Journal of Autoimmunity 46 (2013) 66–73

Contents lists available at SciVerse ScienceDirect

Journal of Autoimmunity

journal homepage: www.elsevier.com/locate/jautimm

Review

Role of the intestinal microbiome in liver disease

Jorge Henao-Mejia [a,1], Eran Elinav [b,1], Christoph A. Thaiss [b,1], Paula Licona-Limon [a,1], Richard A. Flavell [a,c,*]

[a] Department of Immunobiology, Yale University School of Medicine, CT 06520, USA
[b] Immunology Department, Weizmann Institute of Science, Rehovot 70100, Israel
[c] Howard Hughes Medical Institute, USA

ARTICLE INFO

Article history:
Received 1 July 2013
Accepted 2 July 2013

Keywords:
Microbiota
Non-alcoholic fatty liver disease
Primary biliary cirrhosis
Innate immune system

ABSTRACT

The liver integrates metabolic outcomes with nutrient intake while preventing harmful signals derived from the gut to spread throughout the body. Direct blood influx from the gastrointestinal tract through the portal vein makes the liver a critical firewall equipped with a broad array of immune cells and innate immune receptors that recognize microbial-derived products, microorganisms, toxins and food antigens that have breached the intestinal barrier. An overwhelming amount of evidence obtained in the last decade indicates that the intestinal microbiota is a key component of a wide variety of physiological processes, and alterations in the delicate balance that represents the intestinal bacterial communities are now considered important determinants of metabolic syndrome and immunopathologies. Moreover, it is now evident that the interaction between the innate immune system and the intestinal microbiota during obesity or autoimmunity promotes chronic liver disease progression and therefore it might lead to novel and individualized therapeutic approaches. In this review, we discuss a growing body of evidence that highlights the central relationship between the immune system, the microbiome, and chronic liver disease initiation and progression.

1. Introduction

The multiple physiological processes that are dependent on the reciprocal interaction between the liver and the gastrointestinal tract highlight the critical functional relationship between these organs. The portal vein, which conducts venous blood from the intestines and the spleen, provides ~75% of the blood supply to the liver (1000–1200 mL/min). Therefore, the liver is constantly exposed to multiple noxious and beneficial products or microorganisms derived from the small and large intestines [1].

In recent years, it has been widely demonstrated that the intestinal microbiota have critical functions in multiple aspects of mammalian physiology including regulation of body weight and related metabolic homeostasis, instruction of the immune system, and regulation of epithelial cell responses that are essential to maintain mutualism [2,3]. The human gastrointestinal tract hosts 10–100 trillion bacteria containing approximately 500–1500 different bacterial species [4]. The intestinal microflora significantly differs among species and individuals. Host genotype, age, health status, diet and exposure to antibiotics are critical parameters that regulate the configuration of the intestinal microflora [5,6]; moreover, disturbances in the ecosystem of bacterial communities within the gastrointestinal tract can initiate serious metabolic and inflammatory pathologies.

The liver's strategic location confers it with the important role of translating physiological and pathological processes within the gastrointestinal tract into metabolic and immunologic outcomes. Therefore, it is becoming increasingly clear that the intestinal microbiota is a central component of hepatic pathophysiology. Here, we review recent evidence that highlights the influence of the gut microbiota on chronic hepatic diseases, with a special emphasis on how the interactions between the innate immune system and the microbiota determine the progression of liver disease.

2. Interactions between the intestinal microbiota and the innate immune system in the context of liver diseases

A large array of pattern-recognition receptors (PRRs) of the innate immune system mediates the fine interaction between the host and its intestinal microflora [7]. Although originally these receptors were mainly regarded for their role in recognizing invading pathogenic microorganisms and priming adaptive immune

* Corresponding author. Department of Immunobiology, Yale University School of Medicine, 300 Cedar Street, TAC S-569, New Haven, CT 06520, USA. Tel.: +1 203 737 2216; fax: +1 203 737 2958.
E-mail address: richard.flavell@yale.edu (R.A. Flavell).
[1] Equal contributors.

0896-8411/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jaut.2013.07.001

responses, it is now clear that PRRs and their downstream signaling cascades are essential for the recognition of the commensal microflora. The interaction between commensal microorganisms and the host PRRs under homeostatic conditions is necessary to locally contain the microbiota and maintain mutualism [8]; and disruption of multiple innate immune signaling pathways has been associated with a wide array of aberrant pathological processes including abnormal development of the intestinal immune system, altered intestinal epithelial homeostasis and severe intestinal injury [9].

The expression of innate immune receptors has been reported in multiple hepatic (hematopoietic and non-hematopoietic) cells such as biliary epithelial cells, sinusoidal endothelial cells, hepatic stellate cells, Kupffer cells and hepatocytes [10–14]. Innate PRRs expression in the liver provides an additional surveillance system recognizing microbial-derived products that are originated in the gastrointestinal tract. Therefore, the liver has to maintain a delicate balance between its ability to keep tolerance toward translocated microbial-derived products or food antigens and its ability to promote immune responses against persistent or harmful microbial stimulus that result from intestinal breach and are indicative of systemic microbial spread. In the sections below, we will describe and discuss how the interactions between hepatic PRRs with microorganisms contribute to the pathogenesis of chronic of liver disease.

2.1. Toll-like receptors

The first class of PRRs identified was the Toll-like receptors, or TLRs. TLRs recognize a variety of microbial ligands, including bacterial and fungal cell wall components as well as nucleic acids [15]. Multiple cells in the liver express significant levels of multiple TLRs and have long been recognized to be critical determinants in the pathogenesis of chronic liver diseases. Specifically, TLR2, TLR3, and TLR4 are highly expressed in Kupffer cells and respond to endotoxin stimulation leading to the rapid production of TNF-α, IL-6, and IFN-γ. Furthermore, the expression of TLRs has been found on biliary epithelial cells, hepatic stellate cells, hepatocytes and liver sinusoidal endothelial cells [1] (Fig. 1).

The signaling pathway activated through TLR4-MyD88-NF-kB has been found to be fundamental to the pathophysiological processes that drive multiple liver diseases such as viral hepatitis, hepatocellular carcinoma, fatty liver disease, cirrhosis and fibrosis. Activation of TLR4 in Kupffer cells has been shown to promote alcoholic liver disease [16]. In addition, hepatic TLR4 expression is increased in animal models of non-alcoholic steatohepatitis (NASH) [17], Primary Sclerosing Cholangitis (PSC) [18], and Primary biliary cirrhosis (PBC) [14]. Moreover, TLR4-deficient mice fed a high-fat diet have decreased levels of hepatic steatosis [19]. Interestingly, genetic data from humans identified a polymorphism in the gene encoding TLR4, which attenuates the signaling downstream of the receptor in response to LPS stimulation, and has been associated with a decreased risk to developing cirrhosis [20,21].

TLR9-dependent activation of IRF-7 to induce the expression of type I interferons (IFNs), has also been associated with enhanced severity of inflammatory liver disease. Interestingly, type I IFNs were recently described to protect from TLR9-associated liver damage, an effect mediated by the endogenous IL-1 receptor antagonist [22]. A protective role for type I IFNs in a TLR4-driven model of alcoholic liver disease has also been recently reported [23].

