (which has vasodilatory and bi-directional inflammatory effects) has been shown to promote cholangiocytes proliferation and hepatic fibrosis through specific pathways such as Notch/Jagged, thereby exacerbating the degree of cholestasis.<sup>40–42</sup> It has recently been shown that mast cells can also secrete autologous sorting proteins (ATX), which contribute to itching in the model of bile duct ligation.<sup>43</sup> Meanwhile, it has been shown that MC can regulate the enterohepatic circulation of TBA through regulating FXR/FGF15 expression in the biliary and intestinal tracts via MC-FXR.<sup>44,45</sup>

Patients with PBC and PSC have higher amounts of MC accumulated in the biliary tree and gallbladder, especially on the cholangiocyte membrane, suggesting that senescent cholangiocytes may promote MC activation and proliferation. And histamine levels were also found to be elevated, suggesting increased secretory capacity of MC.<sup>46</sup> The reduction of MC in the cholestasis models can reduce liver inflammation<sup>47</sup> and the development of liver fibrosis.<sup>48,49</sup> Therefore, reducing the degree of inflammation in cholestatic liver by decreasing MC levels may be an effective therapeutic target. This is similar to the drug UDCA, which is commonly used clinically to reduce inflammatory damage by interfering with the degranulation process of MC while decreasing macrophage infiltration and reducing oxidative stress.<sup>50</sup>

## Lymphocytes

### T-lymphocytes

T-lymphocytes are major players in specific cellular immunity, killing target cells directly and also releasing lymphokines and regulating the immune process.<sup>51</sup> Monoclonal expansion of the CD4+ and CD8+ T cells can be found in the liver of patients with PBC as well as biliary atresia. The CD4+ T cells play a major role in the development of PBC, causing systemic inflammation through the production of pro-inflammatory cytokines, while the CD8+ T cells mainly mediate the process of bile duct injury.<sup>52,53</sup> The recruitment of CD8+ T cells has been shown to affect BA metabolism, by reducing the expression of the basolateral bile acid uptake transporters NTCP and OATP, and increasing the expression of the transporter BSEP involved in bile acid excretion, so as to reduce the persistent toxic damage of BA to the liver, thereby playing a positive role in regulating cholestasis.<sup>54</sup>

Regulatory T cells (Tregs) are T cells capable of negatively regulating the body's immune response. Earlier studies have suggested that Tregs expression levels are significantly reduced in patients with PBC and BA, suggesting that defects in the Tregs can also promote the development of inflammation in cholestatic liver damage.<sup>55,56</sup> It was found that using a combination of immunosuppressive drug vitamin D3 and dexamethasone induced the differentiation of naïve CD4+ T cells into Tregs that produce IL-10 in vitro, thus acting to reduce inflammation.<sup>57</sup>

## B-Lymphocytes

B-lymphocytes, including memory B cells, plasma blasts and plasma cells are also involved in specific immune processes, which further produce various immunoglobulins (Ig) and their isoforms, including IgM, IgD, IgG (types 1–4), IgA, and IgE.<sup>58</sup> B cells play an immune role by neutralizing antibodies, producing cytokines, participating in complement cascade reactions, and communicating with immune cells, such as macrophages and T cells.<sup>59</sup> Overexpression of serum B-cell activating factor (BAFF) is suggestive of autoimmune hyperactivity, it was found to be highly expressed in patients with PBC and positively correlated with aspartate aminotransferase (AST) and total bilirubin levels, suggesting a possible association with persistent necrotizing inflammation of hepatocytes.<sup>60</sup> In PBC patients, CD20 and CD19 were also found in lymphoid follicle-like aggregates at some distance from the portal bundle, and CD19 levels were positively correlated with ALP levels, further suggesting the presence of B-cell activation in patients with PBC.<sup>61,62</sup> Increased infiltration of B cells is also found in the liver of patients with biliary atresia, with IgM and IgG deposition in the epithelial basement membrane of the bile ducts.<sup>63</sup> Further studies have shown that B cells can directly produce pro-inflammatory factors such as IL-6, IL-19 and TNF- $\alpha$ , triggering the activation of CD4+ and CD8+ T cells, as well as inducing apoptosis of Treg cells, and decreases the expression of the anti-inflammatory cytokines IL-10 and TGF- $\beta$ . And CD38 plasma cells produced by B cells can be directly involved in the bile duct injury process.<sup>64,65</sup> However, therapeutic approaches to ameliorate inflammatory damage by depleting B cells are not necessarily effective, and an earlier experimental study showed that anti-CD20/CD79-treated mice had more CD4 and CD8+ T cell infiltration in the portal region and more severe bile duct inflammation, suggesting that simply depleting a kind of inflammatory cell may cannot achieve the desired anti-inflammatory effect.<sup>66</sup>

