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REVIEW

Alteration of Gut Microbiota and Its Impact on Immune Response in Patients with Chronic HBV Infection: A Review

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Abstract: Chronic hepatitis B virus infection is a source of substantial global health problems, particularly in economically underdeveloped and/or developing countries. It is the primary cause of severe liver disorders such as liver fibrosis, cirrhosis, and hepatocellular carcinoma. The liver is connected by the bile duct to the small intestine that carries bile produced in the liver to the intestine. The liver is the initial organ exposed to materials originating from the gut including dietary compounds, bacteria, and their products. Human intestines harbor a wide diversity of the community of microbes which are collectively termed as gut microbiota. In chronic infection with the hepatitis B virus, microbial alteration of the gut is a source of systemic immune activation. Besides, gut permeability is altered in hepatitis B virus-infected patients with an increased bacterial translocation and endotoxin load in the portal vein that caused toll-like receptor activation in the liver, which facilitates immune-mediated liver injury. Toll-like receptors further triggered the host-wide inflammatory response by inducing signaling cascades such as nuclear factor-kappa B-linked pathways and by accelerating cytokine secretion like tumor necrosis factor-alpha, which evokes chronic inflammation and leads to liver lesion formation, fibrosis progression, and cirrhosis and hepatocellular carcinoma development. In conclusion, changes in intestinal flora play an important role in encouraging the production of chronic infection with the hepatitis B virus. Therefore, careful attention should be given to the maintenance of intestinal microecology of patients which can provide a sound foundation for the treatment of chronic infection with the hepatitis B virus.

Keywords: hepatitis B virus, gut microbiota, immune response

Introduction

Chronic hepatitis B (CHB) infection is a global epidemic disease that results from hepatitis B virus (HBV) infection and may progress to severe liver disorders, including liver fibrosis, cirrhosis, and hepatocellular carcinoma.[1] Hepatitis B virus (HBV) is a noncytopathic, hepatotropic virus of the family Hepadna viridae; it is an enveloped virus with circular partially double-stranded DNA representing the highly compact organization. HBV is the smallest known DNA virus, having a spherical shape of approximately 42 nm in diameter and a genomic length of about 3200 base pairs.[2,3]

Hepatitis B virus has four overlapping open reading frames: preC/C that encodes for e antigen (HBeAg) and core protein (HBcAg), P for polymerase (reverse transcriptase), S for surface proteins, and X for a transcriptional trans-activator protein.

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HBV is replicated by reverse transcription of pre-genomic RNA, which is transcribed from the covalently closed-circular form of HBV DNA in the hepatocyte nucleus by cellular RNA polymerase II. Sequence heterogeneity is a characteristic of HBV since POL cannot proofread.[4] According to phylogenetic analysis, HBV can be classified into ten, A-J, based upon an inter-group divergence.[4,5]

HBV surface antigen (HBsAg) indicates an acute or chronic infection, and it is commonly regarded as a serological marker for HBV infection. Infection is considered chronic if it persists for greater than 6 months. HBsAg disappearance and HBV surface antibody (HBsAb) emergence indicate the recovery from infection and the development of HBV immunity.[6] HBV core antigen (HBcAg) consists of the core particle of the virus that is surrounded by HBsAg. HBV e antigen (HBeAg), the soluble secretory form of HBcAg, serves as an important serological marker indicating viral replication. HBeAg to e antibody (HBeAb) conversion suggests host immune control and low replication of HBV.[7] Adult infections have a relatively low chronicity rate (around 5%); typically, neonatal infections have a high persistence rate.[8–10] Chronic infections are often asymptomatic, but carriers of HBV are at risk of developing life-threatening cirrhosis and later on hepatic carcinoma.[11]

Standard treatment regimens with pegylated interferon (IFN)-α and nucleoside/nucleotide analogs are used to treat chronic hepatitis B, but unfortunately, it neglects the role of gut microbiota (GM) balance in patients with chronic hepatitis B virus-infected. Increasing pieces of evidence have shown that gut microbiota plays an important role in the development of liver disease, pathogenesis, and response to treatment.[1,12]

Gut microbial alteration is a cause of systemic immune activation in chronic HBV infection. Translocation of gut microbial products, bacterial peptidoglycan, flagellin, and metabolic by-products has been suggested to exacerbate the clinical course of patients with chronic HBV infection, and intestinal dysfunction in liver cirrhosis is well known.[13,14] A better understanding of the pathophysiological connection between gut microbiota alteration and its impact on the hepatic immune response is crucial for the development of new therapy to treat chronic hepatitis B virus infection.

