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Microbiome dysbiosis and alcoholic liver disease⋆

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Abstract

Microbiome dysbiosis is strongly associated with alcoholic liver disease (ALD). Recent studies on comprehensive analyses of microbiome compositional and functional changes have begun to uncover the mechanistic relation between microbiome and the pathogenesis of ALD. Importantly, targeting the microbiome has become a potential strategy for the prevention and treatment of ALD. In this review, we summarize the clinical evidence of microbiome dysbiosis in ALD patients, and experimental advances in microbiome and metabolomic functional changes in animals with different species and genetic backgrounds in ALD. We also summarize the studies in humanized intestinal microbiome and fecal microbiota transplantation in mice. We introduce new developments in the studies on the role of the circulating bacterial microbiome, oral bacterial microbiome and fungal microbiome in the development of ALD. We highlight the potential mechanisms by which microbiome dysbiosis contributes to ALD, including short chain fatty acid changes, bile acid metabolism, intestinal barrier function, release of bacterial and fungal products, and inflammation. In addition, we summarize the recent developments targeting the microbiome in prevention and treatment of ALD, including dietary nutrient interference, herbal medicine, antibiotics, anti-fungal agents, probiotics, engineered bacterial therapy, fecal transplantation and oral hygiene. Although recent preclinical studies have advanced our understanding of the microbiome and ALD, clinical studies, especially prospective studies with large samples, are needed to better understand the cause-effect of microbiome dysbiosis in ALD. Identifying new precision-based strategies targeting the microbiome are expected to be developed as more effective therapies in ALD.

Keywords

Microbiome; Dysbiosis; Alcoholic liver disease (ALD)

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Authors' contributions

F. Li and W. Feng drafted the manuscript. C. J. McClain and W. Feng contributed to the critical revision of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

1. Introduction

Bacteria, fungi and other microorganisms create the human microbiome, a community of various microbial communities located in different areas of the human body including intestine, mouth, vagina and skin.[1] The bacterial microbiome is the most studied of these regarding its functions in the mammalian host. There are over 3 million bacterial genes, approximately ten times more than human host genes. The gut bacterial microbiome consists of more than ten phyla, and 90% of the bacteria belong to two phyla, Bacteroidetes and Firmicutes, and most of the rest belong to four phyla Actinobacteria, Fusobacteria, Proteobacteria and Verrucomicrobia. The human gut microbiome is created in utero and becomes relatively stable in early childhood. The microbiome composition and function affect the host by enhancing food metabolism,[2] supporting the barrier for defense against dangerous pathogens and providing the host with essential metabolites.[3,4] Recent studies have demonstrated that an imbalanced microbiome (dysbiosis) is associated with many diseases and disorders such as cancer,[5-8] cardiovascular diseases,[9-11] liver diseases,[12-20] and other metabolic disorders.[21-23] Investigation of the relation between the microbiome and these diseases and disorders provides opportunities for unique treatment strategies. The studies on intestinal, skin, oral and vaginal microbiome have begun to support the development of unique approaches to potentially modulate microbiome-related diseases.

Alcoholic liver disease (ALD) encompasses a broad spectrum of stages including fatty liver (steatosis), steatohepatitis, fibrosis, cirrhosis, and even liver cancer.[24] Although approximately 95% of heavy drinkers develop hepatic steatosis, only a small portion progresses to advanced liver disease, indicating that factors other than the amount of alcohol consumed may contribute to ALD development/progression. Despite extensive research, the cellular and molecular mechanisms underlying the development/progression of ALD are not fully understood.

Accumulating evidence demonstrated that microbiome dysbiosis is a risk factor in ALD development/progression. The majority of studies on relation between the microbiome and ALD have been focused on intestinal bacterial microbiome dysbiosis. Recent studies also indicate that oral bacterial dysbiosis and intestinal fungal dysbiosis are risk factors for ALD. The aim of this review is to summarize recent advances in ALD studies related to the microbiome, including the intestinal microbiome, oral microbiome, circulating microbiome, intestinal fungal microbiome, potential mechanisms of toxicity, and microbiome targeted treatment strategies.

2. Intestinal bacterial dysbiosis

Intestinal bacterial dysbiosis is defined as an imbalance of the different microbial entities in the intestine with a disruption of symbiosis.[25] There are three types of dysbiosis including pathobiont expansion, reduced diversity, and loss of beneficial microbes, and they are not mutually exclusive.

Both chronic and acute alcohol consumption induce small and large intestinal bacterial dysbiosis, particularly the overgrowth of Gram-negative bacteria and alterations in bacterial

diversity. Alcohol metabolism-generated aldehydes in the intestine produce reactive oxygen species that trigger pro-inflammatory responses and cause intestinal epithelial barrier dysfunction. This results in increased bacterial transcytosis and translocation of bacterial products, including endotoxin (lipopolysaccharide, LPS), bacterial deoxyribonucleic acid (DNA), and other pathogen-associated molecular patterns (PAMPs), from the gut lumen to the liver, causing liver injury. Alcohol consumption-induced leaky gut has been demonstrated in human subjects with ALD, in which higher levels of serum bacterial products were found compared to healthy controls.[26,27]

2.1. Gut bacterial dysbiosis and functional changes in ALD patients

Gut bacterial dysbiosis has long been observed in human alcoholic subjects. That alcohol abuse causes small intestinal bacterial overgrowth was documented over three decades ago.[28] Bacterial cultures of samples from jejunal juice displayed significantly higher bacterial counts in alcoholics compared to healthy controls. The small intestinal bacterial overgrowth may contribute to gut leakiness in patients with chronic alcohol abuse. Similar results were found by the same group of investigators using a breath test showing a higher prevalence of small intestinal bacterial overgrowth in chronic alcoholics compared to controls.[29] This observation was later confirmed in alcoholic patients with hepatic cirrhosis by other groups.[30-32]

Development of sequencing techniques greatly advanced gut bacterial research. Alcohol consumption, either excessive or moderate, caused small intestinal bacterial overgrowth.[33] In a study involving 244 alcoholic cirrhotic patients, investigators found that intestinal dysbiosis was more severe in decompensated cirrhotics compared to compensated cirrhotics.[34] A comparative metagenomic study of 99 patients with alcohol dependence syndrome with or without liver cirrhosis found that alcoholic dependence and liver cirrhosis were associated with profound shifts in gut bacterial community structures and metabolic potential across the patients, suggesting a strong negative influence of alcohol dependence and associated liver dysfunction on gut microbiota.[35] Colon biopsy sample analysis revealed that colonic mucosa-associated bacteria were persistently altered in a subset of alcoholics, and this was correlated with serum LPS levels.[36] This clinical finding was also documented in the authors' preclinical observations in rats.[37]

Although studies on bacterial overgrowth and dysbiosis are increasing, as yet there is no specific intestinal bacterial pattern identified to have an etiological role in the development of ALD. However, the fact that alcohol consumption causes intestinal bacterial dysbiosis provides an opportunity for the treatment and/or prevention of ALD by targeting intestinal microbiota.

Alcohol-dependent subjects who had gut leakiness on admission were more likely to exhibit psychological symptoms following three weeks of abstinence compared to those without gut leakiness. Moreover, subjects with initial altered gut permeability had greater alteration in gut microbiome and fecal metabolites.[38] The functional changes in microbiota (gut bacteria-mediated metabolite changes) have also been studied in ALD patients. Alcohol-dependent subjects with high intestinal permeability showed higher phenol and lower 4-methyl-phenol concentrations in feces than the subjects with low intestinal permeability, while indole and

3-methyl-indole concentrations were lower in subjects with high gut permeability. Volatile organic compounds (VOC) were analyzed in fecal samples collected from alcoholics and non-alcoholic healthy controls. Several notable metabolite alterations were found in the alcoholics that were different from healthy controls. While propionate and isobutyrate (beneficial short chain fatty acids (SCFAs)), caryophyllene (alcohol consumption natural suppressant) and camphene (hepatic steatosis attenuator) were decreased, tetradecane (oxidative stress biomarker) was elevated in alcoholics.[39] These metabolite alterations could be associated with liver injury from alcohol abuse.

2.2. Gut bacterial dysbiosis and functional changes in animal models of ALD

2.2.1. Rodent models—Studies in experimental animal models of ALD provide more detailed mechanistic information on gut dysbiosis compared to clinical observations. Yan *et al.*[40] showed that 3 weeks of intragastric alcohol ingestion led to bacterial dysbiosis in mice, which was associated with the reduction of antimicrobial peptides, regenerating islet-derived 3 (Reg3) beta (Reg3B) and gamma (Reg3G). Alcohol-feeding increased Bacteroidetes and Verrucomicrobia and decreased Firmicutes levels compared to pair-feeding. Interestingly, an overgrowth of Akkermansia muciniphila was observed in one mouse model of ALD, and this bacterium is believed to be responsible for mucin degradation. Moreover, the population of *Lactobacilli* was depleted in alcohol-fed mice, which is generally considered as a beneficial bacterial group.