## Natural Killer (NK) Cells

NK cells are natural lymphocytes that play an important immunomodulatory role in multiple liver diseases, such as PBC, chronic hepatitis B, chronic hepatitis C, alcoholic liver disease, and hepatocellular carcinoma (HCC).<sup>67,68</sup> In patients with cholestatic liver injury, the NK cells in the portal region and around the hepatic bile ducts usually show stronger expression activity than the controls, and its recruitment from the blood to the liver is associated with the stimulation of a large number of chemokines, such as CCL3, CCL5, CCL7, CCL8, CXCL11-3, and CXCL30.<sup>69,70</sup> In contrast, a study in bile duct-ligated mice with combined MCMV infection found reduced recruitment of NK cells to the liver, a phenomenon that may be related to cholestasis impairing the expression of pathogen-induced chemokines in the liver.<sup>71</sup> After the recruitment process is completed, NK cells are able to trigger non-specific cytotoxicity in the cellular epithelial lining, destroy normal hepatic duct cells and bile duct cells (through perforin/granzyme B-dependent and TRAIL-dependent mechanisms),<sup>72,73</sup> and stimulate inflammation by producing both IFN- $\gamma$  and TNF and granulocyte-macrophage colony-stimulating factors (GM-CSF)<sup>74</sup> and bind to Toll-like receptors to induce lysis of biliary epithelial cells, thus aggravating liver damage.<sup>75</sup> While, NK cells can also play a negative regulatory role in liver inflammation in PBC, a process that may be achieved by inhibiting the proliferation of the CD4+ T cells.<sup>76,77</sup>

## Humoral Immunity

### Immunoglobulin

#### IgM

IgM is the first immunoglobulin expressed during B-cell development and plays a role in the direct encapsulation and destruction of antigens and immobilization of the complement cells.<sup>78</sup> Most cases of autoimmune hepatitis are dominated by elevated IgG levels. Elevated IgM levels are predominant in the serum of PBC patients; however, the mechanism for this elevation is unclear and may be related to methylation of CD40L, the promoter of CD4 + T cells, which affects immunoglobulin class switching.<sup>79</sup> High levels of autophagy in the B cells of patients with PBC play an important factor in the synthesis.<sup>80</sup> Patients with biliary atresia develop IgM deposits around the basement membrane of the hepatobiliary epithelium and novel IgM autoantibodies against cholangiocyte-associated proteins (CHI3L1, DLL-4, and SFTPD) have been identified, which are involved in the formation of antigen-antibody complexes, complement activation, and cholangiocyte injury, and are positively correlated with serum bilirubin levels and the incidence of liver transplantation.<sup>81,82</sup> Thus, IgM may play a major driving role in cholestatic liver injury and its autoantibodies may be a potential target for future therapeutic interventions.

## IgG

IgG activates a cascade of complement reactions and has antibody-dependent cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). Elevated levels of IgG has been found in various cholestatic liver injury.<sup>83</sup> In patients with PBC, elevated levels of the IgG subclasses (IgG3 and IgG5) can be found, and the IgG3 subtype of anti-mitochondrial antibodies in PBC-specific autoantibodies may be associated with the severity of PBC.<sup>84</sup> IgG deposit can also be found on the biliary epithelial basement membrane in patients with biliary obstruction, with a positivity rate of up to 56.5%,<sup>85</sup> and the biliary epithelial target protein that has been shown to react strongly with IgG is human alpha enolase ( $\alpha$ ENOL), which is also one of the antigenic targets of the anti-neutrophil cytoplasmic antibodies present in autoimmune liver disease.<sup>86</sup> The formation of an immune complex with IgG by  $\alpha$ ENOL induces endothelial damage via the complement classical pathway and cell death via the apoptotic process, thus suggesting higher liver failure-related mortality in patients with PBC.<sup>87</sup> A recent study suggests that IgG can also contribute to the development of inflammation in PBC by binding to Fc $\gamma$ RIIB (a negative regulatory receptor for lymphocytes, but which may have a different role in B cells) and thus in turn modulating B cell function, but the exact mechanism of this regulatory role needs to be further explored.<sup>88</sup>