Host Immunity and Chronic HBV Infection

Chronic HBV infection is one of the major global health problems, especially in economically underdeveloped or developing countries. It is the main cause of severe liver disorders, like liver fibrosis, cirrhosis, and hepatocellular carcinoma. According to World Health Organization (WHO) report, there are approximately 257 million people worldwide with chronic HBV infection, 887,000 of whom die of complications caused by chronic HBV infection.[15]

HBV immune response generally involves innate immunity and adaptive immunity; however, the successful innate immune response can not only directly eradicate the virus but can also have a substantial impact on unique immunity to HBV.[16] The primary response to HBV infection is initiated by the innate immune system. Immunocytes of the innate immune system, including monocytes, macrophages, dendritic cells (DCs), neutrophils, natural killer cells (NK), and natural killer T cells (NKT), orchestrate innate immune systems and activate adaptive immune responses.[17] The first important role of the immune response is appropriate sensing and recognition of invading microbes by Toll-like receptors (TLRs).[18]

Toll-like receptors are a group of highly conserved molecules that play a key role in recognizing pathogen-associated molecular patterns (PAMPs) and triggering innate immune responses to infectious agents.[19] TLRs play a significant role in interferon and pro-inflammatory cytokine synthesis as well as immune cell mobilization to suppress viral replication.[16,20]

In the early stage of infection, toll-like receptors, especially TLR3 to detect intracellular HBV virions and TLR2/4 that bind to extracellular HBV components, are likely to be involved. Against HBV invasion, dendritic cells, Kupffer cells, NK cells, and NKT cells cooperate. Then, HBV-specific T-cells of CD4+ and CD8+ T-cells, the major HBV clearance effectors, induce IFN-γ production and proliferation of CD8+ T cells, as well as the production of multiple HBV antibodies and other cytokines.[17] Generally, the key mechanisms by which HBV replication is suppressed in liver cells are usually the activation of cell signaling pathways by TLRs and the release of antiviral cytokines.[19]

Generally, host immune systems play an important role in the outcome of HBV infection. However, the immunological mechanisms by which HBV establishes and maintains chronic infection, cirrhosis, and hepatocellular carcinoma are still under debate.[17] However, the immune responses to HBV antigens are responsible for both viral clearances during acute infection and disease pathogenesis. The cause of liver injury is currently thought not to be the replication of HBV in liver cells, but rather the

immune response caused by HBV. Chronic HBV-infected patients fail to develop HBV-specific immune responses; for example, inappropriate, excessive, and nonspecific effector responses might be involved in persistent HBV replication and pathogenesis of HBV-associated liver inflammation.[21]

Another study has shown that HBeAg in patients with chronic HBV infection can minimize TLR2 expression in liver Kupffer cells and mononuclear cells.[18] A recent study also found that the viral load of HBV DNA is negatively associated with the expression of TLR7 in biopsy samples, suggesting that HBV can inhibit TLR7 expression in liver cells.[22] Obviously, TLR-mediated signaling pathways induce immune responses to HBV infection, but the expression of TLRs on the immune cells is down-regulated by HBV itself. A significant number of studies on the gut–liver axis have currently shown that improvements in intestinal microbiota play a key role in the induction and development of chronic hepatitis B infection.[23,24]

The involvement of gut microbiota (GM) in HBV clearance was demonstrated in animal models. The research found that, after six weeks of infection, adult mice with mature GM managed to clear up HBV, which is the opposite of young mice without GM who remained HBV positive. The fact that adult mice did not clear HBV after antibiotic sterilization of the gut (6 to 12 weeks) emphasizes the importance of GM in anti-HBV immunity.[25]

Acute HBV infection can progress to chronic hepatitis B in just 5% of adult patients, while the proportion in children is very different because more than 90% of exposed neonates and 30 to 50% of children aged 1 to 5 years fail to clear HBV.[8–10] It shows that adults have the highest rates of new infections and acute disease, but chronic infection is more likely to occur in infants or young children. It is due to their immune immaturity and unstable flora of the intestines. However, the adult epithelial cell surface is protected by the antimicrobial peptide, which can play an important role against the virus and experiencing an abrupt onset after HBV infection.[21,26]

It also implies a new therapeutic approach for patients with HBV infection.[14] Fecal microbiota transplantation (FMT), in addition to standard antiviral, is effective in HBeAg clearance.[27] Consequently, the frequency and production of chronic HBV infection depend not only on the direction of HBV invasion, virulence, immune system maturity, and viral load but also on the stability of GM.

