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Review

Outside the liver box: The gut microbiota as pivotal modulator of liver diseases☆

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ABSTRACT

The gut microbiota affects host physiology and has evolved as an important contributor to health and disease. Gut and liver are closely connected and communicate via the portal vein and the biliary system so the liver is constantly exposed to gut-derived bacterial products and metabolites. The intestinal barrier is important for maintaining physical and functional separation between microbes in the gut and the interior of the host and disruption of the barrier function can lead to bacterial translocation and increased leakage of bacterial metabolites. Liver diseases have been associated with dysbiotic changes in the gut microbiota and impaired gut barrier integrity, thus a future strategy to treat liver disease may be to target the gut microbiota and thereby restore the gut barrier function. This review will summarize and discuss studies that have shown a link between the gut microbiota and liver disease with the main focus on non-alcoholic fatty liver disease and alcoholic liver disease.

1. Introduction

Our gut microbiota consists of trillions of bacteria which are important contributors to health and disease [1]. Microbial metabolites produced in the gut signal to distal organs and thereby affect numerous processes in our body [2]. The liver, which is a central organ with many essential functions (e.g. synthesis of proteins such as albumin and clotting factors, detoxification of various metabolites, lipid metabolism, hormone and bile production and glycogen production and storage) is constantly exposed to gut-derived bacterial products and metabolites and it has become clear that these compounds can contribute to liver damage, which can lead to chronic liver disease and eventually to liver failure [3]. Currently, there are limited treatment options for patients with liver failure and the only curative treatment is liver transplantation.

Already in 1967, the group of Hans Popper showed in a model with germ-free rats that the intestinal microbiota has an influence on liver injury [4] and several human studies have shown that different types of liver disease are associated with an altered gut microbiota [5,6] but more studies are required to obtain evidence for a causal effect of the microbiota. Animal models in which the microbiota can be manipulated are important tools to provide such evidence and an emerging picture shows that the gut microbiota might have a role in the development and progression of liver disease and that targeting the gut microbiota could be a new treatment option for liver disease. This review will summarize and discuss rodent models that have shown a link between the gut microbiota and liver disease. The main focus will be on non-alcoholic fatty liver disease (NAFLD) and alcoholic liver disease (ALD), which are the most common causes of chronic liver disease in Western countries [7,8]. Other conditions such as cholestatic liver disease, cirrhosis and hepatocellular carcinoma (HCC) will also be discussed.

2. The gut microbiota

The gut microbiota is the sum of all microorganisms (bacteria, archaea, eukaryotes and viruses) that reside in the gastrointestinal tract and this review will focus on the bacteria in the gut. The dominating bacterial groups (phyla) in the human gut microbiota are Bacteroidetes and Firmicutes followed by Actinobacteria, Proteobacteria and Verrucomicrobia [9–11]. Microbiota composition can be determined by various methods and the two most commonly used are 16S rRNA sequencing, (sequences PCR amplicons from the 16S ribosomal RNA gene) or shot-gun metagenomics (sequences the whole microbial genome) [12]. 16S rRNA sequencing is relatively quick, simple and cheap whereas whole genome analysis is more expensive and requires more complex sample preparation and can directly provide the relative abundance of microbial functional genes. For a more detailed overview and comparison of microbiota analyses, see the excellent review by

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Knight et al. [13]. The microbiota composition vary in different compartments of the gut but the fecal microbiota is used as a proxy of the overall gut microbiota [14]. The microbial genome encodes at least 100-times more genes than the human genome and is assumed to have a great impact on health and disease [15–17]. The composition of the gut microbiota is influenced by many different factors both within the host (genotype) and in the environment (e.g. diet, exercise, cold exposure, infections and antibiotics) [8,18]. Studies have shown that events early in life such as mode of delivery, breast- or formula feeding, use of antibiotics can affect the microbiota composition and likely the health status later in life [19,20].

Human studies have provided information about bacterial groups or species associated with different disease conditions but more mechanistic information is still required. Gnotobiotic mice (i.e. germ-free mice colonized with different bacterial communities or specific bacteria) are essential tools to identify and study bacteria that can prevent or cause disease. However, it is important to be precautious when interpreting and translating results from animal studies into human settings [21–25]. One major concern when using mouse models is the differences in bile acid profiles between humans and mice, specifically the high levels of the FXR antagonist tauro-β-muricholic acid (TβMCA) in germ-free mice [26,27]. The crosstalk between bile acids and the gut microbiota is important not only in cholestatic liver disease but in other liver diseases as well and an improved mouse model to study these interactions would be mice with a humanized bile acid profile that can be colonized with human bacteria. A potential approach would be to generate germ-free mice without CYP2C70, which has been identified as the enzyme responsible for the formation of murine bile acids [28].

