



The gut-liver axis in liver disease: Pathophysiological basis for therapy

Agustín Albillos^{1,*}, Andrea de Gottardi^{2,3}, María Rescigno^{4,5}

Keywords: Microbiome;
Intestinal barrier; Cirrhosis;
Bile; Farnesoid X receptor.

Received 10 July 2019; received
in revised form 14 September
2019; accepted 2 October 2019

Summary

The gut-liver axis refers to the bidirectional relationship between the gut and its microbiota, and the liver, resulting from the integration of signals generated by dietary, genetic and environmental factors. This reciprocal interaction is established by the portal vein which enables transport of gut-derived products directly to the liver, and the liver feedback route of bile and antibody secretion to the intestine. The intestinal mucosal and vascular barrier is the functional and anatomical structure that serves as a playground for the interactions between the gut and the liver, limiting the systemic dissemination of microbes and toxins while allowing nutrients to access the circulation and to reach the liver. The control of microbial communities is critical to maintaining homeostasis of the gut-liver axis, and as part of this bidirectional communication the liver shapes intestinal microbial communities. Alcohol disrupts the gut-liver axis at multiple interconnected levels, including the gut microbiome, mucus barrier, epithelial barrier and at the level of antimicrobial peptide production, which increases microbial exposure and the proinflammatory environment of the liver. Growing evidence indicates the pathogenetic role of microbe-derived metabolites, such as trimethylamine, secondary bile acids, short-chain fatty acids and ethanol, in the pathogenesis of non-alcoholic fatty liver disease. Cirrhosis by itself is associated with profound alterations in gut microbiota and damage at the different levels of defence of the intestinal barrier, including the epithelial, vascular and immune barriers. The relevance of the severe disturbance of the intestinal barrier in cirrhosis has been linked to translocation of live bacteria, bacterial infections and disease progression. The identification of the elements of the gut-liver axis primarily damaged in each chronic liver disease offers possibilities for intervention. Beyond antibiotics, upcoming therapies centred on the gut include new generations of probiotics, bacterial metabolites (postbiotics), faecal microbial transplantation, and carbon nanoparticles. FXR-agonists target both the gut and the liver and are currently being tested in different liver diseases. Finally, synthetic biotic medicines, phages that target specific bacteria or therapies that create physical barriers between the gut and the liver offer new therapeutic approaches.

© 2019 European Association for the Study of the Liver. Published by Elsevier B.V. All rights reserved.

Introduction

The gut-liver axis refers to the bidirectional relationship between the gut, along with its microbiota, and the liver, arising as a result of interactions between signals generated by dietary, genetic and environmental factors. The interdependence of the gut and the liver explains why disturbances in the intestinal barrier result in increased portal influx of bacteria or their products to the liver, where they cause or worsen a range of hepatic diseases. The part played by intestinal microbes in liver diseases such as alcohol-related liver disease (ALD) or bacterial infection in advanced liver disease has long been known.^{1,2} However, the critical role of a disrupted gut-liver axis in the pathogenesis of many liver diseases has only recently been accepted, as knowledge has gradually accumulated on intestinal microbiome composition and function, intestinal barrier homeostasis and the role of bile in gut-liver communications.

This Seminar article focuses on the most recent advances in our understanding of i) homeostasis of the gut-liver axis in health, including the role of

epithelial, immunological and vascular barriers; ii) distinctive gut-liver axis disruption patterns in the prevalent chronic liver diseases, ALD and non-alcoholic fatty liver disease (NAFLD), including their end-stage form cirrhosis; and iii) innovative therapies to restore gut-liver axis homeostasis. Most of the advances were brought up during the EASL Monothematic Conference held in Leuven in June 2018 on the state of the art of gut/liver communication and liver disease, and a complete view of the contents of the conference can be accessed at <https://livertree.easl.eu/>.

The gut-liver axis in health

The reciprocal interaction between the microbiome and the liver is established through the portal vein, which carries gut-derived products to the liver, and the feedback of bile and antibodies from the liver to the intestine. In fact, besides interacting with nuclear receptors to regulate metabolic functions, bile acids critically control the gut microbiota. The interface between the liver and

¹Servicio de Gastroenterología y Hepatología, Hospital Universitario Ramón y Cajal, Universidad de Alcalá, IRYCIS, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Instituto de Salud Carlos III, Madrid, Spain;

²Hepatology, Inselspital and Department of Biomedical Research, University of Bern, Switzerland;

³Servizio di Gastroenterologia e Epatologia, Ente Ospedaliero Cantonale, Università della Svizzera Italiana, Lugano, Switzerland;

⁴Department of Biomedical Sciences, Humanitas University, 20090 Pieve Emanuele (Mi), Italy;

⁵Humanitas Clinical and Research Center, IRCCS, 20089 Rozzano (Mi), Italy

the microbiome is the gut mucosal barrier, which comprises intestinal epithelial cells that maintain gut homeostasis by segregating gut microbiota and host immune cells.

Stratification of the microbiota by the mucus barrier

The mucus barrier physically separates the microbiota from the epithelial lining, providing protection against an exaggerated inflammatory response (Fig. 1). Only in a few cases, such as segmented filamentous bacteria which are only present in the human gut in early life,³ are microbes capable of crossing the mucus barrier and interacting with epithelial cells in a host-specific manner.⁴ For the rest of the microbes, the interaction with the host is indirect and is mediated by their metabolic products, also called postbiotics, that are released during food fermentation.^{5–7}

The thickness of the mucus barrier varies in the different segments of the gut and is higher where the microbiota resides, such as in the terminal ileum and colon.⁸ In the colon, the mucus barrier is formed of 2 layers, the inner layer is firmer and is in close proximity to the epithelium, while the outer layer is colonised by bacteria.⁸ Microbial strains can attach to the outer mucus layer, to avoid being washed out of the gut by peristaltic movements. Bacteria that are unable to bind to the mucus layer can attach via the intervention of mucin-IgA interactions.⁹ The inner mucus layer is almost sterile because of the restricted size of the meshes and because of the presence of antimicrobial peptides¹⁰ and of microbiota-excluding proteins such as lypd8 and the lectin-like protein ZG16 (zymogen granule protein 16).¹¹ These proteins interact with several groups of bacteria and hinder their penetrance in the inner mucus layer.

The composition of the mucus barrier is dictated by the microbiota as shown by experiments in germ-free mice that, when colonized with the microbiota, develop mucus similar to that of the mice from which the microbiota was originally collected.¹² This is probably due to the capacity of goblet cells to sense the presence of bacterial products and to produce Muc2 after activating the NLRP6-inflammasome pathway.¹³ Conversely, the mucus barrier is a source of nutrients for several bacteria, for instance *Akkermansia muciniphila* can degrade mucins and uses them for its growth.¹⁴ In the absence of fibres in the diet, mucin-degrading bacteria overgrow at the expense of mucus thickness.¹⁵ In addition, the balance between *Bacteroides* and *Firmicutes* can alter mucin glycosylation.¹⁶ Hence, both the mucus barrier and the diet can affect microbiota composition. This can have huge consequences on health as it has been shown that alterations in mucus composition, such as those observed in patients with inflammatory bowel disease, and in particular ulcerative colitis, can lead to a direct interaction between the microbiota and the epithelium, thus contributing to the

establishment and maintenance of the inflammatory response.⁸ Thus, the mucus barrier is not only a first line of defence, but it also serves as a nutrient source and as a niche for microbiota colonisation, enabling the microbiota to avoid wash out by peristaltic movements.

Physical elements of the intestinal barrier: the epithelial and the gut-vascular barriers

Just below the mucus layer the gut barrier is formed of a monolayer of epithelial cells that are composed of enterocytes, goblet cells, tuft cells and enterochromaffin cells.¹⁷ All of these cells cooperate to protect the gut from insults, arising from the microbiota and infectious agents. This barrier is simultaneously physical, as adjacent epithelial cells are sealed together by tight junctions; electrical, as the brush border is negatively charged and opposes a negative charge on the microbiota; and chemical, as a series of antimicrobial peptides are released by epithelial cells. In addition, a series of mucosal immune cells patrol the epithelium, described later. Further, the lamina propria is enriched in plasma cells that release IgA to further protect the barrier. In case the epithelium is breached by bacteria, either via active mechanisms employed by invasive pathogens or pathobionts, or after an injury, then bacteria are found within the lamina propria.

However, only a minority of them will be able to disseminate systemically. Some will reach the mesenteric lymph nodes that act like a firewall to prevent microbes from entering the systemic blood circulation. This is because of the existence of another barrier, the gut vascular barrier (GVB) that prevents bacteria from entering the portal circulation and reaching the liver.^{18,19} However, some pathogenic bacteria and probably some pathobionts have evolved strategies to elude this barrier. Indeed, during *Salmonella* infection, this barrier is disrupted and bacteria are found systemically.¹⁹ Interestingly, this barrier is disrupted in some pathologic conditions such as coeliac disease,¹⁹ ankylosing spondylitis,²⁰ and non-alcoholic steatohepatitis (NASH).²¹

Immune system control of the microbiota

The intestinal mucosal barrier is further reinforced by the presence of a series of immune cells that contribute to the establishment of the barrier. These cells can be grouped into intraepithelial cells and lamina propria cells. Intraepithelial cells consist of intraepithelial lymphocytes including conventional and unconventional $\alpha\beta$ and $\gamma\delta$ T cells and intraepithelial mononuclear phagocytes.^{22–25} Intraepithelial lymphocytes share common properties as they all express type I cytokines, are cytolytic and release antimicrobial peptides upon activation by intestinal epithelial cell-released cytokines or engagement with natural killer (NK) cell receptor-activating ligands.²² Intraepithelial mononuclear phagocytes extend protrusions that

* Corresponding author.
Address: Servicio de Gastroenterología y Hepatología, Hospital Universitario Ramón y Cajal, Universidad de Alcalá, IRYCIS, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Instituto de Salud Carlos III, Madrid, Spain.
E-mail address: agustin.albillo-s@uah.es (A. Albillos).