Gut Microbiota

The gut microbiota (GM) is a complex ecosystem composed of a distinct array of bacteria that have a symbiotic relationship with each other and other microorganisms. From an immunological perspective, microorganisms are considered as pathogens by the host immune system that recognizes and destroys them. However, the immune system has coevolved to live in a collaborative relationship with the healthy microbiota.[28]

The GM colonizes the human intestinal tract and has a vital role in health and disease. The intestine of a healthy adult harbors 100 trillion microbial cells and has a complex genome of 150-fold more genes than the human genome and consists of 6–10 major phyla and 3000–5000 species.[29] Bacteroides and Firmicutes are the major phyla, followed by Proteobacteria and Actinobacteria, together representing more than 97% of the total microbiota.[9]

Gut microbiota can supplement the nutritional needs of the host by breaking down and absorbing complex dietary carbohydrates that cannot be digested by human enzymes, as well as synthesizing some essential substances, such as vitamins.[30] Besides, it helps maintain the integrity of the intestinal epithelial barrier through the production of short-chain fatty acids, particularly butyrate, that play an important role in providing energy for cellular metabolism and regulating apoptosis, cellular differentiation, and chemical modification of nuclear proteins and nucleic acid.[31]

The role of gut microbiota in the regulation of immune cell homeostasis is increasingly recognized. Intestinal bacterial signals are responsible for priming systemic immune responses, including T regulatory cells (Tregs) and T helper 17 (Th17) cells, and for controlling pro- and anti-inflammatory host immune responses. Commensal species such as fragilis and Clostridial species promote Treg differentiation in the intestine. Furthermore, segmented filamentous bacteria are responsible for the induction of Th17 cytokines and initiate the maturation of natural killer T cells.[14,32] Different genetic and environmental factors affect the composition of GM among individuals, but the human body can eventually develop a stable structure of intestinal flora and monitor the health of the body.[33]

Thus, the intestinal immune function cannot be neglected. In chronic HBV-infected patients, gram-negative bacteria and inflammatory cascade were increased; however, anti-inflammatory and beneficial bacteria were depleted. In addition to this, the different studies also demonstrated that disruption of intestinal homeostasis

and alterations in the intestinal microbiome contribute to a variety of diseases such as inflammatory bowel disease (IBD) and autoimmune liver disease. Moreover, the gut microbiota is implicated in non-alcoholic liver fat disease (NAFLD) and is associated with NAFLD progression to non-alcoholic hepatitis, cirrhosis, or hepatocellular carcinoma (HCC).[34,35]

Gut–Liver Axis

The "gut–liver axis" is defined as a close relationship between the gut and the liver because of its structural, functional, and bidirectional characteristics. The portal system gained approximately 75% blood from the gut, and intestinal blood content activates liver functions. The liver, in turn, affects intestinal functions through bile secretion into the intestinal lumen.[14] The liver is the initial organ that is exposed to products originating from the gut, including not only the alimentary but also bacteria and their products. In healthy conditions, the protective mechanism for preserving hepatic immune homeostasis relies primarily on the roles of the intestinal barrier and the detoxifying ability of the liver.[36]

There is a profound impact on the immunological axis between the liver and intestine against microbe invasion. In other words, the interplay between the gut microbiota and liver pathology through the gut–liver axis plays an important role in determining the disease severity and prognosis of chronic HBV liver diseases.[17]

Gut Microbiota in Chronic HBV Infection

Now, there is emerging evidence that chronic liver disease has been related to imbalanced intestinal microbiota homeostasis, which has qualitative (dysbiosis) or quantitative (overgrowth) variations.[29,37] Recently, some researchers are beginning to investigate the relationship between the diversity and composition of human gut microbiota and chronic hepatitis B virus infection. Chronic hepatitis B virus infection exhibits alterations in the relative levels of "beneficial" and potentially "harmful" bacteria compared to healthy subjects; however, the dynamic alteration of the gut microbiota following HBV infection is still unknown.[14,38,39]

There are mutually causal structural changes in the intestinal microflora and the incidence of liver disease. The gut microbiota in chronic hepatitis B and liver cirrhosis patients have a reduced ratio of Bifidobacteriaceae/Enterobacteriaceae (B/E), based on low levels of Bifidobacteria and Lactobacillus and high levels of Enterococcus and Enterobacteriaceae. The outcome suggested that the B/E ratio is useful for following the extent of intestinal microecological disorder in the course of liver disease progression.[1] In addition, these authors also found that the intestinal microflora composition of patients with CHB had changed compared to that of patients with chronic HBV infection before the serious liver injury, suggesting that changes in intestinal flora played a possible pathogenic role in patients with chronic HBV infection.[1] A study reported that GM of patients with HBV-related cirrhosis contained lower levels of Bacteroidetes and increased levels of Enterobacteriaceae, Veillonellaceae and Streptococcaceae compared to the healthy group.[39]

