A story of liver and gut microbes: how does the intestinal flora affect liver disease? A review of the literature

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Giuffrè M, Campigotto M, Campisciano G, Comar M, Crocè LS. A story of liver and gut microbes: how does the intestinal flora affect liver disease? A review of the literature. *Am J Physiol Gastrointest Liver Physiol* 318: G889–G906, 2020. First published March 9, 2020; doi:10.1152/ajpgi.00161.2019.—Each individual is endowed with a unique gut microbiota (GM) footprint that mediates numerous host-related physiological functions, such as nutrient metabolism, maintenance of the structural integrity of the gut mucosal barrier, immunomodulation, and protection against microbial pathogens. Because of increased scientific interest in the GM, its central role in the pathophysiology of many intestinal and extraintestinal conditions has been recognized. Given the close relationship between the gastrointestinal tract and the liver, many pathological processes have been investigated in the light of a microbial-centered hypothesis of hepatic damage. In this review we introduce to neophytes the vast world of gut microbes, including prevalent bacterial distribution in healthy individuals, how the microbiota is commonly analyzed, and the current knowledge of the role of GM in liver disease pathophysiology. Also, we highlight the potentials and downsides of GM-based therapy.

chronic liver diseases; dysbiosis; fecal transplantation; gut-brain-liver axis; gut-liver axis; liver cirrhosis; microbiome; oral microbiota; probiotics

INTRODUCTION

The human gastrointestinal system is inhabited by a large group of 2,000 distinct species of bacteria, in addition to archaea, fungi, microbial eukaryotes, and viruses, that exist in a symbiotic relationship with each other and their human host. This motley collection of microbes is called microbiota, whereas their genetic material is known as the microbiome (192).

Bacteria flourish on every surface of the human body that is exposed to the outer environment. The most densely colonized organ is the gastrointestinal tract: the colon alone contains >70% of microbiota (120). Until recently, a misconception was that the ratio of cells of the entire human microbiota to cells in the human body was 10:1. This notion was based on a rough estimation of 40 years ago (171); since then, more accurate evaluations proved that this ratio is much closer to 1:1, with a balance slightly in favor of our microbes (29). Despite this ~1:1 ratio, the microbiome is far more complex and variegated than our own genetic information. In fact, the gut is colonized by ~100 trillion bacteria, which cumulatively possess a genome that is 150 times greater than that of their human host (3,000,000 vs. ~23,000 genes) (40, 152).

The gut microbiota (GM) is strictly involved in human physiology. It acts as a critical regulator of digestion: commensal bacteria synthesize, extract, and absorb many metabolites, including lipids, amino acids, vitamins, and bile acids (BAs). In addition, the GM can directly prevent colonization of foreign bacteria by inhibiting their growth through the appropriation of available resources and/or production of antibacterial molecules. Millions of years of coevolution with our microbes have ensured a beneficial symbiotic relationship that combines the host and its microbial guests in a "superorganism" (82, 127) that performs synergetic immune and metabolic functions (189). In healthy conditions, the two parts of this superorganism benefit from each other's functions in a homeostatic balance of "eubiosis," whereby the various species of beneficial bacteria cohabit peacefully and provide health benefits to the host. However, what constitutes a "healthy" microbial composition is yet to be clarified, given that gut microbes exist in such a heterogeneous state that they can be influenced by a multitude of factors (62, 119). In fact, the GM represents a dynamic ecosystem that is severely tested by many factors, such as medications (e.g., antibiotics), unbalanced diet, and stress (209). Despite these premises, shifts to an "abnormal"

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microbiota are referred to as dysbiosis (i.e., loss of keystone taxa, pathogen proliferation, and changes in metabolic capacity) (99). The idea of dysbiosis derived from the discovery of variations in GM composition in individuals suffering from a multitude of diseases, such as cardiovascular disease (206), obesity (33, 119), diabetes (117), inflammatory bowel disease (80), irritable bowel syndrome (151), and diverticular disease (59). However, the postulation of direct correlations between dysbiosis and the onset of a specific disease should be undertaken with extreme caution because of the current lack of information.

In this review we introduce neophytes to the vast world of gut microbes, including bacterial distribution in healthy individuals, how the microbiota is commonly analyzed, and the current knowledge of the role of the GM in liver disease pathophysiology.

MICROBIOME ANALYSIS

For years, scientists have been interested in the study of GM composition, but most of these bacteria cannot be grown as purified cultures derived from fecal samples (138). The advent of next-generation sequencing (NGS) techniques has enabled investigations of the GM with unprecedented resolution and throughput. The most widely used method for the taxonomic and phylogenetic identification of bacterial community composition relies on 16S rRNA gene amplicon analysis. The ~1.5-kilobase-long 16S rRNA gene consists of nine variable (V1–V9) regions separated by conserved regions. The variable regions are used as a barcode that allows differentiation of bacteria on the genus level, but they are not so often helpful in species/strain differentiation. The taxonomic assignment is based on the comparison of clustered reads with specific databases of known 16S sequences, i.e., Ribosomal Database Project, Greengenes, and SILVA (51, 67, 153).

Because many species are identical along the full sequence of the 16S rRNA gene, this technique is not always useful (203). To improve the taxonomic assignments, a specific human intestinal database has been developed (160). Another possible approach to overcome this limitation is operational taxonomic unit (OTU) clustering, which is implemented in some popular tools for metagenomics, such as QIIME (Quantitative Insights into Microbial Ecology) (35). This clustering is based on comparison of sequences using different algorithms based on sequence length or pairwise alignment.

Recently, alternative methods to control sequencing errors have been developed. Namely, the amplicon sequence variants can resolve exactly, down to the level of single-nucleotide differences over the sequenced gene region. The benefits of finer resolution are immediately apparent, eliminating the OTU-inflated outputs of NGS (32).

Although 16S sequencing has enabled a great deal of scientific research on the GM, the mere knowledge of their genera and relative abundance is not useful for clinical purposes, because each genus can have a wide range of strains that could exert different pathological or beneficial effects (145). To overcome 16S rRNA gene sequencing limitations, a shotgun strategy, able to analyze the entire genomic content of a community, can be used. The molecular approach is similar to that used for the analysis of a single bacterial genome (54, 55), but, in this case, sequences from both the host and all the microbes (bacteria, archaea, fungi, and viruses) in the studied environment are obtained.

Despite the success and high efficiency of NGS, the detected composition of bacterial communities could be affected by experimental design and procedures, including sampling and storage of the fecal material (36, 38, 49, 75), as well as DNA extraction protocols (133). In addition, one of the significant criticisms of GM studies is related to the fact that not all gut bacteria (e.g., mucosally adherent bacteria and those residing in the small intestine) are present in the stools. Also, stools are often distant from the region in the gastrointestinal system related to the pathology, because fecal material is stored in the rectum, where active dehydration and selective fermentation allow the growth of bacteria that are not present in other parts of the gastrointestinal lumen.

Great potential to better understand the impact of the GM on host health resides in the survey of the functional activity of the microorganisms. For this reason, metagenomics studies are often associated with metatranscriptomics, which allows us to gain a dynamic picture of a specific microbial niche (22, 87). Data from metatranscriptome analyses enrich metagenomics studies by elucidating which of the microbial genes are actively transcribed and to what extent, which enables demonstration of the metabolic functions from a potential repertoire of microbial genes. Indeed, metatranscriptomics consists of the analysis of the total mRNA, with or without a mRNA enrichment step, which is basically obtained by subtractive techniques aimed at depletion of the more abundant and less functionally informative rRNA (122). In so doing, only the active genes and, thus, the metabolic pathways are described. Therefore, metatranscriptomics enables us to gain more insights into the underlying differences between different clinical conditions.

GUT MICROBIOTA COMPOSITION

The adult human microbiota includes trillions of bacteria, but how does the gut colonization begin?