In another study using the Lieber DeCarli alcohol feeding mouse model,[41] metagenomic analysis demonstrated a decline in the abundance of both Bacteroidetes and Firmicutes phyla, with a proportional increase in the Gram-negative Proteobacteria and Gram-positive Actinobacteria phyla. Genera analysis showed the greatest expansion in Gram-negative alkaline tolerant *Alcaligenes* and Gram-positive *Corynebacterium*. These alterations were accompanied by the increases in colonic pH and levels of liver steatosis.[41]

Chronic ethanol consumption leads to alterations in fecal bacterial microbiome, as well as gastrointestinal content of metabolites. One of the major functions of gut bacteria is to metabolize food to produce metabolites that are beneficial (or harmful) to the host. A metabolomic study revealed profound alterations in metabolite contents within the gastrointestinal tract in rats following four or eight weeks of ethanol exposure.[42] Major metabolic pathways that are critical for host physiology were affected, including markedly altered bile acids (BAs), increased levels of certain fatty acids and steroids, decreased carnitines and metabolites involved in lipid metabolism, a significant decrease of all amino acids and branched chain amino acids, and significantly decreased SCFAs except for acetic acid, which rapidly elevated as a product of ethanol metabolism.[42] Metabolomic studies by our group also revealed changes in metabolites in the liver and intestine. Levels of diet-derived long chain fatty acids increased in mouse livers and decreased in mouse feces when mice were chronically exposed to alcohol. Several amino acids including branched chain amino acid L-Isoleucine were down-regulated in the liver and fecal samples from animals exposed to alcohol.[43] Interestingly, heptadecanoic acid (C17:0), a long chain fatty acid produced only by bacteria, was reduced by alcohol ingestion, indicating alcohol exposure changes gut fatty acid-metabolizing bacteria. Supplementation of saturated long-chain fatty

acids maintains intestinal eubiosis and reduces alcohol-induced liver injury.[44] The effect of the types of dietary fat on gut microbiota homeostasis was evaluated in mice with ALD. Saturated fat (medium chain triglycerides enriched) feeding reduced Proteobacteria and Actinobacteria and increased Bacteroidetes in feces of mice exposed to 8-weeks of alcohol feeding.[45] A reduction in endotoxemia and liver steatosis and injury was observed in animals fed a saturated fat diet as compared to a comparable amount of unsaturated fat (mainly linoleic acid) diet.

More recent studies analyzed polar metabolites in the feces from mice fed with or without alcohol.[46] Taurine, nicotinic acid, and several major SCFAs, were significantly decreased in mice fed alcohol. Moreover, we showed that the deficiency of an antimicrobial peptide, cathelicidin, in mice (*Camp*$^{-/-}$ mice) led to similar alterations in intestinal metabolites to that observed in alcohol-fed wild type mice.[46]

The translocation of bacteria and bacterial products from the intestine to the liver is a hallmark of ALD.[47] In a recent study in which mice were subjected to chronic ethanol feeding for eight weeks, researchers found that alcohol exposure changes bacterial α-diversity in the ileum and the liver and leads to compositional changes, especially in the ileum which is largely driven by an increase in the endotoxin-producing Gram-negative phyla. Among these phyla, *Prevotella* was not only increased in the mucus layer of the ileum but also in liver samples.[47]

2.2.2. Mouse models with altered genetic background—Alcohol exposure-associated gut bacterial alteration was also studied in mice with various genetic backgrounds. Mucin production from Goblet cells in the intestine is important for the integrity of intestinal mucus layer, which forms a physical barrier between the underlying epithelium and the gut lumen. Mucin 2 is the dominant mucin in the small and large intestine. Surprisingly, wild-type mice have higher alcohol-induced hepatic steatosis and injury than mucin 2 knockout mice (Muc2$^{-/-}$).[48] Muc2$^{-/-}$ mice are protected from intestinal bacterial overgrowth after alcohol feeding compared to wild-type mice. This protective effect may be due to the higher expression of antimicrobial peptides, Reg3B and Reg3G lectins, and less mucus layer thickness that allows antimicrobial peptides to readily enter into the gut lumen and enhance intraluminal killing of bacteria. Interestingly, overexpression of Reg3G in the intestinal epithelial cells restricts bacterial colonization of the mucosal surface, reduces bacterial translocation, and protects mice from alcohol-induced steatohepatitis.[49]

The importance of antimicrobial peptides Reg3B and Reg3G was further demonstrated in *Reg3b*$^{-/-}$ and Reg3g$^{-/-}$ mice. Compared to wild-type mice, the knock-out mice had a similar change in fecal microbiota after alcohol feeding. However, *Reg3b*$^{-/-}$ and *Reg3g*$^{-/-}$ mice showed significantly higher numbers of mucus-associated bacteria in the mucus and epithelial layer of the small intestine and an enhanced bacterial translocation to the mesenteric lymph nodes and liver.[49] Alcohol appears to impair control of the mucosa-associated microbiota, and the subsequent breach of the mucosal barrier facilitates the progression of ALD.

Alcohol consumption also decreased other types of antimicrobial peptides. Cathelicidin-associated antimicrobial peptides (CRAMP) is a mouse ortholog of LL-37, the only member of the human cathelicidin family. Intestinal CRAMP is down-regulated by alcohol feeding, which was associated with gut dysbiosis and increased circulating LPS levels.[19,50] CRAMP knock-out exacerbated ALD,[51] which was associated with decreased butyrate-producing bacteria population (unpublished data) and reduced fecal butyric acid concentration.[46] The Paneth cell-produced antimicrobial peptide, α-defensin, is another important group of antimicrobial peptides. Although expression of α-defensin 5 did not differ significantly in mice fed alcohol intragastrically for 3 weeks,[40] dysfunction of α-defensin 5 exacerbated alcohol-induced luminal and mucus-associated microbiota and resulted in a severe liver injury.[52] Zinc supplementation increased α-defensin 5 production and reduced ALD.

Alcohol consumption has been correlated with intestinal epithelial expression of hypoxia-inducible factor 1 (HIF1), which regulates a number of barrier-protective genes including intestinal trefoil factor (ITF/TFF3), CD73 (NT5E), P-glycoprotein (P-gp/ABCB1), cathelicidin, claudin-1, mucin-3, and β-defensin-1 (DEFB1). Intestinal specific *Hif-1α*$^{-/-}$ mice displayed an exacerbated gut dysbiosis and liver steatosis and injury after alcohol feeding compared to wild-type mice.[19] It seems that intestinal HIF-1α is essential for the adaptive response to alcohol-induced changes in intestinal microbiota and barrier function associated with elevated endotoxemia and hepatic steatosis and injury.

Alcohol-associated metagenomic changes are associated with alterations in bile acid profiles with significant decreased taurine-conjugated BAs in intestine and in the serum in rat model of ALD.[42,53] Alcohol feeding increased abundance of bacteria harboring cholylglycine hydrolase (CGH),[54] a major enzyme that deconjugates bile acid.[55] Interestingly, serum conjugated BAs were markedly elevated in alcoholic cirrhosis patients.[56] The mechanism underlying the difference of conjugated bile acid in human and rodents are unknown, but the changes in gut microbiota may be a causative factor for these changes. In contrast, recent study showed increased amount of unconjugated BAs in the small intestine of mice fed alcohol for 8 weeks.[54] Increased unconjugated BAs are associated with lower farnesoid X receptor (FXR) activity in enterocytes, lower fibroblast growth factor (FGF)-15 protein secretion and increased hepatic cytochrome P450 enzyme (Cyp)-7A1 protein expression and circulating bile acid levels. Depletion of the commensal microbiota with non-absorbable antibiotics attenuated hepatic Cyp7A1 expression and reduced ALD in mice, suggesting an important role of gut bacteria in bile acid *de novo* synthesis. Pharmacological intervention with the intestine-restricted FXR agonist, fexaramine, to restore intestinal FXR activity protected mice from ethanol-induced liver injury.[54] While inhibition of bile acid synthesis by activating intestinal FXR is beneficial in ALD, genetic knock out of Cyp7a1 exacerbated ALD in mice subjected to binge-on-chronic alcohol feeding.[57] These contradictory results are likely due to the differences in animal species, feeding protocol and animal sex difference in these observations. Interestingly, clinical study showed an increase in serum total BAs, while a decrease in *de novo* bile acid synthesis in alcoholic hepatitis (AH) patients compared to healthy controls.[58]

2.2.3. Monkey and fish models—The relation between ALD and the gut microbiome was also explored in other animal models. The rhesus monkey provides great translational

validity in preclinical studies due to its similarities to humans in genetic, physiological, metabolic and behavioral aspects. Wang *et al*.[59] demonstrated that rhesus monkeys fed alcohol displayed a change in the intestinal bacterial community structure at phylum, order, family, genus and species levels, along with liver steatosis.[59] Firmicutes, Proteobacteria, and Verrucomicrobia tended to increase whereas Bacteroidetes and Actinobacteria decreased. At the genera level, *Lactobacillus* and *Streptococcus* decreased in ALD monkeys compared with normal controls.

