The role of intestinal microbiota, bile acids, and Th17/IL17 axis in hepatitis B virus-related liver fibrosis

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Hepatitis B virus (HBV) infection, which affects 90 million people in China, remains a prominent cause of liver cancer and liver cirrhosis.[1] Chronic HBV infection has a complicated course, which is a dynamic process formed by the interaction between the virus and the immune system.[2,3] Hepatic fibrosis is the basis of liver cirrhosis and liver cancer.[4] In recent years, accumulating studies have indicated potential roles of intestinal microbiota, bile acids, and T helper (Th)17/ interleukin (IL)-17 axis in the process of HBV-related liver fibrosis. Gut microbiota actively communicates with bile acids and plays an important role in the pathogenesis of HBV-related liver fibrosis. In the following content, we are going to summarize current evidence of the role of intestinal microbiota, bile acids, and Th17/IL-17 axis in HBV-related liver fibrosis.

Age at the time of infection is an important factor affecting the immune response after HBV infection, and its mechanism may be related to the establishment of mature intestinal microbiota. In HBV transfected mouse models, virus clearance was substantially dependent on the formation of mature intestinal microbiota. Adult mice with mature and stable intestinal microbiota could completely clear the virus within 6 weeks after HBV plasmid injection, while young mice with immature intestinal microbiota could have the virus persistent after 6 weeks. Antibiotics treatment could delay the clearance of HBV in adult mice.[5] From another perspective, controlled clinical study of chronic HBV infection patients, the combination of nucleoside antiviral drugs with fecal microbiota transplantation could significantly reduce HBV viral load, increase HBV E antigen conversion, and improve liver function.[6] Taken together, it could be inferred that intestinal microbiota is involved in the immune response process of HBV virus clearance, while the specific mechanism remains unclear.

Th17 cells, as a subset of helper T cells, play an important role in chronic inflammation and immune-related diseases. Th17 cells could secrete inflammatory cytokines such as IL-17 and IL-22, which can activate hepatic stellate cells and Kupffer cells. This may promote the synthesis and accumulation of extracellular matrix, and further contribute to the process of liver fibrosis.[7] Chronic hepatic fibrosis diseases, such as alcoholic liver disease, autoimmune hepatitis, primary sclerosing cholangitis, primary biliary cirrhosis, biliary atresia, nonalcoholic fatty liver disease, and viral hepatitis, have been observed with increased peripheral Th17 cell count.[8] A number of studies have found that chronic HBV infected patients are accompanied by increased number of Th17 cells in peripheral circulation and liver tissue, together with alterations of related cytokines. In chronic HBV infected patients, the number of peripheral Th17 cells was significantly higher than that of the control group, and was positively correlated with the level of serum alanine aminotransferase (ALT).[9] The level of Th17 cell infiltration to liver tissues of chronic HBV infection patients is also positively correlated with the severity of liver inflammation and fibrosis.[10] After antiviral therapy with nucleoside analogs, the level of Th17 cells and IL-17 in peripheral blood of chronic HBV infection patients declines with viral load.[11]

Th17 cells in liver tissue might not differentiate *in situ*, but mainly migrate based on local signals in the liver parenchyma. HBV infection can induce Th17 cells to

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migrate from peripheral to hepatic tissue by upregulation of chemokines such as chemokine C-C motif ligand (CCL) 17, CCL20, and CCL22 in hepatocytes. In comparison with uninfected controls, the mRNA levels of hepatocellular chemokines CCL17 and CCL22 were significantly increased in patients with chronic HBV infection, as well as increased expression of chemokine receptors 6 (CCR6) and chemokine receptor 4 (CCR4) on Th17 cells. *In vitro* experiments also confirmed that HBV DNA could induce hepatocytes to express Th17 chemokines CCL17 and CCL22.[12]

The most possible source of Th17 cells is the intestinal lamina propria, which contains a large number of CD4+ T cells, primarily Th17 cells and regulatory T cell (Treg) cells.[13] Intestinal microbiota maintains immune regulation function by changing the balance of Th17/Treg cells.[14] Th17 cells of specific pathogen-free (SPF) mouse were mainly observed in the ileum and colonic lamina, which also contain the highest density of bacteria. There are relatively few Th17 cells in the duodenum, jejunum, mesenteric lymph nodes, spleen, or liver.[15] In comparison with SPF mice, germ-free mice contained fewer Th17 cells in the intestinal lamina propria; moreover, after transplanting intestinal microbiota from normal mice to germ-free mice, the intestinal Th17 cell content increased accordingly.[16] Low-dose penicillin exposure early after birth can change the composition of intestinal microbiota, thus influencing the differentiation of Th17 cells and the expression of pro-inflammatory factor IL-17.[17] On the other hand, vancomycin added to drinking water significantly reduced Th17 cell levels in the lung.[18]

The differentiation of Th17 cells depends on the stimulation of symbiotic intestinal bacteria. However, whether the microbes interact with CD4+ T cells directly remains unclear. Some intestinal microbes have been found to be related to the differentiation of Th17 cells directly, such as segmented filamentous bacteria, *Citrobacter Rodentium*, and *Escherichia coli O 157*.[19] Besides, twenty mixed strains isolated from fecal flora could also stimulate the differentiation of intestinal Th17 cells. Then, further 16S rRNA sequencing analysis showed that the 20 strains were derived from *Clostridium*, *Bifidobacterium*, *Ruminococcus*, and *Bacteroides*.[20] Intestinal commensal *Bacteroides* species peptide derivatives could specifically stimulate the differentiation of intestinal Th17 cells and participate in the development of myocarditis.[21]

