

## REVIEW

## The Gut Microbiota and Liver Disease



Cristina Llorente and Bernd Schnabl

Department of Medicine, University of California San Diego, La Jolla, and Department of Medicine, VA San Diego Healthcare System, San Diego, California

## 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>)

**Keywords:** Dysbiosis; Leaky Gut; Alcoholic Liver Disease; NASH; NAFLD; Cirrhosis; Microbiome; PAMPs.

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.<sup>1</sup> 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.<sup>2-4</sup> 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.<sup>4,5</sup> Disruption of the microbial homeostasis is associated with obesity,<sup>6</sup> malnutrition,<sup>7</sup> inflammatory bowel diseases (IBD),<sup>8</sup> neurologic disorders,<sup>9</sup> cancer,<sup>10</sup> and liver diseases,<sup>11-16</sup> 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.<sup>17</sup> 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.<sup>3</sup> 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  $\approx 150$  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.<sup>18</sup> 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.<sup>19</sup>

Alcohol consumption is another environmental factor affecting the composition of the intestinal microbiome. Alcohol causes intestinal bacterial overgrowth in humans<sup>20,21</sup> and in animal models of chronic alcohol administration.<sup>12,14</sup> Alcohol also results in alcohol-associated qualitative changes of the microbiota in humans<sup>20–22</sup> and experimental animal models.<sup>12,14,23–25</sup> In particular, beneficial commensal bacteria including *Lactobacillus* are relatively lower after chronic alcohol consumption.<sup>12,20</sup> 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.<sup>25</sup> A decrease in beneficial commensal bacteria contributes to a tight junction barrier disruption.<sup>26</sup> 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.<sup>25</sup>

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<sup>27</sup> 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.<sup>28</sup> 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.<sup>29–31</sup> Other environmental factors that shape microbiota are delivery mode,<sup>32</sup> smoking,<sup>33–35</sup> and parasitic infections.<sup>36</sup>

### Genetic Factors Leading to Intestinal Dysbiosis

The microbiota from family members has more similarities than that from unrelated individuals,<sup>37,38</sup> 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.<sup>39</sup> It is important to consider that studies that have failed to find significant genotype effects on microbiome diversity<sup>3,17</sup> 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).<sup>40</sup> Adding Christensenellaceae to an obese-associated microbiome resulted in reduced weight gain in the recipient mice.<sup>40</sup> 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.<sup>41</sup>

Concerning liver disease, NOD2 variants increase the risk for culture-positive spontaneous bacterial peritonitis and bacterascites in cirrhosis and may affect survival.<sup>42,43</sup> Genetic polymorphisms of NOD2 are associated with increased mortality in nonalcoholic liver transplant patients.<sup>44</sup> Inflammasomes recognize pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) that cleavage proinflammatory cytokines such as pro-interleukin (IL)-1 $\beta$ <sup>44</sup> 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.<sup>45</sup> Intestinal dysbiosis causes inflammation of the colon, which is mediated by chemokine (C-C motif) ligand 5 (CCL5).<sup>46</sup> 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.<sup>46</sup> 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.<sup>47</sup>

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,<sup>48</sup> 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

(IgA and antimicrobial peptides and proteins [AMP]), and physical barriers (the mucus layer and the tightly interconnected intestinal epithelial lining).<sup>49–51</sup>

Host IgA response is primarily directed toward specific bacteria within the mucosa, and this bias is disrupted in animals deficient for MyD88 in T cells (T-MyD88<sup>-/-</sup>). T-MyD88<sup>-/-</sup> animals showed increased dissimilarity in both their IgA-bound and mucosa-associated communities and increased total bacterial load at the mucosa. Thus, innate signaling by T cells dictates IgA specificity to constrain the composition of the microbial community, while also limiting mucosal association of commensal microbes.<sup>52</sup> MyD88 signaling in T cells directs IgA-mediated control of the microbiota to promote health. In addition, gut microbiota-derived flagellin is recognized by dendritic cells in lamina propria via TLR-5, inducing the differentiation of B cells into IgA-producing plasma cells.<sup>5</sup> IgA-deficient mice produce gut microbiota-specific serum IgG antibodies due to mucosal barrier disruption and activation of the systemic immune system.<sup>53</sup> Furthermore, the host protein programmed cell death 1 (PD1) partially regulates the microbial modulation of IgA homeostasis; PD1-deficient mice had an altered IgA repertoire and changes in microbial communities of the gut.<sup>54</sup> The importance of IgA for microbiota composition and mucosal defense remains to be studied in chronic liver diseases.

