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Targeting Dysbiosis for the Treatment of Liver Disease

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Abstract

The gut microbiome is composed of a vast number of microbes in the gastrointestinal tract, which benefit host metabolism, aid in digestion, and contribute to normal immune function. Alterations in microbial composition can result in intestinal dysbiosis, which has been implicated in several diseases including obesity, inflammatory bowel disease, and liver diseases. Over the past several years, significant interactions between the intestinal microbiota and liver have been discovered, with possible mechanisms for the development as well as progression of liver disease and promising therapeutic targets to either prevent or halt the progression of liver disease. In this review the authors examine mechanisms of dysbiosis-induced liver disease; highlight current knowledge regarding the role of dysbiosis in nonalcoholic liver disease, alcoholic liver disease, and cirrhosis; and discuss potential therapeutic targets.

Keywords

microbiota; dysbiosis; liver disease; therapy

Chronic liver diseases leading to cirrhosis and hepatocellular carcinoma are a major cause of morbidity and mortality worldwide.¹ The burden of illness due to chronic liver diseases is linked to three major etiologies: viral hepatitis (B, C, and D), alcoholic liver disease, and nonalcoholic fatty liver disease (NAFLD).^{1,2} With the widespread use of vaccination for hepatitis B and the advent of more potent directly acting antiviral therapies against hepatitis C, the prevalence of end-stage liver disease (ESLD) due to viral hepatitis is expected to decline in the coming decades.^{1,3,4} Although there have been dramatic advances in antiviral therapies, treatment options remain limited for both alcoholic liver disease and NAFLD. In contrast, due to the rising rates of obesity and metabolic syndrome that are commonly associated risk factors for NAFLD, it is expected to be the leading cause of cirrhosis and ESLD in the coming decades, especially in the developed world.^{5,6} Over the past several years, significant interactions between the intestinal microbiota and liver have been discovered with possible mechanisms for the development as well as progression of liver

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disease; these discoveries have provided invaluable insight into promising therapeutic targets to either prevent or halt the progression of liver disease.

Our understanding of human gut microbiome is still in its infancy, especially as it relates to liver disease. Greater than 10^{14} microorganisms live in the human gastroenterological tract, including bacteria, viruses, fungi, and archaea; this collective microbial community is known as the *gut microbiome*. Most of the bacteria are anaerobic, with varying numbers and composition according to the site in the gut, increasing from the stomach to the small intestine to the colon. Over 90% of the intestinal bacteria belong to the phyla Firmicutes and Bacteroidetes.⁷ Although these main phyla are consistently seen, the species present and their relative proportions vary considerably between individuals.⁸ Due to the presence of a large number of anaerobic, fastidious organisms, the analysis of gut microbiota has evolved beyond culture-based techniques to enable sequencing of whole genomes.⁹

The gut microbiota is beneficial for host metabolism, aids in digestion, and contributes to normal immune function, thereby creating a symbiotic relationship with the host.⁷ *Intestinal dysbiosis* is defined as a disruption in symbiosis due to an imbalance in the microbial composition. This can present as quantitative (intestinal bacterial overgrowth) and/or qualitative changes in the intestinal microbiota.¹⁰ The gut microbiota plays a critical role in the gut and systemic immune system and has been potentially implicated in several different diseases, including obesity, neurologic diseases, inflammatory bowel disease, cancer, and liver disease.⁸

Our aim here is to examine mechanisms of dysbiosis-induced liver disease; highlight current knowledge regarding the role of dysbiosis in NAFLD, alcoholic liver disease, and cirrhosis; and review potential therapeutic targets.

Mechanisms of Liver Disease in Dysbiosis

The liver is particularly susceptible to potential effects of dysbiosis. Greater than 70% of the liver's blood supply is via the portal vein, resulting in frequent exposure of gut-derived toxins and microbial products. In normal conditions, small amounts of bacteria and bacterial metabolites enter the liver and are rapidly cleared. However, when the normal gut barrier is disrupted such as in intestinal dysbiosis, large amounts of bacteria and bacterial products enter the liver resulting in the activation of the immune cascade, production of proinflammatory cytokines, and subsequent liver damage.¹¹ Mechanisms of dysbiosis-induced liver disease include increased intestinal permeability, bacterial/metabolite translocation, and immune activation (► Fig. 1). Increased intestinal permeability is attributed to tight junction disruption, possibly from metabolites or dysbiosis-induced inflammation.^{12–15} Augmented gut permeability results in increased translocation of bacteria, bacterial products (such as lipo-polysaccharide/endotoxin), and metabolites into the portal circulation. Metabolites such as trimethylamine (produced from bacterial enzymatic cleavage of dietary choline) and alcohol (produced by enteric bacteria) can have direct toxic effects on the liver, whereas bacterial products cause liver damage through activation of the innate immune system.¹⁶ Foreign pathogens (such as bacteria, viruses, and fungi) are recognized by pathogen recognition receptors, such as toll-like receptors (TLRs)

and inflammasomes. TLRs are expressed on cells in the hepatic sinusoids (including Kupffer cells and hepatic stellate cells) and recognize pathogen molecular patterns expressed on cell membranes.^{17,18} TLR-mediated signaling results in the production of inflammatory cytokines, which have antimicrobial effects. However, sustained production of these cytokines could cause or enhance hepatic injury.¹⁹ Toll-like receptor-4 (TLR-4) is the receptor for lipo- polysaccharide, the cell wall component of Gram-negative bacteria, and has been implicated in hepatic damage in NAFLD and alcoholic liver disease.^{20,21} Although TLRs recognize cell membrane components, inflammasomes are cytoplasmic multiprotein complexes that are critical in cytoplasmic surveillance of pathogen-associated molecular patterns and endogenous (noninfectious) damage-associated substances.²² Activation of inflammasomes results in the production of inflammatory cytokines that can cause hepatic injury.