## IgA

Cholangiocytes can actively secrete secretory IgA in the biliary tree and participate in the process of mucosal immunity. At the same time, IgA is actively deposited in the hepatobiliary epithelium of patients with PBC and may enter the cells via recombinant pyruvate dehydrogenase complex (PDC-E2)-specific IgA binding to the human polymeric immunoglobulin receptor (pIgR) to activate cysteine asparaginase (Caspase), which induces hepatocyte apoptosis and biliary tract injury.<sup>89,90</sup> Deposition of circulating immune complexes of IgA is found in bile duct ligated mice, which may be associated with reduced hepatic clearance of IgA and increased renal secretory IgA production.<sup>91</sup>

## IgE

IgE is a key effector in the type I allergic immune response, and its activation by B cells leads to the differentiation of IgE-secreting cells.<sup>92</sup> Elevated IgE levels were found in approximately 40% of patients with PSC, but there is no relationship between the prognosis of cholangiocarcinoma, liver transplantation, or death.<sup>93</sup> Whereas earlier studies have suggested that this is more likely to be related to the presence of hypereosinophilic syndromes and anaphylactic reactions during the pathogenesis of PSC, the question of whether IgE acts as a positive or a negative regulator of the progression of cholestasis needs to be explored further.<sup>94</sup> We know that the mast cells play an important role in cholestasis and that the IgE receptor Fc $\epsilon$ RI is present on the surface of the mast cells; hence, IgE may be involved in the process of cholestatic liver damage by inducing an immune response in the mast cells.<sup>95</sup> Furthermore, the liver X receptor (LXR) is a member of the nuclear receptor superfamily in bile acid metabolism, and activation of LXR was found to reduce the levels of IgE secreted by the B cells and activated by the CD68 and IL-11, suggesting that activation of LXR can inhibit IgE and the inflammatory and allergic responses.<sup>96</sup>

## Cytokines

Pro-inflammatory cytokines, such as TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 and IL-8, produced by the inflammatory cells, such as KCs and bile duct cells stimulated by LPS, can contribute to the development of cholestasis by inducing a sustained decrease in the levels of bile acid transport proteins.<sup>97–99</sup> In the early stages of inflammation, TNF- $\alpha$  and IL-1 $\beta$  are involved in the downregulation of NTCP, while in the later stages of inflammation, they are regulated by IL-6.<sup>100,101</sup> Higher levels of IL-8 may be associated with the development of PBC and reflect the progression of the disease towards cirrhosis.<sup>102</sup> Elevated levels of TNF- $\alpha$  are often thought to reflect a more severe disease in patients with PBC,<sup>103</sup> while IL-12 and IFN- $\gamma$  have also been found to be associated with the severity of PBC, contributing to inflammation by inducing a Th1 immune response during the initial stages of the disease.<sup>104</sup> Also, the inflammatory cytokines can slow bile flow by inhibiting chloride and bicarbonate ion transport via the bile duct cells.<sup>105</sup>

KCs secrete the cytokines with anti-inflammatory functions, such as IL-10, IL-4, and IL-13.<sup>106,107</sup> Decreased levels of IL-10 can be found in patients with PBC, which inhibits the production of the pro-inflammatory factors, IFN- $\gamma$  and TNF- $\alpha$ , by the NK cells and inhibits the proliferation of the Th1 cells, reducing the degree of liver inflammation by blocking



the process of antigen presentation.<sup>108,109</sup> Recently, a large case-control study found that some of the immune-related genes of IL-10 were associated with Biliary Atresia, further emphasizing its protective role.<sup>110</sup> Meanwhile, IL-6 has been found to act as an anti-inflammatory cytokine in ICP patients, inhibiting TNF- $\alpha$  and IL-1 while activating IL-10.<sup>111</sup> Thus, in models of cholestatic liver damage, cytokine networks may drive disease progression and may also be protective against inflammation. More cytokines need to be tapped as well as further explored, and there is a need to further define which cytokines play a major role in cholestatic liver disease and how we will slow disease progression by intervening in the cytokine network.