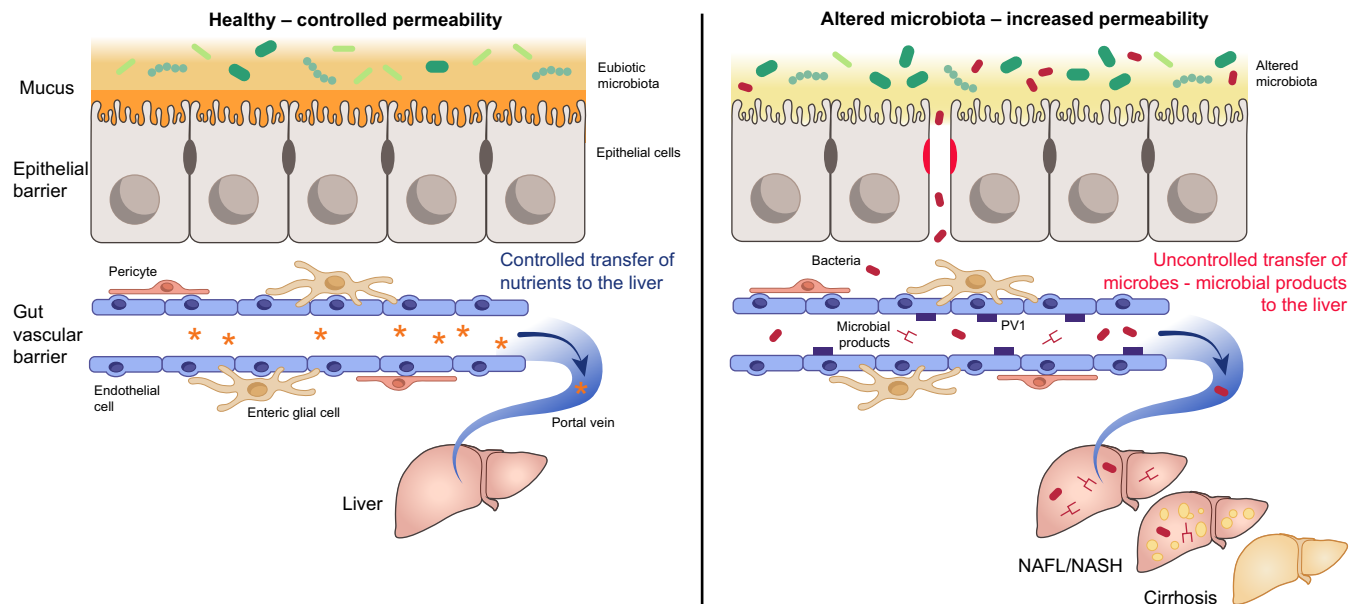


Fig. 1. The intestinal barrier of the colon is composed of several layers of defence. Left: Under healthy/homeostatic conditions, the most external layer of defence is the mucus which is composed of an outer, microbiota-colonised layer, and an inner sterile layer. Just below, one can find the epithelium which is a monolayer of cells sealed one to the other by tight junctions. A further layer of defence is provided by the GVB, which controls the systemic dissemination of microbial metabolites and the microbiota through the portal circulation. Right: Under inflammation, the intestinal barrier can be disrupted at several places, when the GVB is also damaged as demonstrated by increased detection of the fenestrated marker PV1, then the translocation of inflammatory microbial metabolites or microbes can occur to systemic sites, including the liver where they can induce local inflammation and promote liver disorders, such as non-alcoholic fatty liver disease. GVB, gut vascular barrier.

allow direct sensing of the intestinal lumen^{23–25} and development of oral tolerance after delivering antigenic peptides from food to dendritic cells in the lamina propria.²⁶ Together, these cells act like front line soldiers in the battle against infection and induce tolerance to food and microbial antigens. In the lamina propria, immune cells act as a second line of defence and foster tissue regeneration in case of damage. Besides lymphocytes (mostly CD4⁺ T cells) one can distinguish innate-like cells such as invariant NKT cells and mucosa-associated invariant T cells. These cells are highly specialised in the type of microbial antigens or metabolites that they recognise. NKT cells recognise lipids presented on CD1 molecules,²⁷ while mucosa-associated invariant T cells recognise riboflavin metabolites presented by MR1 molecules.^{28–30} CD4⁺ T cells comprise primarily T helper 17 (Th17) cells and T regulatory cells. Th17 cells release IL-17A, IL-17F and IL-22 which is involved in reinforcing tight junction molecules between epithelial cells and fostering epithelial cell regeneration.³¹ Th17 cells are induced after adhesion of segmented filamentous bacteria to the intestinal epithelium.^{4,32,33} They also drive IgA production and the expression of pIgR which enables their translocation into the intestinal lumen.^{4,34} T regulatory cells can be distinguished into thymus-derived and peripherally derived T regulatory cells.³⁵ The first recognise self-antigens and control the function of autoreactive T cells, while the second recognise food antigens (small intestine) or microbial antigens (large intestine) where they control tolerance to innocu-

ous non self-antigens.³⁵ In the intestine one can also find innate lymphoid cells which can release type 1, 2 and 3 cytokines in a rapid response to infection before the action of adaptive T cells.^{36,37} Hence the whole intestinal barrier is formed in response to the microbiota by the coordinated action of structural elements (mucus, epithelial cells), immune cells (intraepithelial and lamina propria immune cells) and soluble mediators (IgA, antimicrobial peptides). Any changes in these elements can alter the intestinal barrier. The microbiota can also affect treatment efficacy by acting on the immune system. For instance during immune checkpoint blockade (anti-PD1) in cancer immunotherapy, the composition of the microbiota of treated patients has an impact on the outcome of therapy.^{38,39}

Postbiotic control of the intestinal barrier

As mentioned earlier the intestinal epithelium is covered by the mucus barrier, whose thickness and tightness depends on the segment of the gut being analysed.⁸ In particular in the colon, where most of the bacteria reside, the mucus layer comprises an external structure which is colonised by the microbiota and offers nutrients for their growth and metabolic activity, and a firmer inner structure which is almost devoid of the microbiota, confers protection to the host^{40–42} and is termed the “demilitarized zone”.

Thus, most of the interactions occurring between the host and the microbiota are indirect and are mediated by soluble factors which include bacterial products and metabolites, also called

postbiotics.^{7,43} Examples of postbiotics are short-chain fatty acids (SCFAs) such as acetate, propionate and butyrate that are released during the degradation of dietary fibres, peptides with immunomodulatory activities, biosurfactants, vitamins, *etc.* These can have several activities on both the intestinal epithelial barrier, the immune system and the microbiota. SCFAs, for instance, have been shown to control the differentiation of several immune cells and in particular T regulatory cells,³⁶ the microbicidal activity of macrophages⁴⁴ and several other immune cell functions.⁴⁵ The fibres and their postbiotics can also impact on the brown versus white adipose tissue ratio.⁴⁶

Bile acid control of microbiota: role of farnesoid X receptor signalling

Bile acids are molecules synthesised in the liver from cholesterol that are then released in the gut where they can be further metabolised by the microbiota (beautifully reviewed in 47). The amount of bile acids produced depends on an active feedback loop from the gut to the liver called the enterohepatic circulation. Before being excreted, primary bile acids are conjugated to the amino acid glycine and to a lesser extent taurine; they are then released in the bile.⁴⁸ Bile then reverses direction in the terminal ileum after a meal and the conjugated bile acids are reabsorbed through the gut epithelium and recycled in the liver after entering the portal circulation. Besides their function in micelle formation and absorption of fat and fat-soluble vitamins, bile acids have a key role in shaping the microbiota. The crosstalk between bile acids and gut microbiota can take place in several places, but importantly it is a two-way interaction as the microbiota affects bile acid metabolism⁴⁹ and bile acids affect microbiota composition.⁴⁷ Further, while bile acids were initially thought to simply recirculate from the gut to the liver, it is now clear that after their transformation into secondary bile acids they signal in the intestinal epithelium primarily via the farnesoid X receptor (FXR).

FXR engagement can enhance epithelial barrier properties,⁵⁰ repair GVB damage,²¹ and control the metabolic syndrome.⁵¹ Contrasting results have been obtained when using mice with constitutive or epithelial-specific *Fxr* knock-out, suggesting different roles of FXR engagement in NASH development. FXR-deficient mice are resistant to diet-induced obesity⁵² which seems to be mediated by intestinal FXR⁵³ and the microbiota.⁵⁴

The gut-liver axis in disease

Growing evidence indicates that crosstalk between the gut microbiome, its derived metabolites, the immune system and the liver plays a key role in the pathogenesis of ALD and NAFLD. In both diseases, gut barrier dysfunction in the form of increased intestinal permeability is the factor that

facilitates portal influx of pathogen-associated molecular patterns (PAMPs), *e.g.* lipopolysaccharide (LPS or endotoxin), and microbiome-derived metabolites to the liver, triggering a proinflammatory cascade that worsens hepatic inflammation.⁵⁵ In this setting of gut barrier dysfunction, the intestinal load of luminal bacteria determines the level of PAMPs that translocate to the portal and systemic circulation, and as a consequence, the severity of liver inflammation (Fig. 1). Progression from compensated to decompensated chronic liver disease is associated with damage at the different levels of intestinal defence, which results in further functional impairment of the gut barrier.

Alcohol-related liver disease

Current data indicate that besides the direct toxic effect of alcohol on liver parenchymal cells, abnormal microbiota, loss of intestinal barrier function and the resultant activation of toll-like receptors (TLRs) on liver immune cells contribute to the pathogenesis of ALD (Fig. 2A). Notably, the contribution of an altered gut microbiota to ALD begins before there is evidence of liver disease.

Gut microbiome

Alcohol intake has direct effects on the gut microbiome, which appear long before fibrosis occurs.⁵⁶ Intestinal overgrowth of aerobic and anaerobic microorganisms has been widely recognised in jejunal aspirates of individuals presenting with chronic alcohol abuse.⁵⁷ Recent metagenomic analysis of the intestinal microbiome of individuals with a history of chronic alcohol abuse/mice fed alcohol has revealed reduced bacterial diversity and a shift in phyla towards a greater abundance of Proteobacteria and lower abundances of Bacteroidetes and Firmicutes, as well as of *Lactobacillus* species.^{58–60} Interestingly, a specific microbiota pattern involving large amounts of *Bifidobacteria* and *Streptococci* has recently been identified in the gut of patients with severe alcoholic hepatitis.⁶¹