Small-sized medaka fish have been used to study the changes of intestinal microbiome by alcohol exposure.[60] Exposing the fish to alcohol for two months caused liver steatosis and injury, along with changes of fecal microbiota composition. Decreased numbers of *Fusobacterium*, *Tenericutes* and *Firmicutes*, and increased abundance of *Proteobacteria* and *Bacteroides* were observed. Moreover, at the species level, *Alcaligenes* incertae sedis and *Cloacibacterium* incertae sedis decreased while *Cetobacterium* incertae sedis and *Erysipelotrichaceae* incertae sedis increased with ethanol administration. These changes were similar to those observed in humans.[60]

2.3. ALD models using germ-free mice and microbiota humanized mice

The germ-free rodent model provides a unique tool to study the contribution of intestinal microbiota to disease. Germ-free mice/rats can be protective in autoimmune hepatitis and liver cancer induced by chemicals,[61-63] while they can exacerbate acute liver injury and liver fibrosis.[64-66] The protective effects may be due to the deficient natural killer T cells in germ-free mice, which have lower levels of presented bacterial glycolipid antigens compared to conventional mice, and/or due to the absence of bacterial LPS and lack of Toll-like receptor 4 (TLR4) signaling in germ-free mice. On the other hand, germ-free mice are more sensitive to acute liver injury and liver regeneration. These effects may be also due to the absence of LPS, since LPS has been shown to stimulate liver regeneration.[67] In ALD, germ-free status can be associated with worsened or improved hepatic phenotype depending on the type of animal and the experimental models. Feeding germ-free Swiss mice alcohol for 7 days resulted in a reduced liver injury compared to conventional mice.[68] This was associated with a reduced intestinal permeability and lower neutrophil accumulation. On the other hand, C57BL/6 germ-free mice showed significantly more pronounced liver injury, inflammation and steatosis than conventional mice treated with acute alcohol administration.[69] The increased susceptibility to acute alcohol-induced liver injury in germ-free mice may be related to a more efficient xenobiotic metabolism, elevated baseline levels of inflammatory factors and up-regulated genes involved in lipid synthesis. The discrepancy between these two studies could be due to the different animal strains and germ-free status maintenance. Studies using different rodent strains in ALD have shown that the strain can have a significant effect on multiple aspects of ALD pathogenesis, including alcohol metabolism, inflammation, fat accumulation and stress response. It is also very difficult to sterilize the liquid Lieber DeCarli diet every day and to keep germ-free status in animal facilities.

To overcome the limitations of germ-free mice, microbiota humanized mice have been used in studies of ALD.[70] Humanization of mice using human intestinal microbiota transplants from ALD patients showed significant differences between germ-free and conventional

mice.[18] The germ-free mice receiving the intestinal microbiota from a patient with severe AH developed more severe liver inflammation, greater liver necrosis, greater intestinal permeability and more translocation of bacteria to the liver than in mice transplanted with intestinal microbiota from an alcoholic patient without AH. Interestingly, in conventional mice humanized with the intestinal microbiota from a severe AH patient, a second subsequent transfer of intestinal microbiota from patients without AH improved alcohol-induced liver lesions. Although such a strategy cannot be used in the clinical setting due to the ethical concerns, humanized microbiota animal models can offer a better understanding of the associations between microbiota and ALD. The importance of intestinal microbiota in ALD is further demonstrated by fecal microbiota transplantation (FMT) between mice that are housed in different facilities.[71] Two groups of mice were housed in two nearby facilities, and the mice in one facility developed ALD (alcohol-sensitive) and the mice in other facility did not (alcohol-resistant), despite a similar alcohol intake. Alcohol induced hepatic steatosis and liver inflammation, which were associated with gut dysbiosis in the alcohol-sensitive mice. Importantly, transplantation of intestinal microbiota from the resistant mice to the sensitive mice restored gut flora homeostasis and prevented the development of ALD.

3. Circulating microbiota dysbiosis and ALD

Microscopic studies using bacterial culture have demonstrated that many bacteria can survive in a dormant form in blood and inside of red blood cells, and these findings were further confirmed by next-generation sequencing techniques.[72-75] Changes in blood microbiota profiles have been linked to liver fibrosis in obese patients.[76] Recent study by Puri and colleagues[77] determined the circulating blood bacterial microbiota by qualitative and quantitative analyses of bacterial DNA in human subjects with moderate AH or severe AH, and compared the results with those from heavy drinkers without liver disease and non-drinkers. Multiple changes in the circulating microbiome were found in heavy alcohol consumers with AH and in heavy drinkers without obvious liver disease, and these changes are associated with a shift in the metabolic functions of bacteria. Patients with AH had higher levels of circulating bacterial DNA compared to non-drinkers and the drinkers without liver disease. All alcohol consumers had a significantly decreased Bacteroidetes, and an enrichment of Fusobacteria. Functional metagenomics analysis revealed that alcohol consumption associated with an increase of bacteria with genes related to methanogenesis and denitrification. Heavy alcohol consumption appears to be the primary driver of changes in the circulating microbiome associated with a shift in its inferred metabolic functions. More studies are required to elucidate the role of the circulating microbiome and its related metabolites in the pathogenesis of ALD. Especially, well-designed preclinical animal studies on the circulating microbiome will further help to gain insights into the mechanisms of the changes produced by alcohol consumption and liver diseases. Nevertheless, the blood microbiome analysis provides potential biomarkers for the detection of AH and other forms of liver disease.

4. Oral microbiota dysbiosis and ALD

Bacterial infection is frequently observed in patients with ALD. The oral cavity is home to several million bacteria that can cause periodontitis and systemic disease.[78] The oral

microbiota plays an important role in maintaining our health by forming a protective layer in the mouth, which prevents colonization by pathogens. If the microbial composition is disturbed, the oral microbiota may promote a state of disease in the oral or even extra-oral tissues such as the liver. The relation between periodontopathic bacteria and liver diseases has been explored in several studies. Oral microbiota dysbiosis is associated with autoimmune liver disease,[79] non-alcoholic fatty liver disease (NAFLD),[80] non-alcoholic steatohepatitis (NASH),[81] and liver cirrhosis.[82] The oral cavity could be an important under-defined source of inflammation in liver cirrhosis. The emerging role of the oral-gut axis in the development and progression of cirrhosis was highlighted by Bajaj *et al.*[83] in a recent review article. It is possible that the link between the liver and the oral cavity could be via the gut through impaired intestinal integrity that, in turn, could allow direct translocation of bacteria and/or their products and inflammatory mediators from the oral cavity to the systemic circulation.

Porphyromonas gingivalis (*P. gingivalis*) is a major pathogen of severe periodontal disease (PD). It functions as a keystone pathogen that not only sets the stage for the entire cascade of PD by altering the local immune microenvironment, but also enters the blood circulation and contributes to multiple systemic diseases.[84,85] *P. gingivalis* can also be swallowed with saliva and enter the intestine,[86,87] causing liver disease via the gut-liver axis.[88] Periodontitis is associated with increased hepatic fibrosis in human subjects with NAFLD and *P. gingivalis* worsens steatohepatitis in mice fed a high-fat diet. Interestingly, a program of dental hygiene therapy improved NAFLD in humans.[80,81,89]

Recent studies explored the association of oral disease and oral microbiome with alcohol consumption. Alcoholic patients have a pathogenic oral microbiome and worse PD than non-alcoholic patients,[90] and alcoholic patients with a smoking history have higher odds ratio of having PD.[91] More recently, a study showed an association of *P. gingivalis* with acute alcoholic hepatitis (AAH) development/progression.[88] Patients with severe AAH showed significantly higher plasma levels of IgG, IgA, and IgM against two *P. gingivalis* strains (W83 and 33277) compared to healthy controls. Patients with moderate AAH also had significantly elevated anti-*P. gingivalis* IgA concentrations of both strains compared to healthy controls. Male patients with moderate AAH showed a significant inverse association in lifetime drinking history (LTDH) and anti-*P. gingivalis* IgM. The aspartate aminotransferase (AST): alanine aminotransferase (ALT) ratio was positively associated with IgM of both *P. gingivalis* strains in male patients with moderate AAH. Female patients with severe AAH showed a significant association between MELD scores and W83 IgM. *P. gingivalis* may be associated with ALD and may function as a confounding factor in AAH. This study supports the concept that infection with oral *P. gingivalis* is associated with both progression and severity of AAH, and this association was modestly impacted by sex. Further studies are indicated to determine whether treatment of PD may help to prevent or attenuate ALD in humans and to understand the cause-effect relation between oral *P. gingivalis* and ALD.

