Intestinal Permeability and Liver Cirrhosis

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An alteration of gut microbiota and their products, particularly endotoxins may play a major role in the pathogenesis of liver diseases. Gut dysbiosis caused by a high-fat diet and alcohol consumption induces increased intestinal permeability, the so-called “leaky gut.” Clinical studies have found that plasma endotoxin levels are elevated in patients with chronic liver diseases. The decreased diversity of gut microbiota in cirrhotic patients before liver transplantation is also related to a higher incidence of posttransplant infections and cognitive impairment. The exposure to endotoxins activates macrophages via toll-like receptor 4 (TLR4), leading to a greater production of proinflammatory cytokines and chemokines including tumor necrosis factor–alpha, interleukin (IL)–6, and IL-8, which play key roles in the progression of liver diseases. TLR4 is also a major receptor activated by the binding of endotoxins in hepatic stellate cells, which play a crucial role in liver fibrogenesis that could develop into hepatocarcinogenesis, suggesting the importance of the interaction between endotoxemia and TLR4 signaling as a target for preventing liver disease progression.

Keywords: leaky gut ; endotoxins ; alcoholic liver disease ; nonalcoholic steatohepatitis ; liver cirrhosis ; hepatocarcinogenesis ; Toll-like receptor 4 pathway

1. Introduction

The interaction between the intestine and liver via microbiologic features from the gut, referred to as the “gut–liver axis”, has been recently demonstrated as contributing to the development of liver diseases that develop liver cirrhosis and hepatocellular carcinoma (HCC). Although there are many mechanisms underlying the pathogenesis of liver inflammation, fibrosis, and carcinogenesis, microbial metabolites and products derived from the intestinal tract are considered to be some of the major factors that accelerate the progression of liver diseases due to the close links between the liver and intestine.

In patients with liver diseases, the gut bacteria themselves, as well as their components, often translocate into the portal blood flow and directly reach the liver as a result of the disrupted intestinal barrier, or so-called bacterial translocation. In chronic liver disease (CLD) patients, dysbiosis is often observed and increases alongside the stages of liver fibrosis, leading to bacterial translocation including endotoxins. Endotoxins (lipopolysaccharides; LPS) are a well-known component of Gram-negative bacteria and work as pathogen-associated molecular patterns (PAMPs) for Toll-like receptor 4 (TLR4), which recognizes unique structural components of bacteria and drives innate immunity-producing inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin (IL)-6 [1]. In the liver, Kupffer cells (KCs) are regional macrophages that control the innate immune response and are activated by LPS binding, but LPS also stimulate hepatic nonimmune cells, including hepatic stellate cells (HSCs) and sinusoidal endothelial cells as they also have TLR4 [2][3][4].

2. Mechanisms of Endotoxemia Derived from Gut Microbiota

Recent studies have demonstrated that the alteration in the gut microbiome referred to as “dysbiosis” is closely associated with the clinical stages of CLDs, including liver cirrhosis, the end stage of chronic hepatitis [5][6][7]. The role of dysbiosis in patients with CLDs has been reported by a study investigating the alteration in gut microbial diversity by 16S sequencing and a meta-analysis in some diseases such as liver cirrhosis, colorectal cancer, inflammatory bowel disease, obesity, and type 2 diabetes mellitus [5]. This study also suggested that liver fibrosis, the F4 stage of fibrosis, is the condition in which dysbiosis is linked to the pathogenesis, including in patients with nonalcoholic steatohepatitis (NASH) [8][9]. Furthermore, patients with alcoholic liver disease demonstrated an increase inVeillonellaceae,which has been reported to induce highly systemic inflammation [10].

Small intestinal bacterial overgrowth (SIBO) is a part of dysbiosis that occurs in the small intestine. Recently, it has become known to be associated with various diseases and not just liver diseases. SIBO is considered as an important phenomenon that leads to an increased intestinal permeability via the impairment of tight junctions (TJs) by intestinal

epithelial cell damage [11][12]. Since the intestinal overgrowth ofEscherichia coliin patients with liver cirrhosis was reported in 2016, a recent study was the first to demonstrate the change in gut microbial diversity in cirrhotic and HCC patients and its possibility as a novel, noninvasive surrogate marker for the detection of HCC in patients with HBV infection [13][14].