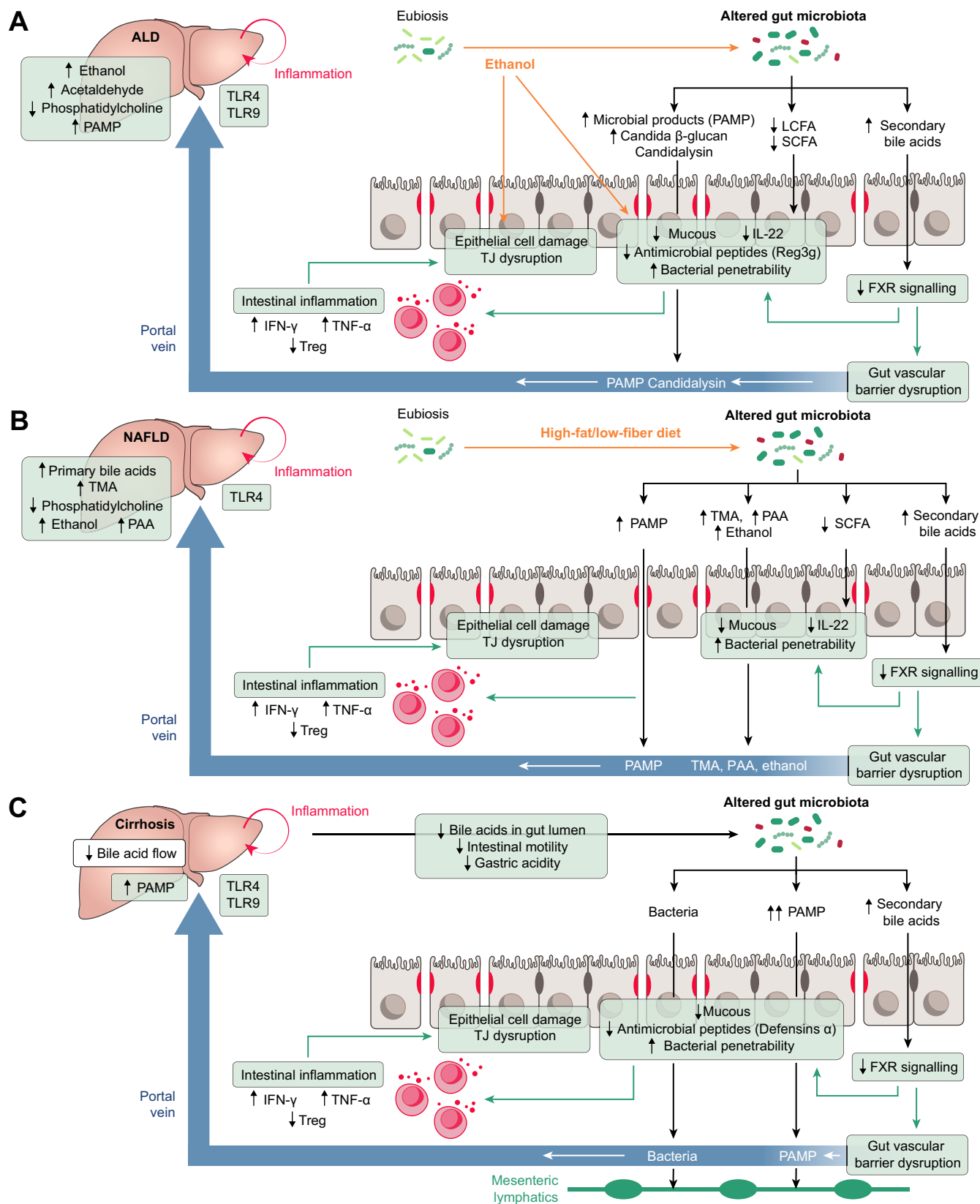
Besides the microbiome, the gut mycobiome (*i.e.* yeast and fungi) is also altered in ALD, and systemic exposure to mycobiota correlates with the severity of liver damage. Chronic alcohol abuse in humans/feeding alcohol to mice causes reduced intestinal fungal diversity and *Candida* overgrowth, along with *Candida* β -glucan translocation to the systemic circulation with a subsequent immune response.⁶² In response to the treatment of mice with antifungals, intestinal fungal overgrowth is reduced, β -glucan translocation decreases and ALD improves.

Intestinal barrier dysfunction

Gut barrier dysfunction, *i.e.* increased intestinal permeability, is a well-recognised feature of alcohol bingeing, chronic abuse, alcohol mouse models, and patients with ALD at pre-cirrhotic and cirrhotic stages.^{63,64} Besides the toxic effects of alco-

Key points

Interactions between the gut microbiome, the immune system and the liver are increasingly implicated in the development of chronic liver diseases.



hol and/or its metabolites on intestinal epithelial cells, more and more lines of evidence point to the contribution of dysbiosis-associated intestinal inflammation to gut barrier dysfunction and translocation of microbial products in ALD. Chronic alcohol abuse in humans/feeding alcohol to mice leads to subclinical intestinal inflammation and increased numbers of monocytes and macrophages producing tumour necrosis factor (TNF)- α in the intestinal lamina propria.⁶⁵ In these circumstances, loss of epithelial tight junction proteins through TNF receptor 1-mediated activation of myosin light-chain kinase results in increased intestinal permeability, translocation of bacterial products and liver inflammation.⁶⁵ Interestingly, endotoxin and bacterial DNA increase in the serum of both humans and mice after acute and chronic alcohol abuse, yet intestinal inflammation is severe after chronic but minimal after acute alcohol abuse.^{66,67}

Alcohol also damages specific components of the intestinal barrier such as proteins involved in innate antibacterial defence. Chronic alcohol use in humans/feeding alcohol to mice suppresses the intestinal regeneration of islet-derived 3- β (Reg3b) and 3- γ (Reg3g) expression,^{59,68} leading to increased bacteria adhesion to the mucosal surface, intestinal bacterial overgrowth, enhanced translocation of viable bacteria, and worsened liver inflammation. These consequences of Reg3 lectin suppression are reversed by Reg3g overexpression in intestinal epithelial cells and can be partially rescued with prebiotics.^{59,69} A further example of the protective role of Reg3g and Reg3b in ALD is the fact that a compensatory increase in these lectins protects mice lacking mucin-2 from liver inflammation; this being the main mucin found in the intestinal mucous layer.⁶⁸

Bacterial products and metabolites

In a seminal study showing that LPS-induced release of transforming growth factor (TGF)- β was MYD-88-NF- κ B mediated, LPS was linked to proinflammatory and profibrogenic signals in the liver.⁷⁰ Besides immunological responses to barrier dysfunction, the course of ALD is also marked

by system-wide changes in many bioactive compounds. Alcohol-induced abnormal microbiota in mice reduces the microbiome's capacity to synthesise saturated long-chain fatty acids (LCFAs), thus reducing the proportion of microbes whose growth depends on LCFAs, such as *Lactobacillus* species.⁵⁸ In this last study, dietary supplementation with saturated LCFAs was found to restore eubiosis, stabilise the intestinal gut barrier and improve ethanol-induced liver injury.

Bile acids

Alcohol-induced alterations in gut microbiota also result in changes in bile acid homeostasis, increasing the intestinal deconjugation of bile acids and exposure of hepatocytes to more toxic bile acids. In effect, intestinal microbiota abnormalities induced by alcohol lead to overrepresentation in the intestine of bacteria encoding cholyglycine hydrolase and disruption of the FXR activation pathway, leading to low FXR activity in enterocytes, low fibroblast growth factor 15 (FGF15, the murine equivalent of FGF19) plasma levels, and increased hepatic Cyp7a1 expression. Depleting commensal bacteria with non-absorbable antibiotics or improving bile acid-FXR-FGF15 signalling via hepatic Cyp7a1 modulation has been reported to improve ALD in mice.⁷¹

Non-alcoholic fatty liver disease

NAFLD encompasses a spectrum of liver diseases that can be broadly classified into non-progressive and progressive phenotypes called NAFL and NASH, respectively. NAFLD is strongly associated with obesity and shares mechanisms with insulin resistance, type 2 diabetes mellitus and cardiovascular risk factors. Current data indicate that a high-fat diet alters the microbiome, which in turn impairs the intestinal barrier and the GVB,²¹ and facilitates the portal influx of bacterial products, worsening non-hepatic inflammation and metabolic abnormalities (Figs. 1 and 2B). The liver, as a 'first pass' organ exposed to the highest concentration of portal system products such as PAMPs, is the most vulnerable to their effects, particularly if pre-conditioned by a

Fig. 2. Disruption of the gut-liver axis in alcoholic liver disease (ALD), non-alcoholic liver disease (NAFLD) and cirrhosis. An altered gut microbiota is the cornerstone of gut-liver axis disruption in chronic liver diseases. (A,B) Ethanol and a high-fat diet have direct effects on gut microbiota composition in ALD and NAFLD, respectively. (C) In cirrhosis, abnormalities in intestinal microbiota primarily result from reduced bile acid flow with deficient luminal levels of bile acids and intestinal hypomotility. Levels of secondary bile acids are increased in the gut lumen as a consequence of alterations to the microbiota and an abundance of 7- α -dehydroxylating bacteria. These elevated secondary bile acids lead to reduced intestinal FXR-signalling, which compromises mucous and antimicrobial peptide synthesis and gut-vascular barrier integrity. Ethanol also directly impairs the synthesis of antimicrobial peptides. Abnormalities in intestinal microbiota jeopardise the availability of energy substrates for epithelial cells such as SCFAs and LCFAs. Consequences are loosening of epithelial cell intercellular junctions, mucous layer thinning and reduced synthesis of antimicrobial peptides all of which facilitate bacterial penetrability and the interaction of pathobionts with mucosal immune system cells. Ultimately, the result is intestinal inflammation featuring a Th1 regulatory pattern of immune cell activation along with the increased synthesis of IFN- γ and TNF- α , which further contribute to increased intestinal permeability. Alterations in microbiota composition and bacterial overgrowth challenge a hyperpermeable intestinal barrier with an overload of PAMPs or metabolites such as TMA in NAFLD that elicit liver inflammation. An extreme severity of intestinal barrier disruption is reached in advanced end-stage liver disease, i.e., decompensated cirrhosis. At this stage, the concurrence of profound abnormalities in microbiota with an overabundance of Enterobacteriaceae on top of markedly damaged physical, immune and vascular intestinal barriers leads to the massive passage of not only PAMPs but also of viable bacteria, causing systemic and liver inflammation as well as spontaneous bacterial infections. ALD, alcohol-related liver disease; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PAA, plasma phenylacetic acid; PAMPs, pathogen-associated molecular patterns; SCFA, short-chain fatty acids; TMA, trimethylamine; TJ, tight junction.

subclinical pathology such as lipid accumulation in hepatocytes. Although ALD and NAFLD share the basic mechanisms of intestinal barrier dysfunction, subtle differences exist between them, including alterations in intestinal microbial composition, gut permeability, and shifting levels of bile acids, ethanol and choline metabolites (Table 1).

Gut microbiome

Multiple preclinical and clinical studies have highlighted a role of the gut microbiome in NAFLD pathogenesis, although we are still far from finding a causal link. In agreement with preclinical models, the prevalence of intestinal bacterial overgrowth^{72,73} and changes in microbiota composition⁷⁴ are higher in patients with NAFLD than in healthy controls, in whom these 2 factors have been correlated with increased intestinal permeability and metabolic syndrome, but interestingly not with hepatic fibrosis or the severity of inflammation.

Recent research efforts have sought to correlate microbiome signatures with NAFLD phenotypes using culture independent techniques. Thus, shotgun metagenomic sequencing has identified an association between a microbiome signature characterised by an increased abundance of *Escherichia coli* and *Bacteriodes vulgatus* and advanced fibrosis in patients with NAFLD.⁷⁵ Similarly, a greater abundance of the genus *Escherichia* has been observed in obese children with NASH.⁷⁶ In spite of these findings, it is unlikely that single micro-

biome signatures will explain the different NAFLD phenotypes, which probably result from varying impacts of the different microbiome signatures on the host according to its genetic predisposition or environmental factors.

Intestinal barrier dysfunction

Recently, a link has been established between intestinal microbiota abnormalities, barrier damage, and hepatic inflammation and metabolic abnormalities under high-fat diet conditions. Increased intestinal permeability has largely been identified in mice on high-fat or choline-deficient diets and in patients with NAFLD.^{72,77–79,21} Mice fed high-fat or fibre-deprived diets have an abnormal colon microbiome composition that causes increased bacterial penetrability and reduced thickness of the mucous layer, redistribution of tight junction proteins of the epithelial barrier and low-grade gut inflammation.^{15,78,79} The altered microbiota is directly responsible for disrupting the intestinal epithelial and vascular barriers as faecal microbial transplantation from mice fed a high-fat diet to mice fed a standard diet is sufficient to drive gut barrier damage, indicating that it is not the nutritional regimen *per se*, but rather its consequence on microbiota composition that drives epithelial and GVB damage.²¹ The altered microbiota acquires the ability to cross the epithelium, which is disrupted as early as 48 h after the introduction of a high-fat diet (demonstrated by reduced expression of tight junction proteins).²¹ Whether the capacity to cross

Key points

Increased intestinal permeability is a well recognised feature of alcohol-related liver disease that has also been linked to non-alcoholic fatty liver disease.