5. Fungal dysbiosis and ALD

Although intestinal microbiome consists of bacteria, fungi and viruses, almost all the studies on ALD and the microbiome focus exclusively on gut bacteria. Dysbiosis of intestinal fungi, or mycobiota, has been associated with diseases such as colitis.[92-94] A recent study demonstrated that intestinal fungi contribute to the development of ALD.[95] There are significant changes in abundance and composition of intestinal fungi between alcohol-dependent patients and healthy controls. Fungal diversity was lower in alcoholics than controls. A dramatic overgrowth of *Candida*, with concomitant decreases in *Epicoccum*, *Galactomyces* and *Debaryomyces* was observed in alcoholics. Interestingly, the degree of exposure to fungal products correlates with mortality of patients with cirrhosis from alcohol abuse but not viral hepatitis. Although this study supports the importance of the mycobiota for patients with ALD, a larger, prospective study is required to confirm data from this retrospective analysis of human subjects.

Chronic alcohol feeding increased fungi populations and translocation of β-glucan, a fungi cell wall component, into systemic circulation in mice. Once relocated inside the liver, β-glucan binds certain immune cells and triggers inflammation, which ultimately promotes ALD. Treatment of alcohol-fed mice with an antifungal compound, amphotericin B, protects mice from alcohol-induced liver disease.[95] This study demonstrated a potential important role of fungal dysbiosis in the development of ALD. Manipulation of the intestinal mycobiome might be an effective strategy for treatment of alcohol-related liver disease.

6. Treatments targeting microbiome in ALD

Restoration of gut eubiosis is the major aim of gut-microbiota based therapies in ALD. Several approaches including diet modulation, probiotics, prebiotics, antibiotics, antifungals intervention, engineered-bacteria therapy, fecal bacteria transplantation and oral hygiene have proven to be effective in alleviating alcohol-induced liver injury through positively modifying gut microbiota.

Dietary supplements have received increasing interest in ALD management. It has been shown that unsaturated fat together with alcohol consumption is harmful in ALD. Supplementation of saturated fat (medium chain triglycerides enriched) in mice with chronic alcohol feeding restored gut microbiota profiles and reduced alcohol-induced gut barrier dysfunction.[45] Alcohol consumption is often associated with micronutrient deficiency, such as vitamins and zinc. Zinc deficiency occurs as early as after two weeks of alcohol feeding in mice, and alcohol-induced zinc deficiency could also be detected in the small intestine, and this exacerbates gut barrier dysfunction caused by alcohol. Supplementation with zinc restored microbiota and gut barrier function.[96,97]

Many other dietary supplements have been studied for the prevention/treatment of ALD in animal models. Dietary flaxseed oil was shown to protect the liver from alcohol-induced injury, likely through gut microbiota modulation.[98] Herbal supplements have also been used to target gut microbiota in ALD.[99]

It is logical that antibiotic use may restrict pathogenic bacteria in ALD management. Intestinal sterilization was used as a prevention strategy for alcohol-induced liver injury in rats as early in 1995.[100] Fecal culture of stool samples from ethanol-fed rats treated with antibiotics showed virtually no growth of Gram-negative bacteria, and rats receiving antibiotics had reduced endotoxin levels and liver injury.[100] Non-absorbable antibiotics have been used to reduce ALD in mice.[19] In patients with ALD, treatment with antibiotics led to an improvement in the Child-Pugh score and cirrhosis index.[101,102] These findings might be related to the reduction of endotoxemia resulting from intestinal decontamination, and highlighted the therapeutic potential of antibiotics in treating ALD. However, antibiotic treatment frequently induces diverse off-target side-effects by killing a wide set of microbes outside of the desired target, including increased susceptibility to pathogenic bacteria, such as *Clostridium difficile*.[103]

Another approach targeting microbiome dysbiosis in ALD is the use of probiotics. A variety of probiotic strains have been administrated to rodents for the prevention/treatment of experimental ALD.[104] Among those, *Lactobaciilus rhamnosus* GG (LGG) is the most frequently used strain. LGG administration to rodents on chronic alcohol exposure reduced liver injury and steatosis.[105] The beneficial effects of LGG were associated with gut microbiota modification, improved intestinal tight junction protein expression, and reduced serum endotoxin levels. VSL#3 was demonstrated to be effective in modulating gut microbiota and protecting against alcohol-induced intestinal barrier dysfunction.[106] A combination treatment using *Lactobacillus acidophilus*, *Lactobacillus helveticus* and *Bifidobacterium* in rats with alcoholic pancreatitis-related liver damage effectively protected against endotoxin/bacteria translocation, as well as liver damage in the course of acute pancreatitis and concomitant heavy alcohol consumption.[107]

While many reports have studied the effects of probiotics in experimental ALD, clinical data are limited. Effectiveness of the probiotic *Lactobacillus casei Shirota* on the alcoholic cirrhosis patients was evaluated in a small open-labeled study ($N = 12$).[108] Compared to the control group, cirrhotic patients who received the probiotics for 4 weeks had a significantly lower TLR4 expression as well as soluble tumor necrosis factor receptor 1 (sTNFR1) and sTNFR2 levels, along with a restored neutrophil phagocytic activity, suggesting that the probiotic is safe and may be effective in the treatment of patients with defective immunity. Administration of a synbiotic mixture of different bacteria strains improved liver damage and function in 10 alcoholic liver cirrhosis patients.[109] Notably, the effects seemed to be partially persistent. In humans, Kirpich *et al*.[31] demonstrated that after 5 days of treatment with *Bifidobacterium bifdum* and *Lactobacillus plantarum* 8PA3, mild AH patients had a significant end-of-treatment reduction of ALT, AST, lactate dehydrogenase and total bilirubin. These improvements were associated with changes in the fecal commensal bacteria *Bifidobacteria* and *Lactobacilli*.

Probiotic treatments on multiple pathological disorders are not always effective due to the viability of these beneficial bacteria.[110,111] Colonization of the gut is essential for live bacteria in order to confer their beneficial effects. However, in many disease conditions including ALD, there is an augmentation of pathogenic bacteria, which could dampen the ability of probiotics to colonize the gut. Drugs, in particular antibiotics, used by patients may

be harmful to live probiotics. Therefore, an unstable and variable effect of live probiotics may occur. Moreover, the clinically recommended dose of probiotics usually consists of billions of live bacteria, and ingesting such large amounts of bacteria raises safety concerns, especially for patients with compromised intestinal function and immune response.[112,113]

In fact, soluble factors secreted from probiotics and dead probiotics have been shown to be effective in treating several diseases conditions such as inflammatory bowel disease, colitis, and arthritis.[114-116] Yan *et al*.[117] demonstrated that soluble proteins produced by probiotic bacteria regulate intestinal epithelial cell survival and growth. Oral administration of heat-killed *Lactobacillus brevis* SBC8803 has also been shown to ameliorate ALD in mice by inducing the expression of cytoprotective heat shock proteins and improving intestinal barrier function.[118] The effectiveness of LGG culture supernatant in the prevention of acute and chronic alcohol-induced hepatic steatosis and liver injury has been investigated by our group.[50,119,120] Pretreatment with LGG supernatant (LGG-s) reduced hepatic fat accumulation in mice in an acute-binge alcohol model.[50] We also demonstrated that co-administration of LGG-s with alcohol using the Lieber DeCarli liquid diet for 4 weeks significantly prevented alcohol-induced intestinal barrier dysfunction, endotoxemia, fatty liver and inflammation in mice.[119,120] The use of probiotic culture supernatant opens a new avenue for probiotic application. Further characterization of the LGG-s active components will enhance our understanding of the protective effects of probiotics in ALD and advance the development of new therapeutic strategies for ALD.