The critical role of TLRs in chronic liver disease suggests that increased microbial translocation across the gastrointestinal tract and hepatic recognition of microbial products is an important component of liver pathology (Fig. 1); however, direct evidence to support this hypothesis has been lacking until recently. Seki et al. demonstrated that the microbiota is an important component for the development of hepatic fibrosis since deficiency in TLR4 signaling and antibiotic treatment reduced hepatic fibrosis after bile duct ligation. The underlying mechanism driving fibrosis

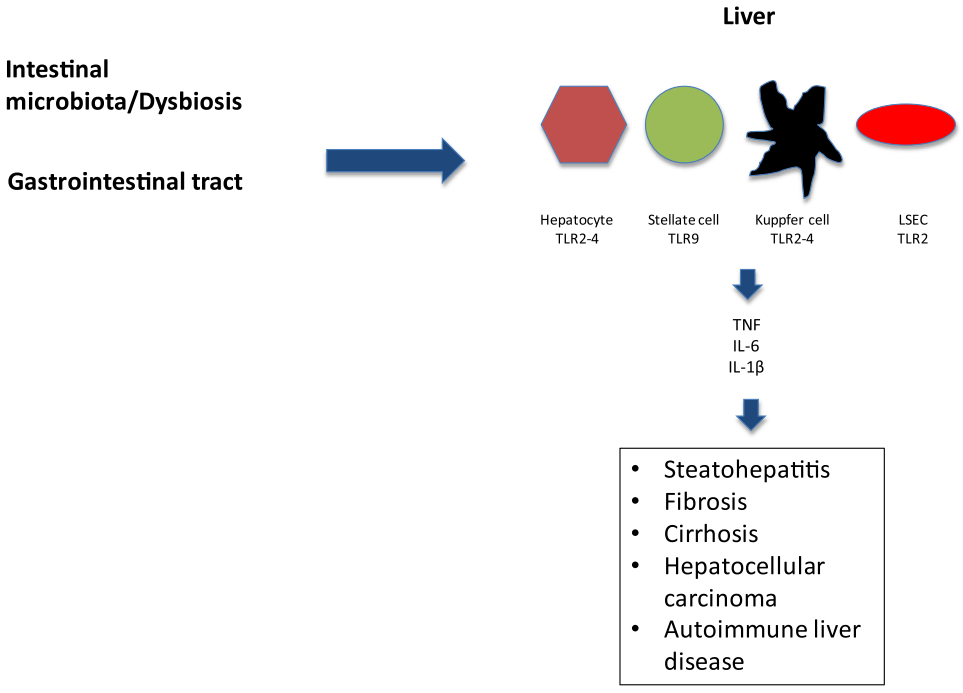

Fig. 1. Pattern recognition receptors expressed in multiple hepatic cells regulate the pathogenesis liver disease. NLRP6 regulates the intestinal microbial ecology. Dysbiosis and intestinal inflammation are associated with increased permeability and influx of PAMPs into the portal circulation. In the liver, TLR activation in multiple liver cells leads to chronic inflammation and disease progression.

through TLR4 relies on the ability of TLR4 signaling on hepatic stellate cells to enhance TGF-β signaling [24].

As highlighted below, we recently reported that in the context of intestinal inflammation induced by dysbiosis, the influx of microbial products into the portal circulation promotes the progression of Non-alcoholic fatty liver disease (NAFLD) through TLR4 and TLR9 activation [25]. In agreement with these results, Lin et al. recently used a model of acute liver injury (concanavalin A (ConA)) to demonstrate that the interaction between TLR4 expressed in intrahepatic hematopoietic cells and the intestinal microbiota is critically involved in ConA-induced hepatitis. Accordingly, treatment of mice with broad-spectrum antibiotics and TLR4 deficiency significantly diminished the ConA-induced liver damage, hepatocyte death and pro-inflammatory cytokine production while administration of LPS enhanced the liver pathology [26].

2.2. Inflammasomes

Inflammasomes are cytoplasmic multiprotein complexes that usually consist of a sensor protein in the NOD-like receptor (NLR) family, the adaptor protein ASC, and the downstream effector caspase-1 [27]. Different NLR proteins have been reported to form inflammasomes upon stimulation with a diverse set of microbial or damage-associated molecular patterns (DAMPs), including NLRP1, NLRP2, NLRP3, NLRP6, NLRP7, NLRC4, and the HIN-200 family member AIM2. Inflammasome assembly leads to the autocatalytic cleavage of caspase-1 and processing of pro-IL-1β and pro-IL-18 into their mature and bioactive forms [28]. Inflammasome activity has been shown to be dependent on two sequential stimuli. The first stimulus promotes the transcription of the pro-forms of IL-1β and IL-18, while the second stimulus triggers the protein–protein interaction that is required for the formation of the multiprotein inflammasome complex [29]. Inflammasomes play a critical role as a cytoplasmic surveillance system that recognizes both endogenous damage-associated substances such as ATP or crystal particles and pathogen-associated molecular patterns from bacterial, viral, fungal, and parasitic infections [30]. Furthermore, the inflammasomes have been recently described as fundamental regulators of the intestinal microbial ecology as discussed below.

Inflammasome components are expressed in various cell types in the liver and inflammasomes have been recently described as critical players in the pathogenesis of liver disease. Hepatocytes upregulate NLRP3 expression upon LPS stimulation; in addition, sinusoidal endothelial and Kupffer cells express high levels of NLRP1, NLRP3, and AIM2 [31]. Initial reports demonstrated the key role of the NLRP3 inflammasome in the severity of acetaminophen-induced hepatotoxicity and showed that NLRP3 inflammasome-deficient mice treated with acetaminophen have decreased mortality [32]; however, others could not find a role for NLRP3 in acetaminophen-mediated liver failure [33]. Furthermore, recent studies indicate that hepatic stellate cells express multiple inflammasome components and demonstrated that inflammasomes regulate liver fibrosis pathogenesis in carbon tetrachloride or thioacetamide mouse models [34]. Similarly, decreased production of inflammatory cytokines and NF-kB activity was observed during ischemia-reperfusion injury in mice with specific NLRP3 deficiency [35].

Although early studies mainly focused on the role of the inflammasome in response to DAMPs in the context of mouse models of liver sterile injury, subsequent reports have also demonstrated that activation of the inflammasome by microbial components or live microorganisms triggers liver pathology, such as in a model of *Propionibacterium acnes*-induced sensitization to LPS-induced liver injury [36] and in *Schistosoma mansoni* infection [37]. Importantly, in these studies cooperation between TLRs signaling and inflammasomes is necessary to drive overt liver inflammation, suggesting concomitant recognition events of microbial and damage-associated molecules.

Interestingly, Csak et al. recently reported an important role for the NLRP3 inflammasome in the development and progression of NASH [38]. Expression of inflammasome components was upregulated in the liver upon induction of a mouse model of NASH and inflammasome activation occurred in isolated hepatocytes. In this model, palmitic acid, a saturated fatty acid, was found to activate the inflammasome and sensitized hepatocytes to IL-1β secretion in response to LPS; suggesting that both PAMPs and DAMPs act in concert to induce pathogenic inflammasome responses in the liver. Another study confirmed NLRP3 activation in the liver and showed that LPS stimulation alone is sufficient to drive hepatic production of inflammatory cytokines downstream of NLRP3 inflammasome activation [39].