Another concern is that different mouse strains have different susceptibility to metabolic diseases [29,30]. The interaction between environmental factors such as birth place, host genetics and diet influences the gut microbiota and this is important to consider when designing animal studies and interpreting the results [30–32]. Many studies have shown that alterations in the gut microbiota composition and changes in microbial metabolites are associated with diseases [2] and strong evidence for a causative role of the gut microbiota have been presented for metabolic diseases [21,33–40]. Central obesity, type 2 diabetes mellitus and dyslipidemia are all features of the metabolic syndrome and could lead to the development of NAFLD [8].

3. The gut-liver axis

The gut and liver are closely connected and communicate via the portal vein and the biliary system and the liver is constantly exposed to gut-derived bacterial products and metabolites. The microbes in the gut can affect the liver via different mechanisms such as metabolite production (short chain fatty acid (SCFA), immune modulation (hepatic immune response to gut-derived factors e.g. lipopolysaccharide (LPS)), bile acid metabolism (generation of secondary bile acids) and alterations in the barrier integrity (tight junction proteins)) [6,41]. The integrity of the intestinal barrier is important to maintain physical and functional separation between microbes in the gut and the interior of the host. Under normal conditions there should only be selective passage of substances across the intestinal epithelium but disturbances in the intestinal barrier function can lead to a “leaky gut” resulting in bacterial translocation and increased leakage of bacterial metabolites (Fig. 1).

There are many different initiating factors that can cause liver damage including viral infection, excessive alcohol consumption, metabolic disorders, autoimmune disorders, or cholestatic disorders [42] (Fig. 1). Most liver diseases, regardless of their origin, seem to be associated with a disrupted gut barrier function and there is a link between an altered gut microbiota and increased intestinal permeability [5,6]. However, the cause-consequence is not fully understood. Some studies have shown that alterations in the gut microbiota can lead to changes in permeability [43–45] while others have indicated that increased intestinal permeability may occur independently of changes in the gut microbiota [42,46].

Cani et al. discovered that LPS, which is derived from gram negative bacteria and crosses the intestinal barrier during physiological conditions, can contribute to obesity, insulin resistance and low grade inflammation via toll-like receptors [44]. They coined the term “metabolic endotoxemia”, defined as increased levels of the bacterial LPS, and showed that LPS triggered inflammatory processes and accumulation of fat in the liver via binding to CD14. Later on, they also showed that treatment with prebiotics improved the gut barrier function and decreased systemic and hepatic inflammation in a mouse model with genetically induced obesity [47]. In this study, *ob/ob* mice were treated with oligofructose which induced changes in the gut microbiota composition with increased levels of *Bifidobacterium* spp. *Lactobacillus* spp. and *Clostridium coccoides-Eubacterium* cluster and increased the production of GLP-2. Additional experiments showed that GLP-2 treatment had similar effects on gut permeability and systemic and hepatic inflammation as the prebiotic while a GLP-2 antagonist reduced most of the positive effects induced by the prebiotic treatment.

The mucus degrading bacteria *Akkermansia muciniphila* have also shown positive effects on the barrier function. The abundance of *A. muciniphila* was reduced in mice with obesity and insulin resistance and this was associated with decreased barrier function [43]. Normalization of the levels of bacteria by either prebiotic feeding or treatment with *A. muciniphila* restored the gut barrier function and improved the high-fat diet (HFD)–induced obesity and low-grade inflammation. Underlying mechanisms for the protective effect of *A. muciniphila* has been further studied and Plovier et al. showed that Amuc_1100, a specific protein from the outer membrane of *A. muciniphila,* improved the gut barrier and lowered LPS levels in HFD-fed mice [48]. It was also shown that extracellular vesicles derived from *A. muciniphila* could decrease gut permeability in HFD-fed mice by increasing the expression of the tight junction protein occludin [49]. These findings show that not only live bacteria but also gut-derived bacterial products can have beneficial effects on the gut barrier function.

An important part of the gut-liver axis is the crosstalk between bile acids and the gut microbiota. Bile acids signal via receptors both in the liver and the gut and the most well-studied ones are farnesoid X receptor (FXR) and Takeda G-protein receptor 5 (TGR5). Different bile acids differ substantially in their affinity for these receptors and intestinal bacteria can metabolize primary bile acids into secondary bile acids and thereby changing the signaling properties of the bile acid pool [50]. Conversely, bile acids can modulate the gut microbiota composition both directly and indirectly by signaling via bile acid receptors. Studies using knockout mice for FXR or TGR5 have shown that these receptors affect many metabolic processes in the body and that the gut microbiota plays an important role in this context, which have been reviewed elsewhere [41,50].