Genetically modified-bacteria have also been evaluated for the treatment of ALD. Administration of engineered *Lactobacillus reuteri* to produce interleukin-22 (IL-22) (*L. reuteri*/IL-22) in mice on chronic alcohol exposure reduced liver damage, inflammation and bacterial translocation to the liver compared with mice fed an isogenic control strain. The beneficial effects were associated with up-regulated intestinal expression of Reg3G, which plays a pivotal role in gut microbiota homeostasis.[121]

FMT as a therapeutic strategy has been used in disease treatment involving intestinal bacterial infection.[122] Preclinical studies showed that transplanting feces from alcohol-resistant donor mice to alcohol-sensitive recipient mice resulted in prevention of alcohol-induced gut dysbiosis and hepatic steatohepatitis.[71,123,124] There also have been small studies suggesting beneficial effects of fecal transplantation in AH and in patients with hepatic encephalopathy.[125,126] Nevertheless, this approach should be cautiously applied to the therapy of ALD patients in the future. Recent reports of one patient's death and another patient suffering an invasive infection caused by extended-spectrum β-Lactamase-producing Escherichia Coli after receiving fecal microbiota transplants from the same donor has raised an alert on FMT. The Food and Drug Administration (FDA) has therefore halted clinical trials on fecal microbial transplantation due to this occurrence.

7. Summary

Increasing evidence suggests that the bacterial microbiome has a major role in ALD. Alcohol-induced bacterial microbiome alterations are associated with changes of bacterial pathogen-derived molecules, intestinal immune defenses, Paneth and Goblet cell secretion,

intestinal tight junction protein expression, and integrity of gut barrier. All of these factors appear to play an integrated role in the development/progression of ALD. On the other hand, liver metabolism and immune regulation in ALD seem to affect intestinal bacterial microbiome. Although we have some understanding of the interactions between the intestinal bacterial microbiome and the host, further studies to better characterize microbiome changes and the interactions between these components are needed in additional to animal models to mimic different stages of ALD and in larger cohorts of patients. Routine monitoring of the changes in the bacterial microbiome may help to develop more personalized approach targeting microbiome for the prevention/treatment of ALD in different stages. In addition, the intestinal fungal microbiome and oral bacterial microbiome seem to also play an important role in ALD. Although we are still in the early stages in understanding the role of fungal microbiome and oral bacterial microbiome in ALD, targeting these microbiome communities provides additional opportunities to develop approaches for ALD treatment (Fig. 1).

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References

1. Lloyd-Price J, Abu-Ali G, Huttenhower C. The healthy human microbiome. Genome Med. 2016;8:51. [PubMed: 27122046]
2. Xu Z, Knight R. Dietary effects on human gut microbiome diversity. Br J Nutr. 2015;113:S1–S5.
3. Takiishi T, Fenero CIM, Câmara NOS. Intestinal barrier and gut microbiota: shaping our immune responses throughout life. Tissue Barriers. 2017;5, e1373208. [PubMed: 28956703]
4. Karlsson FH, Ussery DW, Nielsen J, Nookaew I. A closer look at bacteroides: phylogenetic relationship and genomic implications of a life in the human gut. Microb Ecol. 2011;61:473–485. [PubMed: 21222211]
5. Kosumi K, Mima K, Baba H, Ogino S. Dysbiosis of the gut microbiota and colorectal cancer: the key target of molecular pathological epidemiology. J Lab Precis Med. 2018;3:76. 10.21037/jlpm.2018.09.05. [PubMed: 30345420]
6. Lazar V, Ditu LM, Pircalabioru GG, et al. Aspects of gut microbiota and immune system interactions in infectious diseases, immunopathology, and cancer. Front Immunol. 2018;9:1830. [PubMed: 30158926]
7. Zhuang H, Cheng L, Wang Y, et al. Dysbiosis of the gut microbiome in lung cancer. Front Cell Infect Microbiol. 2019;9:112. [PubMed: 31065547]
8. Zou S, Fang L, Lee MH. Dysbiosis of gut microbiota in promoting the development of colorectal cancer. Gastroenterol Rep (Oxf). 2018;6:1–12. [PubMed: 29479437]
9. Lau K, Srivatsav V, Rizwan A, et al. Bridging the gap between gut microbial dysbiosis and cardiovascular diseases. Nutrients. 2017;9:E859. 10.3390/nu9080859. [PubMed: 28796176]
10. Tang WH, Kitai T, Hazen SL. Gut microbiota in cardiovascular health and disease. Circ Res. 2017;120:1183–1196. [PubMed: 28360349]
11. Yoshida N, Yamashita T, Hirata KI. Gut microbiome and cardiovascular diseases. Diseases. 2018;6. 10.3390/diseases6030056. E56.
12. Schnabl B, Brenner DA. Interactions between the intestinal microbiome and liver diseases. Gastroenterology. 2014;146:1513–1524. [PubMed: 24440671]
13. Llorente C, Schnabl B. The gut microbiota and liver disease. Cell Mol Gastroenterol Hepatol. 2015;1:275–284. [PubMed: 26090511]

14. Acharya C, Bajaj JS. Gut microbiota and complications of liver disease. Gastroenterol Clin N Am. 2017;46:155–169.
15. Sarin SK, Pande A, Schnabl B. Microbiome as a therapeutic target in alcohol-related liver disease. J Hepatol. 2019;70:260–272. [PubMed: 30658727]
16. Bajaj JS. Alcohol, liver disease and the gut microbiota. Nat Rev Gastroenterol Hepatol. 2019;16:235–246. [PubMed: 30643227]
17. Hartmann P, Chu H, Duan Y, Schnabl B. Gut microbiota in liver disease: too much is harmful, nothing at all is not helpful either. Am J Physiol Gastrointest Liver Physiol. 2019;316:G563–G573. [PubMed: 30767680]
18. Llopis M, Cassard AM, Wrzosek L, et al. Intestinal microbiota contributes to individual susceptibility to alcoholic liver disease. Gut. 2016;65:830–839. [PubMed: 26642859]
19. Shao T, Zhao C, Li F, et al. Intestinal HIF-1alpha deletion exacerbates alcoholic liver disease by inducing intestinal dysbiosis and barrier dysfunction. J Hepatol. 2018;69:886–895. [PubMed: 29803899]
20. Nistal E, Sáenz de Miera LE, Ballesteros Pomar M, et al. An altered fecal microbiota profile in patients with non-alcoholic fatty liver disease (NAFLD) associated with obesity. Rev Esp Enferm Dig. 2019;111:275–282. [PubMed: 30810328]
21. Li X, Watanabe K, Kimura I. Gut microbiota dysbiosis drives and implies novel therapeutic strategies for diabetes mellitus and related metabolic diseases. Front Immunol. 2017;8:1882. [PubMed: 29326727]
22. Aw W, Fukuda S. Understanding the role of the gut ecosystem in diabetes mellitus. J Diabetes Investig. 2018;9:5–12.
23. Sun J, Furio L, Mecheri R, et al. Pancreatic β-cells limit autoimmune diabetes via an immunoregulatory antimicrobial peptide expressed under the influence of the gut microbiota. Immunity. 2015;43:304–317. [PubMed: 26253786]
24. Gao B, Bataller R. Alcoholic liver disease: pathogenesis and new therapeutic targets. Gastroenterology. 2011;141:1572–1585. [PubMed: 21920463]
25. McLoughlin RM, Mills KH. Influence of gastrointestinal commensal bacteria on the immune responses that mediate allergy and asthma. J Allergy Clin Immunol. 2011;127:1097–1107. quiz 1108-1099. [PubMed: 21420159]
26. Parlesak A, Schäfer C, Schütz T, Bode JC, Bode C. Increased intestinal permeability to macromolecules and endotoxemia in patients with chronic alcohol abuse in different stages of alcohol-induced liver disease. J Hepatol. 2000;32: 742–747. [PubMed: 10845660]
27. Bajaj JS, Heuman DM, Hylemon PB, et al. Altered profile of human gut microbiome is associated with cirrhosis and its complications. J Hepatol. 2014;60:940–947. [PubMed: 24374295]
28. Bode JC, Bode C, Heidelbach R, Dürr HK, Martini GA. Jejunal microflora in patients with chronic alcohol-abuse. Hepato-Gastroenterology. 1984;31: 30–34. [PubMed: 6698486]
29. Bode C, Kolepke R, Schäfer K, Bode JC. Breath hydrogen excretion in patients with alcoholic liver-disease-evidence of small intestinal bacterial overgrowth. Z Gastroenterol. 1993;31:3–7. [PubMed: 8447153]
30. Morencos FC, de las Heras Castaño G, Martín Ramos L, López Arias MJ, Ledesma F, Pons Romero F. Small-bowel bacterial overgrowth in patients with alcoholic cirrhosis. Dig Dis Sci. 1995;40:1252–1256. [PubMed: 7781442]
31. Kirpich IA, Solovieva NV, Leikhter SN, et al. Probiotics restore bowel flora and improve liver enzymes in human alcohol-induced liver injury: a pilot study. Alcohol. 2008;42:675–682. [PubMed: 19038698]
32. Bhonchal S, Nain CK, Taneja N, et al. Modification of small bowel microflora in chronic alcoholics with alcoholic liver disease. Trop Gastroenterol. 2007;28: 64–66. [PubMed: 18050841]
33. Gabbard SL, Lacy BE, Levine GM, Crowell MD. The impact of alcohol consumption and cholecystectomy on small intestinal bacterial overgrowth. Dig Dis Sci. 2014;59:638–644. [PubMed: 24323179]
34. Bajaj JS, Hylemon P, Heuman DM, Sanyal AJ, Gillevet PM. The cirrhosis dysbiosis ratio provides insight into gut microbiome changes across the spectrum of cirrhosis: a prospective study of 250 patients. Hepatology. 2013;58:274a.