The gut microbiota also regulates the production of AMP such as defensins, C-type lectins (eg, regenerating islet-derived 3b [Reg3b] and Reg3g), ribonucleases (eg, angiopoietin 4), and S100 proteins (eg, psoriasin) in intestinal epithelial cells and Paneth cells, which rapidly kill or inactivate microorganisms.<sup>51</sup> Altered AMP production is evident in mice deficient for MYD88, NOD2, or metalloproteinase-7 (MMP7), a protease involved in the regulation of defensin activity, as well as in mice that are transgenic for  $\alpha$ -defensin 5.<sup>55–57</sup> We have shown that chronic alcohol administration results in suppression of intestinal Reg3b and Reg3g expression in mice.<sup>12,14</sup> The Reg3g level can be restored using prebiotics, which are associated with suppression of intestinal bacterial overgrowth.<sup>14</sup> Patients who abuse alcohol similarly have decreased Reg3g expression in the duodenum.<sup>14</sup> Cirrhotic rats with ascites and translocation of viable bacteria to mesenteric lymph nodes produce lower levels of defensins and Reg3 molecules compared with cirrhotic rats without bacterial translocation. This reduction is accompanied by reduced antimicrobial activity against Enterobacteriaceae.<sup>58</sup> Further studies are required to determine whether and to what extent lower levels of AMP contribute to liver disease progression by either modulating the composition of the intestinal microbiota or facilitating bacterial translocation.

Furthermore, goblet cells are secreting mucins, which are highly glycosylated and responsible for the formation of the inner and outer mucus layer. Mucin-2 is the major secreted mucin in the small and large intestines of mice and humans.<sup>59</sup> The mucus layer with its expression of certain lectins shapes the bacterial composition. Bacteria express glycosidases and metabolic enzymes, which allows them to bind to or even use the mucus layer as a source of

energy.<sup>60,61</sup> Although the absence of mucin-2 in mice increases the susceptibility to enteric infections,<sup>62</sup> the innate immune system is on high alert and activated to maintain intestinal homeostasis.<sup>63</sup> In fact, mucin-2-deficient mice showed much higher expression of antimicrobial proteins and were protected from intestinal bacterial overgrowth and dysbiosis in response to alcohol feeding. Subsequently, lower amounts of bacterial products such as endotoxin translocated into the systemic circulation, thereby decreasing ALD.<sup>12</sup> This is an example of the interconnection between the different intestinal defense layers and the microbiota.

Interestingly, in patients with liver cirrhosis most taxonomically assigned species are of buccal origin, suggesting an invasion of bacteria from the mouth to the intestine.<sup>64</sup> Liver cirrhosis is known to be accompanied by decreased innate immune system surveillance, including lower gastric acid secretion,<sup>65</sup> reduced bile flow,<sup>66</sup> and impaired AMP production.<sup>58</sup> Compromised host defense might contribute to the migration of the oral to the intestinal microbiome. However, to which degree each individual factor contributes to compositional changes in the microbiota requires further analysis.

## Intestinal Dysbiosis Associated With Liver Disease

We have summarized the bacterial taxonomic changes in ALD, NAFLD/NASH, and cirrhosis in recent reviews.<sup>67,68</sup> Interestingly, most of the precirrhotic liver diseases and cirrhosis are associated with intestinal bacterial overgrowth (described in detail in our aforementioned reviews). No single bacterial species has been mechanistically linked to the onset or progression of liver disease. Dietary habits may induce dysbiosis, characterized by an increased percentage of intestinal Gram-negative bacteria. Indeed, Gram-negative proteobacteria are a transplantable phylum that accelerates cholestatic liver fibrosis.<sup>69</sup> As shown by us and described in more detail previously, the reversal of metabolic intestinal changes improves ALD.<sup>25</sup> Future studies should establish causative correlations between functional rather than taxonomic microbiota changes and the pathogenesis of liver disease.