Nonalcoholic Fatty Liver Disease

Nonalcoholic fatty liver disease is defined as hepatic steatosis on either liver histology or imaging without any secondary causes of hepatic fat accumulation such as alcohol consumption.²³ Nonalcoholic fatty liver disease can be broadly classified into two categories: nonalcoholic fatty liver (NAFL), which is considered to have minimal risk of liver disease progression; and nonalcoholic steatohepatitis (NASH), which is considered to have a substantially higher risk of liver disease progression.²⁴ Nonalcoholic steatohepatitis is defined as the presence of hepatic steatosis typically in zone 3 with lobular inflammation and ballooning with or without peri- sinusoidal fibrosis.²³ Obesity, type 2 diabetes, dyslipidemia, and metabolic syndrome are all risk factors for NAFLD.^{23,25} Although there are many animal and human studies demonstrating the effects of gut microbiota on energy absorption, obesity, and insulin resistance, in this review we will focus primarily on selected studies describing the association of dysbiosis with NAFLD.^{26,27}

Recent studies have suggested that fibrosis progression is substantially higher in NASH versus NAFL; it is likely that the various stages of liver diseases may have the following steps: normal to NAFL to borderline NASH to definite NASH to cirrhosis.²⁴ When patients develop cirrhosis, they may not show all the typical features that are diagnostic of NASH. Various hypotheses have been proposed, but it is commonly accepted that multiple hits may be needed to progress from NAFL to NASH.^{28–31} Lipotoxicity, oxidative stress, inflammatory cytokines, innate immune mechanisms, or toxic metabolites may all contribute in progression from NAFL to NASH.^{28,32} The intestinal microbiota has been implicated in both the development of hepatic steatosis and subsequent progression to NASH.

Multiple animal studies have investigated the first hit (i.e., the development of hepatic steatosis); intestinal microbiota greatly impact hepatic lipid metabolism. Donor mice on a diet-induced obesity protocol who develop microvesicular steatosis, hyperglycemia, and hyperinsulinemia can transmit this phenotype to germ-free mice.³³ However, donor diet-induced obesity mice who did not develop dysmetabolism or hepatic steatosis while on a high-fat diet did not transmit their phenotype. The intestinal microbiota may promote hepatic steatosis through several mechanisms including alterations in energy harvest and

homeostasis,^{26,34} endogenous alcohol production,^{35,36} altered choline metabolism,³⁷ and changes in bile acid metabolism.³⁸

Additional animal studies have improved our understanding of the role of gut microbiota in development of NAFLD and its contributions toward the progression from NAFL to NASH. These studies have suggested multiple mechanisms for liver injury in steatohepatitis. One mechanism is that endotoxin production from gut microbiota results in the activation of Kupffer cells via the TLR-4 complex and subsequent release of inflammatory mediators that promote steatohepatitis.²¹ TLR-4-deficient mice and Kupffer cell-depleted mice have decreased steatohepatitis compared with wild-type mice. In addition, inflammasome plays an important role in preventing the progression of NAFL to NASH. Inflammasome-depleted mice develop dysbiosis that results in more severe steatohepatitis. This phenotype was mediated through an influx of TLR-4 and TLR-9 agonists into the portal vein, ultimately resulting in the activation of tumor necrosis factor α (TNF- α) in the liver.³⁹

Nevertheless, the physiological phenomena observed in animal studies have been difficult to demonstrate in humans. Most human studies investigating the role of intestinal microbiota in NAFLD/NASH have instead focused on the compositional differences between those who have the disease (or trait), and those who do not. Initial real-time polymerase chain reaction analysis of the fecal microbiome among patients with NAFL, NASH, and healthy controls demonstrated a lower percentage of Bacteroidetes among NASH patients, a finding that was independent of body mass index (BMI) and diet.⁴⁰ Subsequent studies, using 16S rRNA sequencing of the fecal microbiome, showed a more nuanced picture of the gut microbiome, with overrepresentation of several Firmicutes genera such as those belonging to *Lactobacillus*, *Dorea*, *Rob- insoniella*, and *Roseburia*, but underrepresentation of other Firmicutes genera such as *Oscillibacter*.^{41,42} Similar findings are reported in a pediatric population.³⁵ ► Table 1 reviews changes in intestinal microbiota composition among human studies of NAFLD. However, these studies did not control for various factors that are now known to have an effect on the composition of the gut microbiome in humans, such as diet in the days immediate to fecal sample collection.⁴³ Furthermore, there is ongoing debate as to whether bacteria residing in the mucous layer, where it more intimately interacts with the host cells, are more likely to affect host physiology than luminal bacteria, found in fecal samples. It should also be noted that *Lactobacillus* genera contains multiple members that are particularly adapted to be commensal gut species. Other studies have found that particular lactobacillus species can be protective against hepatic injury,⁴⁴ hence the diversity of this genera, with both protective and disruptive member species, should be fully appreciated.