Table 1. Differences in the components of the intestinal barrier in alcoholic liver disease, non-alcoholic fatty liver disease and cirrhosis.

Component	Alcohol-related liver disease	Non-alcoholic fatty liver disease	Cirrhosis
Gut microbiome	Proteobacteria↑: Enterobacteriaceae↑* Bacteroidetes↓: Bacteroidaceae ↓ Firmicutes↓: Streptococcaceae↑, Lactobacillaceae↓, Lachnospiraceae↓, Veilloccoccaceae↑ Candida↑	Proteobacteria↑: Enterobacteriaceae↑ Bacteroidetes↑: Prevotellaceae↑, Rikenellaceae ↑ Firmicutes↓: Lactobacillaceae↑, Lachnospiraceae↓, Ruminococcaceae↓	Proteobacteria↑: Enterobacteriaceae↑ Bacteroidetes↓: Bacteroidaceae ↓ Firmicutes↓: Streptococcaceae↑, Clostridiaceae↑, Lachnospiraceae↓, Veilloccoccaceae ↑, Ruminococcaceae↓ Fusobacteria↑: Fusobacteriaceae↑
Intestinal bacterial overgrowth	Present	Present**	Present
Intestinal permeability	Increased	Increased***	Increased (ascites)
Intestinal inflammation	Intestinal TNF-α ↑	Intestinal TNF-α↑, IFN-γ↑, Treg cells↓	Intestinal TNF-α↑, IFN-γ↑, Treg cells↓
Intestinal antimicrobial proteins	Intestinal Reg3 lectins↓	-	Intestinal alpha-defensins
Bile acids pool	Hepatic synthesis and faecal bile acids↑ Faecal secondary bile acids ↑ Intestinal FXR signalling↓	Faecal secondary bile acids ↑ Intestinal FXR signalling↓	Hepatic synthesis and faecal bile acids↓ Faecal secondary bile acids ↑ Intestinal FXR signalling↓
Intestinal bacterial metabolites	Intestinal LCFA ↓ Blood ethanol and acetaldehyde↑	Intestinal trimethylamine↑ Intestinal SCFA↓ Intestinal BCCA and AAA↑ Blood ethanol↑ Blood choline↓	
Translocation of bacteria and bacterial products (PAMP)	Blood PAMP↑	Blood PAMP↑	Blood PAMP↑ Viable bacteria in blood/lymph nodes

*Phylum: Family. ** Only in NASH vs. NAFLD, obese or healthy controls *** Unrelated to the presence of NAFLD or NASH.

AAA, aromatic amino acids; BCCA, branched-chain amino acids; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PAMPs, pathogen associated molecular patterns; SCFA, short-chain fatty acids; Treg, regulatory T.

the disrupted epithelium is an active invasive mechanism shared by the enriched pathobionts or is due to an increased leakiness of the epithelium caused by the downregulation of tight junction proteins remains to be established. Subclinical inflammation in these circumstances is characterised by reduced T regulatory cells, increased IFN- γ -producing Th1 and CD8⁺ T cells, and increased IL-17-producing $\gamma\delta$ T cells in the lamina propria.⁷⁸ Similarly, patients with NAFLD present with colitis and reduced expression of the intestinal epithelial junction adhesion molecule Jam1.⁸⁰ Mice genetically deficient in Jam1 on a high-fat and -fructose diet show increased intestinal permeability, endotoxemia and hepatic inflammation, which emphasises the importance of a healthy intestinal epithelial barrier to halt the portal entry of bacterial products under microbiota disruption. Unlike in the case of ALD, loss of intestinal Reg3 lectins is insufficient to aggravate diet-induced obesity and NASH.⁸¹

Bacterial products and metabolites

Changes in the functional capacity of the gut microbiome are probably more relevant than changes in its composition. Bacterial components (PAMPs) and metabolites derived from the actions of the gut microbiome on exogenous (from diet and environmental exposure) and endogenous (bile acids and amino acids) substrates can reach the liver through the portal vein and promote inflammation. The contribution of PAMPs to liver damage in NAFLD is supported by preclinical studies showing that hepatic steatosis, inflammation and fibrosis are attenuated in TLR-4 or TLR-9 deficient mice under a high-fat or choline-deficient diet.^{82–84} Further, inflammasome deficiency-associated changes in gut microbiota result in hepatic steatosis and inflammation through portal influx of TLR4 and TLR9 agonists in mice, leading to enhanced hepatic TNF- α expression and inflammation, which are especially severe in mice models of hepatic steatosis.⁸⁵

Alterations in intestinal microbiota composition lead to a gut imbalance characterised by reduced bioavailability of choline and increased portal influx of trimethylamine, both of which have been associated with hepatic steatosis in humans and experimental models. A high-fibre diet in mice increases gut microbes that metabolise choline, which reduces its bioavailability and produces different metabolites such as trimethylamine.^{86,87} Additionally, microbial populations in children with NASH have shown an increased ability to produce ethanol.^{76,88} NAFLD has also been linked to gut microbiome-derived products of branched-chain and aromatic amino acid metabolism such as phenylacetic acid and 3-(4-hydroxyphenyl)lactate, both related to insulin resistance. In a cohort of obese, non-diabetic

patients, those with hepatic steatosis and inflammation showed low microbial gene richness, increased microbial genetic potential for processing dietary lipids, increased endotoxin biosynthesis from Proteobacteria, and dysregulated aromatic and branched-chain amino acid metabolism.⁸⁹ The pathogenic role of microbiome-derived metabolites is further demonstrated by the fact that transplantation of faecal microbiota from human donors with hepatic steatosis or chronic administration of phenylacetic acid triggered steatosis in recipient mice.

The production of SCFAs through bacterial fermentation of indigestible carbohydrates (e.g. dietary fibre) is a notable example of the mutualistic relationship between the gut microbiome and the host. Diet provides non-digestible carbohydrates to support bacterial growth, and in return, these bacteria generate SCFAs (e.g. butyrate) that provide an energy substrate for colonocytes, mitigate intestinal inflammation and regulate satiety.^{90,91} In both obesity and NAFLD, incongruous evidence for the association between clinical phenotypes and SCFAs is likely attributable to the differential abundance of individual SCFAs, each of which may have different effects on host metabolism. The microbiome of patients with type 2 diabetes mellitus shows a reduced abundance of butyrate-producing bacteria.⁹² Supplementation with SCFAs improves diet-induced hepatic steatosis in mice. In agreement with these findings, a seminal randomised study has shown that a high-fibre diet in patients with type 2 diabetes mellitus alters gut bacterial fermentation of carbohydrates, promoting a greater diversity and abundance of butyrate-producing bacteria, and improves haemoglobin A1c levels, partly via increased glucagon-like peptide-1 (GLP-1) production.⁹³

Bile acids

Impaired bile acid signalling is another consequence of abnormalities in intestinal microbiota seen in mice on a high-fibre diet and in humans with NAFLD. In these settings, the microbiome is characterised by an abundance of bacteria that produce secondary bile acids, such as deoxycholic acid, an FXR antagonistic bile acid that suppresses FXR- and FGFR4-mediated signalling.⁹⁴ The consequence is augmented synthesis of bile acids with increased serum concentrations of primary and secondary bile acids.^{94,95} Therefore, the composition of the gut microbiome determines the production of the secondary bile acids, and influences FXR-mediated signalling in the intestine and the liver. In this regard, intestinal FXR expression is lowered in mice on a high-fibre diet, and obeticholic acid restores the integrity of the GVB, reducing the portal influx of PAMPs to the liver.²¹

Cirrhosis

Cirrhosis is associated with marked gut barrier impairment paralleling the disease course. In compensated cirrhosis, features of barrier dysfunction are hardly different from those distinctive of each aetiology of chronic liver disease. In contrast, gut barrier disruption in decompensated cirrhosis arises from damage at all levels of intestinal barrier defence, is independent of aetiology and is associated with liver insufficiency, reduced bile flow and impaired immunity (Fig. 2C). Gut microbiome and barrier dysfunction are directly involved in the pathogenesis of compensated cirrhosis, whereas both are related to the frequency and severity of complications in decompensated cirrhosis, namely bacterial infections and encephalopathy.

Key points

In decompensated cirrhosis, gut barrier dysfunction arises from damage at all levels of intestinal barrier defence and is related to both the frequency and severity of complications.

Gut microbiome

For decades, altered intestinal microbiota and bacterial overgrowth have been recognised in humans and experimental models of cirrhosis.⁹⁶ More recently, metagenomic techniques have characterised the faecal microbiome in cirrhosis as one of reduced diversity, increased relative overgrowth of potentially pathogenic taxa (such as Enterococcaceae, Staphylococcaceae and especially Enterobacteriaceae), and decreased relative abundance of potentially beneficial autochthonous taxa (such as Lachnospiraceae and Ruminococcaceae).^{58,97,98} This microbiome profile in cirrhosis accompanies worsening disease, becomes more intense in the setting of decompensation and is associated with poor outcomes.⁹⁹

Alterations in gut microbiota set the stage for gut-liver axis impairment in cirrhosis. Changes in microbiota composition in cirrhosis arise from the disruption of most of the factors involved in microbiome control: i) reduced small bowel motility and transit time, mainly in the ascitic stage, as one of the main contributors to dysbiosis;^{100–103} ii) bile acid abnormalities, including reduced primary bile acid levels and increased secondary bile acid levels in the gut;^{104–106} and iii) impaired intestinal immunity. Experimental cirrhosis with ascites is associated with a deficiency in Paneth cell α -defensins, and impaired function of dendritic cells,^{107,108} both being especially severe in rats with ascites and pathological bacterial translocation. Hypochlorhydria present in cirrhosis, even in the absence of proton pump inhibition, is another factor that contributes to alter the microbiota.^{109–111} Remarkably, the pattern of microbiota abnormalities in cirrhosis is independent of aetiology.^{58,106}

A distinctive feature of cirrhosis is the invasion of the intestine by bacteria from the mouth. Enrichment of patient stools in species taxonomically of buccal origin and Lactobacillaceae seems to be related to the change in salivary microbiota, proton pump inhibitors and relatively low gastric

acid levels.^{97,112} An increase in Lactobacillaceae has been shown in prior studies of the gut microbiota in cirrhosis and could be related to lactulose use.¹¹³

Intestinal barrier dysfunction

Cirrhosis is associated with damage to the physical and immunological layers that comprise the intestinal barrier. Increased gastroduodenal, small intestine, colon and whole intestine permeability, as an expression of gut barrier disruption, are well-established features of cirrhosis, both in patients and experimental models, especially if ascites is present.^{103,114} Gut barrier disruption in cirrhosis leads not only to the increased passage to the systemic circulation of macromolecules, including bacterial components such as LPS or bacterial DNA, but also of viable bacteria (i.e. bacterial translocation) which reach the systemic milieu.¹¹⁵ Intestinal barrier damage parallels cirrhosis progression and is particularly severe when ascites and gut bacterial translocation have developed.^{103,116,117}