An alteration of bile acid secretion is a major factor in inducing bacterial overgrowth. In patients with liver cirrhosis, as the secretion of bile acid from the liver is decreased, bacterial overgrowth and the change in the bacterial composition are promoted. Another study reported an association between proximal small intestinal motility and SIBO in patients with liver cirrhosis [15]. As gastric acid affects the regulation of microbiota and prevents the translocation of oral bacteria through the stomach, the inhibition of gastric acid production with the use of a proton pump inhibitor could also be implicated in the development of SIBO [16].

The epithelium of the intestine has occlusive intracellular junctions, which are the so-called tight junctions (TJs) [17]. TJs are composed of three types of transmembrane proteins associated with scaffolding proteins that link to the actin cytoskeleton such as occludin, claudins, and junctional adhesion molecules [18]. In particular, claudins are the main molecules within the TJ structure and produce charge-selective pores that regulate the transportation of ions and small particles across the epithelial layers. These proteins bind scaffolding proteins such as zonula occludins 1 (ZO-1), ZO-2, and ZO-3, which connect transmembrane proteins including claudins and occludins to the actin cytoskeleton [19][20][21].

The causes of intestinal permeability vary and remain to be elucidated. Physical damage to the intestinal epithelium, disruption to the TJ, and alterations in the thickness of the mucus layer can result in increased intestinal leakiness [22]. Dysbiosis can also promote inflammation in the intestinal epithelium and barrier dysfunction [23]. The increased PAMPs such as LPS, bacterial RNAs, and viral RNAs translocate from the intestine through the intestinal barrier and enter the liver, and they stimulate immune cells such as KCs via binding to TLR4 on their membrane, resulting in the induction of hepatic inflammation that progresses to liver injury and diseases

3. The Role of Endotoxemia in the Progression of Liver Pathogenesis

Excessive consumption of alcohol is an important cause of chronic hepatitis that develops into cirrhosis and the occurrence of HCC based on cirrhosis [24][25]. Moreover, drinking alcohol induces bacterial translocation into the portal vein, which plays a crucial role in alcoholic liver injury [26]. Leclercq et al. indicated that almost half of the patients with alcohol dependence have increased intestinal permeability, even at the early stage of liver fibrosis (F0–1) Kakiyama et al. also reported thatBacteroidaceaeandPorphyromonadaceaewere decreased andVeillonellaceaewas increased in patients with alcoholic liver cirrhosis as compared with those with cirrhosis due to other etiologies [10].

Endotoxemia derived from the Gram-negative bacteria of theProteobacteriaphylum is considered to be a driver of increased hepatic inflammation. Dysbiosis, namely, a decrease in commensal bacteria, induces a disruption in the intestinal TJ barrier [27]. Experimentally, supplementation of the probioticLactobacillus rhamnosus GGimproved downregulated TJ protein expression and attenuated endotoxemia in alcohol-fed mice [28]. This finding could corroborate that improvement of dysbiosis contributes to preventing the disruption of the gut barrier function.

Gut-derived endotoxins from gut bacteria play a crucial role in the pathogenesis of alcoholic liver injury [29][30]. When endotoxins enter into the liver, they initially activate KCs, which are regional macrophages in the liver, via binding of TLR4 on their membrane, leading to an induction of inflammatory cytokines and chemokines including TNF-α, IL-1, and IL-6, mediated by the activation of the nuclear factor–κB LPS–TLR4 binding on the surface of KCs also induces reactive oxygen species (ROS) production, which in turn induces migration of T lymphocytes and neutrophils and HSC activation [1][2]. Likewise, because nonimmune cells such as HSCs and liver sinusoidal endothelial cells have TLR4, the exposure of endotoxins to their cells further promotes fibrotic activity via the activation of these cells [4].

Nonalcoholic fatty liver disease (NAFLD) is considered to be the manifestation of metabolic syndrome in the liver. Recently, it has been suggested that the definition and term NAFLD itself should be changed to “metabolic-associated fatty liver disease” [31]. Various studies have indicated that the gut microbiota are closely associated with the pathogenesis of the development of NASH [32].