Nevertheless, there have been observations in humans that indicate that the compositional differences observed in humans can play a role in the pathophysiology of NAFLD/NASH. For example, shifts in the fecal microbiome composition correlated with an increase in fecal ester volatile organic compounds among obese patients with NAFLD compared with healthy lean control patients.⁴¹ Volatile organic compounds are metabolic products that may have direct toxic effects on the liver via the portal circulation. In addition, there is an increase in small intestinal permeability and small intestinal bacterial overgrowth (SIBO) among

patients with NAFLD compared with controls, which may influence the development of hepatic steatosis.¹²

Gut Microbiome and Bile Acid Signaling

The observed compositional changes in humans have also been reported in animal models of NAFLD. Mice with obesity, dysmetabolism, and hepatic steatosis also have a rise in *Lactobacillus* species and a decrease in *Oscillibacter* species.^{45,46} These compositional changes in the gut microbiome have been linked to altered luminal secondary bile acid profiles, specifically by an increase in bile acid hydrolases in *Lactobacillus* species.^{47,48} The modulation of the luminal bile acid profiles, in turn affect bile acid signaling by the farnesoid X receptor⁴⁹ and the G protein-coupled bile acid receptor 1.⁵⁰ Interestingly, agents that modulate bile acid signaling, such as obeticholic acid, are being vigorously investigated as novel treatments for NAFLD/NASH.^{51,52} Future NAFLD/NASH therapies that target the microbiome will likely mediate their effects by a combination of modulating host metabolism by bile acid signaling and host inflammation through TLR-4/inflammasome pathways.

In summary, animal and human studies have demonstrated differences in gut microbiota with NAFLD. Furthermore, there is emerging evidence to suggest that the gut microbiota may play a key role in the development of hepatic steatosis and progression from NAFL to NASH. However, whether the gut microbial dysbiosis is causally related to liver disease progression in patients with NASH cirrhosis remains to be elucidated.

Alcoholic Liver Disease

Alcoholic liver disease encompasses a spectrum of disease including hepatic steatosis, acute alcoholic hepatitis, chronic hepatitis with fibrosis, and cirrhosis. Development and progression of alcoholic liver disease is influenced by several factors including quantity of alcohol, consumption pattern, presence of obesity, gender, nutritional status, genetic polymorphisms, and the presence of chronic viral hepatitis.⁵³ Recently, data have emerged demonstrating an important role of intestinal microbiota in the development of alcoholic liver disease.

Alcohol use has both quantitative as well as qualitative effects on the gut microbiota. Alcohol use leads to decreased intestinal motility that can lead to the proliferation of intestinal bacteria.⁵⁴ Several mouse and human studies have also demonstrated alterations in the composition of the intestinal microbiota with alcohol consumption. Chronic alcohol exposure in mice leads to significant changes in the intestinal microbiota.⁵⁵ Chronic alcohol feeding resulted in a decline in Bacteroidetes and Firmicutes with an increase in Proteobacteria and Actinobacteria. Furthermore, the alcohol-fed mice had increased plasma endotoxin levels, higher fecal pH, and greater liver inflammation. Similar findings were observed in patients with chronic alcohol use. These patients had a lower quantity of Bacteroidetes and higher quantity of Proteobacteria when compared with control participants.⁵⁶

In addition to alterations in intestinal microbiota, alcohol use has other effects on the gastrointestinal tract, which may contribute to liver toxicity such as increasing intestinal permeability.¹³ Acetaldehyde, a metabolic product of alcohol, induces increased gut permeability through the disruption of epithelial tight junctions and adherens junctions.^{57–60} Increased gut permeability has also been attributed to increased intestinal inflammation through the production of TNF- α in the lamina propria, which results in disruption of tight junctions.^{61,62}

Overall, chronic alcohol use results in changes in the composition of intestinal microbiota and induces bacterial overgrowth. Furthermore, intestinal dysbiosis and metabolic products of alcohol promote gut hyperpermeability. As a result, bacteria and microbial products such as gut-derived lipopolysaccharides (endotoxin) can translocate into the portal circulation. Compared with control participants, patients with chronic alcohol use have an increase in plasma endotoxin levels in all stages of alcoholic liver disease. Furthermore, alcoholic cirrhotics also have higher levels of endotoxin when compared with patients who have cirrhosis from other causes.^{63,64} Endotoxins stimulate the innate immune system via TLR-4 and CD14, which activate hepatic stellate cells and Kupffer cells resulting in the release of proinflammatory mediators resulting in liver inflammation and damage. Mouse studies show TLR-4-deficient mice that are fed alcohol have less liver steatosis, inflammation, and cell death compared with wild-type mice.²⁰