Controversy exists regarding the route by which gut bacteria gain access to the internal milieu in cirrhosis. Pathological translocation of viable bacteria from the gut lumen to the mesenteric lymph nodes and to the systemic circulation has been well established.^{103,118,119} Recent evidence indicates that in cirrhosis the lymphatic route of translocation coexists with the portal-venous passage to the liver of bacteria and bacterial products due to disruption of the GVB.¹²⁰ Vascular hyperpermeability is independent of the lymphatic route as well as of portal hypertension, since it is only present in models incorporating liver insufficiency. Interestingly, obeticholic acid was able to restore reduced ileum FXR signalling, improve the mucus machinery and stabilise the GVB in rats with cirrhosis, which supports the notion that the nuclear receptor FXR partly modulates mucus- and GVB in cirrhosis.¹²⁰ Additionally, obeticholic acid and other FXR-agonists reconstitute microbiota composition, improve intestinal innate defence mechanisms, reduce intestinal inflammation and decrease bacterial translocation and endotoxemia in experimental cirrhosis.^{108,120–122} In fact, reduced ileal FXR signalling has been described in experimental cirrhosis as a likely consequence of a reduction in primary bile acids and an increase in secondary bile acids in the lumen, as well as of intestinal inflammation.^{108,120,123}

The aforementioned abnormalities in intestinal barrier function in cirrhosis have been linked to structural changes in the intestine, including submucosal oedema, minimal infiltration by immune system cells, and disorganisation of interepithelial tight junction proteins in humans and experimental models of cirrhosis.^{108,116,118,124–126} According to a recent study, subclinical intestinal inflammation driven by modifications in microbiota composition contributes to worsen barrier dysfunction in

advanced cirrhosis. As cirrhosis progresses to the ascitic stage, the intestinal immune system of cirrhotic rats is characterised by a switch to a Th1 regulatory pattern with expansion of TNF- α - and IFN- γ -expressing lymphocytes, and concomitant Th17 depletion in the lamina propria.¹¹⁶ Bowel decontamination redistributes microbiota composition, reduces pro-inflammatory activation of mucosal immune cells, and diminishes intestinal permeability and bacterial translocation, supporting the key role of changes in microbiota in intestinal inflammation in cirrhosis.

Bacterial products, metabolites and bile acids

Reduced bile flow, decreased faecal bile acids, and increased serum bile acids are features of cirrhosis which also worsen in parallel with cirrhosis severity.^{104,106} Liver insufficiency impairs the synthesis and excretion of bile acids, resulting in deficient levels of total bile acids in the gut lumen and augmented levels in the serum, respectively. The reduction in faecal bile acids relates to secondary rather than primary bile acids, and is due to the collapse of 7 α bile acid-dehydroxylating bacterial populations, e.g., the *Clostridium* cluster XIVa that occurs in situations of reduced bile flow.^{104,106,113,127–128} In alcoholic hepatitis, the extreme expression of these abnormalities is observed.⁶¹

Since bile acids and the microbiome reciprocally influence each other, it is tempting to speculate that reduced secretion of bile acids into the intestine contributes to severe dysbiosis and an abundance of pathobionts in patients with cirrhosis. As cirrhosis progresses, alterations in microbiota promote intestinal inflammation, intestinal barrier damage and liver inflammation, which in turn further suppress bile acid secretion by the liver. The decline in intestinal FXR-signalling that results from decreased bile flow disrupts intestinal barrier function by reducing mucous thickness and antibacterial protein synthesis, and damaging GVB.

The gut liver axis and cirrhosis complications

Ample data support the contribution of enteric bacteria and PAMPs to the pathogenesis of immune cell activation and the inflammatory state of cirrhosis.¹¹⁵ The causal link between systemic inflammation and gut microbiota has recently been reinforced in patients with cirrhosis undergoing transjugular intrahepatic portosystemic shunting by demonstrating compartment-specific patterns of circulating bacteria, i.e. different genera in central, hepatic, portal, and peripheral venous blood, and inflammatory cytokine clusters specific to the abundance of blood microbiome genera in each patient.¹²⁹ Similarly, compartmentalisation of microbiota composition and immune response is also observed between ascites and blood in patients with decompensated cirrhosis.¹³⁰

Beyond their contribution to systemic inflammation, evidence has linked the abnormal gut microbiome to complications and outcomes in cirrhosis. The microbiome in decompensated cirrhosis is characterised by a relative abundance of potentially pathogenic taxa, mainly Enterobacteriaceae, but also Staphylococcaceae and Enterococcaceae, and a relative decrease in potentially beneficial commensal autochthonous taxa, particularly Lachnospiraceae, Ruminococcaceae and Clostridiales XIV.^{98,113} These changes can be found in the stools, sigmoid colon mucosa, and the saliva and serum of patients with cirrhosis, and mirror the severity of disease.^{98,112,113,128} While the gut microbiome remains unchanged in stable outpatients, the ratio of pathogenic to autochthonous taxa rises in decompensated cirrhosis, especially in patients with bacterial infection and encephalopathy.^{98,113} Interestingly, gut microbiome profiles differed in in- and out-patients, in non-infected and infected patients and in patients with poor outcomes (organ failure and death).⁹⁸ Moreover, these studies have shown that the greater the abundance of pathogenic taxa, the greater the level of endotoxemia, as an expression of gut barrier dysfunction.

Collectively, these data point to a direct influence of abnormalities in gut microbiota on complications and outcomes in cirrhosis, making the microbiota a promising target for therapy (Fig. 3). Our rationale is reinforced by the fact that gut microbial diversity was independently associated with a lower risk of 90-day hospitalisations in patients with cirrhosis on a western-diet. Notably, a diet rich in fermented milk, vegetables, cereals, coffee, and tea has been found to increase microbial diversity and reduce hospitalisations.⁹⁹

Novel tools to target the gut-liver axis

The Leuven EASL conference was planned as a platform to facilitate interaction and research networking among academia and industry. In this regard, we will describe novel tools to target the gut-liver axis that were presented by industry partners and academic researchers during the conference. Interventions can be systematised according to whether they target the intestinal content and mucus, the intestinal microbiome, the intestinal mucosa, or targets outside the intestine (Table 2). A thorough review of the armamentarium available to target the gut-liver axis is beyond the scope of this Seminar and this topic was the subject of a recent Clinical Trial Watch article in *Journal of Hepatology*.¹³¹

Interventions targeting intestinal content and mucus

Hydrogel technology

Fibres and high-viscosity polysaccharides have been employed as a strategy to improve glycaemic control, suppress appetite, and facilitate weight

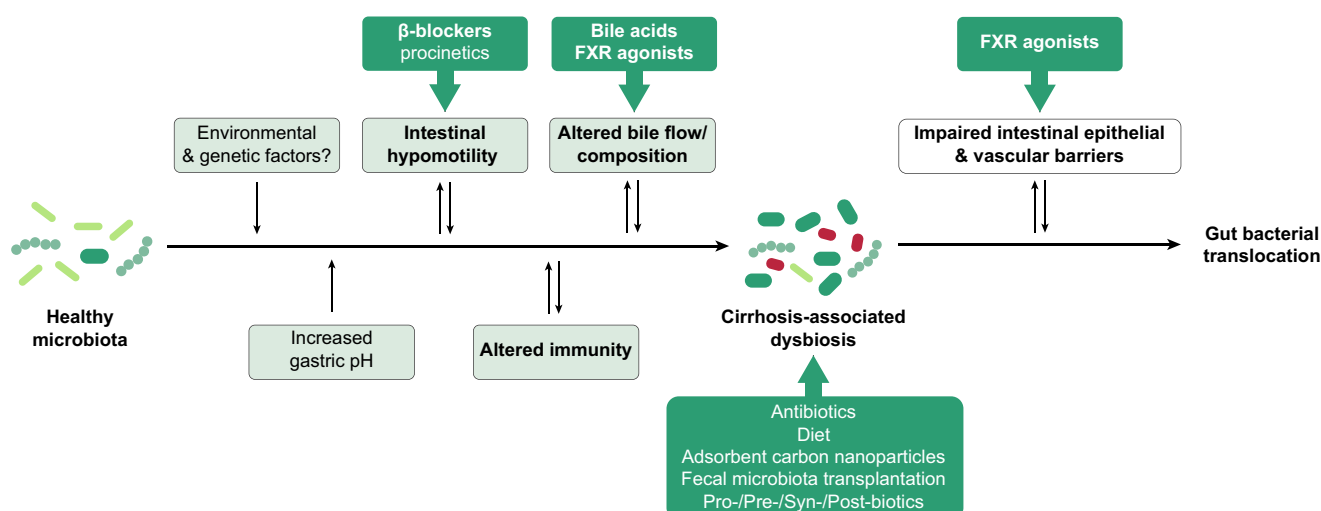


Fig. 3. Targeting the gut-liver axis in cirrhosis. In cirrhosis, abnormalities in microbiota composition and bacterial overgrowth are the hallmark of gut-liver axis disruption and are the consequence of intestinal hypomotility, changes in bile flow and composition, and impaired intestinal immunity. Gastric hypoacidity favoured by proton pump inhibitors use or environmental factors such as a western-diet will also contribute. In this setting of marked disruption of intestinal microbiota composition, concurrent damage to the epithelial and vascular intestinal barriers enables passage to the systemic circulation of PAMPs and viable bacteria through the vascular or lymphatic routes. A topic of active research is the search for alternatives to antibiotics for bowel decontamination designed to halt PAMPs and bacterial translocation in cirrhosis. Intestinal transit time can be sped up by beta-blockers or prokinetics, *e.g.* cisapride, to reduce bacterial overgrowth and translocation. Exogenous conjugated bile acids increase bile acid secretion and reduce bacterial overgrowth, endotoxemia and translocation. Obeticholic acid and other FXR agonists reconstitute microbiota composition, restore epithelial and vascular intestinal barrier function, improve intestinal innate defence mechanisms, reduce intestinal inflammation and decrease bacterial translocation and endotoxemia in experimental cirrhosis. A diet rich in fermented milk, cereals, coffee and tea increases microbial richness and reduces hospitalisations in patients with cirrhosis. Adsorbent carbon nanoparticles absorb gut-derived toxins and bacterial products reducing liver injury in experimental cirrhosis and are a target of on-going proof of concept studies. Faecal microbial transplantation in patients with recurrent hepatic encephalopathy corrects abnormalities in intestinal microbiota, increases antimicrobial peptide expression, and improves hepatic encephalopathy performance. PAMPs, pathogen-associated molecular patterns.

Table 2. Selected therapeutic interventions along the gut-liver axis.