### Chemokines

Inflammatory chemokines are able to act specifically on the inflammatory cells during the adaptive immune response and have the ability to induce cell migration. In senescent BEC from PBC patients, upregulated expression of various chemokines and chemotactic activity, including CCL2 and CXCL1, CXCR3, and increased expression of chemokine receptors, such as CX3CR1, can be found,<sup>112,113</sup> and the levels of chemokines increase with disease progression.<sup>114</sup> CCL11, CCL24, CCL26, and other eosinophils CCL11, CCL24, and CCL26 are also found in PBC as potent chemokines for acidophilic granulocytes, with CCL11 and CCL26 being shown to be associated with fibrosis progression in PBC.<sup>115</sup> As mentioned earlier, these chemokines help to activate the innate immune system around the damaged bile ducts and mediate the migration of various inflammatory cells, which in turn leads to persistent inflammation and further exacerbates bile duct injury. However, the role of chemokines and their receptors is not limited to inducing inflammation. In recent years, CXCR2 has been found to be capable of directly damaging hepatocytes in bile duct ligated livers. Thus chemokines may play an important role in inflammatory cell recruitment and subsequent immune-mediated hepatocyte damage.<sup>116</sup>

### Complement

Complement is synthesized predominantly in the liver and mediates immune complex formation, clearance of apoptotic cells and tissue regeneration, and is activated predominantly via the classical, alternative and lectin pathways.<sup>117</sup> Activation of the complement system triggers a cascade reaction that leads to the rupture of C3, the core molecule of the complement system, which in turn leads to the rupture of C5 downstream, resulting in the production of the products C3a, C5a.<sup>118,119</sup> Complement activation results in the production of membrane attack complexes (MACs), which lead to hepatocyte lysis and induce cellular release of inflammatory cytokines.<sup>120</sup> C3 and C4 levels were significantly higher in the congenital biliary atresia model than in the healthy population, and there was a significant positive correlation with GGT levels.<sup>121</sup> Deficiency of complement C3 attenuates cholestatic liver injury in mice and reduces neutrophil and macrophage infiltration and activation in the liver by regulating the expression of adhesion factors, further confirming the role of elevated complement levels in driving the progression of cholestatic liver disease.<sup>122</sup> In contrast, cholestasis directly induces complement activation, and FXR has been shown to directly encode the C3 gene, increasing C3 mRNA and protein levels and thereby exacerbating liver inflammation.<sup>123</sup>

### Inflammatory Vesicles

Inflammatory vesicles are polymorphic complexes of proteins, including NLR family members NLRP1, NLRP3, NLRC4, as well as AIM2 and pyrin. Activation of inflammatory vesicles can induce or promote inflammation through further activation of the shear cysteine asparaginase-1 that cleaves pro-interleukin-1 $\beta$  (pro-IL-1 $\beta$ ) to IL-1 $\beta$ , which then triggers activation of NF- $\kappa$ B signaling via receptors IL-1R and IL-18R.<sup>124</sup> This process is seen in a variety of liver diseases: PBC, alcoholic hepatitis, ischemia-reperfusion injury.<sup>125–127</sup> Activation of NLRP3 inflammatory vesicles in the cholestatic model can be activated by the production of galactoglucan-3 by macrophages,<sup>128,129</sup> whereas bile acids inhibit the activation of NLRP3 inflammatory vesicles via the TGR3-cAMP-PKA axis, thus controlling inflammation and metabolic disturbances. This may therefore contribute to the different roles of NLRP3 in acute and chronic cholestatic liver injury, with a lack of NLRP3 leading to reduced inflammation in chronic cholestatic liver injury, whereas hepatic inflammation is exacerbated in acute patients.<sup>130</sup>

## Bile Acids and Inflammation

Bile acids are closely associated with the inflammatory response and biliary stasis that leads to the accumulation of toxic bile acids, which directly activate inflammatory and pro-fibroblastic cells and stimulate the release of pro-inflammatory and pro-fibrotic mediators from the hepatocytes and bile duct cells. This, in turn, leads to persistent biliary stasis and promotes the development of liver fibrosis. For example, bile acids are known to trigger a neutrophil-mediated inflammatory response and can act synergistically with LPS to promote inflammatory vesicle activation.<sup>131,132</sup> In addition, bile acids activate various isoforms of early growth response protein 1 (Egr-1), protein kinase C family, p38, c-Jun N-terminal kinase, and pregnane X receptor, stimulating the upregulation of pro-inflammatory genes by activating one or more of these pathways.<sup>133–135</sup> Recently, it has been found that goose deoxycholic acid (CDCA) can trigger the excessive accumulation of mitochondrial reactive oxygen species and promote the activation of inflammatory vesicles by targeting heme oxygenase-1 (HO-1), which in turn promotes the development of inflammation in hepatocellular carcinoma.<sup>135</sup> But bile acids also have anti-inflammatory effects and have been shown to inhibit lymphocyte proliferation, immunoglobulin production, and cytokine secretion,<sup>136,137</sup> and reduce phagocytosis of KCs.<sup>138</sup> Therefore, the mechanisms by which bile acids determine the development of inflammation and their specific role in the injury process still need to be further explored.