Cirrhosis

There are compositional and quantitative changes in gut microbiota of patients with cirrhosis. The fecal microbiome of these individuals have reduced Bacteroidetes and increased Proteobacteria and Fusobacteria at the phyla level when compared with that of healthy controls.⁶⁵ On the family level, potentially pathogenic bacteria such as Enterobacteriaceae and Streptococcaceae were increased and potentially beneficial populations such as Lachnospiraceae were diminished. Severity of liver disease was associated with greater changes in the microbiome. Comparison of the intestinal microbiota among control participants, compensated cirrhotics, and decompensated cirrhotics showed diminishing ratios of autochthonous and nonautochthonous taxa. This calculation, the cirrhosis dysbiosis ratio (CDR), significantly decreased with cirrhotic severity but was stable in compensated cirrhotics over a 4- to 6-month period. Metagenomic sequencing of the intestinal microbiota of cirrhotics and healthy controls revealed specific bacterial genes that are a signature of cirrhosis.⁶⁶ Using 15 genes as biomarkers, Qin et al created a patient discrimination tool, which could accurately distinguish patients with cirrhosis from healthy individuals. Analysis of the intestinal microbiome among cirrhotics can be challenging due to many confounding variables such as differing etiologies of cirrhosis, frequent antibiotic exposure lactulose use, hospital admissions, and dietary effects.

In addition to qualitative changes, quantitative changes are common in the gut microbiota among cirrhotics. Many animal and human studies have demonstrated small intestinal bacterial overgrowth in patients with cirrhosis.^{67–69} Human studies utilizing jejunal aspirates and breath testing has estimated the prevalence of SIBO as 35% to 61% among cirrhotic patients, with increasing SIBO as severity of cirrhosis worsens.^{70–72}

In addition to the proliferation of bacteria and compositional change of microbiota to potentially more pathogenic strains of bacteria, there is increased bacterial translocation with cirrhosis that may have clinical consequences.¹⁵ This was illustrated by comparing the bacterial cultures in mesenteric lymph nodes from noncirrhotics and cirrhotics, stratified by Childs-Pugh class.⁷³ Enteric organisms grew from mesenteric lymph nodes in 30.8% of Childs C cirrhotics compared with 8.6% of noncirrhotics. Results were similar among noncirrhotics, Childs A cirrhotics and Childs B cirrhotics. Culture-based studies may underestimate true bacterial translocation due to the presence of fastidious anaerobic organisms, which are difficult to grow in a laboratory setting. Thus, more recent studies have measured bacterial DNA as an indicator of bacterial translocation. About 32% of patients with cirrhosis have detectable bacterial DNA in their blood and ascitic fluid, whereas no bacterial DNA was detected in the blood of healthy controls.⁷⁴ Interestingly, all patients had nonneutrocytic sterile ascites and negative blood cultures. Factors promoting bacterial translocation in cirrhosis include increased intestinal permeability, SIBO, and host immunological alterations.¹⁵

Potential clinical consequences of dysbiosis and increased bacterial translocation in cirrhosis are worsened hyperdynamic circulation, infection, and hepatic encephalopathy.⁷⁵ Patients with bacterial DNA in their ascites had significantly lower mean arterial pressure, lower systemic vascular resistance, and signs of worsened hepatic endothelial function compared with patients without ascitic fluid bacterial DNA.⁷⁵ In terms of infection, it remains unclear if dysbiosis is a risk factor for the development of infections. However, patients with cirrhosis have an increased presence of potentially pathogenic species that are commonly involved in systemic infections with a decrease in commensal bacteria, raising the concern for an interaction of dysbiosis and infection. A comparison of 38 infected cirrhotic patients to uninfected cirrhotic patients found increased dysbiosis (reflected by lower CDR) and higher serum endotoxin levels among infected patients.⁶³

Targeting Dysbiosis

The gut microbiome can be modulated in different ways, and targeting intestinal dysbiosis has been investigated as a way to combat liver disease. Therapies include prebiotics, probiotics, synbiotics, antibiotics, and fecal microbiota transplantation (►Table 2).⁷⁶ Prebiotics are nondigestible carbohydrates that promote beneficial changes in the activity and composition of gastrointestinal microflora. Probiotics are living microorganisms (bacteria, fungi) that present a health benefit for the host. Synbiotics contain both prebiotics and probiotics.^{76,77}

Prebiotics

Prebiotics are complex carbohydrates including lactulose, inulin, fructo-oligosaccharides (FOS), and galacto-oligosaccharides (GOS), which stimulate the growth of certain bacteria, most commonly *Bifidobacteria* and *Lactobacilli*.⁷⁸ There are limited data describing the effects of other prebiotics in liver disease. Although prebiotics are nondigestible by the host, they are fermented by colonic microbes to form short-chain fatty acids (SCFAs) and lactate.⁷⁹ The production of SCFAs has been shown to modulate cytokine production and may have