Altogether, the role of PRRs of the innate immune system in pathogenesis and progression of chronic liver diseases has so far been restricted to responses to local endogenous signals of damage. While PRR-mediated recognition of DAMPs unquestionably plays a critical role in liver disease, recent evidence from mouse and human indicates that microbial ligands should also be considered as important drivers of hepatic inflammatory disorders.

2.3. Dysbiosis induced by innate immune deficiency and its impact in liver disease

Microbial recognition within the liver has been clearly associated with disease pathogenesis; however, recent reports have demonstrated an important role for the interactions between the innate immune system and the intestinal microflora in the initiation and progression of chronic liver disease. Distorted configuration of the intestinal microbiota and development of several features of metabolic syndrome have been observed in TLR5-deficient mice, the PRR that recognizes bacterial flagellin [40]. Although familial transmission rather than genetic deficiency has been recently reported to be the dominant driver of dysbiosis in mice [41], the intriguing notion that defective host–microbiome interactions in the gastrointestinal tract might have systemic consequences such as impaired metabolism and liver dysfunction has prompted further investigation.

We recently demonstrated that mice deficient in the inflammasome components Nlrp3, Nlrp6, ASC and caspase-1 and the downstream effector cytokine IL-18, develop a pro-colitogenic microbiota characterized by the overrepresentation of anaerobic bacterial species of the *Prevotellaceae* family and the candidate phylum TM7 [42]. Our data indicates that inflammasome activity in the intestine is required for the maintenance of a stable microflora composition, a mechanism partially dependent on IL-18 secretion. Importantly, the altered microbiota found in inflammasome-deficient mice was horizontally transferred to wildtype cohoused mice in the same cage, demonstrating a dominant population effect, which was reversible upon broad-spectrum antibiotic treatment. The particular microenvironment enabling the outgrowth of *Prevotellaceae* correlated to the area close to the colonic epithelial layer and the colonic crypts, a region that is normally less densely colonized with microbes due to mechanisms involving antimicrobial peptide production and mucus secretion. The aforementioned altered intestinal flora leads to mild chronic inflammation and increases susceptibility to develop experimental colitis. A detailed analysis reveals that the colitogenic bacteria present in inflammasome-deficient mice lead to enhanced epithelial production of the chemokine CCL5, which in turn promotes the recruitment of pro-inflammatory immune cell populations to the intestinal lamina propria [42].

Intriguingly, we found that the inflammatory processes induced by the colitogenic flora were not restricted to the regulation of local immune responses and could influence systemic responses. Inflammasome-deficient mice fed with a methionine/choline-deficient diet (MCDD), a model commonly used to induce NAFLD, showed an exacerbated outgrowth of bacterial species of the *Porphynomonadaceae* family and enhanced translocation of microbial products, particularly TLR4 and TLR9 ligands, to the portal circulation [25]. Similar to our initial observation, increased microbial translocation across the gastrointestinal tract was dependent on dysbiosis-induced CCL5 production and intestinal inflammation. Increased stimulation of TLR4 and TLR9 in the liver, induced MyD88/TRIF-dependent TNF-α secretion, which promoted an inflammatory process leading to the development of NASH. Transfer of the altered microbiota from inflammasome-deficient or IL-18-deficient mice to wild-type recipient mice, was sufficient to recapitulate enhanced susceptibility to NASH in a CCL5-, TLR4, TLR9, MyD88/TRIF and TNF-α dependent manner, demonstrating that dysbiosis, rather than genetic deficiency, was responsible for increased disease susceptibility. Our data also supports the idea that metabolic disease might feature infectious, i.e. transmissible microbial, components. Accordingly, antibiotic treatment of inflammasome-deficient mice fed an MCD diet, ameliorated NASH severity and inhibited transmission of the phenotype to wild-type recipients.

The abnormal microflora observed in inflammasome-deficient mice, also influenced additional manifestations of metabolic syndrome in other mouse models of disease. Leptin receptor-deficient mice (genetically obese mice) showed accelerated weight gain when co-housed with inflammasome-deficient mice. The same effect was also observed in ASC-deficient mice and co-housed wild-type mice fed a high-fat diet. Antibiotic treatment revealed the strong influence of the microbial component on systemic metabolic parameters, reversing not only weight gain, but also fasting plasma insulin amounts and glucose intolerance to normal levels [25].

Altogether, our results demonstrated that homeostatic extra-hepatic expression of PRRs is necessary to prevent the development of dysbiosis in the gastrointestinal tract, which can predispose to liver disease (Fig. 1). These data also provide an example where multi-stage host–microbial interactions via different kinds of PRRs and their downstream signaling are involved in disease progression, both at distal (inflammasomes) and proximal sites (TLRs). Importantly, the changes induced by the colitogenic microflora affect inflammatory processes locally (induction of CCL5 and leukocyte recruitment to the intestine), at the most proximal sites draining the intestine (inflammatory cytokine production in the liver), and even beyond (multi-organ regulation of weight gain and insulin sensitivity).

3. Chronic liver pathologies and the intestinal microbiota

3.1. Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is one of the most common human cancers worldwide. Around 80–90% of HCCs are preceded by chronic liver disease, hepatic fibrosis and cirrhosis [43]. Hence, microbial-derived products have been proposed as essential determinants of HCC initiation and progression. Indeed, in a mouse model of HCC, activation of the innate immune receptor TLR4 in non-bone-marrow-derived liver cells is essential for hepatic tumorigenesis. Interestingly, TLR4 and the gut microbiota are required for HCC progression but not HCC initiation as intestinal sterilization delayed late stages of disease progression [44]. The role of the specific intestinal bacterial communities on human HCC is an area that still needs to be explored and warrants further investigation in the near future.

3.2. Autoimmune liver disease

Primary Sclerosing Cholangitis (PSC) is an autoimmune liver disease that results from chronic inflammation and the obstruction of biliary ducts [45]. Despite that the pathogenesis of PSC remains largely undetermined, the human intestinal microflora is considered to be an important determinant in its etiology. The influence of intestinal bacterial communities in Ulcerative Colitis (UC) pathogenesis is well recognized; importantly, nearly 75% of patients with PSC have UC and nearly 3% of patients with UC have PSC as a concomitant comorbidity [46–52]. Furthermore, the prevalence of PSC among UC patients is significantly increased in those patients with total colonic involvement suggesting a strong positive association between intestinal inflammation and PSC pathogenesis [46,52].

Multiple lines of circumstantial evidence suggest that intestinal bacteria is a common and key factor promoting liver and intestinal inflammation in this disease. Accordingly, enteric bacteria such as *Escherichia coli* and *Candida* are often found in the bile of PSC patients [53]. Moreover, the expression of genes involved in innate immune pathways is significantly increased at the late stages of PSC [1]. In addition, serum atypical perinuclear antineutrophil cytoplasmic antibodies (pANCA) are frequently found in patients with PSC [54,55]. More recently, the auto antigen of this atypical pANCA has been reported to be β-tubulin, which cross-reacts with the bacterial cytoskeletal protein FtsZ [56]. Thus, a clinically relevant problem that warrants further investigation in the near future is the identification of the bacterial species that trigger PCS and pANCA.