Recent research has compared patients with asymptomatic carriage of hepatitis B virus (HBV), chronic hepatitis B (CHB), decompensated HBV cirrhosis, and healthy controls. *Faecalibacterium prausnitzii, Enterococcus faecalis*, and Enterobacteriaceae showed a marked increase in asymptomatic carriers compared to healthy controls, and the increased range was much greater in patients with chronic hepatitis B and those with decompensated HBV cirrhosis. The numbers of bifidobacteria and lactic acid bacteria (*Lactobacillus, Pediococcus, Leuconostoc*, and *Weissella*) dropped dramatically in both patient groups. In addition, gut permeability is altered in HBV-infected patients with an increase in bacterial translocation and endotoxin load in the portal vein that caused Toll-like receptor (TLR) activation in the liver, which facilitates immune-mediated liver injury.[40,41]

Constantly, functional gene array data showed that in both the HBV-related cirrhosis group and the alcoholic cirrhosis group, the abundance of functional genes important to nutrient metabolism, including amino acid metabolism, lipid metabolism, nucleotide metabolism, and isoprenoid biosynthesis, was significantly decreased compared to normal controls.[42]

In another study, patients with alcohol-related and HBV-related cirrhosis showed reduced GM diversity, predominantly *Enterobacteriaceae* and Streptococcaceae, compared to healthy individuals.[43] Generally, the overgrowth of intestinal pathogenic bacteria leads to improved permeability of the mucosa, which facilitates immune-mediated liver injury and the cause of systemic immune activation in chronic HBV infection. Therefore, these accumulating studies indicate that changes in gut

microbiota composition play an important role in the induction and furthering the development of HBV-induced chronic liver disease.[40]

Immune Function of Gut Microbiota in the Development of Chronic HBV Infection

In recent years, different studies indicated that the gut microbiota composition influences the host immune response to HBV, and when the intestinal flora is changed, the infection can easily turn into a chronic infection.[25] Immune injuries triggered by structural changes in the gut microbiota are often caused by inflammatory pathways between the gut microbiota, the immune system, and the liver initiated by crosstalk.[32]

Kupffer cells, hepatic stellate cells, plasmacytoid dendritic cells (pDCs), and hepatocyte cells are capable of expressing TLRs that recognize pathogen-associated molecular patterns (PAMPs) and initiating the innate immune response.[44] Intestinal PAMPs associated with chronic infection with HBV are now considered to consist predominantly of lipopolysaccharide (LPS), bacterial DNA/RNA, teichoic acid (TA), peptidoglycan (PGN) and non-methylated CpG DNA, flagellin, and metabolic by-products.[40]

Lipopolysaccharide (LPS) is a critical component of gram-negative bacteria's outer membrane and is an endotoxin produced primarily by Enterobacteriaceae. Under normal physiological conditions, low concentrations of endotoxin entering the liver via the portal blood flow are almost completely scavenged by Kupffer cells since the liver is the key anti-endotoxin defense organ.[45] However, in chronic HBV-infected patients, pathogenic organisms produce a high amount of endotoxin. This lipopolysaccharide binds to LPS-binding protein, and TLR4 may recognize this combination on the surface of mononuclear macrophages.[11] This recognition then stimulates CD14+ Kupffer cells, triggers the inflammatory cascade impact, activates the pathway associated with nuclear factor kappa B (NFforB), and produces inflammatory cytokines such as tumor necrosis factor-α (TNF-α), IL-1, and IL-6 and thus causes the development of liver fibrosis and cirrhosis.[46,47]

Another study showed that LPS can down-regulate the expression of tight junction proteins (closed proteins) in the intestinal tract, increase the permeability of the intestinal mucosa and reach the bloodstream via the portal venous system.[48] Additionally, TLR4 is also expressed by hepatic stellate cells and can release a large number of LPS-TLR4 pathway-dependent extracellular matrix proteins. In the fibrotic phase, these proteins are involved and may also be among the factors that cause chronic HBV infection to evolve into liver fibrosis.[19] Evidence showed that the level of serum LPS in patients with chronic HBV liver failure is higher, which indicates that LPS may be related to the severity of the disease and a decreased clearance of endotoxin from the blood by the liver.[16] Likewise, the implication of TLR4 in liver disease has also been proven, and it is one of the seven genes linked to an elevated risk of developing cirrhosis in patients with chronic hepatitis C infection.[49]