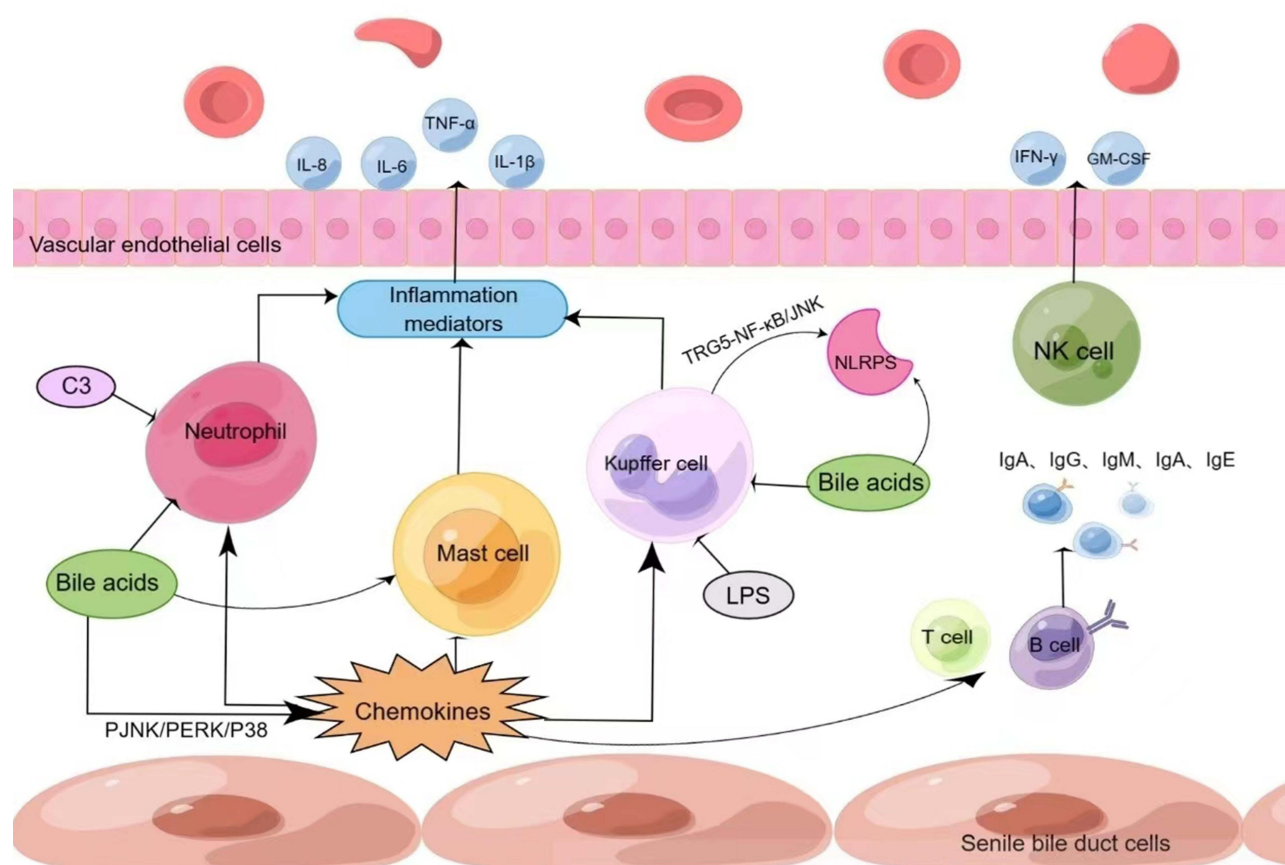
## Sepsis and Cholestasis

Sepsis-associated cholestasis is very common in hospitalized patients with jaundice, most especially infants and immunocompromised populations.<sup>139</sup> Patients with cirrhosis are more likely to develop bacterial infections, possibly related to translocation of intestinal bacteria.<sup>140</sup> In biliary stasis, pathogenic bacteria travel retrograde through the bile ducts to the liver and interact with receptors (especially Toll-like receptors or TLRs) on the immune cells stored in the liver to induce inflammation.<sup>141</sup> At the same time, bacterial toxins and bacterial metabolites cause direct damage to hepatocytes, and several studies have found that LPS and LPS-induced cytokines are associated with cholestasis.<sup>142,143</sup> LPS stimulates the activation of KCs and neutrophils during cholestasis, leading to the production of large amounts of pro-inflammatory cytokines, thus promoting the development of liver inflammation.<sup>144</sup> Notably, this process often occurs in the chronic rather than the acute phase of cholestasis.<sup>145</sup>