35. Dubinkina VB, Tyakht AV, Odintsova VY, et al. Links of gut microbiota composition with alcohol dependence syndrome and alcoholic liver disease. Microbiome. 2017;5:141. [PubMed: 29041989]
36. Mutlu EA, Gillevet PM, Rangwala H, et al. Colonic microbiome is altered in alcoholism. Am J Physiol Gastrointest Liver Physiol. 2012;302:G966–G978. [PubMed: 22241860]
37. Mutlu E, Keshavarzian A, Engen P, Forsyth CB, Sikaroodi M, Gillevet P. Intestinal dysbiosis: a possible mechanism of alcohol-induced endotoxemia and alcoholic steatohepatitis in rats. Alcohol Clin Exp Res. 2009;33:1836–1846. [PubMed: 19645728]
38. Leclercq S, Matamoros S, Cani PD, et al. Intestinal permeability, gut-bacterial dysbiosis, and behavioral markers of alcohol-dependence severity. Proc Natl Acad Sci U S A. 2014;111:E4485–E4493. [PubMed: 25288760]
39. Couch RD, Dailey A, Zaidi F, et al. Alcohol induced alterations to the human fecal VOC metabolome. PLoS One. 2015;10, e0119362. [PubMed: 25751150]
40. Yan AW, Fouts DE, Brandl J, et al. Enteric dysbiosis associated with a mouse model of alcoholic liver disease. Hepatology. 2011;53:96–105. [PubMed: 21254165]
41. Bull-Otterson L, Feng WK, Kirpich I, et al. Metagenomic analyses of alcohol induced pathogenic alterations in the intestinal microbiome and the effect of Lactobacillus rhamnosus GG treatment. PLoS One. 2013;8, e53028. [PubMed: 23326376]
42. Xie G, Zhong W, Zheng X, et al. Chronic ethanol consumption alters mammalian gastrointestinal content metabolites. J Proteome Res. 2013;12: 3297–3306. [PubMed: 23763674]
43. Shi X, Wei X, Yin X, et al. Hepatic and fecal metabolomic analysis of the effects of Lactobacillus rhamnosus GG on alcoholic fatty liver disease in mice. J Proteome Res. 2015;14:1174–1182. [PubMed: 25592873]
44. Chen P, Torralba M, Tan J, et al. Supplementation of saturated long-chain fatty acids maintains intestinal eubiosis and reduces ethanol-induced liver Injury in mice. Gastroenterology. 2015;148:203–214 (e16). [PubMed: 25239591]
45. Kirpich IA, Petrosino J, Ajami N, et al. Saturated and unsaturated dietary fats differentially modulate ethanol-induced changes in gut microbiome and metabolome in a mouse model of alcoholic liver disease. Am J Pathol. 2016;186:765–776. [PubMed: 27012191]
46. He L, Li F, Yin X, et al. Profiling of polar metabolites in mouse feces using four analytical platforms to study the effects of cathelicidin-related antimicrobial peptide in alcoholic liver disease. J Proteome Res. 2019;18:2875–2884. [PubMed: 31188604]
47. Bluemel S, Wang L, Kuelbs C, et al. Intestinal and hepatic microbiota changes associated with chronic ethanol administration in mice. Gut Microb. 2019: 1–11. 10.1080/19490976.2019.1595300 ([Epub ahead of print]).
48. Hartmann P, Chen P, Wang HJ, et al. Deficiency of intestinal mucin-2 ameliorates experimental alcoholic liver disease in mice. Hepatology. 2013;58: 108–119. [PubMed: 23408358]
49. Wang LR, Fouts DE, Stärkel P, et al. Intestinal REG3 lectins protect against alcoholic steatohepatitis by reducing mucosa-associated microbiota and preventing bacterial translocation. Cell Host Microbe. 2016;19:227–239. [PubMed: 26867181]
50. Wang Y, Liu Y, Sidhu A, Ma Z, McClain C, Feng W. Lactobacillus rhamnosus GG culture supernatant ameliorates acute alcohol-induced intestinal permeability and liver injury. Am J Physiol Gastrointest Liver Physiol. 2012;303: G32–G341. [PubMed: 22538402]
51. Li F, Shao T, McClain C, Feng W. CRAMP deletion exacerbates chronic-binge alcohol-induced liver steatosis and injury via regulation of gut microbiota and hepatic inflammation. FASEB J. 2017;31:994–997.
52. Kirpich IA, Warner DR, Feng W, et al. Mechanisms, biomarkers and targets for therapy in alcohol-associated liver injury: from genetics to nutrition: summary of the ISBRA 2018 symposium. pii: S0741-8329 Alcohol. 2019;30069–2(19). 10.1016/j.alcohol.2019.05.004 ([Epub ahead of print]).
53. Xie G, Zhong W, Li H, et al. Alteration of bile acid metabolism in the rat induced by chronic ethanol consumption. FASEB J. 2013;27:3583–3593. [PubMed: 23709616]
54. Hartmann P, Hochrath K, Horvath A, et al. Modulation of the intestinal bile acid/farnesoid X receptor/fibroblast growth factor 15 axis improves alcoholic liver disease in mice. Hepatology. 2018;67:2150–2166. [PubMed: 29159825]