Intestinal flora may be determined before birth. Until a few years ago, a central dogma asserted that the fetus is preserved in a sterile environment and that the newborn gains its microbiota only after birth (161). Increasing evidence hints that gut colonization may be initiated before delivery (149). The current view is that progressive gut microbial colonization may be initiated during the gestation period by specific resident phyla, mainly Proteobacteria, in the placenta and amniotic fluid (52). Early gut colonizers appeared to be enterobacteria and bifidobacteria (115), and it seems that these pioneer microbes can modulate gene expression in the host to create a more suitable environment for themselves and prevent excessive growth of other bacteria that are later introduced to the ecosystem (213). Autochthonous microbiota is shaped in early life, and it is determined by gestational age at birth, type of delivery (vaginal vs. cesarean section), milk feeding (breast vs. formula), and weaning age. At 3 yr of age, GM composition and diversity are comparable to those of adults, which are primarily inhabited by five bacterial phyla: Firmicutes (79.4%), Bacteroidetes (16.9%), Actinobacteria (2.5%), Proteobacteria (1%), and Verrumicrobia (0.1%) (187). Less represented phyla include Fusobacteria, Tenericutes, Spirochaetes, and Cyanobacteria (103). The Firmicutes phylum is composed of >200 genera, of which the *Clostridium* genera is the predominant (95%) one.

Bacteroides and *Prevotella* are the predominant genera in the Bacteroidetes phylum. The Actinobacteria phylum, even if less abundant, is mainly represented by the *Bifidobacterium* genus. Examples of taxonomy and phylogeny of common constituents of the gastrointestinal flora are reported in Table 1.

Given the importance and complexity of the gut ecosystem, scientists have tried to identify shared patterns in microbial composition, which eventually led to the idea of enterotypes. Accordingly, individuals were stratified based on the most predominant cluster of bacteria that inhabited their gut into enterotype 1 (*Bacteroides*), enterotype 2 (*Prevotella*), and enterotype 3 (*Ruminococcus*) (5, 159). The proposal for such stratification was met with both excitement and controversy. In fact, this stratification neglected species- and strain-level variation and function (e.g., clustering deadly *Streptococci* to useful fermenting *Streptococci*). In addition, the genetic diversity between one gut bacterium and another may be greater than that between a human and a goldfish (8). Therefore, relying solely on enterotype classification could hide significant microbial variation. Direct clinical association and microbial species and function analysis should be preferred, where possible (53).

Although bacteria are the most represented biological entities, fungi, archaea, and viruses create the "rare biosphere" (0.1% of the GM) (208). Fungi represent only 0.03% of the fecal microbiota (146). Cultivation-based analyses have typically identified *Candida* as the most common fungal genus (172), followed by *Saccharomyces, Cladosporium*, and *Malassezia* (88, 98). The available information on archaea and viral communities is limited. The most commonly reported genera of archaea discovered in GM are *Methanobrevibacter, Methanosphaera*, *Nitrososphaera, Thermogynomonas*, and *Thermoplasma* (97). The most represented viruses are bacteria-infecting phage families (90%), while eukaryotic viruses are less abundant (10%) (156). It is still unclear how phages may influence the ecology of bacterial ecosystems (114). The roles of fungi, archaea, and viruses have not been examined throughout; however, they are known to play essential functions in the host immunological physiology (58).

Bacterial Concentration Along the Gastrointestinal Tract

The concentrations of gut microbes vary across the gastrointestinal lumen according to pH, O_2 tension, digestion flow rates, and composition of digestive products. Bacterial density, expressed in colony-forming units (CFUs) per milliliter, increases from the stomach and duodenum (10^1–10^3 CFU/mL) to the jejunum/ileum (10^4–10^7 CFU/mL) and is maximal in the colon (10^{11}–10^{12} CFU/mL), as shown in Fig. 1. The small intestine provides a more challenging environment for microbial colonizers, given the fairly short transit times (3–5 h) and the high bile concentrations. The large intestine, which is characterized by slower flow rates and neutral-to-mildly acidic pH, harbors by far the largest microbial community (dominated by obligate anaerobic bacteria) (144). Also, there is a clear distinction between surface-adherent and luminal microbial populations, expressed by the ratio of anaerobes to aerobes,

Table 1. *Examples of taxonomy and phylogeny of common constituents of gastrointestinal flora*

Phylum	Class	Order	Family	Genus	Species
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	*Faecalibacterium*	*F. prausnitzii*
				Ruminococcus	*R. faecis*
			Lachnospiraceae	*Roseburia*	*R. intestinalis*
			Clostridiaceae	*Clostridium*	*Clostridium* spp.
			Eubacteriaceae	*Eubacterium*	*E. hallii*
	Negativicutes	Veillonellales	Veillonellaceae	*Dialister*	*D. invisus*
		Selenomonadales	Selenomonadaceae	*Megamonas*	*M. funiformis*
	Bacilli	Lactobacillales	Lactobacillaceae	*Lactobacillus*	*L. reuteri*
			Enterococcaceae	*Enterococcus*	*E. faecium*
		Bacillales	Staphylococcaceae	*Staphylococcus*	*S. leei*
	Erysipelotrichi	Erysipelotrichales	Erysipelotrichidae	*Catenibacterium*	*C. mitsuokai*
				Coprobacillus	*C. catenaformis*
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	*Bacteroides*	*B. fragilis*
					B. vulgatus
					B. uniformis
			Porphyromonadaceae	*Tannerella*	*T. forsythia*
				Parabacteroides	*P. distasonis*
			Rikenellaceae	*Alistipes*	*A. finegoldii*
			Prevotellaceae	*Prevotella*	*Prevotella* spp.
Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae	*Corynebacterium*	*C. accolens*
		Bifidobacteriales	Bifidobacteriaceae	*Bifidobacterium*	*B. longum*
					B. bifidum
	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	*Atopobium*	*A. rimae*
				Collinsella	*C. intestinalis*
				Olsenella	*O. profusa*
Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	*Escherichia*	*E. coli*
				Shigella	*S. flexneri*
	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	*Desulfovibrio*	*D. intestinalis*
				Bilophila	*B. wadsworthia*
	Epsilonproteobacteria	Campylobacterales	Helicobacteraceae	*Helicobacter*	*H. hepaticus*
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Akkermansiaceae	*Akkermansia*	*A. muciniphila*

Five of the most-represented phyla are reported, with Firmicutes and Bacteroidetes representing 97% of gut microbiota (GM). This information is intended to orient researchers who are exploring the GM for the first time to a "whole-picture" view; these are just examples.