As compared with lean NAFLD individuals, NAFLD patients with obesity have a tendency to have SIBO and leaky gut. A greaterFirmicutes/Bacteroidetesratio and increasedProteobacteriawere associated with negative health effects, such as induction of systemic inflammatory activity by the increased gut permeability [33]. An experimental study using obese mice demonstrated that increased intestinal permeability via the downregulation of occludins and ZO-1 on the intestinal epithelium is closely associated with portal endotoxemia as well as the elevation of serum inflammatory cytokine levels in obese mice [34]. Moreover, a high-fat diet also promotes the translocation of bacterial products including living bacteria through the intestinal mucosa, suggesting the importance of the gut–liver axis in liver injury in NAFLD patients [35].

To date, various studies have already demonstrated the importance of the gut–liver axis and the role of TLR4 signaling on the progression of NAFLD. It was previously reported that mice fed a methionine choline-deficient diet demonstrated steatohepatitis, portal endotoxemia, and elevation of TLR4 expression in the liver, whereas TLR4 mutant mice showed less tissue damage and lipid accumulation in the liver [36]. Our study demonstrated that rats fed a choline-deficient L-amino acid-defined diet, which mimics the NASH liver, showed greater α-smooth muscle actin expression and enhanced LPS binding protein mRNA levels in the liver tissue, increased intestinal permeability, and decreased TJ protein expression in the intestine [37]. In contrast, oral medication with poorly absorbable antibiotics inhibited LPS–TLR4 signaling and suppressed the progression of liver fibrosis [37].

Hepatitis A and E viral infections cause acute hepatitis, which can be self-clearing. Both A and E hepatitis viruses are transmitted through the mouth into the gastrointestinal tract and may affect the diversity of the gut microbiota. Although supplementation of beneficial bacteria such asEnterococcus faeciumwas shown to contribute to the removal of the hepatitis E virus from the intestine of pigs, this effect remains unclear in humans [38].

In contrast, hepatitis B and C viruses are major viruses that cause chronic hepatitis. It is also observed that patients with chronic hepatitis have higher bacterial translocation from the gut [39]. It was recently reported that commensal microbiota play an important role in both the viral host cell interaction and viral replication. Some bacterial species includingNeisseria,E. coli,Enterobacteriaceae

Dysbiosis can influence the progression of disease pathogenesis, resulting in liver failure. In patients with HBV, bacteria producing LPS are enriched. It has been demonstrated that the proportions ofF. prausnitzii,E. faecalis,Enterobacteriaceae,Bifidobacteria, andLactobacilluswere markedly changed in HBV cirrhotic patients [40]. Another study suggested the positive correlation ofNeisseriaceaewith the level of serum HBV DNA [41].

In contrast, Lu et al. reported that cirrhotic HBV patients exhibited a marked decrease in the ratio ofBifidobacteriaceaetoEnterobacteriaceae. Another study of cirrhotic patients with HBV also found a decrease inBifidobacteriaandLactobacillus, whereas the levels ofEnterococcusandEnterobacteriaceaewere significantly increased as compared with the healthy population.

In most patients with hepatitis C virus (HCV), the amount ofEnterobacteriaceaeandBacterioidetesincreased, whereas that ofFirmicutesdecreased. Previous studies found that plasma levels of LPS in HCV patients are elevated due to the promotion of bacterial translocation and intestinal inflammation [42][43]. As the production of bile acids is important for the gut microbial composition [44], several pathogenic bacteria includingEnterobacteriaceae,Enterococcus,andStaphylococcusprevent the production of bile acid in patients with HCV, and the use of oral direct-acting antivirals reversed the alteration in the gut microbiota. That study further revealed that oral direct-acting antivirals could be helpful in improving the gut microflora through a reduction in intestinal inflammation via mediating the TNF-α level [45].

Several beneficial bacteria have been reported to have a protective effect during HCV infection. These bacteria includingBifidobacteriumspp. andL. acidophilusplay a supportive role in the antiviral effect, even in antibacterial activities [46]. The immune response by natural killer cells can be extended by protective bacteria such as probiotics in HCV-infected patients, and they also promote the cytotoxic effect of natural killer cells to inhibit HCV replication [47][48].