55. Ridlon JM, Kang DJ, Hylemon PB. Bile salt biotransformations by human intestinal bacteria. J Lipid Res. 2006;47:241–259. [PubMed: 16299351]
56. Yang Z, Kusumanchi P, Ross RA, et al. Serum metabolomic profiling identifies key metabolic signatures associated with pathogenesis of alcoholic liver disease in humans. Hepatol Commun. 2019;3:542–557. [PubMed: 30976744]
57. Donepudi AC, Ferrell JM, Boehme S, Choi HS, Chiang JYL. Deficiency of cholesterol 7α-hydroxylase in bile acid synthesis exacerbates alcohol-induced liver injury in mice. Hepatol Commun. 2017;2:99–112. [PubMed: 29404516]
58. Brandl K, Hartmann P, Jih LJ, et al. Dysregulation of serum bile acids and FGF19 in alcoholic hepatitis. J Hepatol. 2018;69:396–405. [PubMed: 29654817]
59. Wang H, Yan Y, Yi X, et al. Histopathological features and composition of gut microbiota in rhesus monkey of alcoholic liver disease. Front Microbiol. 2019;10:165. [PubMed: 30800107]
60. Fujisawa K, Takami T, Nagatomo T, et al. Usefulness of adult medaka fish as a model for the evaluation of alcoholic fatty liver. Alcohol. 2019;77:147–154. [PubMed: 30660600]
61. Wei Y, Zeng B, Chen J, et al. Enterogenous bacterial glycolipids are required for the generation of natural killer T cells mediated liver injury. Sci Rep. 2016;6: 36365. [PubMed: 27821872]
62. Laqueur GL, Matsumoto H, Yamamoto RS. Comparison of the carcinogenicity of methylazoxymethanol-beta-D-glucosiduronic acid in conventional and germfree Sprague-Dawley rats. J Natl Cancer Inst. 1981;67:1053–1055. [PubMed: 6946247]
63. Dapito DH, Mencin A, Gwak GY, et al. Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. Cancer Cell. 2012;21:504–516. [PubMed: 22516259]
64. Sumi Y, Miyakawa M. Susceptibility of germ-free rats to the hepatotoxic effects of dimethylnitrosamine or dimethylamine plus sodium nitrite administered orally. Cancer Res. 1983;43:2942–2946. [PubMed: 6850604]
65. Bauer H, Paronetto F, Porro RF, Einheber A, Popper H. The influence of the microbial flora on liver injury and associated serum gamma-globulin elevation. A study in germ-free rats treated with 3'-methyl-4-dimethylaminoazobenzene. Lab Investig. 1967;16:847–857. [PubMed: 4168350]
66. Mazagova M, Wang LR, Anfora AT, et al. Commensal microbiota is hepatoprotective and prevents liver fibrosis in mice. FASEB J. 2015;29: 1043–1055. [PubMed: 25466902]
67. Cornell RP, Liljequist BL, Bartizal KF. Depressed liver regeneration after partial hepatectomy of germ-free, athymic and lipopolysaccharide-resistant mice. Hepatology. 1990;11:916–922. [PubMed: 2194922]
68. CCC M, LL N, MF C, et al. Comparing the effects of acute alcohol consumption in germ-free and conventional mice: the role of the gut microbiota. BMC Microbiol. 2014;14:240. [PubMed: 25223989]
69. Chen P, Miyamoto Y, Mazagova M, Lee KC, Eckmann L, Schnabl B. Microbiota protects mice against acute alcohol-induced liver injury. Alcohol Clin Exp Res. 2015;39:2313–2323. [PubMed: 26556636]
70. Wrzosek L, Ciocan D, Borentain P, et al. Transplantation of human microbiota into conventional mice durably reshapes the gut microbiota. Sci Rep. 2018;8: 6854. [PubMed: 29717179]
71. Ferrere G, Wrzosek L, Cailleux F, et al. Fecal microbiota manipulation prevents dysbiosis and alcohol-induced liver injury in mice. J Hepatol. 2017;66: 806–815. [PubMed: 27890791]
72. Nikkari S, McLaughlin IJ, Bi W, Dodge DE, Relman DA. Does blood of healthy subjects contain bacterial ribosomal DNA? J Clin Microbiol. 2001;39: 1956–1959. [PubMed: 11326021]
73. McLaughlin RW, Vali H, Lau PC, et al. Are there naturally occurring pleomorphic bacteria in the blood of healthy humans? J Clin Microbiol. 2002;40: 4771–4775. [PubMed: 12454193]
74. Potgieter M, Bester J, Kell DB, Pretorius E. The dormant blood microbiome in chronic, inflammatory diseases. FEMS Microbiol Rev. 2015;39:567–591. [PubMed: 25940667]
75. Damgaard C, Magnussen K, Enevold C, et al. Viable bacteria associated with red blood cells and plasma in freshly drawn blood donations. PLoS One. 2015;10, e0120826. [PubMed: 25751254]
76. Lelouvier B, Servant F, Païssé S, et al. Changes in blood microbiota profiles associated with liver fibrosis in obese patients: a pilot analysis. Hepatology. 2016;64:2015–2027. [PubMed: 27639192]

77. Puri P, Liangpunsakul S, Christensen JE, et al. The circulating microbiome signature and inferred functional metagenomics in alcoholic hepatitis. Hepatology. 2018;67:1284–1302. [PubMed: 29083504]

78. Kumar PS. Oral microbiota and systemic disease. Anaerobe. 2013;24:90–93. [PubMed: 24128801]

79. Abe K, Takahashi A, Fujita M, et al. Dysbiosis of oral microbiota and its association with salivary immunological biomarkers in autoimmune liver disease. PLoS One. 2018;13, e0198757. [PubMed: 29969462]

80. Yoneda M, Naka S, Nakano K, et al. Involvement of a periodontal pathogen, Porphyromonas gingivalis on the pathogenesis of non-alcoholic fatty liver disease. BMC Gastroenterol. 2012;12:16. [PubMed: 22340817]

81. Furusho H, Miyauchi M, Hyogo H, et al. Dental infection of Porphyromonas gingivalis exacerbates high fat diet-induced steatohepatitis in mice. J Gastroenterol. 2013;48:1259–1270. [PubMed: 23307045]

82. Bajaj JS, Matin P, White MB, et al. Periodontal therapy favorably modulates the oral-gut-hepatic axis in cirrhosis. Am J Physiol Gastrointest Liver Physiol. 2018;315:G824–G837. [PubMed: 30118351]

83. Acharya C, Sahingur SE, Bajaj JS. Microbiota, cirrhosis, and the emerging oral-gut-liver axis. JCI Insight. 2017;2:94416. 10.1172/jci.insight.94416. [PubMed: 28978799]

84. Hajishengallis G, Darveau RP, Curtis MA. The keystone-pathogen hypothesis. Nat Rev Microbiol. 2012;10:717–725. [PubMed: 22941505]

85. Hajishengallis G. Periodontitis: from microbial immune subversion to systemic inflammation. Nat Rev Immunol. 2015;15:30–44. [PubMed: 25534621]

86. Saygun I, Nizam N, Keskiner I, et al. Salivary infectious agents and periodontal disease status. J Periodontal Res. 2011;46:235–239. [PubMed: 21261620]

87. Atarashi K, Suda W, Luo C, et al. Ectopic colonization of oral bacteria in the intestine drives TH1 cell induction and inflammation. Science. 2017;358: 359–365. [PubMed: 29051379]

88. Zhou Y, Vatsalya V, Gobejishvili L, Lamont RJ, McClain CJ, Feng W. Porphyromonas gingivalis as a possible risk factor in the development/severity of acute alcoholic hepatitis. Hepatol Commun. 2018;3:293–304. [PubMed: 30766965]

89. Han P, Sun D, Yang J. Interaction between periodontitis and liver diseases. Biomed Rep. 2016;5:267–276. [PubMed: 27588170]

90. Fan X, Peters BA, Jacobs EJ, et al. Drinking alcohol is associated with variation in the human oral microbiome in a large study of American adults. Microbiome. 2018;6:59. [PubMed: 29685174]

91. Lages EJ, Costa FO, Lages EM, et al. Risk variables in the association between frequency of alcohol consumption and periodontitis. J Clin Periodontol. 2012;39:115–122. [PubMed: 22092940]

92. Chiaro TR, Soto R, Zac Stephens W, et al. A member of the gut mycobiota modulates host purine metabolism exacerbating colitis in mice. Sci Transl Med. 2017;9. 10.1126/scitranslmed.aaf9044.eaaf9044. [PubMed: 28275154]

93. Iliev ID, Leonardi I. Fungal dysbiosis: immunity and interactions at mucosal barriers. Nat Rev Immunol. 2017;17:635–646. [PubMed: 28604735]

94. Mogilnicka I, Ufnal M. Gut mycobiota and fungal metabolites in human homeostasis. Curr Drug Targets. 2019;20:232–240. [PubMed: 30047327]

95. Yang AM, Inamine T, Hochrath K, et al. Intestinal fungi contribute to development of alcoholic liver disease. J Clin Investig. 2017;127:2829–2841. [PubMed: 28530644]

96. Zhong W, McClain CJ, Cave M, Kang YJ, Zhou Z. The role of zinc deficiency in alcohol-induced intestinal barrier dysfunction. Am J Physiol Gastrointest Liver Physiol. 2010;298:G625–G633. [PubMed: 20167873]

97. Vatsalya V, Kong M, Cave MC, et al. Association of serum zinc with markers of liver injury in very heavy drinking alcohol-dependent patients. J Nutr Biochem. 2018;59:49–55. [PubMed: 29960116]

98. Zhang X, Wang H, Yin P, Fan H, Sun L, Liu Y. Flaxseed oil ameliorates alcoholic liver disease via anti-inflammation and modulating gut microbiota in mice. Lipids Health Dis. 2017;16:44. [PubMed: 28228158]