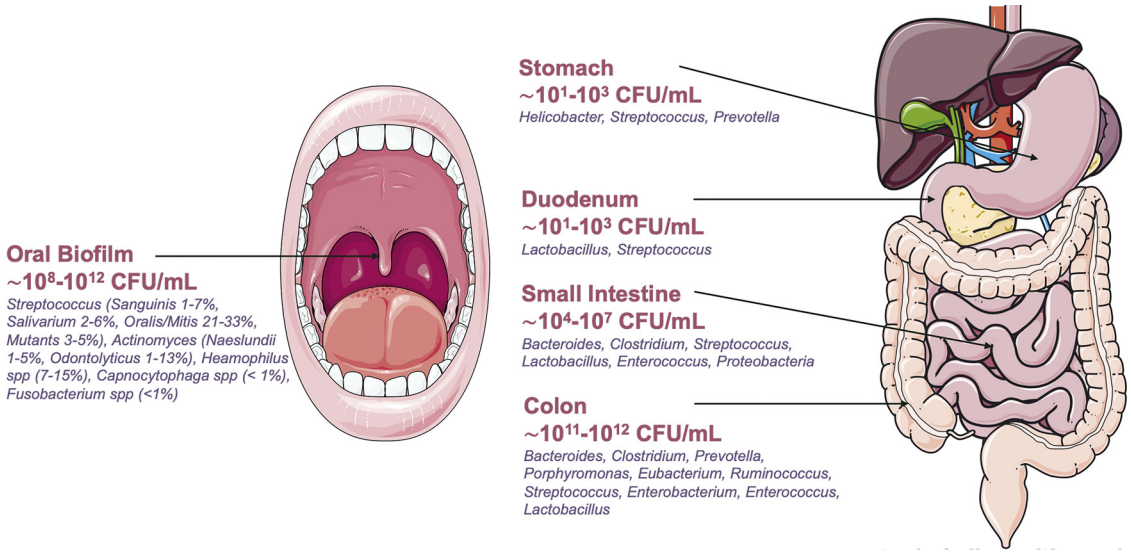

Fig. 1. It is estimated that >600 species of bacteria (from ≥12 phyla) reside in the oral cavity, but most of the resident flora derives from Firmicutes (~36%), Actinobacteria (~24%), Proteobacteria (~22%), Bacteroidetes (~12%), and Fusobacteria (~4%). The most predominant phyla in the human gut are Firmicutes (~79.4%), Bacteroidetes (~16.9%), Actinobacteria (~2.5%), Proteobacteria (~1%), and Verrumicrobia (~0.1%). Along the gastrointestinal tube, there is marked diversity and concentration of bacteria, which is expressed in colony-forming units (CFU) per milliliter. The dominant bacterial families of the small intestine and colon reflect physiological differences along the length of the gut. For example, a gradient of O_2, antimicrobial products (including bile acids), and pH limits the bacterial density in the small intestine community, whereas the colon microenvironment allows higher bacterial loads.

which appears to be lower at the mucosal surface than in the lumen (69).

GUT-LIVER AXIS

Among the most critical physiological connections between the GM and an extraintestinal organ is given by the gut-liver axis, because of the close bidirectional interface between the intestine and the liver, which occurs through the biliary tract, portal vein, and systemic circulation. The liver communicates with the intestine by releasing BAs into the biliary tract and systemic circulation. In the gut, the host and GM metabolize endogenous (BAs) and exogenous (diet and environmental) substrates, the products of which are transported to the liver through venous tributaries of the portal vein (181). GM-hepatic interaction through the gut-liver axis is summarized in Fig. 2.

Enterohepatic Circulation of Bile Acids

BAs are amphipathic molecules synthesized from cholesterol. Pericentral hepatocytes produce primary BAs, which are successively conjugated to taurine or glycine and then released in the biliary tract. BAs, upon their arrival at the small intestine, facilitate the emulsification and absorption of fat-rich molecules and fat-soluble vitamins. Nearly 95% of BAs are actively reabsorbed by enterocytes in the terminal ileum and transported back to the liver (47, 198). The residual 5% is converted to secondary BAs by the colonic microbiota (via deconjugation, dehydrogenation, and dehydroxylation) and passively reabsorbed into the portal circulation (198). Once in the liver, BAs are recycled and then secreted back into the biliary tract, completing the so-called enterohepatic circulation. Intestinal intraluminal BAs regulate hepatic BAs synthesis by interaction with the farnesoid X receptor (FXR), which induces transcription of an enterokine known as fibroblast growth factor (FGF) 19 (FGF19); that for the sake of clarity, represents is the human ortholog of FGF15, which is found in rodents (219). FGF19 downregulates BAs synthesis by inhibiting cholesterol 7α-monooxygenase (the rate-limiting enzyme for BAs synthesis) in hepatocytes, thus creating a negative-feedback system. Another function of the FXR is related to GM control: the BAs-FXR interaction induces secretion of antimicrobial peptides (AMPs), which can directly inhibit bacterial overgrowth and prevent intestinal epithelial dysfunction (104, 148). In turn, intestinal microbes can influence the composition of the BAs pool by promoting a disproportion toward secondary BAs, which have a different affinity to the FXR and a less powerful antibacterial effect (140).

Choline, BAs, and Fatty Liver

Choline is a water-soluble nutrient essential for human metabolism. In the form of phosphatidylcholine, it is a fundamental component of the cell membrane and very-low-density lipoprotein (VLDL) envelope. Choline deficiency reduces VLDL assembly, which blocks export of triglycerides from the liver, resulting in the development of fatty liver (162). Gut microbes (e.g., *Desulfovibrio desulfuricans*) may actively contribute to diminished bioavailability of choline (178) by metabolizing it to trimethylamine (206), which is further metabolized by liver flavin monooxygenases to trimethylamine-*N*-oxide (TMAO) (101). TMAO affects BAs by decreasing their synthesis and limiting their enterohepatic circulation (46). Therefore, it is possible that choline deficiency, either through insufficient dietary intake or excessive GM conversion, may eventually end in fat accumulation in the liver.

Intestinal Barrier

The gastrointestinal tract represents the most extensive interface in the body that is in direct communication with the external environment. It is, therefore, a significant line of defense in which epithelial cells create a physical barrier that works in concert with immune and stromal cells to

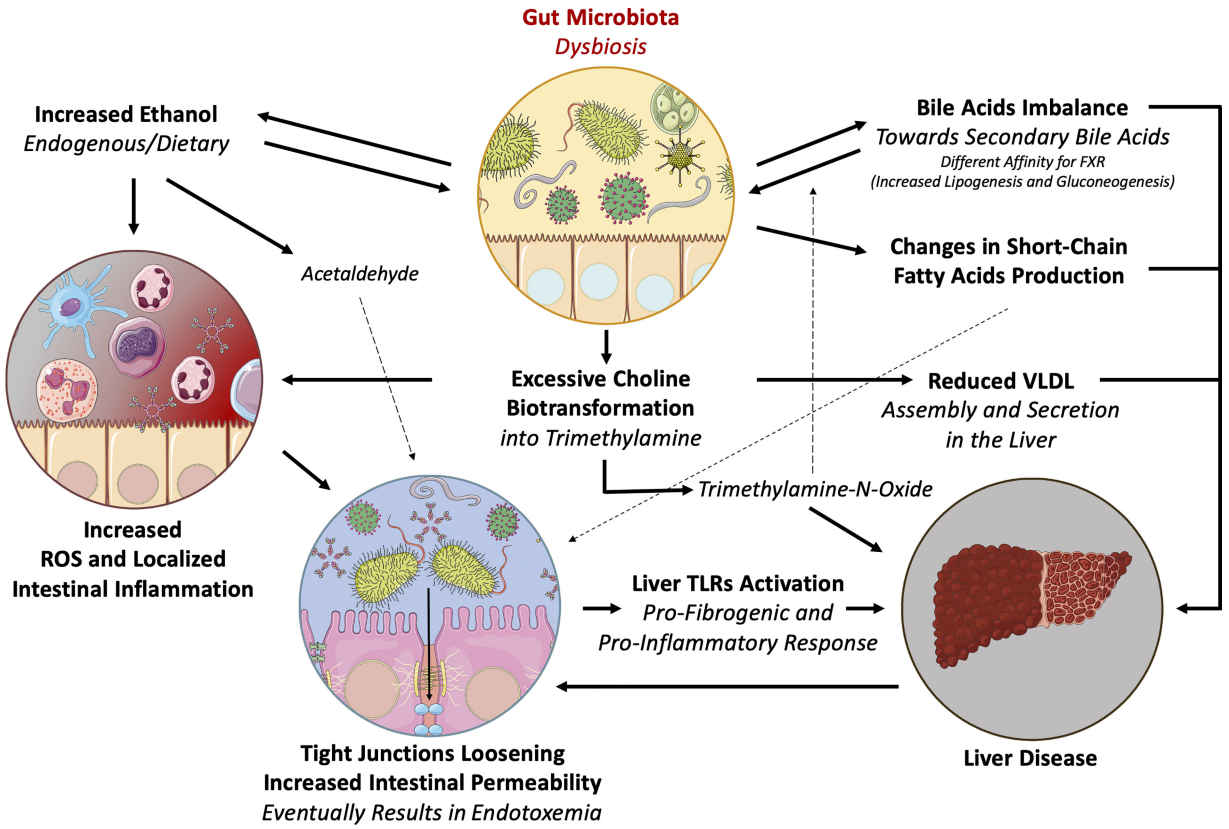

Fig. 2. Most important mechanisms through which the gut microbiota can promote liver disease. FXR, farnesoid X receptor; ROS, reactive oxygen species; SCFAs, short-chain fatty acids; TLR, Toll-like receptor, VLDL, very-low-density lipoprotein.