Although previous results have suggested a direct interaction between intestinal bacteria and Th17 cells, several lines of evidence have proposed that the stimulation might be mediated by the intestinal bile acids pool. Intestinal bacteria could produce choline dehydrogenase and steroid dehydrogenase, which can affect bile acids metabolism, covalent modification and isomerization, and participate in the formation of secondary bile acids.[22] Intestinal secondary bile acids can stimulate intestinal epithelial or lamina propria immune cells to produce inflammatory regulatory factors, which directly influence subsequent immune response.[23] Hang *et al*[24] screened more than 30 bile acid metabolites and identified two lithocholic acid derivatives, 3-oxo lithocholic acid (LCA) and isoallo LCA, which can reduce the differentiation of Th17 cells and increase the differentiation of Treg cells. The regulatory

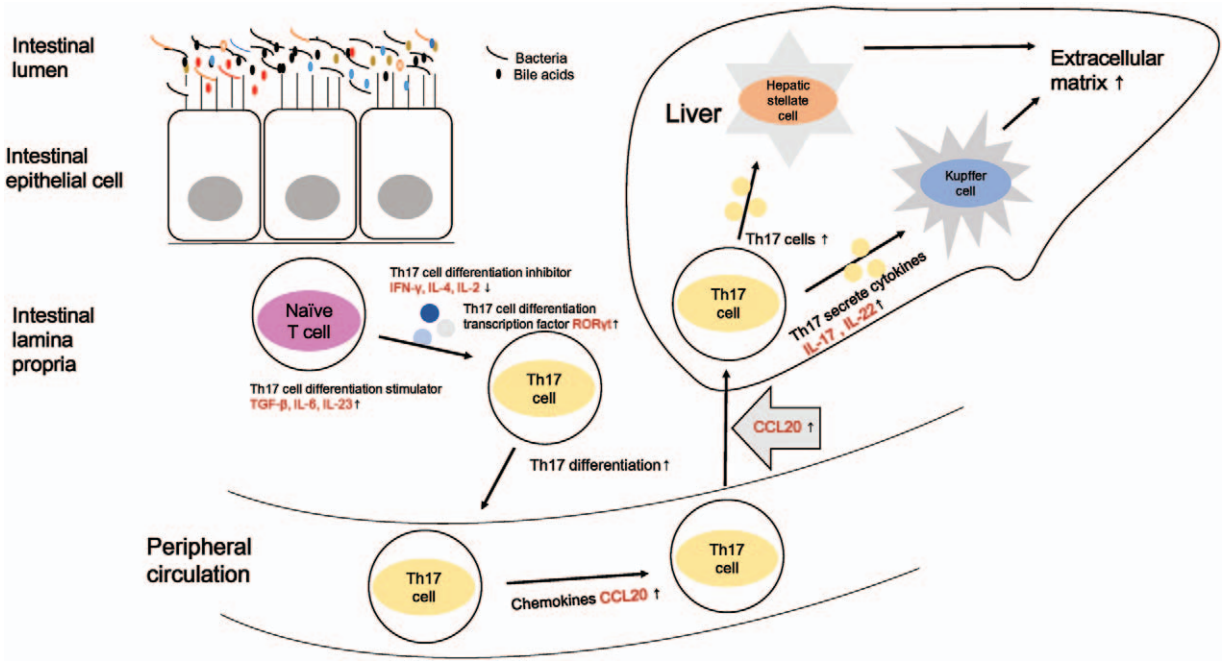

Figure 1: Possible relationship among intestinal microbiota, bile acids, and Th17/IL-17 axis in HBV-related liver fibrosis. Distinct gut microbiota in chronic HBV infection may lead to alterations of gut bile acids pool, then stimulate naive T cells to differentiate into Th17 cells, which then enter peripheral circulation and migrate to the liver via chemokine CCL20. Intrahepatic Th17 cells then secrete IL-17 and IL-22, which would activate hepatic stellate cells and Kupffer cells, lead to over-synthesis of the extracellular matrix. Th: T helper; IL: interleukin; HBV: Hepatitis B virus; CCL: C-C motif ligand.

effect of intestinal bile acids pool on adaptive immunity was also observed in another study, which found that the composition of the intestinal bile acids pool can regulate the expression of the transcription factor retinoic acid receptor-related-orphan-receptor-C (RORγ) on colonic forkhead box protein P3+ (FOXP3+) Treg cells. The deletion of bile acids metabolic pathway significantly reduced the number of intestinal Treg cells, while the restoration of the intestinal bile acids pool showed increased the number of ROR+ Treg cells in the colon and improvement of the host colon inflammation.[25] Thus, it can be inferred that the intestinal bile acids pool may be involved in mediating the regulatory role of intestinal bacteria on Th17 cell differentiation.

Based on the evidence presented, we proposed a potential scientific hypothesis on the mechanism of HBV-related liver fibrosis [Figure 1]. Distinct gut microbiota in chronic HBV infection may lead to alterations of gut bile acids pool, then stimulate naive T cells to differentiate into Th17 cells, which then enter peripheral circulation and migrate to the liver via chemokine CCL20. Intrahepatic Th17 cells then secrete IL-17 and IL-22, which would activate hepatic stellate cells and Kupffer cells, and then lead to over-synthesis of the extracellular matrix. Although, there is still much to uncover for the specific mechanisms. With further research, the therapeutic potential of targeting the bile acids-intestinal microbiota-Th17/IL-17 axis will allow us to better discern the pathogenesis and treatment of HBV.

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Conflicts of interest

None.

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