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REVIEW

The Gut Microbiota and Liver Disease

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SUMMARY

Composition of healthy microbiota can be compromised by certain factors leading to dysbiosis, gut barrier dysfunction, and liver disease. A more detailed picture of the intestinal microbiota contributing to liver disease beyond increasing intestinal permeability has started to evolve.

The leaky gut hypothesis links translocating microbial products with the onset and progression of liver disease, and for a long time they were considered one of its major contributors. However, a more detailed picture of the intestinal microbiota contributing to liver disease started to evolve. The gut is colonized by trillions of microbes that aid in digestion, modulate immune response, and generate a variety of products that result from microbial metabolic activities. These products together with host-bacteria interactions influence both normal physiology and disease susceptibility. A disruption of the symbiosis between microbiota and host is known as dysbiosis and can have profound effects on health. Qualitative changes such as increased proportions of harmful bacteria and reduced levels of beneficial bacteria, and also quantitative changes in the total amount of bacteria (overgrowth) have been associated with liver disease. Understanding the link between the pathophysiology of liver diseases and compositional and functional changes of the microbiota will help in the design of innovative therapies. In this review, we focus on factors resulting in dysbiosis, and discuss how dysbiosis can disrupt intestinal homeostasis and contribute to liver disease. *(Cell Mol Gastroenterol Hepatol 2015;1:275–284; http://dx.doi.org/10.1016/j.jcmgh.2015.04.003)*

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The gut microbiota is composed of 100 trillion bacteria of diverse taxonomy (2000 distinct species). The microbiota has a collective genome (microbiome) that has 150-fold more genes than the human genome as determined with high-throughput DNA sequencing.[1] Bacteria provide a variety of beneficial products that result from metabolic activities. These products are essential nutrients and maximize the efficiency of energy harvest from ingested food and together with host-bacteria interactions influence both normal physiology and disease susceptibilities.[2–4] Gut microbiota homeostasis is tightly regulated by environmental and genetic factors and a specialized mucosal immune system. Host immunity and microbiota are dependent on each other.[4,5] Disruption of the microbial homeostasis is associated with obesity,[6] malnutrition,[7] inflammatory bowel diseases (IBD),[8] neurologic disorders,[9] cancer,[10] and liver diseases,[11–16] among others.

Here we describe recent advances in understanding gut microbiota composition and review how disruption of microbial and intestinal homeostasis contributes to the most prevalent chronic liver diseases: nonalcoholic fatty liver disease (NAFLD) and steatohepatitis (NASH), which are both commonly associated with obesity, and alcoholic liver disease. We will also discuss cirrhosis as end-stage liver disease.

Factors Contributing to Intestinal Dysbiosis

Environmental Factors That Shape the Microbiota in Liver Disease

Differences in intestinal microbial composition in dizygotic as well as monozygotic twins suggest that the environment is in fact an important factor shaping the microbiome.[17] Obesity is associated with phylum-level changes in the microbiota, less bacterial diversity, and different expression of bacterial genes and metabolic pathways. For instance, the microbial inhabitants from populations with similar cultural factors such as hygiene, exposure to chemicals and/or antibiotics, and especially diet share more similarities in the microbiome structure when compared with populations in other countries.[3] Diet is therefore an important environmental factor.

Bacteria extract energy from the diet. The observed associations between gut microbes and nutrient absorption indicate that human gut microbiota regulates nutrient harvest. A 20% increase in Firmicutes and a corresponding decrease in Bacteroidetes were associated with an increased

Abbreviations used in this paper: **ALD, alcoholic liver disease; AMP, antimicrobial peptides and proteins; Fiaf, fasting-induced adipocyte factor; HFD, high-fat diet; IBD, inflammatory bowel disease; IL, interleukin; LCFA, long-chain fatty acid; LPS, lipopolysaccharide; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NLRP, nucleotide-binding domain and leucine rich repeat-containing protein; NOD2, nucleotide-binding oligomerization domain 2; PAMPs, pathogen-associated molecular patterns; Reg3, regenerating islet-derived 3; TLR, Toll-like receptor; TNF, tumor necrosis factor; TNFR, tumor necrosis factor receptor.**

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energy harvest of ≈150 kcal. Furthermore, the efficiency of extracting energy from various dietary ingredients favors the growth and/or colonization of certain bacterial strains, thereby contributing to this complex and competitive environment.[18] Conventionally raised mice have a 40% higher body fat content and 47% higher epididymal fat content than germ-free mice even though they consumed less food than their germ-free counterparts. Fecal transplantation to gnotobiotic mice resulted in a 60% increase in body fat within 2 weeks without any increase in food consumption or obvious differences in energy expenditure.[19]