## Leaky Gut Hypothesis—Truth or Myth?

Many of the precirrhotic liver diseases are associated with increased intestinal permeability. For example, a high-fat diet (HFD) increases intestinal permeability in mice<sup>70</sup> and humans.<sup>71</sup> Patients with NAFLD have significantly increased intestinal permeability and more disrupted tight junctions than healthy individuals.<sup>72</sup> Chronic alcohol abuse results in a disruption of the intestinal barrier.<sup>73</sup>

How is the gut barrier being disrupted, and is this dependent on the microbiome and dysbiosis? Although there is a possibility that liver disease itself increases intestinal permeability, intestinal inflammation might directly cause a gut barrier dysfunction and the translocation of microbial products. Intestinal inflammation is commonly

found in patients with chronic liver disease. Colorectal mucosa inflammation is characteristic in obesity.<sup>74</sup> HFD increases the activity of the transcription factor nuclear factor- $\kappa$ B (NF $\kappa$ B) and expression of tumor necrosis factor- $\alpha$  (TNF $\alpha$ ) in the small intestine of mice. HFD-induced intestinal inflammation depends on the enteric microbiota, because germ-free mice are protected from inflammatory changes.<sup>75</sup> As discussed earlier, NLRP3 and NLRP6 deficiency promotes intestinal dysbiosis, triggers colonic inflammation via chemokine (C-C motif) ligand 5 (CCL5), and increases intestinal permeability. Microbial products translocate to the liver, enhance hepatic inflammation, and cause NAFLD to progress to steatohepatitis.<sup>46</sup> As a marker of a disrupted mucosal barrier, the systemic levels of LPS are elevated in HFD-fed mice<sup>70</sup> and humans after a high-fat meal.<sup>76,77</sup> Endotoxemia is observed in children with NAFLD<sup>78</sup> and in patients with NASH.<sup>79</sup>

Similarly, chronic alcohol feeding results in subclinical intestinal inflammation in mice. The number of TNF $\alpha$ -producing monocytes and macrophages in the small intestine increases in mice chronically administered alcohol. These findings were confirmed in duodenal biopsy samples from patients with chronic alcohol abuse. Intestinal decontamination with nonabsorbable antibiotics reduced intestinal bacterial overgrowth, decreased intestinal inflammation and permeability, and reduced ALD in mice. These findings suggest that dysbiosis causes intestinal inflammation and gut barrier dysfunction, although the triggering microbial metabolite or product is currently not known.

In tissue-specific genetically manipulated mice, the TNF-receptor-1 (TNFR-1) expressed on intestinal epithelial cells was identified as a target for TNF $\alpha$  secreted from inflammatory cells in the lamina propria. Our results suggest that dysbiosis-induced intestinal inflammation and TNFR-1 signaling on enterocytes are mediating a disruption of the intestinal barrier and most importantly ALD. Therefore, intestinal TNFR-1 is a crucial mediator of ALD.<sup>16</sup>

Both, experimental cirrhosis and human cirrhotics, show features of intestinal inflammation.<sup>80–82</sup> Whether intestinal inflammation associated with cirrhosis depends on the gut microbiome is not known. Microbial products or PAMPs translocate through disrupted tight junctions using the paracellular route from the intestinal lumen to extraintestinal tissues and organs, but living bacteria appear to translocate via the transcellular route (transcytosis).<sup>83</sup> During decompensated cirrhosis, a further increase in intestinal permeability could be triggered by intestinal inflammation and may contribute to enhanced translocation of viable bacteria.<sup>84</sup>

PAMPs reach the liver via the portal system and activate specific pattern recognition receptors including TLRs. TLR signaling promotes liver inflammation and damage.<sup>67</sup> Mice that express nonfunctional molecules in the TLR signaling pathway are often protected from liver disease.<sup>85–88</sup> Signaling via the LPS receptor TLR4 in hematopoietic-derived cells is required for the development of liver steatosis but not for the development of obesity in mice.<sup>89</sup> Mice deficient in sensing PAMPs or downstream signaling are resistant to NASH.<sup>90,91</sup>