Primary biliary cirrhosis (PBC) is an autoimmune liver disorder affecting approximately 40 per 100,000 people in the United States. PBC is characterized by immune cell activation and directed damage of cholangiocytes, which results in cholestasis that ultimately leads to hepatic fibrogenesis and liver failure in 26% of patients within 10 years of diagnosis [57]. A hallmark of PBC patients is the presence of antimitochondrial antibodies (AMA) in serum. These antibodies are detected in approximately 95% of human PBC samples and their cross-reaction with bacterial components, including *E. coli* proteins, is proposed as a critical event for PBC early pathogenesis [58,59]. IgG3 antibodies cross-reacting with β-galactosidase of *Lactobacillus delbrueckii* has been reported in approximately 50% of PBC patients. Similarly, reactive serum against proteins of *Novosphingobium aromaticivorans* from stool specimens, has been found in 25% of PBC patients [60,61]. The previous observations suggest an important association that will require further study to determine if modulation of gut microbiota might aid in the treatment of this catastrophic disease.

3.3. Non-alcoholic fatty liver disease

In Western societies, Non-alcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease. Its prevalence ranges from 20 to 40% in the general population and up to 75–100% in obese individuals [62,63]. NAFLD represents the hepatic manifestation of metabolic syndrome [64], with many patients co-developing other significant clinical alterations such as hyperlipidemia, cardiovascular disease, insulin resistance, polycystic ovary syndrome and obstructive sleep apnea [65,66]. While most patients with NAFLD remain asymptomatic, 20–30% progress to develop non-alcoholic steatohepatitis (NASH), which in turn can lead to cirrhosis, hepatocellular carcinoma and increased mortality [67–69]. NASH can be classified according to the underling comorbidities. Primary NASH is associated with obesity and type 2 diabetes,

whereas secondary NASH occurs in association with Wilson's disease, pharmacological interventions, parenteral nutrition or jejunoileal bypass surgery. It is interesting to note that despite the high prevalence of this disease, factors that promote NAFLD progression remain poorly understood [70,71].

NAFLD/NASH progression has been proposed to be driven by a "two hit" model [72]. The first hit, hepatic steatosis, is closely associated with multiple metabolic abnormalities such as insulin resistance and with enhanced intrahepatic lipotoxic-induced mitochondrial aberrations that predispose the hepatocytes to additional pro-inflammatory insults (second hits) which in turn promote disease progression. Second hits include increased lipid peroxidation that eventually leads to the increased accumulation of reactive oxygen species (ROS) and intestinal-derived factors. The coordinate and concomitant action of these hepatic tissue insults work in concert to promote the development of steatohepatitis [73]. Recently, a growing amount of evidence unequivocally links alterations in the composition of the intestinal microbiota and defects in the intestinal barrier function, to the development of steatosis (first hit) and the progression to NASH (second hit).

3.3.1. *Role of the intestinal microbiota on obesity and insulin resistance (first hits)*

Obesity is the most prevalent risk factor for NAFLD in humans [74]. Multiple lines of evidence point to the intestinal bacterial communities as a critical modulator of body weight and body fat composition (Fig. 2). Germ-free mice have a lower body fat content than specific pathogen free (SPF) mice; moreover, the colonization of germ-free mice with microbiota from SPF mice leads to a significant increase in the accumulation of body fat [75]. A great percentage of the intestinal microbiota composition is represented by the phyla Bacteriodetes and Firmicutes in humans and mice; however, the relative abundance of these bacteria significantly impacts the individual body composition [76,77]. The ratio of Bacteriodetes to Firmicutes is significantly increased in genetically obese mice (*ob/ob*) when compared to lean SPF controls; more importantly, weight gain was faster and calories were more efficiently harvested in germ-free mice colonized with microbiota from *ob/ob* mice than mice colonized with intestinal microflora from lean mice [75]. The previous findings suggest that the composition of the microbiota directly influences weight gain and body fat composition through the regulation of calorie extraction.

Multiple lines of evidence support the notion that the composition of the intestinal microbiota correlates with multiple inflammatory and metabolic parameters as well as dietary habits in humans [5,77,78]. In concordance to the observed phenotypes in mice, increased levels of Bacteriodetes are detected in the intestinal microflora of obese individuals and the reduction of this phylum is significantly associated with weight loss [77]. Metagenome-wide association studies have recently shown that intestinal microbial dysbiosis, increases in various opportunistic bacterial pathogens and reductions in the abundance of butyrate-producing bacteria are characteristic of type 2 diabetes (T2DM) patients, indicating that specific microbial communities play critical roles on the pathogenesis of T2DM and associated disorders [79]. Calorie intake of Western society diets is an important determinant of metabolic syndrome. Profound alterations on the human intestinal microflora are associated with long-term dietary habits resulting in potential deleterious immunological and metabolic consequences. Although it is still highly debated, it is proposed that the human gut microbiota can be divided into three compositions (enterotypes). Each enterotype is characterized by a dominant presence of a different genus — *Ruminococcus*, *Bacteroides*, *Prevotella* — [80]. Strikingly, individuals under a diet rich in protein and animal fat (Western diet) have an enterotype characterized by *Bacteriodes*, while diets rich in carbohydrates/fiber are associated with *Prevotella*-dominated enterotypes [81,82], suggesting that energy extraction is maximized by the diet-induced changed in the gut microbiota. Finally, recent evidence from animal studies showed that a specific composition of the intestinal bacterial communities is absolutely required for NAFLD development [83]. Altogether, these reports show that the configuration of the microbiota plays an essential role in metabolic processes and its disruption might lead to metabolic abnormalities that are associated with the "first hit" (steatosis) during NAFLD pathogenesis.

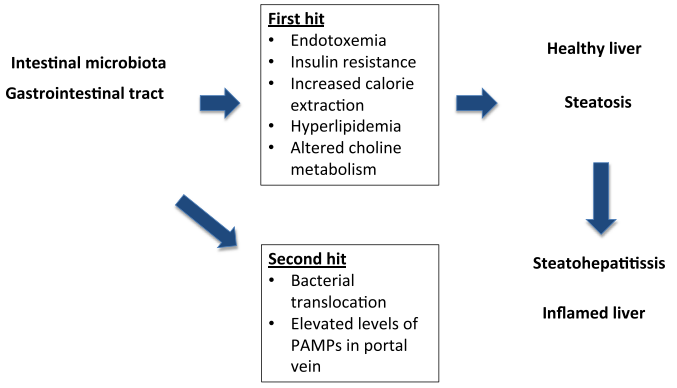

Fig. 2. NAFLD progression is regulated by the intestinal microbiota. The configuration of the intestinal bacterial communities regulates a wide array of metabolic processes that induce steatosis (first hit) and eventually can promote NAFLD progression to NASH.