immunomodulatory effects. Lactulose is probably the best-studied prebiotic in liver disease and is commonly used for the treatment of hepatic encephalopathy. However, studies have shown different compositional outcomes in patients with cirrhosis who are taking lactulose. In one study, lactulose administration resulted in decreased stool pH and increased *Lactobacilli* in the stool.⁸⁰ In another study, however, there was greater dysbiosis, reflected by a decline in CDR, without a change in *Lactobacilli*.⁶³ In the latter study, only seven patients were included and these results may be limited by a small sample size. In patients with alcoholic liver disease, there is strong evidence in animal models that prebiotics could play a protective role.⁸¹ Chronic alcohol consumption affects bacterial synthesis of saturated long-chain fatty acids (LCFAs). When LCFAs were added to the diet in mice models of alcoholic liver disease, there was an improvement in the function of the epithelial barrier and reduction in liver injury. Furthermore, changes were also noted in the composition of the gut microbiome with an increase in *Lactobacilli* species. Therefore, prebiotics that may augment LCFA production may be helpful in the treatment of alcoholic liver disease. Randomized, controlled clinical trials along with mechanistic studies are needed to better assess the role of prebiotics in improving alcoholic hepatitis and alcoholic cirrhosis.

Probiotics

Probiotics are living microorganisms that confer a health benefit on their host through antimicrobial effects, enhancement of mucosal barrier integrity, and immunomodulation.⁷⁶ Antimicrobial effects of probiotics are related to the production of antimicrobial products (such as bacteriocins and hydrogen peroxide), competitive colonization with other microbes, and production of organic acids that acidify the lumen, inhibiting growth and colonization of pathogenic bacteria.⁸² Enhancement of the mucosal barrier is accomplished through stimulation of mucin production and enhanced tight junction function through the actions of butyrate, a SCFA produced by probiotics.^{76,77,83} Immunomodulation by probiotics occurs through effects on epithelial cells, dendritic cells, Treg cells, natural killer (NK) T cells, and immunoglobulin-A-producing B cells.⁷⁷ These immunomodulating interactions result in changes in cytokine production that can inhibit epithelial cell apoptosis.⁸⁴ Commonly used and studied probiotics include *Lactobacillus GG (LGG)*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus johnsonii*, *Bifidobacterium lactis*, and *Saccharomyces boulardii* and VSL#3, which is a probiotic combination consisting of eight strains of *Lactobacilli*, *Bifidobacteria*, and *Streptococcus*.

Over the past few years, there have been an increasing number of studies evaluating the effect of probiotics in liver disease. Several small clinical trials have evaluated the effect of probiotics (predominantly *Lactobacillus*, VSL#3, *Bifidobacterium*) in patients with NAFLD with varying results.^{85–89} The main outcomes in these studies were improvement in liver enzymes (serum alanine [ALT] and aspartate [AST] aminotransferases), inflammatory markers, and anthropometric measurements. Two studies measured changes in steatosis via magnetic resonance spectroscopy but none of the studies assessed liver histological response to probiotics.^{87,89} A meta-analysis found significantly improved levels of ALT, AST, TNF- α , and cholesterol with probiotic use among patients with NAFLD but included two studies which used synbiotics.⁹⁰ Overall, clinical data remain limited to recommend the use of probiotics as therapy for patients with NAFLD and larger randomized controlled trials with

either resolution of NASH or improvement in hepatic fibrosis on histology as endpoint are needed

In alcoholic liver disease, animal studies have shown probiotics can reduce endotoxemia and liver injury associated with alcohol consumption. A recent study has evaluated liver histology and serum endotoxin levels in rats fed alcohol compared with rats fed alcohol and *LGG*. The group treated with *LGG* had minimal histological liver damage and significantly lower serum endotoxin levels.⁹¹ In addition, *LGG* was associated with less liver injury and diminished gut leakiness in a steatohepatitis rat model.⁹² Administration of oats as a prebiotic or *LGG* as a probiotic prevented alcohol-induced dysbiosis in rats.⁹³ There are a limited number of human studies evaluating the effects of probiotics on alcoholic liver disease. The effect of 5 days of *Bifidobacterium bifidum* and *Lactobacillus plantarum 8PA3* has been investigated in an open-label randomized trial evaluating males with alcoholic psychosis in Russia.⁹⁴ Probiotic-treated patients had significantly increased levels of Bifidobacteria and Lactobacilli and lower ALT and AST compared with patients treated with standard therapy.