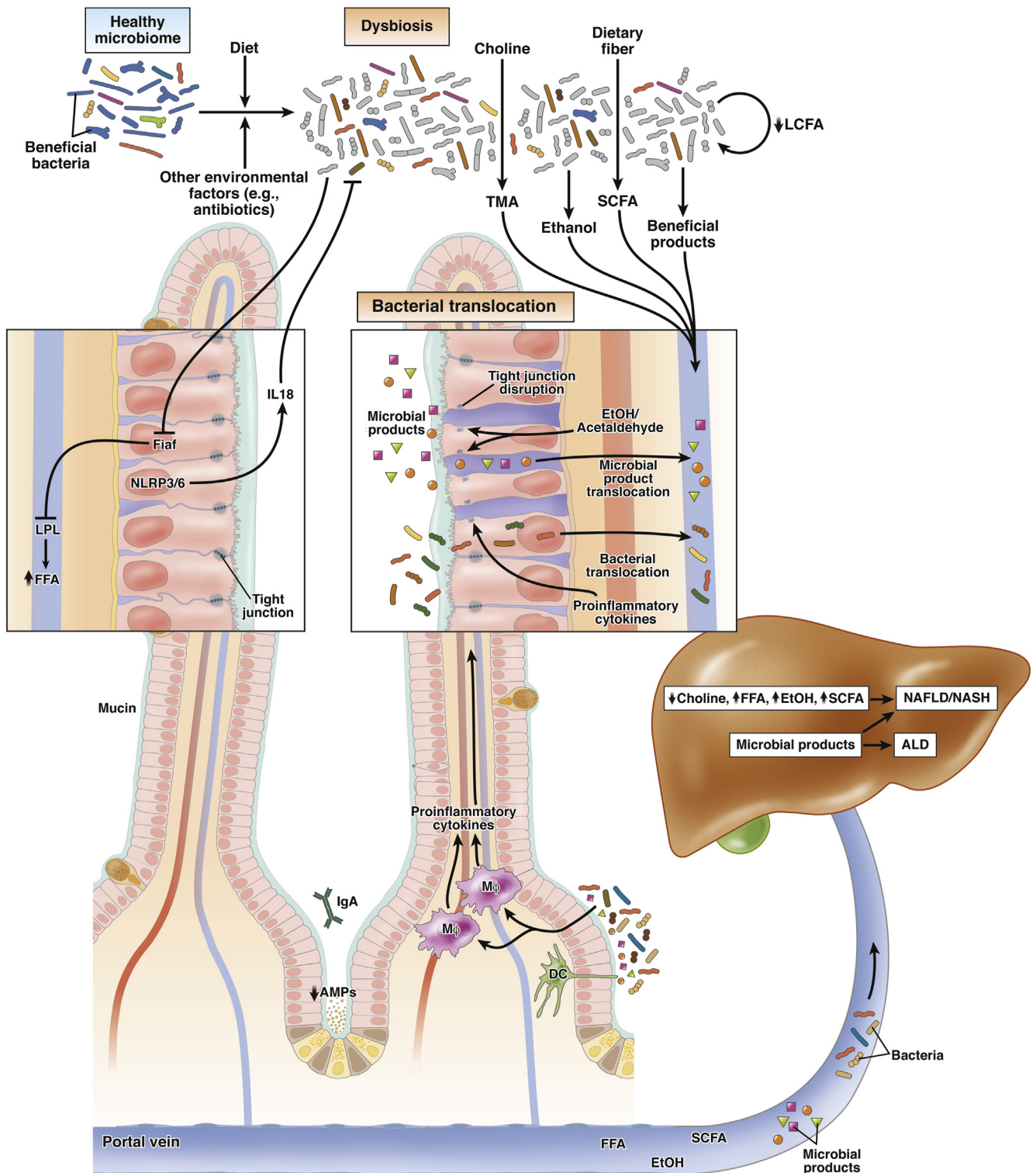
Bacterial overgrowth correlates with an increase in the luminal amount of PAMPs. Thus, in the presence of an intestinal barrier dysfunction, the luminal bacterial load determines the amount of PAMPs that translocate to the systemic circulation and hence the degree of liver damage.<sup>12</sup> Antibiotics seem to have a beneficial effect by reducing the intestinal bacterial burden, which in turn reduces the amount of translocating PAMPs. Intestinal decontamination improves several diseases that are dependent on increased intestinal permeability and elevated systemic levels of PAMPs. Treatment of obese mice with antibiotics improves glucose metabolism and reduces adiposity and adipose inflammation.<sup>92</sup> Intestinal decontamination improves experimental ALD as a preventive strategy<sup>93</sup> and as an intervention.<sup>16</sup> Nonabsorbable antibiotics have a beneficial effect on toxic and cholestatic liver fibrosis<sup>87</sup> and NASH.<sup>94</sup> A clinical trial in patients with ALD using the nonabsorbable antibiotic paromomycin did not show an improvement in liver damage compared with placebo-treated patients.<sup>95</sup> Because systemic endotoxemia was not reduced after 4 weeks of treatment, antibiotic therapy might not have been effective, or it might have induced dysbiosis. Cirrhotics suffering from hepatic encephalopathy are commonly treated with nonabsorbable antibiotics, which decreases the number of ammonia-producing intestinal bacteria and improves the mental status of patients with end-stage liver disease.<sup>96</sup> In addition, antibiotics reduce the translocation of viable bacteria from the intestinal lumen to extraintestinal space such as mesenteric lymph nodes in experimental cirrhosis.<sup>94</sup>