3.3.2. *Role of the intestinal microbiota on NAFLD progression (second hit)*

Despite the fact that body weight gain and fat accumulation are clearly regulated by the intestinal microflora, the role of gut-derived factors on NAFLD progression has just begun to be investigated. Progression from steatosis to steatohepatitis is an inflammatory process that likely reflects the concomitant harmful effects of multiple deleterious stimuli. Multiple lines of evidence have shown that the configuration of the intestinal microflora plays a critical role in this process. Small intestinal diverticulosis, jejunoileal bypass, intestinal failure and total parenteral nutrition are associated with NASH progression [84–88]; interestingly, a proposed key determinant factor for NAFLD progression in these morbidities is small intestinal bacterial outgrowth (SIBO) secondary to low intestinal motility [84,85,89]. In agreement, SIBO and steatohepatitis are reversed by surgical removal of the bypassed section or treatment with antibiotics. Likewise, rats fed under total parenteral nutrition are characterized by severe liver injury secondary to bowel hypomotility, which causes increased hepatotoxic mediators such as endotoxin or tumor necrosis factor induced by the expansion of Gram-negative bacterial populations [89].

The role of particular intestinal microflora configurations or microbial-derived products in the more prevalent primary NASH is less clear. Obese individuals have significantly increased levels of SIBO as compared to healthy lean subjects [90], however its role in NAFLD progression to NASH has been largely unnoticed. Nevertheless, a recent study by Miele et al. correlated NAFLD stage disease with SIBO and intestinal permeability [91]. Importantly, significantly elevated levels of SIBO were associated to increased gut permeability in patients with NAFLD when compared with healthy individuals, implying that bacterial translocation of previously underrepresented bacterial communities might lead to portal endotoxemia and eventually hepatic injury [91]. Accordingly, high

levels of SIBO have been reported in different cohorts of NASH patients [92,93]; moreover, we recently demonstrated that inflammasome-mediated dysbiosis characterized by an expansion of the *Prevotellaceae* and *Porphyromonadaceae* families as well as the TM7 taxa, promotes NAFLD progression in different mouse models [25]. Taken together, these studies indicate that different configurations of the intestinal microflora might regulate NAFLD progression in humans. Characterization of the bacterial communities associated to different stages of NAFLD and the exact role of metabolites derived from the bacterial microflora on disease progression should be the focus of research in the near future.

3.4. Cirrhosis and associated comorbidities

The final clinical stage of multiple liver diseases is cirrhosis. The major complications of liver cirrhosis, such as hepatic encephalopathy (HE), spontaneous bacterial peritonitis and esophageal variceal bleeding are characterized by severe alterations in the intestinal microflora [94–98]. Dysbiosis and dysfunctions in the intestinal epithelial barrier are promoted by liver fibrogenesis through multiple physiopathological processes. Reduced portal vein blood flow and intestinal vascular congestion are characteristic abnormalities of cirrhotic patients, which ultimately leads to increased intestinal permeability [99,100]. Furthermore, decreased bile acid production and low intestinal motility that results in SIBO are associated to compromised liver function and significant changes in intestinal bacterial communities [101]. Hence, it is now well recognized that dysbiosis in combination with defects in innate immunity and altered vascular/liver physiology are key pathological processes that promote bacterial translocation to the peritoneum.

Hepatic encephalopathy is a general term that includes a variety of neuropsychiatric abnormalities observed in patients with liver dysfunction [102]. Forty five percent of patients with cirrhosis show signs of overt HE, while minimal HE is observed in 60–80% of the patients [102]. A healthy liver converts ammonia to urea protecting the brain from this neurotoxic metabolite. Thus, ammonia becomes a critical driver of HE pathogenesis during liver failure and the intestinal microflora is its more important source [103]. Particularly, *Klebsiella* and *Proteus*, two Urease-producing bacteria species, play a key role on increased ammonia production and HE development [94]. In agreement with the notion that HE is a bacterial-driven disease, treatment with Neomycin and Rifaximinis (non-absorbable antibiotics) leads to a significant decrease in the risk of breakthrough episodes of HE, relapses, or hospitalization due to this neuropsychiatric complication [104–106].

The composition of the intestinal microbiota and their role in cirrhotic patients has recently begun to be elucidated. Two different studies have determined the composition of the intestinal microflora through 16S ribosomal DNA pyrosequencing in patients with cirrhosis and HE. Both studies found a significant overrepresentation of *Streptococcaceae* and an underrepresentation of *Lachnospiraceae* in cirrhotic individuals [107,108]. Interestingly, cirrhotic patients with confounded HE seem to have a significant increase in the abundance of multiple bacterial families (*Enterobacteriaceae*, *Alcaligenaceae*, and *Streptococcaceae*) when compared to cirrhotic patients without HE [108]. Furthermore, data obtained by standardized cognitive tests showed a positive correlation between cognitive dysfunction and the presence of *Alcaligenaceae* and *Porphyromonadaceae* [108]. The characterization of the wide array of repercussions that dysbiosis might cause in the context of cirrhosis and its severe complications is still in its early stages; however, the identification of specific bacterial species that promote disease progression will greatly improve our understanding of the complex pathogenesis of these hepatic diseases.

4. Concluding remarks

The mesenteric lymph node represents the first firewall for microbial-derived products or food antigens entering the lymphatic system. Therefore, it represents a key site for tolerance induction to harmless food and microbial substances, but at the same time it is the first line of defense once the intestinal barrier has been breached. Likewise, the liver is exposed to all substances leaving the gastrointestinal tract via the portal blood circulation; therefore, it represents a critical firewall for intestinal components that have entered the vascular compartment. Thus, the liver must also balance tolerance to innocuous particles and immune responses to potentially harmful microbial substances or microorganisms. In contrast to the mesenteric lymph node, the liver is the principal metabolic regulator of the body, and any hepatic aberrations that result from the alterations in the homeostatic state of host–microbial interactions in the gastrointestinal tract could potentially lead to severe metabolic and inflammatory pathologies. We are certain that the recognition of the intestinal microbial composition and host–microbial interactions as critical components influencing hepatic pathophysiologic processes will guide future therapeutic efforts and promises more efficient approaches aimed to be used in the clinical settings.