Another interesting player in the gut-liver axis is immunoglobulin A (IgA), which can be produced by the liver and secreted into the gut via bile. The production of IgA seems to be dependent on a normal microbiota since is it almost absent in germ-free mice and the production is induced upon colonization [51,52]. In turn, IgA can modulate the composition of the microbiota by binding to bacteria and it protects the host against pathogens [53,54].

Increasing evidence shows that there is a bilateral interaction between the liver and the gut microbiota and targeting this intriguing crosstalk might provide a new treatment option for liver diseases.

4. Non-alcoholic fatty liver disease

NAFLD occurs in over 30% of the general population in Western countries and is often considered as the hepatic manifestation of the metabolic syndrome. The initial signature of NAFLD is accumulation of fat as triglycerides in hepatocytes in combination with low grade inflammation (simple steatosis) that can progress into non-alcoholic

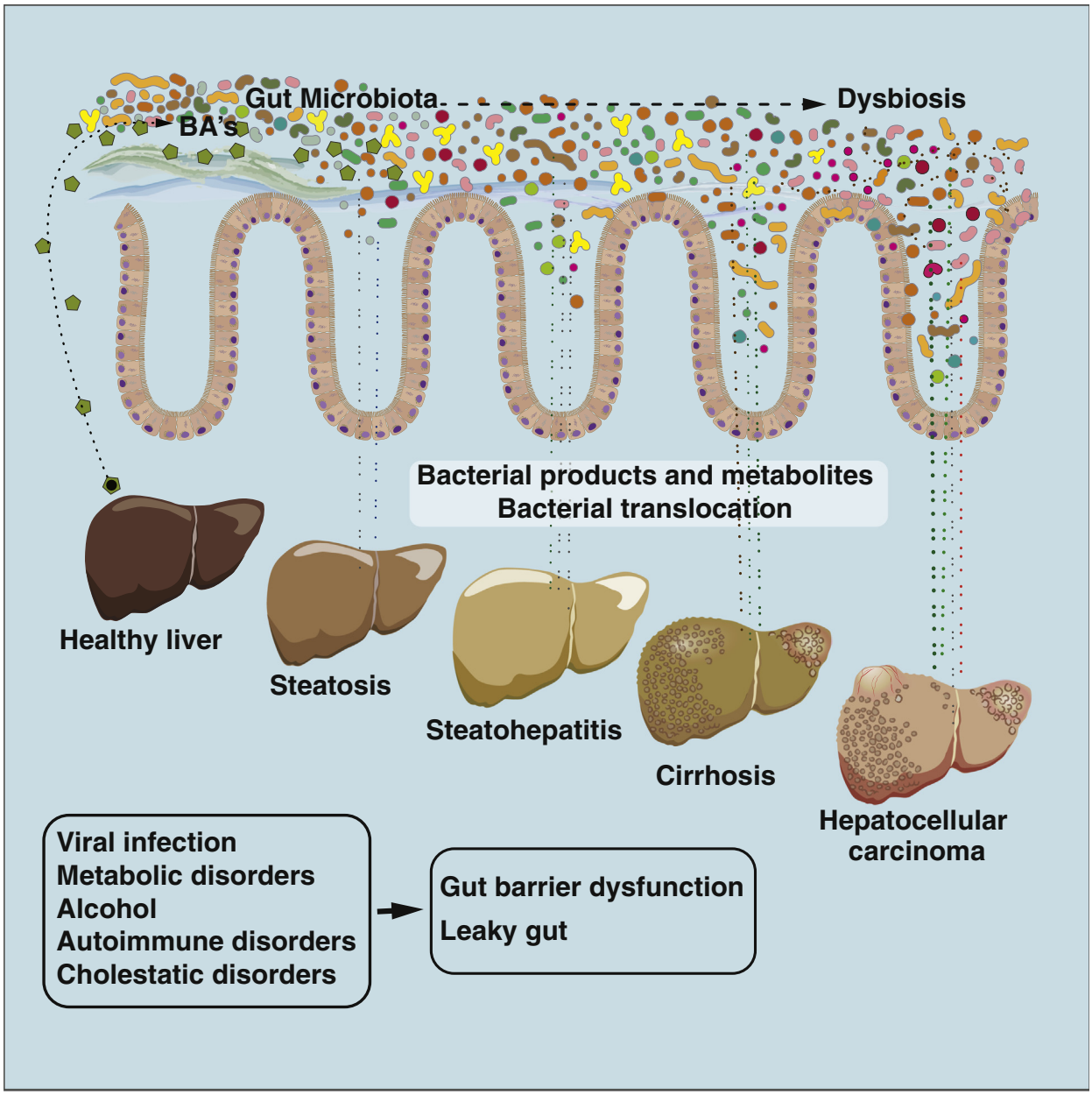

Fig. 1. Development and progression of liver disease. Liver damage can be induced by different factors such as viral infection, metabolic disorders, excessive alcohol intake, autoimmune disorders or cholestatic disorders. Many liver diseases are associated with disrupted intestinal barrier function resulting in a leaky gut which is characterized by bacterial translocation and increased leakage of bacterial products. Dysbiotic changes in the gut microbiota are associated with liver disease and might contribute to the development and progression of disease. Non-alcoholic fatty liver disease is initiated when the liver starts to accumulate fat (simple steatosis) and can progress into steatohepatitis and further into cirrhosis and hepatocellular carcinoma.