All the above evidence strongly argues for an important role of microbial products that translocate from the intestine to the liver. Interestingly, not all patients with alcohol dependence show increased intestinal permeability. Alcoholics could be divided into low and high gut permeability groups, with the low permeability group having a similar permeability as healthy controls. Alcoholics with high intestinal permeability also had an altered composition and activity of the gut microbiota such as lower amounts of *Bifidobacterium* spp., Clostridiales Family XIV *incertae sedis*, and Ruminococcaceae when compared with healthy controls. Levels of these bacteria were not changed in alcoholics with a low intestinal permeability. Importantly, higher intestinal permeability was associated with higher scores of depression, anxiety, and alcohol craving after 3 weeks of abstinence.<sup>20</sup> Whether liver disease correlates with the degree of intestinal permeability in humans requires further study.

To confirm the importance of the intestinal microbiota for chronic liver disease, we induced liver fibrosis in germ-free and conventional mice by administration of thioacetamide via the drinking water. To our surprise, germ-free mice showed more liver fibrosis than conventional mice. To confirm our results we used a second model of liver fibrosis by repeated intraperitoneal injections of carbon tetrachloride, switched the source of germ-free mice, and used a different gnotobiotic facility, but we still found exacerbated liver fibrosis in the absence of the microbiota. Using an additional genetic model with mice lacking

Myd88/Trif and hence downstream innate immunity signaling, we identified hepatocytes to be more susceptible to toxin-induced cell death.<sup>97</sup> Thus, although microbial products are related to inflammation and progressive liver disease, the complete absence of PAMPs also has a deleterious effect on the liver. It is feasible that metabolic products from the microbiota might provide hepatoprotection and might ameliorate chronic liver diseases.

For example, indole-3 propionic acid is a deamination product of dietary tryptophan, and its production is completely dependent on the presence of the gut microbiota.<sup>98</sup> Indole-3 propionic acid was recently shown to protect the intestinal barrier through the xenobiotic sensor pregnane X receptor (PXR).<sup>99</sup> Meta-transcriptomic and metabolomic studies are needed for further analysis and investigation.