Other translocated bacterial products can also activate an inflammatory response by activating TLRs. TLR-2 recognize peptidoglycan (PGN) and lipoteichoic teichoic acid (TA), which are components of the cell wall of gram-positive bacteria, and TLR5 are mainly activated by bacterial flagellin, whereas TLR-9 is recognized as unmethylated CpG DNA, and TLR3 can combine with dsRNA in bacteria, and ssRNA can activate TLR7 and TLR8 receptors.[50,51]

The final downstream effect is the production of inflammatory cytokines, such as TNF-alpha, IL-1-beta, and IL-6, via the NF-kB pathway. However, if the immune response lasts for a long time or reaches an excessive intensity, this mechanism can also exacerbate liver damage, resulting in the release of a large number of cytokines in the body.[50] Besides the gut microbiota is altered, the Th17 lymphocytes have been detected in the serum of chronic hepatitis B patients. It is found occasionally in the liver and comes mostly from the gut. It is associated with poor prognosis, possibly due to abnormal secretion of IL-17A that increases angiogenesis and inflammatory cytokine production.[52]

Generally, alterations in gut microbiota and colonization by opportunistic bacteria can increase the risk of developing comorbid conditions in patients with HBV-induced chronic liver disease. Restructuring of those gut microbiota is a successful treatment for those patients.

The Future Therapeutic Target for Chronic HBV Infection

The first step in the development of a new treatment is to set targets such as reversal or prevention of fibrosis/cirrhosis progression, improvement or maintenance of liver homeostasis, or prevention of transplantation or death. Many different antiviral drugs are used in the treatment of chronic HBV infection, for various reasons, and it seems likely that chronic HBV infection will evolve into

liver cirrhosis, liver failure, or liver cancer. Currently, a new therapeutic target has been established by studies on gut microbiota.[11]

Fecal microbiota transplantation (FMT) refers to the process of infusing fecal suspension from a healthy donor to the intestinal tract of a recipient to normalize the intestinal microbiota's composition and functionality. FMT can be applied to normalize the composition of the gut microbiota and increase the proportion and diversity of beneficial bacteria. FMT also provides the necessary signals for epithelial regeneration, induces mucins and antimicrobial peptide production, and reduces intestinal permeability to preserve the integrity of the epithelial barrier.[53]

Currently, FMT is emerging as one of the more hopeful treatments for chronic HBV infection. FMT has been conducted in HBeAg-positive chronic hepatitis B patients combined with ongoing entecavir (ETV) and tenofovir disoproxil fumarate (TDF) therapy, and 80% of them have reached HBeAg clearance. In contrast to the direct and indirect costs of repeated antiviral therapy courses, the cost of transplanting intestinal microbiota is bound to be lower.[27]

In addition to this, several reviews are summarizing the beneficial effect of probiotics on liver diseases.[54–58] Probiotics are defined as live microorganisms that are ingested as food or medicinal supplements with beneficial effects on health.[59,60] In chronic liver diseases, probiotics may improve the unhealthy state of the gut microbiota, as well as chronic inflammation.[55] Sometimes, probiotics are species-specific or even strain-specific.[61] Therefore, the option of strain or combination of strains is essential for therapeutic effectiveness.

Lactobacilli and bifidobacteria produce intestinal benefits that influence the immunity of the host, thus restoring gut dysbiosis, increasing the number of beneficial bacteria, and preserving the integrity of the intestinal barrier by preventing endotoxin translocation in chronically viral-infected patients. In this respect, it was found that antibacterial activity against common pathogenic bacteria is provided by Lactobacilli and bifidobacteria.[62]

Conclusion

Chronic hepatitis B virus infection is a global concern that affects human health. Some serious complications, such as liver failure, cirrhosis, and even hepatocellular carcinoma, may easily result from chronic HBV infection (HCC). Several studies have verified that there is a unique GM profile in chronic hepatitis B virus infection, closely correlated with immune responses and specific metabolic status. It increases intestinal permeability, impaired gut barrier, bacterial overgrowth, and translocation of bacteria, which facilitates liver injury mediated by immunity. Therefore, the role of gut microbiota should not be underestimated; rather careful attention to patients' intestinal micro-ecology should provide a sound basis for the treatment of chronic HBV infection. Many different antiviral drugs are used in the treatment of chronic HBV infection; for various reasons, it seems likely that chronic HBV infection well evolves into liver cirrhosis, liver failure, or liver cancer. So, we have been moving forwards with the use of FMT and probiotics. FMT is emerging as one of the most promising treatments for chronic HBV infection by manipulating human commensal bacteria. However, the available data in this field remains limited and further research is urgently needed. A detailed understanding of the mechanism of action between gut microbiota and HBV-related diseases is needed. It should be further studied in the future.

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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