steatohepatitis (NASH), characterized by steatosis with ballooning, necroinflammatory changes and various degrees of fibrosis, which can further progress into cirrhosis and/or eventually liver cancer [55–57] (Fig. 1). Simple steatosis is often asymptomatic and it would therefore be desirable to find markers that could identify patients at high risk of developing NASH and cirrhosis.

In the late 1990s, studies using rat models showed that prebiotic feeding with oligofructose had an impact on lipid metabolism in the liver, indicating that the gut microbiota might have a role in the development of liver steatosis [58,59]. The suggested mechanism was increased production of SCFAs (predominantly propionate) by microbial fermentation of the non-digestible carbohydrates in the prebiotics, which decreased lipogenesis and reduced steatosis and liver inflammation [60]. It was later shown that the prebiotic inulin could decrease hepatic lipogenesis and lower plasma triacylglycerol concentrations in human subjects [61].

A direct link between the microbiota and liver steatosis was later demonstrated by Bäckhed et al. who colonized germ-free mice with a mouse microbiota and found that the microbiota induced hepatic de novo lipogenesis measured by expression of several key enzymes in the liver, such as ACC and FAS, and it increased the levels of liver triglycerides in addition to the increased body weight gain and insulin resistance [33]. A recent study confirmed previous findings that germ-free mice are protected against diet-induced obesity and also showed that the microbiota plays a role in lipid metabolism [62]. In this study, Martinez-Guryn et al. showed that germ-free mice exhibited impaired lipid absorption and digestion compared with conventionally raised mice. They also showed that a HFD altered the small intestinal

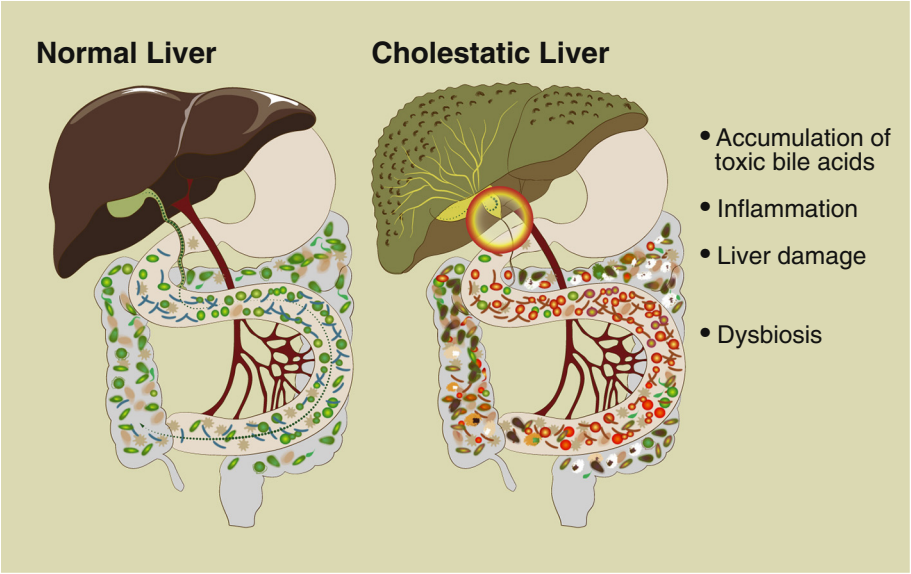

Fig. 2. Cholestatic liver disease. Cholestatic liver disease is characterized by blockage in the bile flow resulting in accumulation of toxic bile acids and hepatic and biliary inflammation. Bacterial dysbiosis and disturbances in the cross-talk between bile acids and the gut microbiota might contribute to the development and progression of cholestatic liver disease.

microbiota and that these changes promoted lipid absorption when the high-fat-induced microbiota was transferred into germ-free mice even on a low-fat diet.