overcome possible pathogen invasion. The epithelium consists of a single layer of intestinal cells, bound by tight junction complexes that create a seal between two adjacent cells. Enterocytes and Paneth cells produce AMPs [α-defensins, lysozyme C, C-type lectin (as regenerating islet-derived protein IIIγ), and phospholipases], which are crucial in the control of bacterial growth (104). Also, goblet cells secrete heavily glycosylated proteins, such as mucin-2, which create a mucous layer that lubricates and impedes the interaction between the luminal content and the underlying epithelial layer (93). Under the intestinal epithelium, the lamina propria hosts dendritic cells and gut-associated lymphoid tissue, which are pivotal induction sites that harbor all the immune-competent cells required to provoke antigenic responses (185). Also, the adaptive immune system contributes to the intestinal barrier by secreting effector factors such as secretory immunoglobulin A (IgA), which blocks microbial attachment to the epithelial layer (131).

Recently, it was pointed out that the intestinal barrier not only fights off gut microbes but that its integrity and function are strictly linked to the same bacteria from which it shields us (23, 24, 37). In fact, the GM produces an extremely diverse repertoire of metabolites that can drive changes in the various lines of defense of the intestinal barrier. Among these metabolites are short-chain fatty acids (SCFAs), which are mainly represented by acetate, propionate, and butyrate, whose synthesis is obtained from saccharolytic fermentation of dietary fibers. Butyrate, in particular, not only functions as an energy source for enterocytes but, also, improves their barrier function by inducing tight junction proteins and mucins production, specifically mucin-2 (78) and claudin-1 (200), and by its local anti-inflammatory properties that result in reduced colonic paracellular permeability (112). However, butyrate displays the most diverse effects on cellular proliferation, differentiation, and apoptosis, which could lead to simultaneous pro- and antitumorigenic effects, a phenomenon defined as the "butyrate paradox" (196). In particular, it seems that butyrate can induce suppression of epithelial stem cell activity, which may result in harmful situations in which high rates of proliferation are needed (e.g., mucosal wound healing in ulcerative colitis) (180). Also, butyrate affects the proliferation of hepatocytes. In particular, when administered at low doses to a murine hepatocyte cell line, it exerted mitogenic effects and enhanced cellular proliferation (179).

As stated before, bacterial products can exert disruptive effects on the intestinal barrier. In particular, ethanol, which comes primarily from the diet, can also be produced by some commensal gut bacteria (such as *Escherichia coli* and *Klebsiella pneumoniae*) (61, 218). Ethanol can directly disrupt the intestinal protective mucus layer by altering mucin glycosylation (90). At the same time, acetaldehyde, an intermediate product of alcohol metabolism, has been shown to weaken tight junctions (41, 155) to downregulate the expression of several AMPs (139). These changes can increase the number of bacteria that come in contact with the epithelial barrier and may lead to local inflammation and bacterial translocation.

Impact of Oral Bacteria

Nearly half of the subjects in the Human Microbiome Project showed overlaps in oral and stool bacteria. Oral flora can reach the stomach through the continuous swallowing of saliva, which contains a conspicuous number of oral microbes. Generally, these bacteria are poor intestinal colonizers (175). However, individuals with periodontitis can ingest up to 10^{10} cells of a periodontal pathogen, *Porphyromonas gingivalis* (197), which can tolerate the harsh pH of the stomach, thus leading to active migration and proliferation in the intestine (199). *P. gingivalis* was linked to reduced expression of tight junction protein 1, thus weakening the most valuable line of defense against intestinal microbes (142). Also, experiments in murine models showed that *P. gingivalis* drastically accelerated the progression of liver disease (216). In general, the study of oral flora and its impact on liver disease is still in its infancy, and further investigation is required. However, particular caution should be exercised in administration of proton-pump inhibitors (PPIs), since the increase in gastric pH may favor oral flora translocation and gut colonization from alien microbes (31).

The Gut Leaks, the Liver Answers

Altered intestinal permeability, also referred to as the "leaky gut," is present in ~50% of patients with both alcoholic liver disease (ALD) and nonalcoholic fatty liver disease (NAFLD) (143). Although liver translocation of intact and functional bacteria (i.e., atopobiosis) (150) is extremely rare (20), the products of these bacteria are easily identified in trace amounts in the liver (30, 71) and peripheral blood (70). Bacterial products include cell wall components such as gram-negative bacteria lipopolysaccharides (LPS), also referred to as endotoxin, and fungal β-glucan, as well as their genetic material (mostly DNA and mRNA).

However, the liver is not only a passive recipient of intestinal-escaped bacterial products; it also actively controls their access to the systemic circulation (28). The recruitment and activation of the hepatic immune response is initiated by bacterial products that promote localized inflammation through a series of Toll-like receptors (TLRs), a subclass of pattern-recognition receptors localized on the outer Kupffer cells and hepatic stellate cells membrane: endotoxin activates TLR4 (176), methylated DNA is recognized by TLR9 (77), and gram-positive bacteria stimulate TLR2 (95). TLR signaling activates proinflammatory and profibrogenic responses mediated by Kupffer and stellate cells. These steps lead to severe oxidative stress induced by inflammatory cytokines, which eventually promotes liver damage and liver fibrosis (26, 134, 182).

Endotoxemia and TLR4

LPS are a group of macromolecules with a median mass of 10–20 kDa. They are relatively heat-resistant and share a common structure, which consists of three components: *1*) a hydrophobic lipid portion (lipid A), which is responsible for the toxic properties of the molecule, *2*) a hydrophilic polysaccharide core, and *3*) a repeating hydrophilic O-antigen oligosaccharide side chain that is specific to the bacterial serotype (92). Gut LPS derives primarily from Bacteroidetes (79%), while Proteobacteria are minor contributors, with *E. coli* accounting for 14% of the total gut-derived LPS (56). When the intestinal permeability increases, LPS can cross the intestinal mucosa more easily reaching the portal circulation. The LPS concentration is ~10 times higher in the portal than the peripheral blood (68). In fact, 90% of free LPS that enters the portal bloodstream is intercepted by resident Kupffer cells within 1 h upon arrival in the liver (177). LPS bind to the LPS-binding protein (LBP). The LPS-LPB complex has a high affinity for myeloid differentiation factor 2 (MD2) and cluster of differentiation 14 (CD14), which, together, bind TLR4. Once activated, TLR4 initiates an intracellular signaling cascade, which ends with the transcription of many inflammatory cytokines, including tumor necrosis factor-α (TNF-α) and interleukin (IL)-1, IL-6, IL-12, and IL-18 (215), which may promote hepatic carcinogenesis through an everlasting tissue inflammation. In addition, TLR4 signaling was found to favor epithelial-to-mesenchymal transition in human hepatocytes, a process in which epithelial cells lose polarity and cell-to-cell contacts, which results in increased metastatic capacity (108).