References

[1] Miyake Y, Yamamoto K. Role of gut microbiota in liver diseases. Hepatol Res 2012.
[2] Maynard CL, Elson CO, Hatton RD, Weaver CT. Reciprocal interactions of the intestinal microbiota and immune system. Nature 2012;489:231–41.
[3] Tremaroli V, Backhed F. Functional interactions between the gut microbiota and host metabolism. Nature 2012;489:242–9.
[4] Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. Nature 2012;489:220–30.
[5] Claesson MJ, Jeffery IB, Conde S, Power SE, O'Connor EM, Cusack S, et al. Gut microbiota composition correlates with diet and health in the elderly. Nature 2012;488:178–84.
[6] Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, et al. A core gut microbiome in obese and lean twins. Nature 2009;457:480–4.
[7] Carvalho FA, Aitken JD, Vijay-Kumar M, Gewirtz AT. Toll-like receptor–gut microbiota interactions: perturb at your own risk! Annu Rev Physiol 2012;74:177–98.
[8] Slack E, Hapfelmeier S, Stecher B, Velykoredko Y, Stoel M, Lawson MA, et al. Innate and adaptive immunity cooperate flexibly to maintain host–microbiota mutualism. Science 2009;325:617–20.
[9] Michelsen KS, Arditi M. Toll-like receptors and innate immunity in gut homeostasis and pathology. Curr Opin Hematol 2007;14:48–54.
[10] Visvanathan K, Skinner NA, Thompson AJ, Riordan SM, Sozzi V, Edwards R, et al. Regulation of Toll-like receptor-2 expression in chronic hepatitis B by the precore protein. Hepatology 2007;45:102–10.
[11] Hosel M, Broxtermann M, Janicki H, Esser K, Arzberger S, Hartmann P, et al. Toll-like receptor 2-mediated innate immune response in human nonparenchymal liver cells toward adeno-associated viral vectors. Hepatology 2012;55:287–97.
[12] Wang B, Trippler M, Pei R, Lu M, Broering R, Gerken G, et al. Toll-like receptor activated human and murine hepatic stellate cells are potent regulators of hepatitis C virus replication. J Hepatol 2009;51:1037–45.
[13] Yokoyama T, Komori A, Nakamura M, Takii Y, Kamihira T, Shimoda S, et al. Human intrahepatic biliary epithelial cells function in innate immunity by producing IL-6 and IL-8 via the TLR4-NF-kappaB and -MAPK signaling pathways. Liver Int 2006;26:467–76.
[14] Wang AP, Migita K, Ito M, Takii Y, Daikoku M, Yokoyama T, et al. Hepatic expression of toll-like receptor 4 in primary biliary cirrhosis. J Autoimmun 2005;25:85–91.
[15] Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol 2010;11:373–84.
[16] Gao B, Seki E, Brenner DA, Friedman S, Cohen JI, Nagy L, et al. Innate immunity in alcoholic liver disease. Am J Physiol Gastrointest Liver Physiol 2011;300:G516–25.
[17] Thuy S, Ladurner R, Volynets V, Wagner S, Strahl S, Konigsrainer A, et al. Nonalcoholic fatty liver disease in humans is associated with increased plasma endotoxin and plasminogen activator inhibitor 1 concentrations and with fructose intake. J Nutr 2008;138:1452–5.
[18] Mueller T, Beutler C, Pico AH, Shibolet O, Pratt DS, Pascher A, et al. Enhanced innate immune responsiveness and intolerance to intestinal endotoxins in human biliary epithelial cells contributes to chronic cholangitis. Liver Int 2011;31:1574–88.

[19] Li L, Chen L, Hu L, Liu Y, Sun HY, Tang J, et al. Nuclear factor high-mobility group box1 mediating the activation of Toll-like receptor 4 signaling in hepatocytes in the early stage of nonalcoholic fatty liver disease in mice. Hepatology 2011;54:1620–30.

[20] Huang H, Shiffman ML, Friedman S, Venkatesh R, Bzowej N, Abar OT, et al. A 7 gene signature identifies the risk of developing cirrhosis in patients with chronic hepatitis C. Hepatology 2007;46:297–306.

[21] Figueroa L, Xiong Y, Song C, Piao W, Vogel SN, Medvedev AE. The Asp299Gly polymorphism alters TLR4 signaling by interfering with recruitment of MyD88 and TRIF. J Immunol 2012;188:4506–15.

[22] Petrasek J, Dolganiuc A, Csak T, Kurt-Jones EA, Szabo G. Type I interferons protect from Toll-like receptor 9-associated liver injury and regulate IL-1 receptor antagonist in mice. Gastroenterology 2011;140:697–708. e4.

[23] Petrasek J, Dolganiuc A, Csak T, Nath B, Hritz I, Kodys K, et al. Interferon regulatory factor 3 and type I interferons are protective in alcoholic liver injury in mice by way of crosstalk of parenchymal and myeloid cells. Hepatology 2011;53:649–60.

[24] Seki E, De Minicis S, Osterreicher CH, Kluwe J, Osawa Y, Brenner DA, et al. TLR4 enhances TGF-beta signaling and hepatic fibrosis. Nat Med 2007;13:1324–32.

[25] Henao-Mejia J, Elinav E, Jin C, Hao L, Mehal WZ, Strowig T, et al. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. Nature 2012;482:179–85.

[26] Lin Y, Yu LX, Yan HX, Yang W, Tang L, Zhang HL, et al. Gut-derived lipopolysaccharide promotes T-cell-mediated hepatitis in mice through Toll-like receptor 4. Cancer Prev Res (Phila) 2012;5:1090–102.

[27] Henao-Mejia J, Elinav E, Strowig T, Flavell RA. Inflammasomes: far beyond inflammation. Nat Immunol 2012;13:321–4.

[28] Strowig T, Henao-Mejia J, Elinav E, Flavell R. Inflammasomes in health and disease. Nature 2012;481:278–86.

[29] Latz E. The inflammasomes: mechanisms of activation and function. Curr Opin Immunol 2010;22:28–33.

[30] Elinav E, Strowig T, Henao-Mejia J, Flavell RA. Regulation of the antimicrobial response by NLR proteins. Immunity 2011;34:665–79.

[31] Boaru SG, Borkham-Kamphorst E, Tihaa L, Haas U, Weiskirchen R. Expression analysis of inflammasomes in experimental models of inflammatory and fibrotic liver disease. J Inflamm (Lond) 2012;9:49.

[32] Imaeda AB, Watanabe A, Sohail MA, Mahmood S, Mohamadnejad M, Sutterwala FS, et al. Acetaminophen-induced hepatotoxicity in mice is dependent on Tlr9 and the Nalp3 inflammasome. J Clin Invest 2009;119:305–14.

[33] Williams CD, Farhood A, Jaeschke H. Role of caspase-1 and interleukin-1beta in acetaminophen-induced hepatic inflammation and liver injury. Toxicol Appl Pharmacol 2010;247:169–78.

[34] Watanabe A, Sohail MA, Gomes DA, Hashmi A, Nagata J, Sutterwala FS, et al. Inflammasome-mediated regulation of hepatic stellate cells. Am J Physiol Gastrointest Liver Physiol 2009;296:G1248–57.

[35] Zhu P, Duan L, Chen J, Xiong A, Xu Q, Zhang H, et al. Gene silencing of NALP3 protects against liver ischemia-reperfusion injury in mice. Hum Gene Ther 2011;22:853–64.

[36] Tsutsui H, Imamura M, Fujimoto J, Nakanishi K. The TLR4/TRIF-mediated activation of NLRP3 inflammasome underlies endotoxin-induced liver injury in mice. Gastroenterol Res Pract 2010;2010:641865.

[37] Ritter M, Gross O, Kays S, Ruland J, Nimmerjahn F, Saijo S, et al. *Schistosoma mansoni* triggers Dectin-2, which activates the Nlrp3 inflammasome and alters adaptive immune responses. Proc Natl Acad Sci U S A 2010;107:20459–64.

[38] Csak T, Ganz M, Pespisa J, Kodys K, Dolganiuc A, Szabo G. Fatty acid and endotoxin activate inflammasomes in mouse hepatocytes that release danger signals to stimulate immune cells. Hepatology 2011;54:133–44.