Clinical studies of probiotics among cirrhotics show improvements in hyperdynamic circulation, decreased dysbiosis, and possible improvement in liver function. Rincon et al evaluated systemic and hepatic hemodynamic changes in 12 cirrhotic patients with ascites treated with 6 weeks of VSL#3.⁹⁵ Patients experienced a statistically significant reduction in hepatic vein pressure gradient (21.8 to 19.6 mm Hg), reduction in cardiac index (4.6 to 4.2 L/min/m²), reduction in heart rate (83 to 75 bpm) and increase in systemic vascular resistance (803 to 912 d · s · cm⁻⁵) with the use of probiotics. However, the clinical significance of these hemodynamic changes and whether they reduce morbidity or mortality is unclear. A recent study in 42 cirrhotic patients treated with *Escherichia coli Nissle* demonstrated an improvement from dysbiosis and trends toward decreased endotoxemia and decreased Model for End-Stage Liver Disease (MELD) score.⁹⁶ Furthermore, probiotics were investigated in a double-blind randomized control trial among cirrhotics with a recent episode of hepatic encephalopathy. Patients treated with VSL#3 experienced fewer hospitalizations for hepatic encephalopathy (19.7% vs. 42%) and fewer complications of cirrhosis (24% vs. 45%) compared with placebo at the end of the study (6 months).⁹⁷ Furthermore, the MELD score improved significantly (from 14 to 12) during the study period in the probiotic arm compared with the placebo group. Studies evaluating probiotics for hepatic encephalopathy date back to the 1960s.^{98,99} The theoretical benefit of probiotics in hepatic encephalopathy is from decreasing non-urease-producing bacteria, resulting in decreased ammonia production. There are now multiple randomized control trials evaluating the role of probiotics in overt and minimal hepatic encephalopathy. In 2011, a Cochrane Review evaluated seven clinical trials and found that probiotics reduce plasma ammonia levels, but concluded there is insufficient evidence to support efficacy in treating hepatic encephalopathy.¹⁰⁰ The study highlighted methodological issues with concerns for bias and random error. In the past few years, additional clinical trials have been published. An open-label randomized trial evaluating the use of VSL#3 for the primary prophylaxis of hepatic encephalopathy found decreased hepatic encephalopathy (9% vs. 20%) in the probiotic group compared with the control group.¹⁰¹ In addition, a phase I randomized control trial evaluating the safety and tolerability of *LGG* among cirrhotic patients with minimal hepatic

encephalopathy was recently performed.¹⁰² The *LGG* was well tolerated and associated with a reduction in endotoxemia and dysbiosis, but no change in cognition. Most studies have revealed no severe adverse reactions to the use of probiotics.

Although there are now multiple animal and human studies highlighting the promise of probiotics in liver disease, large well-designed randomized clinical trials with either reducing the risk of hospitalization or improving survival as an endpoint are needed to elucidate potential benefits of probiotics in patients with hepatic encephalopathy or decompensated cirrhosis. In addition to determining efficacy, there are major questions regarding the specific strains of effective probiotics, optimal dosing, duration of therapy, and long- term consequences of probiotic use.

Synbiotics

Synbiotics combine prebiotics and probiotics with the theoretical goal of augmenting the activity and prolonging the survival of potentially beneficial probiotics. There are limited clinical studies evaluating the use of synbiotics in liver disease. The effects of Protexin, a synbiotic capsule containing seven bacterial strains (mostly *Lactobacilli*, *Streptococci*, *Bifidobacteria*) and FOS, were recently investigated on patients with NAFLD (based on imaging and laboratory diagnosis).¹⁰³ Patients in the synbiotic group had statistically significant declines in ALT, inflammatory markers, and fibrosis score (based on transient elastography) compared with placebo despite similar changes in BMI and waist-to-hip ratio in both groups. The effects of *Bifidobacterium longum* and FOS have also been evaluated on patients with biopsy-proven NASH. Patients treated with synbiotics had statistically significant declines in AST, inflammatory markers, serum endotoxin levels, and histological NASH activity score compared with a placebo despite similar declines in BMI in both groups.¹⁰⁴ However, ALT was similar between the two groups at the end of the study.

Synbiotics have also been assessed in hepatic encephalopathy. The effects of *Bifidobacterium* and FOS have been compared with lactulose in a cohort of patients with mild- to-moderate hepatic encephalopathy.¹⁰⁵ Patients in the synbiotic group had statistically significant declines in ammonia levels and improvements in psychometric testing compared with the lactulose-treated group. In addition, the effects of a synbiotic preparation, consisting of fermentable fiber (inulin, pectin, β glucan, and resistant starch) and four strains of bacteria (*Pediococcus pentoseceus*, *Leuconostoc mesenteroides*, *Lactobacillus paracasei*, and *Lactobacillus plantarum*) have been evaluated on patients with minimal hepatic encephalopathy.¹⁰⁶ Patients receiving synbiotics had statistically significant improvements in ammonia levels and reduction in endotoxin levels compared with placebo. Furthermore, 50% of patients in the synbiotic group experienced resolution of minimal hepatic encephalopathy based on psychometric tests compared with only 10% in the control group. Although these studies are promising, large well-designed clinical trials are needed to elucidate the efficacy of synbiotic use for patients with liver disease.

Antibiotics

Antibiotics have profound quantitative and qualitative effects on the intestinal microbiota, greatly affecting microbial bio- diversity.^{107,108} The class of antibiotic and mechanism of

action greatly influence the ultimate effect on gut microbiota.¹⁰⁷ In addition to changes in bacterial populations and composition, antibiotics also have significant effects on bacterial metabolic function and virulence.^{107,109} Patients with cirrhosis benefit from antibiotics in several settings such as prophylaxis for spontaneous bacterial peritonitis in high-risk patients, but it is unclear if these benefits are via modulation of gut microbiota.¹¹⁰ Rifaximin is a minimally absorbable oral antibiotic commonly used in the treatment of hepatic encephalopathy.¹¹¹ An analysis of the effects of rifaximin on intestinal microbiota among patients with minimal hepatic encephalopathy found only minimal changes in microbial composition, but significant changes in microbial metabolic function.¹⁰⁹ These findings suggest that rifaximin's primary mechanism of action is altering metabolic function in the microbiota as opposed to promoting beneficial bacteria while decreasing harmful bacteria. Further studies are needed to elucidate the mechanisms by which antibiotics modulate gut microbiota and influence long-term outcomes in chronic liver diseases and decompensated cirrhosis.