However, recent findings demonstrated that LPS with underacylated structure have a potent immunoinhibitory effect on TLR4 signaling. In particular, immunoinhibitory LPS are expressed by *Bacteroides* and *Prevotella* spp. These findings may suggest that gut-derived LPS tend to prevent, rather than favor, inflammation (56).

DYSBIOSIS IN LIVER DISEASE

The interaction between the gut-liver axis and intestinal flora has been investigated for its role in the pathogenesis of numerous chronic liver diseases, such as chronic hepatitis B (CHB), chronic hepatitis C (CHC), alcoholic liver disease (ALD), and nonalcoholic liver disease (NAFLD).

Alcoholic Liver Disease

Excessive alcohol consumption is one of the leading causes of chronic liver disease worldwide. ALD ranges from hepatic steatosis to steatohepatitis, fibrosis, and, eventually, cirrhosis. The involvement of the GM was first assumed in 1995, when Adachi et al. reported that antibiotics protected rats against alcohol-induced liver injury (1). Alcohol consumption was found to cause small and large intestine bacterial overgrowth (39). However, these changes are not permanent and may reverse with alcohol abstinence (63). Studies using both murine models and human participants found that excessive alcohol consumption may induce changes in GM composition (121). In particular, intestinal dysbiosis in ALD is characterized by the abundance of endotoxin-producing bacteria (Enterobacteriaceae and *Streptococcus*) and reduced protective strains such as Bacteroidetes and *Lactobacillus* (13, 17). Also, SCFA-producing strains, such as Lachnospiraceae and Ruminococcaceae, were found to be drastically reduced (42, 113, 118). In addition, *Akkermansia muciniphila* was found to be decreased in proportion to the severity and progression of ALD (89).

Nonalcoholic Fatty Liver Disease

NAFLD is one of the most important causes of liver disease worldwide, with a global prevalence of 25%. About 20% of patients with NAFLD develop nonalcoholic steatohepatitis

(NASH), a chronic hepatic inflammation that can progress to cirrhosis (217). The pathophysiology of NASH is still not comprehended fully. It is often described as a "two-hit" phenomenon, characterized by primary lipid accumulation and altered metabolic homeostasis followed by secondary oxidative stress and retention of inflammatory cells (79). Among the numerous stressors that can result in NAFLD/NASH, the GM has been considered to be one of the crucial drivers and has received a great deal of interest in recent years. Current findings on NAFLD dysbiosis in humans are reported in Table 2. The pathophysiological connections must be sought in loss of equilibrium within the various components of the gut-liver axis. First, individuals with NAFLD show lower expression of a major tight junction protein, zonula occludens 1, which may result in increased intestinal permeability (136). A weakened intestinal barrier can promote bacterial translocation, which may be critical in patients with NAFLD and can predispose to small intestinal bacterial overgrowth (136). In addition, the GM can alter cellular energy influx and expenditure by *1*) phenylacetic acid-derived bacterial metabolites, which promote liver steatosis and triglyceride accumulation (102), *2*) SCFAs, which reduce hepatic cholesterol and fatty acid synthesis and increase lipid oxidation, and *3*) altered BAs signaling, with suppression of FXR activity, which is a key regulator of lipogenesis, fatty acid oxidation, gluconeogenesis, and triglyceride homeostasis and also regulates the expression of

Table 2. *Recent findings on GM in healthy subjects compared with patients with NAFLD and NASH*

Reference	Dysbiosis Characteristics	Population Characteristics and Findings
Wong et al., 2013 (210)	**Genus** Increased: *Parabacteroides, Alisonella* Decreased: *Faecalibacterium, Anaerosporobacter*	Healthy (n = 22), NASH (n = 16). No information about patients' diet regimen
Zhu et al., 2013 (222)	**Phylum** Increased: Bacteroidetes, Proteobacteria Decreased: Actinobacteria, Firmicutes **Family** Increased: Prevotellaceae Decreased: Bifidobacteriaceae, Rikellaceae, Lachnospiraceae, Ruminococcaceae **Genus** Increased: *Prevotella, Escherichia coli* Decreased: *Bifidobacterium, Alistipes, Blautia*	Healthy (n = 16), obese (n = 25), NASH (n = 22). No information about patients' diet regimen Gut microbiota enriched in alcohol-producing bacteria (*E. coli*) constantly produce more alcohol than healthy microbiota and, therefore, provide the liver with a constant source of ROS
Raman et al., 2013 (154)	**Phylum** Increased: Proteobacteria **Family** Increased: Lactobacillaceae, Lachnospiraceae Decreased: Ruminococcaceae **Genus** Increased: *Lactobacillus, Robinsoniella, Roseburia, Dorea* Decreased: *Oscillibacter*	Healthy (n = 30), NAFLD (n = 30). Study participants did not maintain food diaries during the study period. Individuals had no standardized diet
Jiang et al., 2015 (106)	**Genus** Increased: *Escherichia coli*, *Lactobacillus, Anaerobacter, Clostridium* XI, *Streptococcus* Decreased: *Alistipes, Prevotella, Odoribacter, Oscillibacter, Flavonifractor*	Healthy (n = 32), NAFLD (n = 53). No information about patients' diet regimen Duodenum in the healthy group contained intact tight junctions and regularly aligned and extensive microvilli. Widened tight junctions and irregularly arranged microvilli were observed in the NAFLD group. Immunohistochemistry revealed significantly higher expression of occludin protein in intestinal mucosa of healthy subjects than NAFLD patients. There were fewer $CD4^+$ and $CD8^+$ T lymphocytes in lamina propria of the duodenal mucosa in NAFLD patients than healthy subjects
Boursier et al., 2016 (25)	**Family** Increased: Bacteroidaceae Decreased: Prevotellaceae **Genus** Increased: *Ruminococcus, Bacteroides* Decreased: *Prevotella*	NASH (n = 35), no NASH (n = 22). No information about patients' diet regimen *Bacteroides* was independently associated with NASH. Patients with liver fibrosis ($n > 2$) had greater abundance of *Ruminococcus*. Use of the relative abundance of *Bacteroides* and *Ruminococcus* enabled definition of subgroups with increasing NAFLD severity
Del Chierico et al., 2017 (65)	**Phylum** Increased: Actinobacteria Decreased: Bacteroidetes **Family** Decreased: Rikenellaceae **Genus** Increased: *Ruminococcus, Blautia, Dorea, Bradyrhizobium, Anaerococcus, Peptoniphilus, Propionibacterium acnes* Decreased: *Oscillospira*	Pediatric subjects: healthy (n = 54), NAFLD, NASH, or obese (n = 61). No information about patients' diet regimen Combination of a low abundance of *Oscillospira* and high levels of 2-butanote may be a specific intestinal bacteria profile for liver steatosis in children

NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; ROS, reactive oxygen species.

glucose transporter 4 and glucagon-like peptide 1, which are involved in insulin sensitivity (66, 102, 107, 211). Also, bacterial microbes can alter appetite via metabolites produced from food conversion (e.g., γ-aminobutyric acid and serotonin) through interaction with enteroendocrine L cells or with the endocannabinoid receptor systems (205). Higher blood ethanol concentration has been reported in individuals with NAFLD, even in alcohol abstinence (141). This can be related to the peculiar finding of alcohol-producing bacteria in NAFLD dysbiosis (61, 218).