## Additional Intestinal Factors Contributing to Liver Disease

We would like to highlight and present a few examples of other ways the microbiota contributes to liver disease. Both NAFLD and NASH are strongly linked to obesity, type 2 diabetes mellitus, and the metabolic syndrome.<sup>100</sup> A western diet rich in fats and carbohydrates contributes to qualitative microbiota changes.<sup>101</sup> The microbiota may contribute to obesity and NAFLD in a number of ways, some of which are quite unexpected. Alterations in the intestinal microbiota increase energy extraction and fermentation of dietary fibers into oligosaccharides, monosaccharides, and short-chain fatty acids, respectively.<sup>102</sup> For instance, germ-free mice are protected from HFD-induced weight gain and obesity.<sup>103</sup> There may be complex mechanisms responsible for increased energy harvest from the diet by bacteria.<sup>92</sup> Transplantation of fecal microbiota from adult female twin pairs discordant for obesity into germ-free mice fed low-fat mouse chow, as well as diets representing different levels of saturated fat and fruit and vegetable consumption typical of the U.S. diet, increased total body and fat mass as well as obesity-associated metabolic phenotypes.<sup>104</sup> Moreover, despite gaining the same body weight after HFD feeding, one group of mice developed hyperglycemia and had a high plasma concentration of proinflammatory cytokines (called the “responders”), while the second cohort remained normoglycemic and had lower levels of systemic inflammation (“nonresponders”). Germ-free mice colonized with intestinal microbiota from the nonresponder group maintained normoglycemia whereas germ-free mice transplanted with responder microbiota showed hyperglycemia, hepatic macrovesicular steatosis, more hepatic triglycerides, and an increased expression of genes involved in *de novo* lipogenesis after HFD feeding. Both groups developed comparable obesity. These results suggest that gut microbiota contributes to the development of NAFLD independently of obesity.<sup>105</sup>

Bacterial metabolites promote obesity and in particular NAFLD. For instance, microbiota produce endogenous ethanol through the fermentation of carbohydrates, a physiologic pathway that is strongly enhanced in the presence of gut dysmotility (eg, associated with obesity, diabetes, or chronic alcohol abuse) or an excess of carbohydrates in the diet.<sup>106,107</sup> Obese animals have higher blood ethanol levels.<sup>107</sup> Once ethanol reaches the liver via the portal vein, it can promote steatosis,<sup>108</sup> oxidative stress, and liver inflammation.<sup>15</sup> Pediatric patients with NASH have higher blood ethanol concentrations than healthy individuals or pediatric patients with NAFLD, suggesting that endogenous ethanol production by the microbiota might be important in the progression from simple hepatic steatosis to NASH.<sup>15</sup> Future metatranscriptomic and metabolomic studies are required to confirm these findings.

Ethanol can be metabolized into acetaldehyde by microbial fermentation<sup>109</sup> or by the host either in the liver or the intestine.<sup>110,111</sup> Ethanol can reach the microbiome and intestinal epithelia cells from either the luminal side or via diffusion from the systemic blood circulation. Ethanol and in particular its metabolic derivative acetaldehyde can disrupt intestinal tight junctions and increase intestinal permeability.<sup>112</sup>

Conventionalization of adult germ-free C57BL/6 mice with a normal microbiota harvested from the distal intestine (cecum) of conventionally raised animals produces a 60% increase in body fat content and insulin resistance. Fasting-induced adipocyte factor (Fiaf), a member of the angiopoietin-like family of proteins, is selectively suppressed in the intestinal epithelium of normal mice by conventionalization. Analysis of germ-free and conventionalized, wild-type, and Fiaf-deficient mice established that Fiaf is a circulating lipoprotein lipase inhibitor and that its suppression is essential for the microbiota-induced deposition of triglycerides in adipocytes.<sup>19</sup>

A dysbiotic microbiome associated with HFD metabolizes and converts dietary choline into methylamines. Lower hepatic choline levels reduce secretion of very-low-density