A link between the microbiota and the development of hyperglycemia and steatosis, regardless of obesity, was also shown in a study where microbiota from HFD–fed mice was transferred into germ-free mice [63]. The donor mice were selected based on glycemic response to the HFD and identified as responder (with hyperglycemia and increased levels of pro-inflammatory cytokines) or non-responder (with normal glucose levels and lower systemic inflammation) even though they showed the same body weight gain. Microbiota analysis revealed that responder and non-responder mice had different microbiota composition. Furthermore, transfer of the responder and non-responder microbiota into germ-free mice showed that the NAFLD features associated with the distinct microbiota were transmissible. Evidence for a contribution of the gut microbiota in energy homeostasis was shown in a study where the gut microbiota was investigated after cold exposure. Mice that had been exposed to cold exhibited an altered gut microbiota composition and transfer of the cold microbiota led to increased insulin sensitivity in the recipient mice [64]. The cold-exposed and the cold microbiota-transplanted mice also showed increased intestinal absorption capacity and decreased levels of *A. muciniphila,* a bacteria that has shown beneficial effects on obesity and insulin resistance in other studies [43,48]. Zietak et al. confirmed that cold exposure protects against diet-induced obesity via alterations in the gut microbiota and they suggested that microbiota-induced changes in bile acid metabolism could be a contributing factor for the improved phenotype [18].

One of the key features that distinguish NASH from simple steatosis is fibrosis which occurs in different stages F0–F4 [57]. NASH patients with no or negligible fibrosis (F0–F1) have a good prognosis and lifestyle changes such as body weight loss and dietary restrictions are the main interventions [57]. In contrast, advanced fibrosis (F2-F3) may progress to cirrhosis (F4) that can lead to liver failure and/or HCC. It is therefore essential to establish the stage of liver fibrosis to provide accurate treatment strategies. Non-invasive diagnostic approaches, including biomarkers, are under development aiming to replace potentially harmful liver biopsies. Recently, it was shown that the metagenomic signature of the fecal microbiota could be used to distinguish patients with mild to moderate NAFLD from patients with advanced fibrosis, which is promising for future non-invasive detection methods of fibrosis in NAFLD [65]. However, this was a relatively small study with 86 patients and among these only 12 females and 2 men with advanced fibrosis. More evidence for a contributing role of the microbiota in NASH was shown by Chiu and coworkers who colonized germ-free mice with feces from patients with NASH or from healthy individuals and fed the mice a standard diet or HFD [23]. The microbiota from healthy individuals in combination with HFD induced simple steatosis while the microbiota from NASH patients induced NAFLD and inflammation in the colonized mice. This study suggested that the microbiota could be a driving force in the development and progression of NAFLD.

However, further studies will be needed to determine if specific bacteria are driving the development and progression of NASH and if other bacteria could be protective.

5. Alcoholic liver disease

Excessive alcohol consumption is one of the major risk factors for liver disease and can lead to several types of injury. Moderate overconsumption of alcohol can result in a fatty liver (simple steatosis), which resembles the phenotype of NAFLD. This state is reversible and resolves with abstinence but people with alcoholic steatosis who continue to drink are predisposed to hepatic fibrosis and cirrhosis [66]. In addition to alcohol-induced steatosis, there are other forms of ALD such as severe alcoholic hepatitis (AH), which generally occurs after several years of heavy drinking and is recognized by a rapid onset of jaundice and commonly also with fever, ascites and occasionally hepatic encephalopathy [66]. The prognosis for patients with severe AH is poor with high short-term and long-term mortality and it is important to identify the predisposed patients at an early stage. Importantly, some but not all of the heavy alcohol consumers develop liver cirrhosis, which indicates that there are other factors than the quantity of alcohol consumption that influence disease development, e.g. genetic factors, diet and the intestinal microbiota [67].

Several studies have shown that alcohol intake leads to dysbiosis with decreased bacterial diversity and increased intestinal permeability [42,68–70]. However, it is not fully understood how the gut microbiota influence the intestinal permeability and liver injury in ALD although several potential mechanisms have been suggested. Depletion of the microbiota with antibiotics lowered the levels of endotoxins and protected against alcohol-induced liver injury in rats indicating that there are bacteria or bacterial products that are detrimental in the disease development [71]. On the other hand, there are several studies indicating that specific bacteria or groups of bacteria can have beneficial effects. Chen et al., showed that germ-free mice exhibited increased hepatic steatosis and more severe liver injury and inflammation after