Cholangiopathies

Cholangiopathies, such as primary sclerosis cholangitis (PSC) and primary biliary cholangitis (PBC), are characterized by bile duct damage or inflammation that leads to cholestasis and bile duct hyperplasia (170). In particular, PSC is characterized by inflammation and scarring of the bile ducts (111), whereas PBC is characterized by progressive destruction of the bile ducts within the liver (186). Human studies have demonstrated higher intestinal mucosal immune responses (190) and increased small bowel permeability (72) in PSC patients compared with healthy subjects. Also, liver specimens showed endotoxin storage in cholangiocytes in both PSC and PBC patients (169). The combination of these three factors raises the possibility of a potential contribution of the GM to PSC/PBC pathogenesis and/or progression. The microbiota in individuals with PSC is characterized by the abundance of Veillonellaceae (116), Barnesiellaceae (191), *Enterococcus*, *Fusobacterium*, *Lactobacillus*, *Morganella*, and *Streptococcus* (164) and the reduction of *Succinivibrio*, *Desulfovibrio*, *Phascolarctobacterium*, *Coprococcus* (116), *Anaerostipes* (164), and Clostridiales (163). Microbiome analysis in individuals with PBS showed a relative abundance of several opportunistic pathogens, such as *Veilonella*, *Klebsiella*, *Neisseria* (128), *Clostridium, Pseudomonas, Hemophilus, Streptococcus*, and Enterobacteriaceae (158), and innocuous commensals, such as *Bifidobacterium* and *Lactobacillus,* combined with a relative depletion of potentially beneficial bacteria such as *Ruminococcus* (128) and *Bacteroides* (158).

Viral Hepatitis B

CHB infection remains a global burden, despite widespread access to vaccinations and antiviral drugs. Information regarding the effect of hepatitis B virus (HBV) on GM is limited. Age-specific clearance of HBV depends not only on the maturity of the host immune system but, also, on the stability of the gut microbes. Experiments on murine models proved that adult mice with mature GM cleared HBV after only 6 wk, whereas younger mice without gut flora and adult mice with antibiotic-induced gut sterilization failed to clear the infection (50). In addition, fecal microbiota transplantation (FMT) in patients with CHB induced hepatitis B e-antigen clearance after long-term antiviral therapy (157). The GM in HBV carriers is characterized by increased *E. coli* (221), and CHB is distinguished by decreased concentrations of *Bifidobacterium*, *Lactobacillus*, *Bacteroides*, *Pediococcus*, *Weissella*, and *Prevotella*, with higher levels of *Enterococcus* and Enterobacteriaceae (109, 126, 201, 212). In addition, the gut diversity of HBV-related cirrhosis appears to have a 10-fold increase in Proteobacteria and a 13-fold decrease in Bacteroidetes levels compared with healthy subjects (207).

Viral Hepatitis C

Little is known about the effect of hepatitis C virus (HCV) infection on the GM and the relationship between GM alteration and CHC progression. A study in Japanese patients showed less bacterial diversity in people with HCV infection than in healthy individuals (205). Also, Heidrich et al. reported that GM diversity is associated with the stage of fibrosis and that the number of phylotypes decreased in patients with CHC and further decreased at cirrhosis onset (96). Regarding species prevalence in CHC, dysbiosis appeared in the early stages of the disease, with a transient increase in *Bacteroides* and Enterobacteriaceae (105). Aly et al. reported a rise in *Prevotella* and *Feacalibacterium* and a reduction in *Acinetobacter*, *Phascolarctobacterium*, and *Veilonella* in an Egyptian cohort of patients with CHC. The increase in *Prevotella* was linked to *1*) viral infection of both hepatic and gastric cells and B lymphocytes, which are responsible for IgA secretion, which modulates the GM, and *2*) higher intestinal carbohydrate concentration due to malabsorption, which is frequent in patients with CHC (4).

Liver Cirrhosis

Liver cirrhosis is a consequence of long-lasting chronic liver diseases. Prognosis mainly depends on the occurrence of decompensation, such as gastroesophageal varices, ascites, hepatic encephalopathy (HE), and bacterial infections (spontaneous bacterial peritonitis), which increase morbidity and mortality (6, 83–85). Etiology and patients' origin should be taken into account in analysis of the GM in cirrhotic patients, because gut biodiversity varies according to geographic provenance: most of the data derive from Chinese and American patients, which means that general assumptions derived from their results cannot be directly applied to all ethnicities. Available data are reported in Table 3. In general, cirrhotic patients are exposed to a higher risk of dysbiosis because of a multitude of pathological interactions between the liver and the intestine: alteration of intestinal motility, changes in gastric pH, and reduction of BAs in the colon progressively lead to a loss of bacterial growth control. The critical process that occurs during cirrhosis progression is the amplification of inflammation, which can be linked to the higher proportion of Enterobacteriaceae (which produce a potent endotoxin) and the decrease of Bacteroidetes with their immunoinhibitory effect on liver TLR4. In addition, a lower abundance of 7α-dehydroxylating bacteria, such as *Lachonospiraceae*, *Ruminococcaceae,* and *Blautia*, further depletes BAs, which aggravates gut dysbiosis. Once portal hypertension develops, systemic inflammation, oxidative stress, and venous stasis further damage the gut barrier, which becomes more and more permeable. The delayed intestinal transit allows small intestine bacterial overgrowth, which has the greatest potential to function as a source of bacterial translocation (147). According to the dysbiosis rate, researchers have developed a scoring system [*Bifidobacterium*-to-Enterobacteriaceae ratio (BER)] and a prognostic score [cirrhosis dysbiosis ratio (CDR)]. The BER reflects the ability of the bowel to counteract colonization of pathogenic bacteria (126). In contrast, the CDR is a prognostic score that

Table 3. *Recent findings on GM in healthy subjects compared with patients with liver cirrhosis*

Reference	Dysbiosis Characteristics	Population Characteristics and Findings
Chen et al., 2011 (45)	**Phylum** Increased: Proteobacteria, Fusobacteria Decreased: Bacteroidetes **Family** Increased: Enterobacteriaceae, Veillonellaceae, Streptococcaceae, Pasteurellaceae, Prevotellaceae Decreased: Lachnospiraceae, Bacteroidaceae **Genus/Species** Increased: *Enterococcus Faecalis, Clostridium* clusters XI	Healthy (n = 24), cirrhosis (n = 36), HBV (n = 24), alcohol abuse (n = 12) Positive correlation between CP score and Streptococcaceae; negative correlation between Lachnospiraceae and CP score
Lu et al., 2011 (126)	**Family** Increased: Enterobacteriaceae Decreased: Firmicutes **Genus/Species** Decreased: *Prevotella* spp., *Enterococcus faecalis, Faecalibacterium prausnitzii, Clostridium* clusters XI, *Lactobacillus pediococcus*	Healthy (n = 32), HBV, cirrhosis (n = 31) Significant decrease in BER, which may indicate resistance to microbial colonization of the bowel, in patients with CHB and patients with decompensated HBV cirrhosis
Xu et al., 2012 (214)	**Genus/Species** Decreased: *Bifidobacterium catenulatum* group	Healthy (n = 15), HBV cirrhosis (n = 16)
Wu et al., 2012 (212)	**Genus/Species** Increased: *Lactobacillus gasseri* Decreased: *Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus reuteri, Lactobacillus fermentus*	Healthy (n = 38), HBV cirrhosis (n = 61) Additional 74 patients evaluated after live transplant from HBV cirrhosis tended to have less complex fecal *Lactobacillus* composition than healthy controls
Bajaj et al., 2012 (19)	**Family** Increased: Enterobacteriaceae, Lauconostocaceae, Lactobacillaceae, Alcaligenaceae, Fusobacteriaceae Decreased: Lachnospiraceae, Ruminococcaceae, Clostrium-Incertae sedis-XIV	Healthy (n = 10), cirrhosis (n = 25): HCV (n = 12) and alcohol abuse (n = 13) In 17 patients with HE, lactulose withdrawal did not change the microbiome significantly beyond *Fecalibacterium* reduction. Specific bacterial families (Alcaligeneceae, Porphyromonadaceae, Enterobacteriaceae) are strongly associated with cognition and inflammation in HE
Bajaj et al., 2012 (15)	**Genus/Species** Increased: *Clostridium, Acidaminococcus, Enterococcus, Burkholderia, Ralstonia, Proteus* Decreased: *Dorea, Subdoligranumum*	Samples were taken from colonic mucosa. Healthy (n = 17), cirrhosis (n = 60); 24 patients with HE Between HE and no-HE patients, there was no difference in stool microbiota, but mucosal microbiome was different, with lower abundance of *Roseburia* and higher abundance of *Enterococcus, Veillonella, Megasphaera*, and *Burkholderia* in HE
Bajaj et al., 2014 (16)	**Family (pre-omeprazole)** Decreased: Lachnospiraceae, Ruminococcaceae	Healthy (n = 15), cirrhosis (n = 15): HCV (n = 8), alcohol abuse (n = 2), both (n = 5) Patients' GM was evaluated before and 14 days after omeprazole therapy. Omeprazole was associated with microbiota composition and functional changes in distal gut, in particular, a relative Streptococcaceae abundance after therapy, which may suggest gut colonization from oral flora
Bajaj et al., 2014 (13)	**Family** Increased: Enterococcaeae, Staphylococcaceae, Enterobacteriaceae Decreased: Ruminococcaceae, Lachnospiraceae, Veillonellaceae, Porphyromonadaceae	Healthy (n = 25), cirrhosis (n = 219) Progressive changes in gut microbiome accompany cirrhosis and become more severe in the setting of decompensation. There was a significant change in GM after the first HE episode, with a drastic increase in Enterobacteriaceae
Kakiyama et al., 2014 (110)	**Family** Increased: Veillonellaceae Decreased: Bacteroidaceae, Porphyromonadaceae	Healthy (n = 19), alcohol abuse cirrhosis (n = 78) Active alcohol use in cirrhosis is associated with a significant increase in secondary BA formation compared with abstinent alcoholic cirrhotic and nonalcoholic cirrhotic patients. This increase in secondary BAs is associated with a significant increase in expression of inflammatory cytokines in colonic, but not ileal, mucosa, which may contribute to alcohol-induced gut barrier injury
Qin et al., 2014 (152)	**Genus/Species** Increased: *Veillonella, Streptococcus, Clostridium*	Healthy (n = 83), cirrhosis (n = 98) Major change in GM in patients with liver cirrhosis mainly because of a massive invasion of the gut by oral bacterial species. Overrepresentation of products of nitrate and ammonia metabolism, denitrification, and GABA synthesis in microbiome of cirrhotic patients