Fecal Microbiota Transplant

Fecal microbiota transplant (FMT) has gained widespread acceptance as a highly effective therapy for the treatment of recurrent *Clostridium difficile* infection.¹¹² A major mechanism by which FMT influences the microbiota is through limiting the colonization of pathogens, a concept known as colonization resistance. Studies have also shown FMT may also influence microbial metabolic function in addition to microbiota composition.¹¹³ Fecal microbiota transplant has not been evaluated in clinical liver disease, but a recent mouse study demonstrated proof of concept by successfully trans-planting a consortium of eight bacteria with minimal urease gene content to create an enduring new bacterial community that exhibited reduction in fecal urease activity.¹¹⁴

Conclusion

Preclinical and clinical studies show the emerging role of the gut microbiome in the development and progression of chronic liver diseases, particularly in NAFLD, alcoholic liver disease, and cirrhosis. Further research is needed to more clearly elucidate gut-liver homeostasis and mechanisms for dysbiosis induced liver injury in humans. Promising new therapies have emerged by which to modulate gut microbiota and potentially treat liver disease. However, current evidence is inadequate to recommend the use of probiotics, prebiotics, or synbiotics in liver disease. High-quality, well-designed, large, multicenter clinical trials are needed to determine the efficacy, optimal dosing, and duration of therapy of therapeutic agents that specifically modulate dysbiosis, and examine the influence of specific changes in gut microbiota on long-term clinical outcomes.

Abbreviations

ALT	alanine transaminase
AST	aspartate transaminase
BMI	body mass index

CD	cluster of differentiation
CDR	cirrhosis dysbiosis ratio
ESLD	end-stage liver disease
FMT	fecal microbiota transplant
FOS	fructo-oligosaccharides
GOS	galacto-oligosaccharides
LCFA	long-chain fatty acid
LGG	<i>Lactobacillus GG</i>
MELD	Model for End-Stage liver Disease
NAFLD	nonalcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
NK	natural killer
PCR	polymerase chain reaction
rRNA	ribosomal ribonucleic acid
SCFA	short-chain fatty acid
SIBO	small intestinal bacterial overgrowth
TLR	toll-like receptor
TNF	tumor necrosis factor

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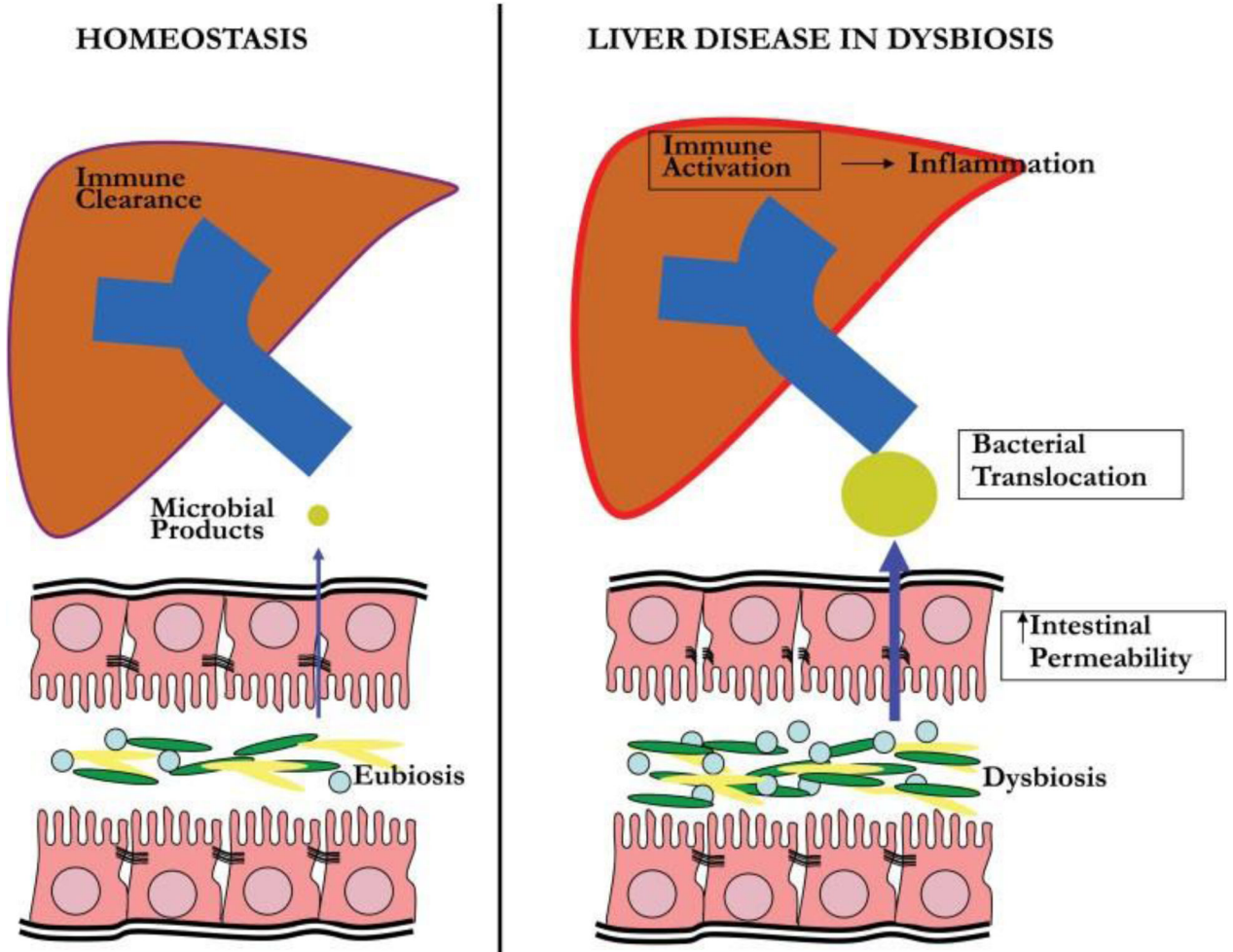