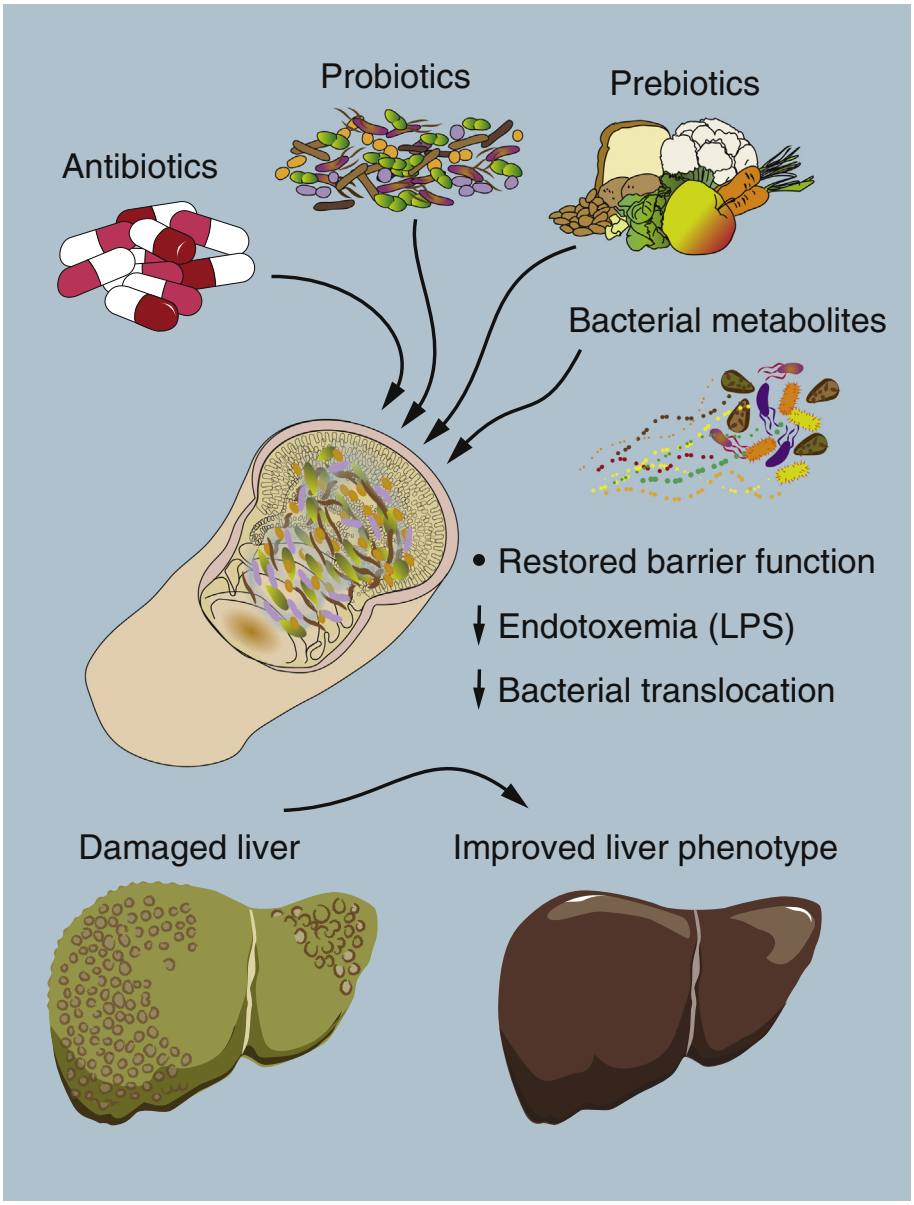

Fig. 3. Potential ways to treat liver disease by modulation of the gut microbiota. Future strategies to treat liver disease might be to target the gut dysbiosis with antibiotics, probiotics, prebiotics and/or bacterially produced metabolites (e.g. bile acids or long-chain fatty acids), and thereby restore the intestinal barrier function and improve liver phenotypes.

ethanol-feeding compared with conventionally raised mice [70]. Furthermore, changing the intestinal microbiota by fecal transplantation with an "alcohol-resistant" microbiota from mice or treatment with the prebiotic pectin reversed intestinal dysbiosis and prevented steatosis and liver inflammation in alcohol-fed mice [72].

Some specific bacteria have shown protective effects against alcohol-induced liver injury. Ethanol-fed mice showed decreased levels of *A. muciniphila* and oral administration of *A. muciniphila* increased its levels in the intestine and improved liver injury and steatosis in mice with ALD likely via restoration of the intestinal barrier [73]. Other bacteria with decreased abundance in ALD patients are *Lactobacillus* spp., *Bifidobacterium* spp., *Ruminococcus* and *Faecalibacterium* [69]. Interestingly, the dysbiosis was only found in the ALD patients with high intestinal permeability, supporting the idea that other factors than the direct toxicity of alcohol may be involved in the disease development [69].