Continued

Table 3.—*Continued*

Reference	Dysbiosis Characteristics	Population Characteristics and Findings
Bajaj et al., 2015 (9)	**Family** Decreased: Lachnospiraceae, Ruminococcaceae, Clostridiaceae	Healthy (n = 32), cirrhosis (n = 102): HCV etiology in 47% of cases Dysbiosis is present in saliva and stool of cirrhotic patients. Cirrhotic patients have impaired salivary defenses
Bajaj et al., 2015 (10)	**Family (hospitalized patients)** Increased: Lactobacillaceae, Enterococcaceae, Enterobacteriaceae, Pasteurellaceae Decreased: Bacteroidaceae, Porphyromonadaceae, Clostridiales XIV, Lachnospiraceae, Ruminococcaceae	Cirrhosis (n = 278): alcohol abuse etiology in 31% of cases; 94 were nonelectively hospitalized within 90 days Concomitant diabetes mellitus impacted GM with increased stool Bacteroidaceae and reduced Ruminococcaceae
Chen et al., 2015 (43)	**Family (cirrhosis with acute-on-chronic liver failure)** Increased: Pasteurellaceae, Streptococcaceae, Enterococcaceae Decreased: Bacteroidaceae, Ruminococcaceae, Lachnospiraceae	Healthy (n = 50), cirrhosis (n = 79): all cirrhosis patients had acute-on-chronic liver failure Patients who developed HE had lower abundance of Lachnospiraceae. IL-6 was negatively correlated with Ruminococcaceae and Lachnospiraceae and positively correlated with Verrucomicrobiaceae and Bifidobacteriaceae
Ahluwalia et al., 2016 (2)	**Family (cirrhosis vs. healthy)** Increased: Lactobacillaceae, Enterococcaceae, Clostridiales XIV, Lachnospiraceae, Enterobacteriaceae **Family (cirrhosis with HE vs. cirrhosis without HE)** Increased: Staphylococcaceae, Enterococcaceae, Porphyromonadaceae, Lactobacillaceae	Healthy (n = 40), cirrhosis (n = 147): 87 patients with HE Specific microbial families correlated (autochthonous taxa negatively and Enterobacteriaceae positively) with MR spectroscopy and hyperammonemia-associated astrocytic changes
Chen et al., 2016 (44)	**Genus/Species** Increased: *Veillonella, Megasphaera, Dialister, Atopobium, Prevotella*	Healthy (n = 28), cirrhosis (n = 30): HBV etiology in 80% of cases Duodenal microbiota analysis. PPI therapy reduced levels of *Cloacibacterium* and increased abundance of *Dialister*
Santiago et al., 2016 (167)	**Genus/Species** Decreased: *Clostridiales, Roseburia faecis, Alistipes putredinis, Oscillospira, Mogibacteriaceae, Dehalobacterium*	Healthy (n = 17), cirrhosis (n = 60) Analysis of serum microbial composition to assess microbial translocation
Sung et al., 2019 (183)	**Genus/Species (in patients with acute HE)** Increased: *Veillonella parvula, Clostridium* XI, *Prevotella, Enterococcus, Schlegelella, Megasphaeae, Lactobacillus* Decreased: *Phascolarctobacterium, Bacteroides, Alistipes*	Healthy (n = 13); cirrhosis (n = 97): 62 with acute HE and 35 with compensated liver cirrhosis

BA, bile acid; BER, *Bifidobacterium*-to-Enterobacteriaceae ratio; CHB, chronic hepatitis B; CP, Child-Pugh; GM, gut microbiota; HE, hepatic encephalopathy; HBV, hepatitis B virus; HCV, hepatitis C virus; PPI, proton pump inhibitor.

measures the ratio of abundance of beneficial bacteria (Lachnospiraceae, Ruminococcaceae, Clostridiales XIV, and Veillonellaceae) to overgrowth of potentially pathogenic taxa (Staphylococcaeae, Enterobacteriaceae, and Enterococcaceae) (15).

Dysbiosis has been linked with most of the complications of liver cirrhosis. For example, HE development is connected to a drastic change in GM composition: stool analyses combined with magnetic resonance imaging (MRI) proved that specific bacterial families were associated with impaired cognition due to astrocytic and neuronal changes (2); in particular, patients with HE and higher concentrations of Veillonellaceae presented poor cognition, endotoxemia, higher ammonia levels, and augmented inflammation compared with cirrhotic patients without HE (19). A direct application of these findings can be found in the concept of the "gut-brain axis." In HE, both inflammatory signals and neuroactive microbial activity can reach the brain and induce inflammation and changes in neuronal transmission (e.g., the effect of *Lactobacillus rhamnosus* on GABA receptor expression), which could explain changes in behavior and sleep patterns (135, 152). In addition, dysbiosis has also been tied to ascites development (167), spontaneous bacterial peritonitis (129), hepatorenal syndrome (73), variceal hemorrhage (188), hepatopulmonary syndrome (184), and acute-on-chronic liver failure (10).

Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) represents the most common hepatic malignant neoplasm and is one of the leading causes of cancer-related deaths worldwide (86). The evidence for the role of the GM in liver cancer is derived from functional studies in rodent models. Drastically reduced rates of carcinogenesis in diethylnitrosamine- and carbon tetrachloride-treated mice, in which the gut remained sterilized, led to speculation of a relationship between the GM and HCC (60). Further studies explained that bacterial translocation and its interaction with TLRs seem to play a pivotal role in hepatic carcinogenesis (202). Despite LPS-TLR4 signaling, which promotes liver damage through inflammation, other TLRs appear to be crucial in hepatic

carcinogenesis. For example, high-fat diets can promote the accumulation of gram-positive bacteria (*Clostridium, Bacteroides, Atopobium,* and *Desulfovibrio)*, which, in turn, are great converters of primary to secondary BAs (such as deoxycholic acid), which can increase TLR2 expression on hepatic stellate cells. Together with increased concentration of lipoteichoic acid, the TLR2 ligand in the gram-positive wall promotes a senescence-associated phenotype in hepatic stellate cells (125). Secondary BAs can also suppress the expression of chemokine ligand 19 (CXCL16), which participates typically in liver recruitment of liver natural killer T cells, which are responsible for killing tumor cells (173).

In addition to explaining liver cancer pathogenesis, some microbial strains may help in diagnosis of HCC at an early stage: analysis of circulating bacterial genetic material of five different genera (*Pseudomonas, Streptococcus, Staphylococcus, Bifidobacterium,* and *Trabulsiella*) can be employed to predict HCC, with an area under the receiver-operating curve (AUC) of 0.879 and an accuracy of 81.6% (48).

CURRENT PERSPECTIVES ON GM-BASED THERAPY

There are different strategies to modulate the GM, including antibiotics, probiotics, prebiotics, and FMT. According to the World Health Organization, probiotics are defined as "live microorganisms, that when administered in adequate amounts, confer a health benefit on the host" (209a), while prebiotics are nondigestible food components, which are selectively fermented by intestinal microorganisms and, thus, able to stimulate the growth of fixed strains of bacteria (81). A formulation that consists of both probiotics and prebiotics is referred to as "synbiotic." The global probiotic market is currently worth 15–36 billion USD/yr and is destined to grow even further (195). Probiotics are regularly administered along with conventional medicine to manage of a wide range of pathological conditions. In addition, everyday media coverage aggressively promotes probiotics as an easy-to-obtain product to maintain (or even ameliorate) consumers' health status. Despite this expanding market and growing medical use of probiotics, there are some concerns regarding their administration and safety, which lead to the first question: can the bacteria in orally administered tablets or powders reach their site of action and start colonizing the gut? Fecal, histological, and immunofluorescence studies showed that some strains survive and their concentrations increase in samples collected from the early days of administration to the end of therapy. However, their overall presence drastically decreases when oral consumption ceases, leading to the assumption that short/medium-term probiotics coverage may not exert a long-term beneficial effect (3, 76, 132, 193). In addition, it is relevant to question whether oral flora may interfere with nontablet probiotic formulations (137). Preliminary studies showed that it is possible to modify oral microbiota with oral dissolving tablets or probiotic toothpaste. However, the real effects of oral microbes on probiotics administration remain unknown.

Treatment with probiotics may involve the consumption of considerable amounts of bacteria, which leads to the second question: is it safe to take probiotics? The principal theoretical risks from introducing bacteria into a human host are related to infections and inflammatory/fatal effects derived from toxins produced either by the probiotic strains or by possible bacterial contaminants (165). Most probiotics in use are obtained from fermented aliments with a long history of safe consumption (94). Also, the majority of clinical trials showed that probiotics do not raise significant safety concerns (165). However, there are a few reports of serious adverse effects, including cases of bacterial sepsis (from supplements containing *Lactobacillus*) (166) and death from gastrointestinal mucormycosis (mold contamination of a probiotic supplement) (194). On the other hand, more invasive methods to alter the GM, by FMT [defined as administration of a solution of a fecal suspension from a donor into the intestinal tract of a recipient to change the GM composition directly (91)], are more susceptible to adverse effects, mainly in the form of nausea, diarrhea, bloating, and abdominal cramping (204). However, FMT raised particular concerns when two patients with the same stool donor experienced β-lactamase-producing *E. coli* bacteriemia, which resulted in the death of one of the patients (64).

With these premises, what is the evidence for GM-targeted therapy in liver disease? Most of the evidence derives from NAFLD studies. A recent meta-analysis (25 studies, 1,309 patients) indicated that probiotics significantly reduced body mass index, transaminases, serum cholesterol, and triglycerides, but not inflammation (measured by TNF-α or C-reactive protein) (124). In addition, synbiotics administration seemed to reduce liver steatosis, measured by ultrasound parameters (7) or by magnetic resonance (174). However, the high degree of heterogeneity (i.e., absence of standardization) in treatment characteristics (i.e., use of different probiotics formulations) and durations and confounding factors (e.g., vitamins and antidiabetic medications), along with discordant baseline characteristics among studies, may account for gaps and inconsistency in treatment response. Despite this ad hoc research, physicians have been studying the effects of GM intervention in liver cirrhosis for decades without being aware of it: we had the indirect effects of lactulose and rifaximin before our eyes without knowing the exact process through which they ameliorate HE. Studies in mice and dogs showed that lactulose could induce dynamic changes in microbial population by increasing the abundance of hydrogen-producing bacteria (Prevotellaceae and Rikenellaceae) and non-urease-producing *Lactobacillus* (74, 220). Conversely, lactulose administration in humans has demonstrated no effect on the microbiome of cirrhotic patients without HE (168) and minimal changes in cirrhotic patients with HE (13), especially after lactulose withdrawal (11), even if the everyday clinical experience tells us the exact opposite because of the proven beneficial effects of lactulose on HE and hepatic decompensation (123). According to recent data, administration of probiotics has proved to be safe and well tolerated in patients with HE, with no reports of probiotic-induced septicemia (12). In addition, two novel meta-analyses (14 trials, 1,132 patients; 21 trials, 1,420 patients) showed that probiotics might reduce endotoxemia, TNF-α, and plasma ammonia levels that, in some studies, were linked to improvement of minimal HE, prevention of overt HE progression and liver function decline, and increased quality of life (34, 57). Even the FMT approach seems to be effective: despite preventing HE recurrence, FMT remarkably reduced hospitalization rates (18). As stated previously, the majority of these trials suffer from a high risk of systematic and random errors; therefore, it is not safe to assume that interfering with gut microbes may have beneficial effects compared with placebo

or no intervention. High-quality randomized clinical trials with standardized outcome and data collection are needed to further clarify these findings.

CONCLUSIONS

The growing interest in the field of the GM has drastically enriched the state of the art over the last decade. Currently, we have clear evidence that the intestinal host-microbiome communications play different roles in the development and progression of several liver diseases. However, the complexity of these interactions remains unclear for most of the cases. Our insight about the clinical relevance of probiotics, prebiotics, and FMT use in the everyday clinical setting is beginning to take shape. Although clinical and experimental data are promising in terms of the therapeutic potential of probiotics in chronic liver diseases, we are missing evidence about the safety of probiotics and their impact on host-microbiota interactions in patients with dysbiosis in the context of liver disease. Recently, many animal studies tried to overcome these gaps, but differences in physiology and variations in the molecular targets between murine models and humans has led to translational limitations. More extensive prospective controlled studies are needed to validate current findings on dysbiosis and to define which strains to use as therapeutics and the duration of probiotics-based treatment, especially with the rapidly growing incidence of NAFLD and the promises of GM-based therapeutic strategies. Only the future will tell if gut microbes will act as the main character in the broad scenery of liver diseases.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

M.G. conceived and designed research; M.G., M. Campigotto, G.C., M. Comar, and L.S.C. prepared figures; M.G., M. Campigotto, G.C., M. Comar, and L.S.C. drafted manuscript; M.G., G.C., M. Comar, and L.S.C. edited and revised manuscript; M.G., M. Campigotto, G.C., M. Comar, and L.S.C. approved final version of manuscript.

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