In patients who have developed sepsis, abnormalities in the biochemical parameters of liver function can be detected, suggesting “hypoxic hepatitis” and “cholestatic liver dysfunction”.<sup>146</sup> At this time, the enlargement in cell size from Kupffer cells in combination with infiltrating polymorphonuclear cells, eosinocytes, and platelets induces the formation of aggregates, which leads to sinus cavity obstruction. At the same time, the loss of actin and myosin microfilament activity due to inflammation and infection during septicemia directly induces apoptosis and necrosis of hepatocytes, leading to paralysis and dilatation of the bile ducts as well as impaired bile secretion.<sup>147,148</sup> However, the liver can rapidly reduce bile formation by adjusting the expression of key molecular components involved in bile flow in response to the infection and inflammatory process, thus elevated biochemical markers of cholestasis do not necessarily persist throughout the course of the disease.<sup>149,150</sup>

## Bile Duct Cell Senescence and Inflammation

Cellular senescence is increasingly recognized as a pathological feature of various inflammatory liver diseases. Bile duct cells in cholestatic liver damage exhibit a senescence-associated secretory phenotype (SASP) and resistance to apoptosis, which contribute to the onset and exacerbation of inflammation by initiating paracrine signaling pathways that enhance the secretion of cytokines (IL-1 and IL-6), chemokines (IL-8, CCR2, MCP-1), growth factors, and pro-fibrotic factors.<sup>151–153</sup> This in turn recruits and/or activates immune cells such as macrophages, NK (NK) cells, and T lymphocytes, to promote the removal of senescent cells. A recent study found that senescent liver cells inhibit autophagy by producing BMP9, a subfamily of TGF- $\beta$ , which downregulate the expression of the ATG3 and ATG7 genes. When autophagic flux is inhibited, macrophages acquire a proinflammatory phenotype and promote tissue damage.<sup>154</sup> Hence, bile duct cell senescence plays an important pro-inflammatory role in the late stages of cholestasis.



**Figure 1** Inflammation is inextricably linked to cholestasis. (By Figdraw).

**Notes:** As shown in the figure above, on the surface of senescent bile duct epithelial cells, neutrophils, macrophages, mast cells, T cells, B cells and other inflammatory cells aggregate under the action of chemokines, and release a large number of inflammatory mediators and cytokines, which jointly promote the progression of inflammation in cholestatic liver damage.

## Conclusions

In summary, the causes of cholestasis are complex, and its pathogenesis remains unclear. Numerous studies have suggested that inflammation plays an important role in cholestasis, including cellular immunity, humoral immunity, and pattern recognition receptors. A large number of inflammatory and immune cells such as neutrophils, macrophages, mast cells, T and B lymphocytes, NK cells, as well as cytokines, inflammatory vesicles, and complement are involved and interact with each other, which not only directly lead to biliary epithelial cell damage, but also regulate disease progression by exacerbating/attenuating biliary inflammation and thereby modulating cholestatic liver damage (see Figure 1). Components of this process with anti-inflammatory effects deserve further exploration to unearth potential therapeutic targets. Cholestasis itself can also have an effect on hepatocyte inflammation; for example, bile acids can act directly as pro-inflammatory agents, inducing inflammatory cells and inflammatory factors causing inflammatory damage. In different models of cholestatic liver damage, cholestasis and inflammation are inextricably linked, and therefore, both should be taken into account in the treatment process; however, the possibility of reducing inflammation to improve the outcome of cholestatic liver damage and delay its progression to liver fibrosis, cirrhosis, or even liver failure remains to be explored.

## Consent for Publication

All authors gave their consent for publication.

## Acknowledgments

The authors would like to express their gratitude to all those who helped during the writing of this thesis.



## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

## Funding

Sponsors include Chongqing Science and Health Joint Medical Research Project (2020FYYX007), Chongqing young and middle-aged medical high-end talent project (2019-181). The funders had no role in study design, data collection and analysis, publication decisions, or manuscript preparation.

## Disclosure

The authors have no relevant financial or non-financial interests to disclose for this work.

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