Primary biliary cholangitis (PBC) is one of the common autoimmune liver diseases characterized by hepatobiliary injury, which shows progressive nonsuppurative destruction of small intrahepatic bile ducts, resulting in cholestasis, inflammation, and fibrosis. An increased diversity ofEubacteriumandVeillonellaand a decreased diversity ofFusobacteriumin the oral microbiota were demonstrated in Japanese patients with PBC as compared with healthy individuals [49]. This report also demonstrated that an increase inVeillonellais positively correlated with IL-1β and IL-8 levels and the relative abundance ofLactobacillalesin feces [49].

eggerthii,andRuminococcus bromii, which are considered to be potentially beneficial bacteria, were reported to be decreased as compared with healthy controls, whereas pathogenic bacteria includingEnterobacteriaceae,Neisseriaceae,Veillonella,Actinobacillus pleuropneumoniae,andHaemophilus parainfluenzaewere increased in the feces of these patients [50]. Another study of PBC patients using 16S rRNA sequence analyses derived from fecal microbiota indicated a decrease inBacteroidetesand an increase inFusobacteriaandProteobacteriaspp. Our recent study using the 16S rDNA sequence also revealed that bacterial diversity was lower in PBC patients with a decreased abundance ofClostridiumand an increase inLactobacillus[51]. As ursodeoxycholic acid (UDCA) is a common medication for PBC, we also investigated the difference in the microbial alteration between UDCA responders and nonresponders.

Autoimmune hepatitis (AIH) is another major autoimmune liver disease, but the pathogenesis of AIH still remains unclear. The first clinical study from Lin et al. revealed that the decreased abundance ofBifidobacteriaandLactobacillusis involved in the development of AIH, along with increasing plasma LPS levels in the later stage of AIH [52]. A recent study reported that as compared with healthy controls, AIH patients had a reduced diversity of intestinal microbiota and a change in bacteria species includingStreptococcus,Veillonella,Klebsiella, andLactobacillus[53]. Another study also reported that AIH patients demonstrated augmented intestinal permeability and bacterial translocation, which were associated with the severity of AIH activity, but not the stage of fibrosis [54].

In the experimental AIH model, germ-free mice demonstrated a much smaller amount of inflammatory cytokines and chemokines, including TNF-α, IL-4, interferon-γ, monocyte chemotactic protein-1, C-X-C motif chemokine 1, granulocyte colony-stimulating factor, and eotaxin. Concanavalin A (ConA)-induced apoptosis of liver cells was also significantly prevented in germ-free BALB/c mice as compared with positive controls [55]. Moreover, in conventional mice with ConA injection, plasma LPS levels were significantly higher than those in germ-free mice with ConA [55]. These data might explain the crucial role of LPS in ConA-induced liver injury in the mimicry of the pathogenesis of AIH.

Liver cirrhosis is the end stage (F4) of liver fibrosis, which is characterized by the conversion of the normal liver architecture and regenerative nodules surrounded by tissue fibrosis and liver dysfunction [56]. As patients with liver cirrhosis are basically in an immunosuppressive state, infections can increase the rate of morbidity and mortality in cirrhotic patients [57]. Likewise, subsequent quantitative endotoxin assays have revealed an elevation in plasma endotoxin levels along with liver fibrosis development to liver cirrhosis [58][59]. Thus, it is important to investigate the change in gut microbial diversity because of the key roles of bacterial translocation and endotoxemia in the pathogenesis of complications resulting from liver cirrhosis [60].

SBP is one of the most important infections in cirrhotic patients with ascitic fluid, which might be caused by bacterial translocation from the intestine. The presence of bacterial DNA in ascites and blood was observed in one third of cirrhotic patients with culture-negative ascites, which could worsen intrahepatic endothelial function and peripheral vasodilation [61] [62]. Proton pump inhibitors (PPIs) are potent inducers of increased bacterial translocation, which might be associated with the promotion of intestinal overgrowth. The evidence from these studies shows that bacterial overgrowth could increase the risk of SBP via bacterial translocation resulting from intestinal hyperpermeability [63][64].