Place of action	Denomination	Principle of action
Intestinal content	Hydrogel technology	Modified cellulose cross-linked with citric acid that mimics natural dietary fibres in vegetables. Hydrogel particles rapidly absorb water in the stomach and increase in volume, thereby improving satiety. Once in the large intestine, the hydrogel is partially digested and releases water, which is then reabsorbed.
	Gut-restricted polymers	Insoluble cross-linked polymeric drugs selectively bind with high-avidity multivalent surface features on bacteria or viruses, toxins, inorganic ions including potassium, phosphate, or bile acids.
	Carbon nanoparticles	Non-absorbable carbon particles exhibit a high adsorptive capacity for bacterial toxins and represent a novel strategy to counteract dysbiosis and translocation of bacterial-derived products.
	Non-selective beta-blockers	Beta-blockers reduce the load of enteric bacteria and inhibit intestinal bacterial overgrowth by fastening intestinal transit time and reducing intestinal permeability
Intestinal microbiome	Non-absorbable antibiotics	Selectively reduce the burden of enteric bacteria that mostly contribute to translocation, <i>e.g.</i> gram-negative bacteria. Rifaximin is a broad-spectrum compound, which exerts endotoxin-lowering and anti-inflammatory effects largely independent from their bactericidal action.
	Bacteriophages	Bacteriophages are viruses that specifically infect and kill intestinal bacterial pathogens. In contrast to antibiotics, phages do not induce resistance.
	Synthetic live bacterial therapeutics	These are engineered probiotics that can selectively consume toxic metabolites in the intestine and convert them into nontoxic forms.
	Fecal microbial transplantation	Fecal microbial transplantation is a method to replenish a healthy gut microbial environment and restore physiological colonization by recolonising the intestine with microbial flora from a healthy donor.
Intestinal mucosa	Pharmacological modulation of gut peptides.	Specific agonists of mucosal gut receptors may elicit responses including the release of regulators of glucose or bile acid metabolism.
	Duodenal mucosal resurfacing	Superficial duodenal mucosal ablation mediates an abnormal signal to endogenous insulin-sensitive tissues by limiting nutrient exposure or contact with the duodenal mucosa.
	Postbiotics	Postbiotics are metabolic products from intestinal bacteria that include short-chain fatty acids, secondary bile acids, proteins polysaccharides, vitamins and organic acids acting as metabolic regulators.
	FXR agonists	FXR-agonists reconstitute microbiota composition, restore epithelial and vascular intestinal barrier function, improve intestinal innate defense mechanisms, reduce intestinal inflammation and decrease bacterial translocation and endotoxemia
Peritoneal cavity	Ammonia uptake particles	This treatment consists in a suspension of large transmembrane pH-gradient liposomes, containing citric acid and designed to rapidly capture ammonia from ascites of cirrhotic patients.

loss in patients with increased metabolic risk. Hydrogel is made from 2 naturally derived building blocks, modified cellulose (carboxymethylcellulose) cross-linked with citric acid, that create a 3D matrix mimicking that of natural dietary fibres in vegetables. Orally administered in capsules with water before a meal, hydrogel particles rapidly absorb water in the stomach and homogeneously mix with ingested foods, but without caloric value.¹³² Once hydrated, the ingested dose of hydrogel occupies about one-fourth of the average stomach, mimicking food in terms of volume and thereby improving satiety. It maintains its 3D structure and mechanical properties during transit through the small intestine and once in the large intestine, the hydrogel is partially digested by enzymes and loses its 3D structure along with most of its absorption capacity. The released water is then reabsorbed, and the remaining cellulosic material is expelled in the faeces. Hydrogel is considered a medical device because it achieves its primary intended purpose through mechanical modes of action.

In an *in vitro* model of gastrointestinal digestion, hydrogels demonstrated viscoelastic profiles that were orders of magnitude superior to those of common processed functional fibre supplements (psyllium, guar gum and glucomannan). Results from the GLOW study demonstrated that hydrogel was an effective, safe and well-tolerated weight loss therapy. A significant association was observed between baseline fasting plasma glucose levels and the effectiveness of the treatment, suggesting a benefit for a higher-risk population otherwise known to be less responsive to therapy.¹³² Hydrogel is currently being tested in preclinical models¹³³ and ongoing

clinical trials in obese patients with and without type 2 diabetes (NCT03622424 and NCT03058029) (Table 3). Currently, no data are available on the effects of this technology on the liver, although it could prevent or improve NAFLD by inducing weight loss and relieving insulin resistance.

Gut-restricted polymers

Polymers have a wide range of applications as therapeutic agents owing to their intrinsic ability to bind and retain target entities.¹³⁴ Insoluble cross-linked polymers are typically administered orally and act in the gastrointestinal tract; the bound substance is then cleared from the body in the faeces, along with the polymer. Polymer sequestrants for binding of inorganic ions including potassium, phosphate, or bile acids have been used clinically for several years. For example, therapy with colestevam has proven efficacy in dyslipidaemia and type 2 diabetes.¹³⁵

Next generation polymer sequestrant development has sought to extend the application of polymeric drugs to achieve more defined physical properties with better moiety binding efficacy and selectivity. Soluble polymers have been investigated as antimicrobial agents, due to potential high-avidity interactions between repeating polymer units and multivalent surface features on bacteria or viruses. Tolevamer, a soluble anionic polystyrene sulfonate, was developed for the treatment of *Clostridium difficile*-associated diarrhoea, due to its ability to bind to toxins A and B which are produced by the bacteria.¹³⁶ An example of a polymeric binder to sequester proteins, such as gliadin, is currently being tested in phase I/II clinical studies in coeliac disease.¹³⁷

Key points

A number of interventions targeting the gut-liver axis are in development and can be systematised according to whether they target the intestinal content and mucus, the intestinal microbiome, the intestinal mucosa, or targets outside the intestine.

Table 3. Selected ongoing studies on novel targets of therapy along the gut-liver axis.

Disease	Title	Reference
Overweight and type 2 diabetes	Effect of Gelesis200 on Body Weight in Overweight and Obese Subjects With Prediabetes and With and Without Type 2 Diabetes (LIGHT-UP)	NCT03058029 NCT03622424
Irritable bowel disease with bile acid malabsorption	Trial to Understand Efficacy of Colesevelam in Diarrhea Predominant IBS Patients With Bile Acid Malabsorption	NCT03270085
Non-alcoholic steatohepatitis	Safety and Tolerability of Yaq-001 in Patients With Non-Alcoholic Steatohepatitis	NCT03962608
Cirrhosis	Safety and Tolerability of Yaq-001 in Patients With Cirrhosis	NCT03202498
Cirrhosis	Safety, Tolerability and Pharmacodynamics of SYN1020	NCT03447730
Decompensated cirrhosis	Efficacy of the Combination of Simvastatin Plus Rifaximin in Patients With Decompensated Cirrhosis to Prevent ACLF Development	NCT03780673
Metabolic syndrome	Fecal Microbiota Transplantation and Fiber in Patients With Metabolic Syndrome	NCT03727321
Alcoholic cirrhosis	Fecal Microbial Transplant for Alcohol Misuse in Cirrhosis	NCT03416751
Type 2 diabetes	Safety & Effectiveness of Duodenal Mucosal Resurfacing Using the Revita™ System in Treatment of Type 2 Diabetes	NCT03653091
Non-alcoholic steatohepatitis	Effect of Duodenal Mucosal Resurfacing in the Treatment of NASH (DMR_NASH_001)	NCT03536650
Non-alcoholic steatohepatitis	Investigation of Efficacy and Safety of Three Dose Levels of Subcutaneous Semaglutide Once Daily Versus Placebo in Subjects With Non-alcoholic Steatohepatitis.	NCT02970942
Non-alcoholic steatohepatitis	Researching an Effect of GLP-1 Agonist on Liver Steatosis (REALIST)	NCT03648554
Non-alcoholic steatohepatitis	Randomized Global Phase 3 Study to Evaluate the Impact on NASH With Fibrosis of Obeticholic Acid Treatment (REGENERATE)	NCT02548351
Type 2 diabetes	Safety & Effectiveness of Duodenal Mucosal Resurfacing Using the Revita™ System in Treatment of Type 2 Diabetes	NCT03653091
Non-alcoholic steatohepatitis	Effect of Duodenal Mucosal Resurfacing in the Treatment of NASH (DMR_NASH_001)	NCT03536650

Carbon nanoparticles

Yaq-001, a new synthetic non-absorbable carbon, has been shown to exhibit a high adsorptive capacity for bacterial toxins and thus represents a novel strategy to counteract gut microbiota alterations and translocation of bacterial-derived products in patients with advanced chronic liver disease. Experimental evidence in a rodent model of secondary biliary cirrhosis showed that Yaq-001 was associated with a significant increase in Firmicutes and a decrease in Bacteroidetes in stool samples.¹³⁸ Moreover, this treatment significantly attenuated the LPS-induced production of reactive oxygen species by monocytes in bile duct-ligated rats. These results showed that gut microbial products are one important target of this therapeutic approach.

The first-in-human clinical investigation with Yaq-001 is an ongoing multicentre, randomised, double blinded, placebo-controlled trial intended to evaluate the safety and tolerability of oral administration of Yaq-001 therapy in cirrhotic patients with diuretic-responsive ascites and Child-Pugh 7–11 (CARBALIVE-SAFETY, NCT 03202498).

Interventions targeting the microbiome*Bacteriophages*

Bacteriophages, viruses that specifically infect and kill bacteria, have been continually evolving to overcome the defence mechanisms developed by bacteria. As phages work through a completely orthogonal mode of action compared to antibiotics, antibiotic resistance does not convey resistance to phages and highly antibiotic resistant bacteria may still be efficiently eliminated by phages. The therapeutic administration of phages under compassionate protocols has recently demonstrated success in terminally ill patients with multidrug resistant infections of *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.^{139,140} Furthermore, an increasing number of controlled and EMA/FDA-regulated clinical trials are being conducted by multiple biotech companies to bring this modality into mainstream medicine.¹⁴¹

While most clinical development activities currently employing phages are directed against infectious diseases, a novel programme of phage therapy for patients with primary sclerosing cholangitis is currently being developed. Recent discoveries show that specific pathogenic gut bacteria infect and penetrate the epithelial lining of the gastrointestinal tract, allowing bacterial translocation and a liver immune response.¹⁴² Ongoing research projects aim to identify specific bacterial targets and to create 'phage cocktails' that are designed to eliminate these particular bacterial strains while leaving the healthy microbiome intact.

Synthetic live bacterial therapeutics

Engineered probiotics may be uniquely designed to consume toxic metabolites in the intestine

and convert them into non-toxic forms. SYNBI020, an *E. coli* Nissle 1917 strain engineered by deleting a negative regulator of L-arginine biosynthesis and by inserting a feedback-resistant L-arginine biosynthetic enzyme, has been demonstrated to improve hyperammonaemia and survival in mice and was well tolerated in a phase I clinical study in hyperammonaemia disorders.¹⁴³ SYNBI020 has recently been tested in a phase Ib/IIa randomised, placebo-controlled study to evaluate the safety, tolerability and pharmacodynamics of SYNBI020 in patients with cirrhosis (NCT03447730). However, its development has been discontinued in favour of other synthetic live bacterial therapeutics due to negative data from an interim analysis (<https://investor.synlogictx.com/news-releases/news-release-details/synlogic-discontinues-development-synbi020-treat-hyperammonemia>). Using the same technique, bacteria expressing genes encoding specific enzymes have been tested to treat phenylketonuria.¹⁴⁴ The same principle was applied to develop an engineered *Lactococcus lactis* strain producing IL-10 that has been evaluated for the treatment of inflammatory bowel disease.¹⁴⁵

Faecal microbial transplantation

Faecal microbial transplantation is used to replenish a healthy gut microbial environment and restore physiological colonisation via different methods of administration; it is currently an accepted treatment for recurrent or refractory *Clostridium difficile* infection and data are arising about its efficacy in improving inflammatory bowel disease, metabolic syndrome and hepatic encephalopathy. Confirming the findings of a previous report, a phase I study has shown that faecal microbial transplantation with oral capsules, following antibiotic pretreatment, is well tolerated and safe over the long-term in patients with cirrhosis and recurrent hepatic encephalopathy.^{146–148} In this study, microbiota transplantation restored microbial diversity and function following antibiotic-associated disruption, led to sustained improvement in cognitive function parameters, and reduced the recurrence of hepatic encephalopathy and liver-related hospitalisations.^{146–148} It is likely that the beneficial effects on the recurrence of encephalopathy could be in part due to amelioration of neuroinflammation and microglial activation, which have been shown to be reduced by stools from human donors in germ-free mice colonised with stools from patients with cirrhosis.¹⁴⁹ Moreover, evidence is growing that suggests faecal microbial transplantation may reverse early portal hypertension, intrahepatic endothelial dysfunction and insulin resistance in rats on a high-fat and high-fructose diet.¹⁵⁰ Despite these promising advances, only a few controlled trials of interventions that alter the microbiome have been performed so far and a number of important questions remain to be

answered, including the safety and the duration of the effect of this intervention.