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**Figure 1. (See previous page). Host-microbiome interactions during liver disease.** Environmental factors (including diet), genetics factors (NLRP3/6 deficiency via IL-18), and the mucosal immune system influence the composition of gut microbiome.<sup>3,45,46</sup> Dysbiosis is often characterized by loss of beneficial intestinal bacteria. Ethanol directly suppresses the capacity of the microbiota to synthesize saturated LCFA, which also contributes to dysbiosis.<sup>25</sup> A dysbiotic microbiota has the ability to metabolize various nutrients, and their fermented products are available to the host, influencing liver disease. The products of bacterial metabolic activity including SCFA from otherwise nondigestible dietary fibers, EtOH and its fermented product acetaldehyde affect immune responses and epithelial integrity (disruption of tight junctions).<sup>106,112</sup> Other factors contributing to tight junction disruption are inflammatory cytokines secreted from activated immune cells in the lamina propria.<sup>16</sup> Microbial products such as LPS translocate through the intercellular space to the systemic circulation.<sup>67</sup> Bacteria can also translocate via transcytosis.<sup>83</sup> These products and bacteria reach the liver via the portal vein, where they are further metabolized or directly act as a ligand of pattern recognition receptors such as TLRs promoting liver diseases as NAFLD, NASH, and ALD.<sup>67,85-88</sup> Dietary choline is metabolized by the commensal bacteria to TMA. Hepatic choline deficiency lowers VLDL efflux resulting in NAFLD and progression to NASH.<sup>113-115</sup> Gene expression of Fiaf in intestinal epithelial cells can be inhibited by intestinal microbiota increasing levels of free fatty acids in plasma via inhibition of LPL.<sup>19</sup> Suppression of AMP secretion might contribute to dysbiosis and facilitate bacteria translocation.<sup>58</sup> In addition, loss of innate (MyD88-dependent) signaling in T cells that coordinates homeostatic IgA-directed targeting against commensal microbes results in dysbiosis and more severe inflammatory disease.<sup>52</sup> As yet unidentified products or metabolites from the microbiota might exert hepatoprotective effects. ALD, alcoholic liver disease; AMP, antimicrobial peptides and proteins; DC, dendritic cells; EtOH, ethanol; FFA, free fatty acids; Fiaf, fasting-induced adipocyte factor; IL, interleukin; LCFA, long-chain fatty acids; LPL, lipoprotein lipase; NAFLD/NASH, nonalcoholic fatty liver disease/steatohepatitis; NLRP, nucleotide-binding domain and leucine rich repeat-containing protein; SCFA, short-chain fatty acid; TMA, trimethylamine; VLDL, very-low-density lipoprotein.

lipoprotein in the liver causing NASH.<sup>113,114</sup> Choline deficiency is linked to NAFLD and NASH<sup>115</sup> and is a common model of experimental NASH in rodents. Healthy subjects develop a fatty liver on a choline-deficient diet only if there is a single-nucleotide polymorphism in the promoter region of phosphatidylethanolamine *N*-methyltransferase, which affects de novo synthesis of phosphatidylcholine.<sup>116</sup> This is an excellent example how environmental and genetic factors synergize to cause liver disease. Causes for changes in the intestinal microbiota composition and consequences of intestinal dysbiosis that are relevant for the pathogenesis of chronic liver diseases are illustrated and summarized in Figure 1.

## Conclusion and Future Directions

The gut microbiome is composed of trillions of microbes that inhabit the human intestine. This complex community influences both normal physiology and disease susceptibilities through its collective metabolic activities and host interactions. We are just starting to understand the mechanisms that result in dysbiosis and its functional consequences for liver diseases. We need to further dissect the mechanisms of how dysbiosis affects intestinal homeostasis and causes the progression of liver disease beyond triggering an increase in intestinal permeability. Genetic factors (single-nucleotide polymorphisms, gene copy numbers, etc.) are involved in shaping the composition of the gut microbiota and modulating disease susceptibility or resistance.<sup>40,43,117</sup> Given that germ-free mice are more susceptible to experimental liver fibrosis, there might be hepatoprotective microbial metabolites. Our ultimate goal should be to design therapies that restore intestinal eubiosis and homeostasis. This could be achieved by either targeting the microbiota or the intestine of the host. This goal is formidable because of the immense diversity among the microbiota, interpersonal variations, and temporal fluctuations in composition, which are especially apparent in disease.

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#### Correspondence

Address correspondence to: Bernd Schnabl, MD, Department of Medicine, University of California–San Diego, Biomedical Research Facility 2 (BRF2), Room 4A22, 9500 Gilman Drive, MC0063, La Jolla, California 92093. e-mail: beschnabl@ucsd.edu; fax: (858) 822-5370.

#### Conflicts of interest

The authors disclose no conflicts.

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