Other factors that potentially can influence the development of ALD are bacterially produced metabolites like secondary bile acids. Hartmann et al. showed that alcohol consumption in mice was associated with changes in bile acid metabolism and reduced FXR signaling [74]. Ethanol-feeding resulted in increased unconjugated bile acid levels in ileum and plasma, reduced plasma FGF15 levels and increased hepatic expression of CYP7A1. Treatment with antibiotics reduced the alcohol-induced increase in bile acid levels likely via increased FXR activation. It was also shown that the intestinal specific FXR agonist fexaramine protected the mice against alcohol-induced liver injury through restoration of the gut barrier indicating that altered intestinal FXR signaling might play a role in the development of ALD. A recent study in humans, also indicated that bile acid metabolism and FXR signaling was altered in AH [75]. However, the alterations seem to be different in humans and mice. Patients with AH showed increased conjugated bile acid levels in serum, and interestingly, the levels of FGF19 (the human equivalent of FGF15), were increased and correlated with disease severity. Furthermore, the levels of the secondary bile acid deoxycholic acid (DCA), which is a potent ligand for TGR5, were almost depleted in the serum of patients with AH indicating that there might be

alterations in the gut microbiota in these patients, however this was not investigated. A novel mechanism for the regulation of bile acid synthesis by the microbiota in ALD was suggested by Kang et al. who colonized mice with microbiota from cirrhotic patients with an active alcohol abuse (Alc-Hum) or cirrhotic patients who had quit drinking (Cirr-Hum). They found elevated hepatic bile acid levels and increased expression of *Cyp7a1* and *Cyp8b1* in the Alc-Hum mice and they suggested that this was due to increase expression of cAMP responsive element-binding protein 3-like protein 3 (CREBH) in the liver [76].

Bacterially produced saturated long-chain fatty acids (LCFAs) belong to another group of metabolites that may influence ALD. Chen et al. showed that ethanol-feeding resulted in reduced bacterial biosynthesis of saturated LCFAs [77]. Dietary supplementation of saturated LCFA reduced the ethanol-induced liver injury and the leakiness of the gut and restored the bacterial dysbiosis mainly by increasing the levels of lactobacilli. The authors suggest that lactobacilli use saturated LCFAs for growth and that lactobacilli are important for the stabilization of the mucosal barrier and thereby preventing translocation of microbial products that can contribute to the development of liver disease. More evidence for the contribution of the diet, potentially in interaction with the gut microbiota, in ALD was shown in a study by Kirpich et al. who found that a diet enriched in unsaturated fat enhanced alcohol-induced liver injury and endotoxemia in contrast to a diet containing saturated fat enriched in medium chain triglycerides [78].

It has also been shown that the gut microbiota can influence the severity of AH. Microbiota from alcoholic patients with different grades of AH was transplanted into germ-free mice and the severity of hepatitis in the human donors could be transmitted into the recipient mice [79]. Mice that received microbiota from patients with severe AH developed more severe liver inflammation than mice that received microbiota from patients without AH. Specific bacterial groups were associated with the severe AH such as *Bilophila wadsvorthia* and species from Clostridium cluster XIVa while others such as *Faecalibacterium* and *Akkermansia* were more abundant in the group without AH. Interestingly, the microbiota from patients without AH could reverse the alcohol-induced liver lesions in the colonized mice. Llopis et al. also showed that there was a difference in bile acid composition with higher levels of CDCA and UDCA in feces of mice colonized with noAH microbiota compared with severe AH microbiota supporting the idea that bile acids can affect the progression of ALD [79].

These findings indicate that susceptibility to ALD could be driven by the intestinal microbiota and that certain groups of bacteria could have a protective role in the development and progression of ALD. In the future we might be able to identify patients at high risk of developing alcohol-induced liver injury based on microbiota composition analysis and possibly find new treatment options through manipulation of the gut microbiota. Of note, non-bacterial microbiota might also contribute to the development of ALD, which was recently shown for fungi [80].

6. Cholestatic liver disease

It has been suggested that the gut microbiota plays a role in biliary inflammatory diseases such as primary biliary cholangitis (PBC) and in particular, primary sclerosing cholangitis (PSC), which is commonly associated with inflammatory bowel disease. Cholestatic liver disease is characterized by impaired bile flow resulting in accumulation of potentially toxic bile acids in the liver (Fig. 2). The close interplay between bile acids and the gut microbiota is affected by the interrupted bile flow into the intestine, and studies have shown that the gut microbiota is altered in patients with cholestatic liver disease [81,82]. However, it is not clear if the gut microbiota is contributing to the disease development or if the altered gut microbiota is a consequence of the disease.