Non-absorbable antibiotics

Rifaximin, as the non-absorbable antibiotic with the most favourable safety profile, is currently being tested to target the microbiome in different clinical trials in chronic liver disease (recently reviewed in¹³¹). Rifaximin is a broad-spectrum compound, which exerts endotoxin-lowering and anti-inflammatory effects that are largely independent of its bactericidal action. The efficacy of the combination of rifaximin and simvastatin, which targets the key mechanisms of cirrhosis progression (*i.e.* the gut-liver axis and the systemic inflammatory response), is currently being tested to prevent acute-on-chronic liver failure development in patients with decompensated cirrhosis in a phase III, multicentre, double-blind placebo-controlled, randomised clinical trial (LIVERHOPE EFFICACY, NCT03780673). This study has been preceded by the LIVERHOPE_SAFETY trial, which has concluded that a dose of simvastatin 20 mg per day plus rifaximin is not associated with a higher risk of liver or muscle toxicity in patients with decompensated cirrhosis (NCT03150459)

Interventions targeting the intestinal mucosa

The duodenum is increasingly recognised as a metabolic signalling centre that plays a role in regulating insulin action and, consequently, insulin resistance. Specialised enteroendocrine cells play a sensing and signalling role throughout the intestine, and the diversity of derived peptides reflects the nuanced manner by which the gut orchestrates many aspects of ingestion, digestion and assimilation of nutrients. Enterocytes have more recently been included in these complex processes following the discovery of FGF19. The pharmacological modulation of the gut-peptide axis offers opportunities that have converged on metabolic homeostasis in obesity, diabetes and NAFLD.

Pharmacological modulation of gut peptides

GLP-1 is produced and stored by L-cells in the gut. It is released into the hepatic portal system in response to luminal sugars, fatty acids and amino acids. Binding of GLP-1 to its cognate G-protein coupled receptor (GLP-1R) increases insulin and decreases glucagon secretion by the pancreas, decreases gastric emptying and elicits a decrease in food intake among many other beneficial effects. The LEAN trial (Liraglutide Efficacy and Action in NASH) was the first randomised controlled trial to report on the efficacy of a GLP-1R agonist in patients with biopsy-proven NASH over a 48-week treatment period. The effects of daily injections of semaglutide on biomarkers have been reported in sub-analyses of patients treated in obesity studies, while long-term efficacy studies in patients with biopsy proven NASH are currently ongoing with daily semaglutide over 72 weeks

(NCT02970942), and are planned with weekly dulaglutide over 52 weeks (NCT03648554) (Table 3).

FGF19 is produced and released by enterocytes of the terminal ileum in response to FXR activation by bile acids. FGF19 flows to the liver through the portal circulation where it binds to a cell surface receptor complex including FGFR4 and β -klotho to elicit pleiotropic effects, including a decrease in bile acid production. Approved FXR agonists drugs such as the bile acid obeticholic acid and investigational medicinal products tropifexor or cilofexor moderately increase circulating FGF19 levels. FXR agonists mediate pleiotropic effects in the liver both directly on the hepatocyte and indirectly via FGF19. Phase III data have shown a beneficial effect of obeticholic acid on fibrosis in patients with NASH.¹⁵¹ Elucidating how much of this effect is due to FXR agonism in the liver versus an effect of FGF19 from the gut poses a clinical challenge. An FGF19 mimetic compound, NGM282, is also in clinical trials, having shown a high effect size on biomarker endpoints in proof of concept studies.^{152,153} One aspect of the FGF19 axis that also requires attention is the association of *FGFR4* mutations with hepatocellular carcinoma and the evidence that transgenic overexpression of *FGF19* induces hepatocellular carcinoma in mouse models.¹⁵⁴ Indeed, blockade of FGFR4 has been investigated in specific forms of hepatocellular carcinoma in humans.¹⁵⁵ Nevertheless, if histological effects reported from an open label study are confirmed, an FGF19 mimetic could offer an attractive therapy for NASH.

Duodenal mucosal resurfacing

Duodenal mucosal resurfacing is a technique that targets the duodenal surface, mediating an abnormal signal to endogenous insulin-sensitive tissues based on the fact that limiting nutrient exposure or contact with the duodenal mucosa exerts powerful metabolic effects. Morphologic changes in the small intestine of patients and rats with diabetes included hyperplasia of enteroendocrine cells and proliferation of endocrine cells which differentiated toward K cells and oversecreted gastric inhibitory polypeptide.^{156,157}

Duodenal mucosal resurfacing can be performed using different techniques including a catheter system that delivers a hydrothermal exchange at the mucosal surface. The resultant superficial mucosal ablation is followed by a re-epithelialisation of the treated duodenal lumen starting within days following the procedure. In the first human study, hydrothermal ablation was safely administered with no evidence of perforation, pancreatitis, or haemorrhage. Duodenal biopsy specimens obtained 3–6 months post-procedure demonstrated full mucosal regrowth, without inflammation and fibrotic scarring, and improvement in glycaemic and hepatic measures.¹⁵⁸

Key points

Pharmacological modulation of the gut-peptide axis holds promise for the treatment of obesity, diabetes and non-alcoholic fatty liver disease.

Postbiotics

As mentioned above, recent data suggest that the underlying mechanism of microbiota-based control of intestinal homeostasis relies on their metabolic products (postbiotics).⁷ Postbiotics are the new frontier in microbiome science and could be key factors in maintaining long-term health benefits. Their composition is variable and depends on bacterial strains and their metabolic status. Examples include SCFAs, secondary bile acids, proteins (e.g. p40 molecule, HM0539), enzymes, peptides, bacteriocins, endo- and exo-polysaccharides, vitamins and organic acids.¹⁵⁹ In general, postbiotic components have been reported to possess immunomodulatory and protective roles on intestinal barrier function. In particular, postbiotics can act on immune cells protecting the gut tissue from immunopathology by increasing the secretion of anti-inflammatory cytokines such as IL-10.^{160,161} Moreover, postbiotics potentiate epithelial tight junction structure by increasing the expression of tight junction proteins (zonula occludens, ZO-1) and intestinal mucin levels, thus favouring the restoration of gut barrier function.¹⁶² In addition, postbiotics also behave as microbiota resilience-inducing factors. They can function as pathogenic bacteria inhibitors and possibly as quorum-sensing signalling molecules to regulate bacterial cell density and biofilm formation, maintaining microbial composition.¹⁶³ Postbiotic mechanisms of action are yet to be fully identified and rigorous clinical trials are needed to

support their health benefit and their possible application in diseases of gut-barrier dysfunction.

Interventions outside the intestine

Ammonia uptake particles

VS-01 is an innovative liposomal fluid that consists of a suspension of large transmembrane pH-gradient liposomes containing citric acid and designed to rapidly capture ammonia.¹⁶⁴ VS-01 can be delivered temporarily into the peritoneal cavity via a paracentesis catheter routinely used in patients with decompensated cirrhosis and ascites, thereby allowing its application at the same time as paracentesis. Following administration of VS-01 into the peritoneal cavity, uncharged ammonia diffuses from the blood into the peritoneal space and across the liposomal bilayer membrane, where it remains trapped due to its positive charge (Fig. 4). VS-01 simultaneously removes urea and toxins that accumulate in these patients, by promoting their passive diffusion into the liposomes' supporting fluid.¹⁶⁵ At the end of the treatment, the fluid containing ammonia-loaded liposomes is withdrawn from the peritoneal cavity by gravity, in the same way ascitic fluid is drained by paracentesis.

In experimental models of cirrhosis and hepatic encephalopathy, VS-01 has demonstrated a fast and efficient clearance of ammonia, up to twenty times superior to conventional peritoneal dialysis solutions, significantly reducing plasma ammonia levels and attenuating the associated brain

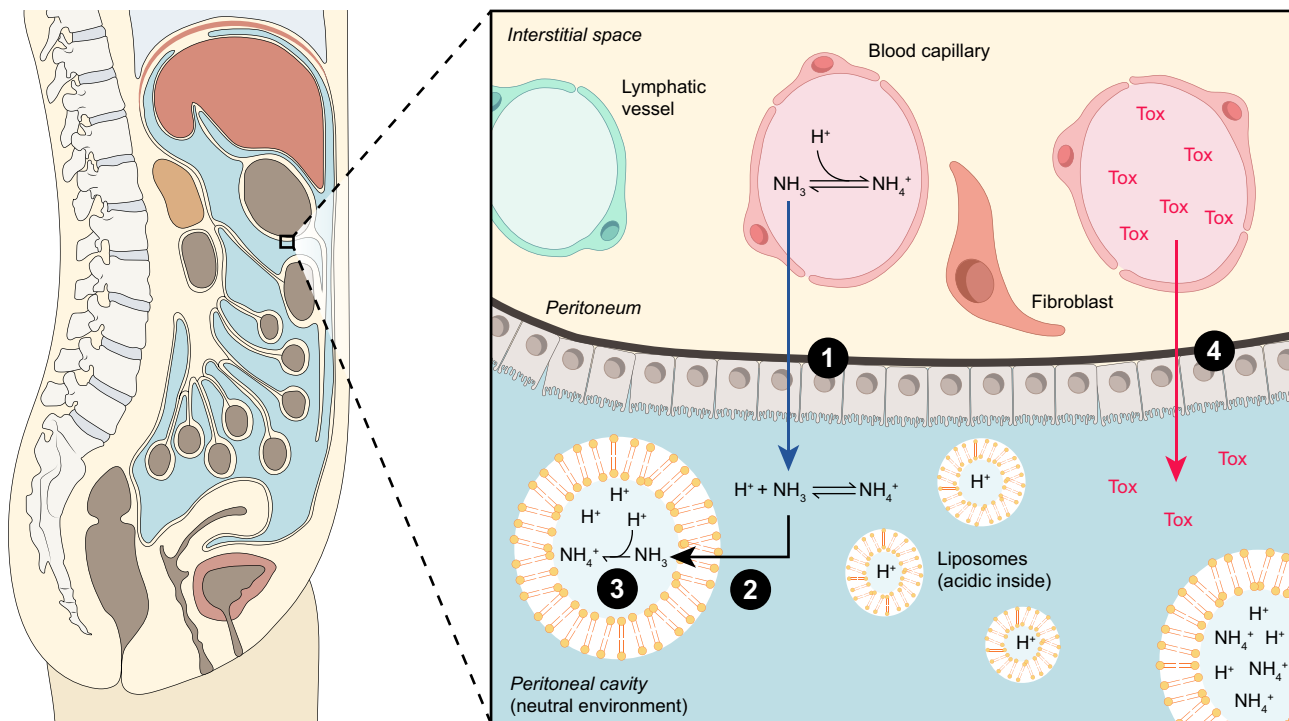


Fig. 4. Mechanism of action of VS-01. VS-01 is a liposomal fluid designed to rapidly capture ammonia once delivered into the peritoneal cavity. Ammonia (NH_3) from the blood passively diffuses to the peritoneal cavity (1) and into the liposomes (2). NH_3 is then protonated due to acidic conditions in the liposomes and charged molecules (NH_4^+) remain trapped (3). Uremic and hepatic toxins in the fluid also passively diffuse and are trapped in the liposomes (4).