Alcohol consumption is another environmental factor affecting the composition of the intestinal microbiome. Alcohol causes intestinal bacterial overgrowth in humans[20,21] and in animal models of chronic alcohol administration.[12,14] Alcohol also results in alcohol-associated qualitative changes of the microbiota in humans[20–22] and experimental animal models.[12,14,23–25] In particular, beneficial commensal bacteria including *Lactobacillus* are relatively lower after chronic alcohol consumption.[12,20] Using metagenomics and metabolomics, we have recently demonstrated that chronic alcohol administration results in a decreased capacity of the intestinal microbiome to produce long-chain fatty acids (LCFA) in both alcoholics and mouse models of alcoholic liver disease. This leads to reduced intestinal levels of saturated LCFA and commensal lactobacilli, which are able to use saturated LCFA as an energy source.[25] A decrease in beneficial commensal bacteria contributes to a tight junction barrier disruption.[26] Supplementing saturated LCFA maintains eubiosis, stabilizes the intestinal gut barrier, and reduces ethanol-induced liver disease in mice. Ethanol exerts a direct effect on the saturated fatty acid biosynthetic gene abundance in intestinal bacteria independently of the host.[25]

Antibiotics are important drugs to treat infectious diseases and other diseases that depend on translocated microbial products. However, antibiotics also can promote dysbiosis, which might not only favor colonization[27] but also affect body physiology by inducing weight gain. Subtherapeutic administration alters the population structure of the gut microbiome and its metabolic capabilities, and increases adiposity in young mice.[28] Thus, dysbiosis induced by antibiotics is an important pathogenic factor in the onset or progression of systemic diseases. This concept has been shown in IBD.[29–31] Other environmental factors that shape microbiota are delivery mode,[32] smoking,[33–35] and parasitic infections.[36]

Genetic Factors Leading to Intestinal Dysbiosis

The microbiota from family members has more similarities than that from unrelated individuals,[37,38] which raises the possibility that genetic factors affect the microbiome composition. This is supported by genetic loci in mice that have been linked to the abundance of gut bacteria.[39] It is important to consider that studies that have failed to find significant genotype effects on microbiome diversity[3,17] were not accounting for environmental conditions and might have been underpowered. Newer, sufficiently powered studies have demonstrated that the Christensenellaceae family is a heritable taxon which forms a co-occurrence network with other heritable taxa and is enriched in individuals with a low body mass index (BMI).[40] Adding Christensenellaceae to an obese-associated microbiome resulted in reduced weight gain in the recipient mice.[40] Similarly, there is a significant association between the nucleotide-binding oligomerization domain 2 (NOD2) risk allele for intestinal bowel disease and an increased relative abundance of Enterobacteriaceae.[41]

Concerning liver disease, NOD2 variants increase the risk for culture-positive spontaneous bacterial peritonitis and bacterascites in cirrhosis and may affect survival.[42,43] Genetic polymorphisms of NOD2 are associated with increased mortality in nonalcoholic liver transplant patients.[44] Inflammasomes recognize pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) that cleavage proinflammatory cytokines such as pro-interleukin (IL)-1β[44] and pro-IL-18. Mice deficient for nucleotide-binding domain and leucine-rich repeat-containing protein 6 (NLRP-6), and hence less intestinal IL-18, showed altered fecal microbiota characterized by an increase of the bacterial phyla Bacteroidetes (Prevotellaceae) and TM7.[45] Intestinal dysbiosis causes inflammation of the colon, which is mediated by chemokine (C-C motif) ligand 5 (CCL5).[46] Intestinal inflammation results in an increase in intestinal permeability, which leads to translocation of microbial products to the liver. Binding of these microbial products to Toll-like receptors (TLRs) in the liver is associated with exacerbated hepatic steatosis driving NASH progression. Furthermore, cohousing of inflammasome-deficient mice with wild-type mice results in exacerbation of hepatic steatosis. NLRP3 and NLRP6 inflammasomes and the effector IL-18 ameliorate NAFLD/NASH progression, thus highlighting the importance of genetic factors for intestinal dysbiosis and systemic diseases.[46] The fact that healthy siblings of patients with Crohn's disease manifest Crohn's disease associated immune and microbiologic features supports the relevance of genetic factors in the composition of microbiota.[47]

Separating the influence of genetics and environment on microbiota composition requires carefully designed studies to identify a microbiota that is associated with liver disease. Variables such as antibiotics usage, diet preferences, and environmental exposures need to be controlled for when the influence of a genetic or environmental factor is evaluated. Whether known genetic risk alleles for liver disease affect the microbiome composition or the mucosal immune system deserves future studies.

Mucosal Immune System

The immune system is very important in maintaining the symbiotic relationship between the host and the intestinal microbiome. Intestinal bacteria develop and regulate the host immune system,[48] and the immune system affects the composition of the intestinal microbiome. In particular, the mucosal immune system ensures a beneficial microbiota composition by restricting the growth of pathogens, controlling bacterial overgrowth, and reacting to pathogens and bacteria that reach the intestinal barriers, chemical barriers