A recent study indicated that the gut microbiota contributes to bile duct inflammation in a mouse model with NOD.c3c4 mice which develop spontaneous biliary inflammation in intra- and extra-hepatic bile ducts [83]. Germ-free NODc3c4 mice were protected from the biliary disease compared with the conventionally raised NODc3c4 mice and antibiotic treatment of the conventionally raised NODc3c4 mice reduced the amount of bile infarcts. However, the antibiotic treatment did neither affect the portal inflammation nor the fibrosis and co-housing experiments showed that the biliary phenotype was not transmissible. In controversy to the improved phenotype of the germ-free mice described above, another study using *Mdr2*$^{-/-}$ mice as a model of PSC showed that the presence of a microbiota might protect against the development of cholestatic liver disease [84]. Germ-free *Mdr2*$^{-/-}$ mice showed a more severe liver phenotype with elevated ALP, ALT and bilirubin in serum as well as more fibrosis than the conventionally raised *Mdr2*$^{-/-}$ mice.

More studies are required to elucidate the role of the gut microbiota in cholestatic liver disease, specifically to determine if microbial dysbiosis can be an initiating factor and/or a contributor to disease progression.

7. Cirrhosis and hepatocellular carcinoma

HCC is the fifth most common cancer form in men and the seventh most common in women and has a very high mortality [85]. The majority of HCC (80–90%) has developed from liver cirrhosis, which is commonly caused by chronic viral hepatitis [85,86]. However, an increasing number of HCC is found in NASH patients with non-cirrhotic livers [57].

The risk of developing HCC in cirrhotic patients is quite variable and it has been suggested that the gut microbiota could play a role in the pathogenesis and progression of disease [87]. It has been shown that HCC is associated with dysbiosis and a leaky gut with increased endotoxemia, bacterial metabolite production and translocation [86] but there are few studies showing a direct causative effect of the gut microbiota on hepatic cancer development. A study by Zhang et al. showed that hepatocarcinogenesis can be affected by the gut homeostasis. Administration of penicillin or dextran sulfate sodium to diethylnitrosamine-treated rats, which is a model for HCC, caused dysbiosis and systemic inflammation respectively and this promoted hepatic tumor formation [88]. Interestingly, probiotic treatment protected the rats against the diethylnitrosamine-induced inflammation and hepatocarcinogenesis and it prevented the development of HCC from cirrhosis. There are studies showing that bacterial metabolites such as the secondary bile DCA can induce hepatocellular carcinoma. In a mouse model of obesity-associated HCC, in which an oncogenic *Ras* mutation was chemically induced by dimethylbenz(*a*)anthracene-treatment, it was shown that dietary or genetically-induced obesity led to alterations in the gut microbiota accompanied by increased levels of DCA [3]. It was also shown that senescence-associated secretory phenotype (SASP) had a crucial role in the HCC development and that DCA induced SASP in hepatic stellate cells. When DCA production was inhibited by antibiotic treatment the tumor burden in the liver was reduced and conversely when the antibiotic-treated mice were given DCA simultaneously for 17 weeks the HCC development was enhanced [3]. This study showed a direct link between the bacterial bile acid metabolite DCA and the progression of HCC. However, it is important to point out that DCA alone was insufficient to induce HCC in lean mice on a normal chow diet indicating that there are additional factors associated with obesity that are required to induce HCC development.

Antibiotic treatment could also improve liver phenotypes and suppress tumor development in another mouse model with a steatohepatitis-inducing HFD (STHD-01), which contains high levels of saturated fat and cholesterol and promotes HCC development without administration of chemical carcinogens [89,90].

So far, it seems reasonable that the gut microbiota has an impact on the development of HCC but more studies are needed to determine the mechanisms to gain more knowledge about how manipulation of the gut microbiota can provide novel ways to treat and/or prevent HCC in

the future.

8. Conclusions and future perspectives

An increasing number of observational studies provide evidence for the pathogenetic role of gut dysbiosis in human liver disease. This review has high-lighted important results from rodent models of different types of liver disease that have provided deeper insights about the role of the gut microbiota in the development and progression of disease. Some studies have even shown a direct causative role for specific bacteria or bacterial groups and there are promising novel treatment options in sight for the future.

Despite differences in physiology and bile acid profiles between humans and mice there are essential benefits with mouse models. One advantage is that the microbiota can easily be analyzed from different compartments of the intestine, which provide more information about the ecology of the gut microbiota, while gut microbiota analysis in humans usually is obtained from fecal samples. Another advantage is that the initiation and progression of disease can be monitored in animals and this will be essential to decipher the connection between microbial dysbiosis and the gut barrier function. Future studies should also focus on the metabolic function of the microbiota with metatranscriptomic and metabolomic approaches. Thereby, we might identify novel bacterially produced metabolites that can provide more mechanistic evidence for the role of the microbiota in liver diseases.

In conclusion, a promising future strategy to treat liver diseases might be to target the gut dysbiosis and restore the intestinal barrier function (Fig. 3). Ideally, we may also be able to use microbiota analysis preventively to identify people at high risk of developing liver disease.

Conflict of interest

I have no conflict of interest to disclose.

Transparency document

The Transparency document associated with this article can be found, in online version.

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