99. Lu KH, Liu CT, Raghu R, Sheen LY. Therapeutic potential of Chinese herbal medicines in alcoholic liver disease. J Tradit Complement Med. 2012;2: 115–122. [PubMed: 24716123]
100. Adachi Y, Moore LE, Bradford BU, Gao W, Thurman RG. Antibiotics prevent liver injury in rats following long-term exposure to ethanol. Gastroenterology. 1995;108:218–224. [PubMed: 7806045]
101. Madrid AM, Hurtado C, Venegas M, Cumsille F, Defilippi C. Long-Term treatment with cisapride and antibiotics in liver cirrhosis: effect on small intestinal motility, bacterial overgrowth, and liver function. Am J Gastroenterol. 2001;96: 1251–1255. [PubMed: 11316178]
102. Bajaj JS, Barrett AC, Bortey E, Paterson C, Forbes WP. Prolonged remission from hepatic encephalopathy with rifaximin: results of a placebo crossover analysis. Aliment Pharmacol Ther. 2015;41:39–45. [PubMed: 25339518]
103. National Academies of Sciences, Engineering, and Medicine, ed. The Chemistry of Microbiomes: Proceedings of a Seminar Series. Washington, DC: The National Academies Press; 2017. 10.17226/24751.
104. Li F, Duan K, Wang C, McClain C, Feng W. Probiotics and alcoholic liver disease: treatment and potential mechanisms. Gastroenterol Res Pract. 2016;2016:5491465. [PubMed: 26839540]
105. Nanji AA, Khettry U, Sadrzadeh SM. Lactobacillus feeding reduces endotoxemia and severity of experimental alcoholic liver (disease). Proc Soc Exp Biol Med. 1994;205:243–247. [PubMed: 8171045]
106. Chang B, Sang L, Wang Y, Tong J, Zhang D, Wang B. The protective effect of VSL#3 on intestinal permeability in a rat model of alcoholic intestinal injury. BMC Gastroenterol. 2013;13:151. [PubMed: 24138544]
107. Marotta F, Barreto R, Wu CC, et al. Experimental acute alcohol pancreatitis-related liver damage and endotoxemia: synbiotics but not metronidazole have a protective effect. Chin J Dig Dis. 2005;6:193–197. [PubMed: 16246229]
108. Stadlbauer V, Mookerjee RP, Hodges S, Wright GA, Davies NA, Jalan R. Effect of probiotic treatment on deranged neutrophil function and cytokine responses in patients with compensated alcoholic cirrhosis. J Hepatol. 2008;48:945–951. [PubMed: 18433921]
109. Loguercio C, De Simone T, Federico A, et al. Gut-liver axis: a new point of attack to treat chronic liver damage? Am J Gastroenterol. 2002;97:2144–2146. [PubMed: 12190198]
110. Wiest R, Chen F, Cadelina G, Groszmann RJ, Garcia-Tsao G. Effect of Lactobacillus-fermented diets on bacterial translocation and intestinal flora in experimental prehepatic portal hypertension. Dig Dis Sci. 2003;48: 1136–1141. [PubMed: 12822876]
111. Bauer TM, Fernández J, Navasa M, Vila J, Rodés J. Failure of lactobacillus spp. to prevent bacterial translocation in a rat model of experimental cirrhosis. J Hepatol. 2002;36:501–506. [PubMed: 11943421]
112. Tandon P, Moncrief K, Madsen K, et al. Effects of probiotic therapy on portal pressure in patients with cirrhosis: a pilot study. Liver Int. 2009;29: 1110–1115. [PubMed: 19490420]
113. Pereg D, Kotliroff A, Gadoth N, Hadary R, Lishner M, Kitay-Cohen Y. Probiotics for patients with compensated liver cirrhosis: a double-blind placebo-controlled study. Nutrition. 2011;27:177–181. [PubMed: 20452184]
114. Yan F, Polk DB. Characterization of a probiotic-derived soluble protein which reveals a mechanism of preventive and treatment effects of probiotics on intestinal inflammatory diseases. Gut Microb. 2012;3:25–28.
115. Zakostelska Z, Kverka M, Klimesova K, et al. Lysate of probiotic Lactobacillus casei DN-114 001 ameliorates colitis by strengthening the gut barrier function and changing the gut microenvironment. PLoS One. 2011;6, e27961. [PubMed: 22132181]
116. Nowak B, Ciszek-Lenda M, Sróttek M, et al. Lactobacillus rhamnosus exopolysaccharide ameliorates arthritis induced by the systemic injection of collagen and lipopolysaccharide in DBA/1 mice. Arch Immunol Ther Exp. 2012;60:211–220.
117. Yan F, Liu L, Dempsey PJ, et al. A Lactobacillus rhamnosus GG-derived soluble protein, p40, stimulates ligand release from intestinal epithelial cells to transactivate epidermal growth factor receptor. J Biol Chem. 2013;288: 30742–30751. [PubMed: 24043629]

118. Segawa S, Wakita Y, Hirata H, Watari J. Oral administration of heat-killed Lactobacillus brevis SBC8803 ameliorates alcoholic liver disease in ethanol-containing diet-fed C57BL/6N mice. Int J Food Microbiol. 2008;128:371–377. [PubMed: 18976829]

119. Zhao H, Zhao C, Dong Y, et al. Inhibition of miR122a by Lactobacillus rhamnosus GG culture supernatant increases intestinal occludin expression and protects mice from alcoholic liver disease. Toxicol Lett. 2015;234:194–200. [PubMed: 25746479]

120. Zhang M, Wang C, Wang C, et al. Enhanced AMPK phosphorylation contributes to the beneficial effects of Lactobacillus rhamnosus GG supernatant on chronic-alcohol-induced fatty liver disease. J Nutr Biochem. 2015;26:337–344. [PubMed: 25622859]

121. Hendrikx T, Duan Y, Wang Y, et al. Bacteria engineered to produce IL-22 in intestine induce expression of REG3G to reduce ethanol-induced liver disease in mice. Gut. 2019;68:1504–1515. [PubMed: 30448775]

122. van Nood E, Vrieze A, Nieuwdorp M, et al. Duodenal infusion of donor feces for recurrent Clostridium difficile. N Engl J Med. 2013;368:407–415. [PubMed: 23323867]

123. Borody TJ, Campbell J. Fecal microbiota transplantation: techniques, applications, and issues. Gastroenterol Clin N Am. 2012;41:781–803.

124. Tian Z, Liu J, Liao M, et al. Beneficial effects of fecal microbiota transplantation on ulcerative colitis in mice. Dig Dis Sci. 2016;61:2262–2271. [PubMed: 26846120]

125. Bajaj JS, Kassam Z, Fagan A, et al. Fecal microbiota transplant from a rational stool donor improves hepatic encephalopathy: a randomized clinical trial. Hepatology. 2017;66:1727–1738. [PubMed: 28586116]

126. Philips CA, Pande A, Shasthry SM, et al. Healthy donor fecal microbiota transplantation in steroid-ineligible severe alcoholic hepatitis: a pilot study. Clin Gastroenterol Hepatol. 2017;15:600–602. [PubMed: 27816755]

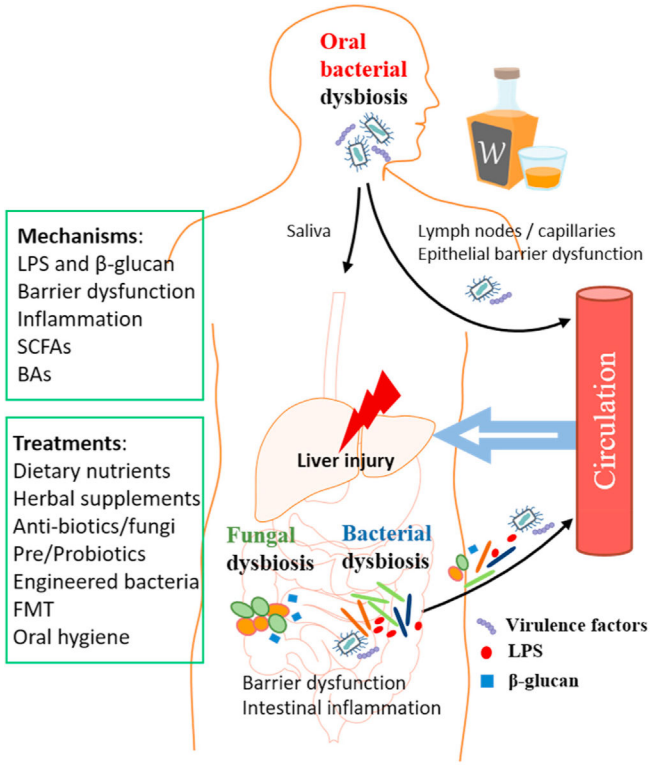

Fig. 1. Bacterial and fungal microbiome in ALD.

Alcohol consumption causes intestinal and oral microbiome (bacterial and fungal) dysbiosis and alterations in the products of these microorganisms. Alcohol-caused damages in intestinal and oral barrier dysfunction allows the translocation of bacteria and fungi and the harmful metabolites to the liver and causes liver injury. Circulating microbiome could serve as biomarker of ALD development/progression. Targeting intestinal and oral microbiome provides a potential strategy for prevention/treatment of ALD. Abbreviations: LPS, lipopolysaccharide; SCFAs, short chain fatty acids; BAs, bile acids; FMT, fecal microbiota transplantation.