Along with the development of liver fibrosis, the portal blood pressure is elevated in patients with CLD as a result of the elevation in liver stiffness. As mentioned above, cirrhotic patients have an impaired gut barrier, resulting in bacterial translocation. An experimental study demonstrated that intraperitoneal injection of LPS can elevate the portal pressure and variceal bleeding, and increased portal hypertension can increase intestinal permeability [65][66][67]. These products also impair the contractility of the mesenteric vessels, which further exaggerates the elevation in the portal blood pressure [68][69].

Clinically, several studies, including our current study, have demonstrated that cirrhotic patients experience an elevation in plasma endotoxin levels and increased intestinal permeability after variceal hemorrhage [70][71]. Moreover, the results of a multivariate statistical analysis revealed that excessive intestinal permeability was an independent risk factor for infections such as SBP [70][72]. In these patients, poorly unabsorbable antibiotics including rifaximin could be useful for preventing SBP and the re-rupture of gastrointestinal varices [73][74]. The accumulating evidence suggests that there is a malignant circulation that induces portal hypertension via LPS stimulation through the gut–liver axis.

Hepatic encephalopathy (HE) is a major complication of end-stage liver cirrhosis, which induces symptoms due to an abnormal elevation in plasma ammonia levels, leading to confusion, lethargy, sleep disturbances, and coma [75]. An experimental animal model using germ-free mice in the mimicry of liver cirrhosis displayed a lower concentration of serum ammonia and reduced the level of neuroinflammation as compared with conventional mice [76]. This study also showed that the enrichment ofLactobacillaeis positively associated with increased neuroinflammation in conventional mice with liver cirrhosis. In addition, LPS permeabilizes the blood–brain barrier in the same manner as the gut barrier and affects the brain microglia through the production of nitric oxide, which induces the swelling of astrocytes in patients with HE [77] [78].

There is no significant difference in the profiles of fecal microbiota between cirrhotic patients with HE and those without HE [79][80]. ofAlcaligeneceaein HE patients was shown to be increased as compared with normal controls. In contrast, none of the healthy controls taking PPIs demonstrated an increase in this alteration [79]. Bajaj et al. reported a higher abundance of fecalEnterobacteriaceae, which is a major bacterium that produces a large amount of endotoxins [81].

Changes in the gut microbiota affect the hepatic tumor environment. Hepatic inflammation caused by leaky gut can drive tumorigenesis in the liver. In this study, TLR4 signaling in the liver cells was partially mediated by the inhibition of hepatocyte apoptosis and growth signals, including epiregulin derived from HSCs [82]. Dysbiosis-induced bacterial metabolites, such as deoxycholic acid, are additional factors that promote hepatocarcinogenesis related to gut-derived endotoxemia, which could enhance liver inflammation via TLR pathways [83].

In clinical studies, a higher abundance of intestinalE. coliwas found to be associated with the presence of HCC and reported to produce endotoxins [14]. Animal studies have also indicated that endotoxins play an important role in the progression of HCC [84]. These studies further suggested that an increase in fecalBacteroidesis probably accompanied by an elevation in proinflammatory cytokine levels such as IL-8 and IL-13, activated monocytes, and monocytic myeloid-derived suppressor cells, which play a role in the hepatocarcinogenesis of NAFLD patients [7].Klebsiella-andHaemophilus-producing LPS are also reported to be increased in patients with HCC in the early stage as compared with non-HCC controls [13][85][86]. This growing evidence may suggest the possibility of a noninvasive predictive marker for the detection of HCC incidence in the future.

4. Conclusions

Lifestyle factors, such as dietary factors and alcohol drinking, are fundamental therapeutic targets for preventing the pathogenesis of liver diseases in terms of the gut–liver axis. Dysbiosis and leaky gut are induced by alcohol and a high-calorie/high-fat diet, which results in liver inflammation and liver fibrosis progression, eventually progressing to liver cirrhosis, especially in patients with alcoholic liver disease and NAFLD. This gut–liver axis resulting from gut dysbiosis might also induce the development of HCC. Considering these findings, the gut microbiota and gut-derived endotoxemia could be useful therapeutic targets in the management of the progression of liver pathogenesis, and further investigation in this field is expected in the future.

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