[39] Ganz M, Csak T, Nath B, Szabo G. Lipopolysaccharide induces and activates the Nalp3 inflammasome in the liver. World J Gastroenterol 2011;17:4772–8.

[40] Vijay-Kumar M, Aitken JD, Carvalho FA, Cullender TC, Mwangi S, Srinivasan S, et al. Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. Science 2010;328:228–31.

[41] Ubeda C, Lipuma L, Gobourne A, Viale A, Leiner I, Equinda M, et al. Familial transmission rather than defective innate immunity shapes the distinct intestinal microbiota of TLR-deficient mice. J Exp Med 2012;209:1445–56.

[42] Elinav E, Strowig T, Kau AL, Henao-Mejia J, Thaiss CA, Booth CJ, et al. NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. Cell 2011;145:745–57.

[43] Nordenstedt H, White DL, El-Serag HB. The changing pattern of epidemiology in hepatocellular carcinoma. Dig Liver Dis 2010;42(Suppl. 3):S206–14.

[44] Dapito DH, Mencin A, Gwak GY, Pradere JP, Jang MK, Mederacke I, et al. Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. Cancer Cell 2012;21:504–16.

[45] Levy C, Lindor KD. Primary sclerosing cholangitis: epidemiology, natural history, and prognosis. Semin Liver Dis 2006;26:22–30.

[46] O'Toole A, Alakkari A, Keegan D, Doherty G, Mulcahy H, O'Donoghue D. Primary sclerosing cholangitis and disease distribution in inflammatory bowel disease. Clin Gastroenterol Hepatol 2012;10:439–41.

[47] Ye BD, Yang SK, Boo SJ, Cho YK, Yang DH, Yoon SM, et al. Clinical characteristics of ulcerative colitis associated with primary sclerosing cholangitis in Korea. Inflamm Bowel Dis 2011;17:1901–6.

[48] Hashimoto E, Ideta M, Taniai M, Watanabe U, Okuda H, Nagasako K, et al. Prevalence of primary sclerosing cholangitis and other liver diseases in Japanese patients with chronic ulcerative colitis. J Gastroenterol Hepatol 1993;8:146–9.

[49] Bergquist A, Montgomery SM, Bahmanyar S, Olsson R, Danielsson A, Lindgren S, et al. Increased risk of primary sclerosing cholangitis and ulcerative colitis in first-degree relatives of patients with primary sclerosing cholangitis. Clin Gastroenterol Hepatol 2008;6:939–43.

[50] Bambha K, Kim WR, Talwalkar J, Torgerson H, Benson JT, Therneau TM, et al. Incidence, clinical spectrum, and outcomes of primary sclerosing cholangitis in a United States community. Gastroenterology 2003;125:1364–9.

[51] Sano H, Nakazawa T, Ando T, Hayashi K, Naitoh I, Okumura F, et al. Clinical characteristics of inflammatory bowel disease associated with primary sclerosing cholangitis. J Hepatobiliary Pancreat Sci 2011;18:154–61.

[52] Joo M, Abreu-e-Lima P, Farraye F, Smith T, Swaroop P, Gardner L, et al. Pathologic features of ulcerative colitis in patients with primary sclerosing cholangitis: a case–control study. Am J Surg Pathol 2009;33:854–62.

[53] Rudolph G, Gotthardt D, Kloters-Plachky P, Kulaksiz H, Rost D, Stiehl A. Influence of dominant bile duct stenoses and biliary infections on outcome in primary sclerosing cholangitis. J Hepatol 2009;51:149–55.

[54] Mulder AH, Horst G, Haagsma EB, Limburg PC, Kleibeuker JH, Kallenberg CG. Prevalence and characterization of neutrophil cytoplasmic antibodies in autoimmune liver diseases. Hepatology 1993;17:411–7.

[55] Terjung B, Herzog V, Worman HJ, Gestmann I, Bauer C, Sauerbruch T, et al. Atypical antineutrophil cytoplasmic antibodies with perinuclear fluorescence in chronic inflammatory bowel diseases and hepatobiliary disorders colocalize with nuclear lamina proteins. Hepatology 1998;28:332–40.

[56] Terjung B, Sohne J, Lechtenberg B, Gottwein J, Muennich M, Herzog V, et al. p-ANCAs in autoimmune liver disorders recognise human beta-tubulin isotype 5 and cross-react with microbial protein FtsZ. Gut 2010;59:808–16.

[57] Washington MK. Autoimmune liver disease: overlap and outliers. Mod Pathol 2007;20(Suppl. 1):S15–30.

[58] Hopf U, Moller B, Stemerowicz R, Lobeck H, Rodloff A, Freudenberg M, et al. Relation between *Escherichia coli* R(rough)-forms in gut, lipid A in liver, and primary biliary cirrhosis. Lancet 1989;2:1419–22.

[59] Bogdanos DP, Baum H, Grasso A, Okamoto M, Butler P, Ma Y, et al. Microbial mimics are major targets of crossreactivity with human pyruvate dehydrogenase in primary biliary cirrhosis. J Hepatol 2004;40:31–9.

[60] Bogdanos DP, Baum H, Okamoto M, Montalto P, Sharma UC, Rigopoulou EI, et al. Primary biliary cirrhosis is characterized by IgG3 antibodies cross-reactive with the major mitochondrial autoepitope and its Lactobacillus mimic. Hepatology 2005;42:458–65.

[61] Selmi C, Balkwill DL, Invernizzi P, Ansari AA, Coppel RL, Podda M, et al. Patients with primary biliary cirrhosis react against a ubiquitous xenobiotic-metabolizing bacterium. Hepatology 2003;38:1250–7.

[62] Sheth SG, Gordon FD, Chopra S. Nonalcoholic steatohepatitis. Ann Intern Med 1997;126:137–45.

[63] Ludwig J, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. Mayo Clin Proc 1980;55:434–8.

[64] Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, et al. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. Hepatology 2003;37:917–23.

[65] Tolman KG, Fonseca V, Dalpiaz A, Tan MH. Spectrum of liver disease in type 2 diabetes and management of patients with diabetes and liver disease. Diabetes Care 2007;30:734–43.

[66] Cerda C, Perez-Ayuso RM, Riquelme A, Soza A, Villaseca P, Sir-Petermann T, et al. Nonalcoholic fatty liver disease in women with polycystic ovary syndrome. J Hepatol 2007;47:412–7.

[67] Caldwell SH, Oelsner DH, Iezzoni JC, Hespenheide EE, Battle EH, Driscoll CJ. Cryptogenic cirrhosis: clinical characterization and risk factors for underlying disease. Hepatology 1999;29:664–9.

[68] Shimada M, Hashimoto E, Taniai M, Hasegawa K, Okuda H, Hayashi N, et al. Hepatocellular carcinoma in patients with non-alcoholic steatohepatitis. J Hepatol 2002;37:154–60.

[69] Propst A, Propst T, Judmaier G, Vogel W. Prognosis in nonalcoholic steatohepatitis. Gastroenterology 1995;108:1607.

[70] Charlton M. Cirrhosis and liver failure in nonalcoholic fatty liver disease: molehill or mountain? Hepatology 2008;47:1431–3.