Fig. 1. Mechanisms of liver disease in dysbiosis. Under normal conditions, gut integrity is preserved with minimal entry of bacterial products into the portal circulation. In the liver, hepatocytes and Kupffer cells rapidly clear microbial products and maintain immunotolerance without inflammation. In dysbiosis, intestinal permeability is increased, resulting in increased translocation of bacteria, metabolites, and microbial products. Metabolites have direct toxic effects on the liver. Activation of the innate immune system via toll-like receptors and inflammasomes by bacteria and bacterial products produces large amounts of inflammatory cytokines and subsequent liver damage.

Table 1

Changes in intestinal microbiota in human studies of nonalcoholic fatty liver disease

Study	Study population	Comparison group ^a	Intestinal microbiota changes in NAFLD group ^b	Technique
Jiang et al, 2015 ³²	Healthy adults (<i>n</i> ¼ 32) Clinical diagnosis or bi-opsy-proven NAFLD (<i>n</i> ¼ 53)	Healthy vs. NAFLD	"Lentisphaerae; "Lactobacillus (genus); "Anaerobacter (genus); "Escherichia (genus); "Streptococcus (genus); "Clostridium XI (genus); ↓Alistipes (genus); ↓Oribacter; ↓Flavoni- fractor (genus); ↓Oscil- ltbacter (genus)	16s rRNA gene sequencing stool sample
Mouzaki et al, 2013 ²⁵	Healthy adults (<i>n</i> ¼ 17) Biopsy-proven simple steatosis (<i>n</i> ¼ 11) Biopsy-proven NASH (<i>n</i> ¼ 22)	Healthy vs. NASH Steatosis vs. NASH	↓ Bacteroidetes ↓ Bacteroidetes; " Clostridium coccoides (Firmicutes phylum)	Real-time PCR; stool sample
Raman et al, 2013 ²⁶	Healthy adults (<i>n</i> ¼ 30) Clinically diagnosed NAFLD (<i>n</i> ¼ 30)	Healthy vs. NAFLD	No change at phylum level; "Lactobacillaceae (Family); ↓Ruminococ- caceae (Family)	16s rRNA gene pyrosequencing; stool sample
Zhu et al, 2013 ²¹	Healthy children (<i>n</i> ¼ 16) Obese children (<i>n</i> ¼ 25) Biopsy-proven NASH (<i>n</i> ¼ 22)	Healthy vs. obese Healthy vs. NASH Obese vs. NASH	" Bacteroidetes; ↓ Firmicutes "Bacteroidetes; ↓Firmi- cutes; ↓Actinobacteria; "Proteobacteria "Proteobacteria	16s rRNA gene pyrosequencing; stool sample

Abbreviations: NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PCR, polymerase chain reaction.

Note: denotes increased in group B compared with group A. ↓ denotes decreased in group B compared with group A.

^aComparison group A versus B.^bChanges at the phylum level unless otherwise noted.

Table 2

Potential therapies for dysbiosis

Therapy	Effect on intestinal microbiota	Examples
Prebiotic	Complex carbohydrates; digested by colonic microbes to form short-chain fatty acids and lactate, which stimulate the growth of beneficial bacteria	Fructo-oligosaccharide (FOS) Galacto-oligosaccharide (GOS) Lactulose Inulin
Probiotic	Living microorganisms that confer a health benefit on their host through antimicrobial effects, enhancement of mucosal barrier integrity, and immunomodulation	<i>Lactobacillus GG (LGG)</i> <i>Lactobacillus casei</i> <i>Lactobacillus plantarum</i> <i>Lactobacillus johnsonii</i> <i>Bifidobacterium lactis</i> <i>Saccharomyces boulardii</i> VSL#3
Synbiotic	Contain prebiotic and probiotic; augment the activity and prolong the survival of potentially beneficial probiotics	<i>Bifidobacterium</i> p FOS Protexin
Antibiotic	Antimicrobial effects; changes in bacterial populations and composition; alterations in bacterial metabolic function and virulence	<i>Lactobacillus</i> p inulin Rifaximin Norfloxacin Neomycin Metronidazole
Fecal microbiota transplant	Colonization resistance (limiting the colonization of patho- gens); modulation of bacterial metabolic function	