[71] Hjelkrem MC, Torres DM, Harrison SA. Nonalcoholic fatty liver disease. Minerva Med 2008;99:583–93.

[72] Day CP, James OF. Steatohepatitis: a tale of two "hits"? Gastroenterology 1998;114:842–5.

[73] Sanyal AJ, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. Gastroenterology 2001;120:1183–92.

[74] Younossi ZM, Stepanova M, Afendy M, Fang Y, Younossi Y, Mir H, et al. Changes in the prevalence of the most common causes of chronic liver diseases in the United States from 1988 to 2008. Clin Gastroenterol Hepatol 2011;9:524–30. e1; quiz e60.

[75] Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 2006;444:1027–31.

[76] Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. Proc Natl Acad Sci U S A 2005;102:11070–5.

[77] Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. Nature 2006;444:1022–3.
[78] Muegge BD, Kuczynski J, Knights D, Clemente JC, Gonzalez A, Fontana L, et al. Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. Science 2011;332:970–4.
[79] Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature 2012;490:55–60.
[80] Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, et al. Enterotypes of the human gut microbiome. Nature 2011;473:174–80.
[81] Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, et al. Linking long-term dietary patterns with gut microbial enterotypes. Science 2011;334:105–8.
[82] De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci U S A 2010;107:14691–6.
[83] Le Roy T, Llopis M, Lepage P, Bruneau A, Rabot S, Bevilacqua C, et al. Intestinal microbiota determines development of non-alcoholic fatty liver disease in mice. Gut 2012.
[84] Quigley EM, Marsh MN, Shaffer JL, Markin RS. Hepatobiliary complications of total parenteral nutrition. Gastroenterology 1993;104:286–301.
[85] Carter BA, Karpen SJ. Intestinal failure-associated liver disease: management and treatment strategies past, present, and future. Semin Liver Dis 2007;27:251–8.
[86] Nazim M, Stamp G, Hodgson HJ. Non-alcoholic steatohepatitis associated with small intestinal diverticulosis and bacterial overgrowth. Hepatogastroenterology 1989;36:349–51.
[87] Corrodi P. Jejunoileal bypass: change in the flora of the small intestine and its clinical impact. Rev Infect Dis 1984;6(Suppl. 1):S80–4.
[88] Vanderhoof JA, Tuma DJ, Antonson DL, Sorrell MF. Effect of antibiotics in the prevention of jejunoileal bypass-induced liver dysfunction. Digestion 1982;23:9–15.
[89] Pappo I, Bercovier H, Berry EM, Haviv Y, Gallily R, Freund HR. Polymyxin B reduces total parenteral nutrition-associated hepatic steatosis by its antibacterial activity and by blocking deleterious effects of lipopolysaccharide. J Parenter Enteral Nutr 1992;16:529–32.
[90] Sabate JM, Jouet P, Harnois F, Mechler C, Msika S, Grossin M, et al. High prevalence of small intestinal bacterial overgrowth in patients with morbid obesity: a contributor to severe hepatic steatosis. Obes Surg 2008;18:371–7.
[91] Miele L, Valenza V, La Torre G, Montalto M, Cammarota G, Ricci R, et al. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. Hepatology 2009;49:1877–87.
[92] Sajjad A, Mottershead M, Syn WK, Jones R, Smith S, Nwokolo CU. Ciprofloxacin suppresses bacterial overgrowth, increases fasting insulin but does not correct low acylated ghrelin concentration in non-alcoholic steatohepatitis. Aliment Pharmacol Ther 2005;22:291–9.
[93] Wigg AJ, Roberts-Thomson IC, Dymock RB, McCarthy PJ, Grose RH, Cummins AG. The role of small intestinal bacterial overgrowth, intestinal permeability, endotoxaemia, and tumour necrosis factor alpha in the pathogenesis of non-alcoholic steatohepatitis. Gut 2001;48:206–11.
[94] Basile AS, Jones EA. Ammonia and GABA-ergic neurotransmission: interrelated factors in the pathogenesis of hepatic encephalopathy. Hepatology 1997;25:1303–5.
[95] Guarner C, Soriano G. Spontaneous bacterial peritonitis. Semin Liver Dis 1997;17:203–17.
[96] Campillo B, Pernet P, Bories PN, Richardet JP, Devanlay M, Aussel C. Intestinal permeability in liver cirrhosis: relationship with severe septic complications. Eur J Gastroenterol Hepatol 1999;11:755–9.
[97] Thalheimer U, Triantos CK, Samonakis DN, Patch D, Burroughs AK. Infection, coagulation, and variceal bleeding in cirrhosis. Gut 2005;54:556–63.
[98] Husova L, Lata J, Husa P, Senkyrik M, Jurankova J, Dite P. Bacterial infection and acute bleeding from upper gastrointestinal tract in patients with liver cirrhosis. Hepatogastroenterology 2005;52:1488–90.
[99] Bauer TM, Steinbruckner B, Brinkmann FE, Ditzen AK, Schwacha H, Aponte JJ, et al. Small intestinal bacterial overgrowth in patients with cirrhosis: prevalence and relation with spontaneous bacterial peritonitis. Am J Gastroenterol 2001;96:2962–7.
[100] Gunnarsdottir SA, Sadik R, Shev S, Simren M, Sjovall H, Stotzer PO, et al. Small intestinal motility disturbances and bacterial overgrowth in patients with liver cirrhosis and portal hypertension. Am J Gastroenterol 2003;98:1362–70.
[101] Sung JY, Shaffer EA, Costerton JW. Antibacterial activity of bile salts against common biliary pathogens. Effects of hydrophobicity of the molecule and in the presence of phospholipids. Dig Dis Sci 1993;38:2104–12.
[102] Bajaj JS. Review article: the modern management of hepatic encephalopathy. Aliment Pharmacol Ther 2010;31:537–47.
[103] Williams R. Review article: bacterial flora and pathogenesis in hepatic encephalopathy. Aliment Pharmacol Ther 2007;25(Suppl. 1):17–22.
[104] Sidhu SS, Goyal O, Mishra BP, Sood A, Chhina RS, Soni RK. Rifaximin improves psychometric performance and health-related quality of life in patients with minimal hepatic encephalopathy (the RIME Trial). Am J Gastroenterol 2011;106:307–16.
[105] Bajaj JS, Heuman DM, Wade JB, Gibson DP, Saeian K, Wegelin JA, et al. Rifaximin improves driving simulator performance in a randomized trial of patients with minimal hepatic encephalopathy. Gastroenterology 2011;140:478–87. e1.
[106] Bass NM, Mullen KD, Sanyal A, Poordad F, Neff G, Leevy CB, et al. Rifaximin treatment in hepatic encephalopathy. N Engl J Med 2010;362:1071–81.
[107] Chen Y, Yang F, Lu H, Wang B, Lei D, Wang Y, et al. Characterization of fecal microbial communities in patients with liver cirrhosis. Hepatology 2011;54:562–72.
[108] Bajaj JS, Ridlon JM, Hylemon PB, Thacker LR, Heuman DM, Smith S, et al. Linkage of gut microbiome with cognition in hepatic encephalopathy. Am J Physiol Gastrointest Liver Physiol 2012;302:G168–75.