oedema.¹⁶⁵ Additionally, untargeted metabolomic studies revealed that the vast majority of toxins known to be present in excess in patients with liver dysfunction were removed by VS-01 concomitantly with ammonia. Hence, VS-01 can provide prompt detoxification and thereby support the 3 primary organs (brain, liver and kidney) that can fail in advanced cirrhosis. VS-01 is currently being evaluated in a first-in-human phase Ib clinical trial in patients with mild hepatic encephalopathy and ascites requiring paracentesis.

Conclusions and future trends

The gut-liver axis refers to the reciprocal interaction that takes place between the gut and its microbiota on the one hand, and the liver on the other. Although independent, the key players, namely diet, microbiota and intestinal mucosa are interrelated and are connected to the host through the bile and portal blood. Effectively, bile acids produced in the liver regulate microbiota composition and intestinal barrier function, and gut products regulate bile acid synthesis and glucose and lipid metabolism in the liver. Further, there is growing evidence that gut-liver axis disruption leads to the progression of most forms of chronic liver disease, including cirrhosis. The main features of a disrupted gut-liver axis are shared by ALD, NAFLD and cirrhosis itself. These features include an altered intestinal microbiota, gut barrier damage and consequently increased permeability, and changes in luminal levels of bile acids. In turn, shifting levels of bile acids lead to reduced intestinal FXR-signalling, which compromises intestinal mucous and antimicrobial peptide synthesis along with gut-vascular barrier integrity. The relative contribution of each of these abnormalities to gut-liver axis disruption depends on the aetiology and stage of liver disease. Hence it is during advanced end-stage liver disease, *i.e.*, decompensated cirrhosis, that the greater severity of barrier damage is observed. The functional consequences of a disrupted gut-liver axis are also shared by different chronic liver diseases and involve repetitive exposure of liver innate immune cells to gut-derived bacterial products such as endotoxin, and metabolites like ethanol and trimethylamine, resulting in liver inflammation.

Advances in knowledge of the gut-liver axis are driving the development of diagnostic, prognostic and therapeutic tools based on microbiota for the management of liver disease. Identifying a set of microorganisms that will serve as indicators of liver damage and disease progression in ALD and NAFLD, as well as helping to predict hospitalisation, bacterial infection and liver-related complications in cirrhosis is an active area of research. Microbiome analysis via multi-omic profiling could provide a mechanistic picture to understand and identify the different microbiota phenotypes

observed in ALD and NAFLD. In effect, this type of analysis in serum could facilitate testing. A promising therapeutic approach is the modification of bile acid signalling to strengthen intestinal barrier function and modulate the gut-liver axis. For this purpose, FXR agonists could reduce intestinal FXR signalling and improve antibacterial protein synthesis, reversing GVB damage. Modifying intestinal contents with targeted faecal microbial transplantation or specific probiotics isolated from human faeces is another therapeutic modality worth exploring. Indeed, the increasingly recognised role of gut microbiota in the pathogenesis and progression of liver diseases opens up a wide gateway for microbiome-based tools to effectively diagnose and treat liver disease.

Abbreviations

AAA, aromatic amino acids; ALD, alcohol-related liver disease; BCCA, branched-chain amino acids; FGF, fibroblast growth factor; FXR, farnesoid X receptor; GIP, glucose-dependent insulinotropic polypeptide; GVB, gut vascular barrier; LCFAs, long-chain fatty acids; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NK, natural killer; PAA, plasma phenylacetic acid; PAMPs, pathogen-associated molecular patterns; SCFAs, short-chain fatty acids; TGF, transforming growth factor; Th, T helper; TJ, tight junction; TLR, toll-like receptors; TNF, tumour necrosis factor; Treg, regulatory T.

Financial support

Supported by grants from the Spanish Ministry of Science, Innovation and Universities (SAF 2017-86343-R, to A.A.), the Swiss National Science Foundation (31003A_163143 to A.D.G.), and The Italian association for Cancer Research (IG, AIRC 5x1000 to M.R.) and the European Research Council (HomeoGUT to M.R.). CIBEREHD is funded by the Instituto de Salud Carlos III with grants cofinanced by the European Development Regional Fund “A way to achieve Europe” (ERDF).

Conflict of interest

Agustín Albillos has received grant and research support from Gilead, Advisory Boards from Gilead, Pfizer, Intercept and Martin Pharmaceuticals, and Speaking and teaching from Gilead, Intercept. Maria Rescigno has received grant and research support from Intercept Pharmaceuticals, Gelesis and Heinz; advisory board of Gelesis and MillBo.

Please refer to the accompanying [ICMJE disclosure](#) forms for further details.

Authors' contributions

AA, AG, MR contributed to this manuscript by selecting and summarizing the information

delivered at the EASL monothematic conference on “The gut-liver axis” held in Leuven in June 2018

BiomX, Ness Ziona, Israel, Vincent Foster at Versantis, Zurich, Switzerland.

Acknowledgments

The authors would like to thank Michael Badman at Novartis, Basel, Switzerland, Sharon Kred-Russo at

Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jhep.2019.10.003>.

References

- [1] Tarao K, So K, Moroi T, Ikeuchi T, Suyama T. Detection of endotoxin in plasma and ascitic fluid of patients with cirrhosis: its clinical significance. *Gastroenterology* 1977;73:539–542.
- [2] Trigger DR, Boyer TD, Levin J. Portal and systemic bacteraemia and endotoxaemia in liver disease. *Gut* 1978;19:935–939.
- [3] Chen B, Chen H, Shu X, Yin Y, Li J, Qin J, et al. Presence of segmented filamentous bacteria in human children and its potential role in the modulation of human gut immunity. *Front Microbiol* 2018;9:1403.
- [4] Atarashi K, Tanoue T, Ando M, Kamada N, Nagano Y, Narushima S, et al. Th17 cell induction by adhesion of microbes to intestinal epithelial cells. *Cell* 2015;163:367–380.
- [5] Blacher E, Levy M, Tatirovsky E, Elinav E. Microbiome-modulated metabolites at the interface of host immunity. *J Immunol* 2017;198:572–580.
- [6] Levy M, Blacher E, Elinav E. Microbiome, metabolites and host immunity. *Curr Opin Microbiol* 2017;35:8–15.
- [7] Tsilingiri K, Rescigno M. Postbiotics: what else?. *Benef Microbes* 2013;4:101–107.
- [8] Johansson ME. Fast renewal of the distal colonic mucus layers by the surface goblet cells as measured by in vivo labeling of mucin glycoproteins. *PLoS ONE* 2012;7:e41009.
- [9] Gibbins HL, Proctor GB, Yakubov GE, Wilson S, Carpenter GH. IgA binding to mucosal surfaces is mediated by mucin-mucin interactions. *PLoS ONE* 2015;10:e0119677.
- [10] Vaishnava S, Yamamoto M, Severson KM, Ruhn KA, Yu X, Koren O, et al. The antibacterial lectin RegIIIγ promotes the spatial segregation of microbiota and host in the intestine. *Science* 2011;334:255–258.
- [11] Bergstrom JH, Birchenough GM, Katona G, Schroeder BO, Schutte A, Ermund A, et al. Gram-positive bacteria are held at a distance in the colon mucus by the lectin-like protein ZG16. *Proc Natl Acad Sci U S A* 2016;113:13833–13838.
- [12] Jakobsson HE, Rodriguez-Pineiro AM, Schutte A, Ermund A, Boysen P, Bemark M, et al. The composition of the gut microbiota shapes the colon mucus barrier. *EMBO Rep* 2015;16:164–177.
- [13] Birchenough GM, Nystrom EE, Johansson ME, Hansson GC. A sentinel goblet cell guards the colonic crypt by triggering Nlrp6-dependent Muc2 secretion. *Science* 2016;352:1535–1542.
- [14] Derrien M, Van Baaren P, Hooiveld G, Norin E, Muller M, de Vos WM. Modulation of mucosal immune response, tolerance, and proliferation in mice colonized by the mucin-degrader *Akkermansia muciniphila*. *Front Microbiol* 2011;2:166.
- [15] Desai MS, Seekatz AM, Koropatkin NM, Kamada N, Hickey CA, Wolter M, et al. A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. *Cell* 2016;167:1339–1353.
- [16] Wrzosek L, Miquel S, Noordine ML, Bouet S, Joncquel Chevalier-Curt M, Robert V, et al. Bacteroides thetaiotaomicron and Faecalibacterium prausnitzii influence the production of mucus glycans and the development of goblet cells in the colonic epithelium of a gnotobiotic model rodent. *BMC Biol* 2013;11:61.
- [17] Kurashima Y, Kiyono H. Mucosal ecological network of epithelium and immune cells for gut homeostasis and tissue healing. *Annu Rev Immunol* 2017;35:119–147.
- [18] Spadoni I, Fornasa G, Rescigno M. Organ-specific protection mediated by cooperation between vascular and epithelial barriers. *Nat Rev Immunol* 2017;17:761–773.
- [19] Spadoni I, Zagato E, Bertocchi A, Paolinelli R, Hot E, Di Sabatino A, et al. A gut-vascular barrier controls the systemic dissemination of bacteria. *Science* 2015;350:830–834.
- [20] Ciccia F, Guggino G, Rizzo A, Alessandro R, Luchetti MM, Milling S, et al. Dysbiosis and zonulin upregulation alter gut epithelial and vascular barriers in patients with ankylosing spondylitis. *Ann Rheum Dis* 2017;76:1123–1132.
- [21] Mouries J, Brescia P, Silvestri A, Spadoni I, Sorribas M, Wiest R, et al. Microbiota-driven gut vascular barrier disruption is a prerequisite for non-alcoholic steatohepatitis development. *J Hepatol* 2019;71:1216–1228.
- [22] McDonald BD, Jabri B, Bendelac A. Diverse developmental pathways of intestinal intraepithelial lymphocytes. *Nat Rev Immunol* 2018;18:514–525.
- [23] Rescigno M, Urbano M, Valzasina B, Francolini M, Rotta G, Bonasio R, et al. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat Immunol* 2001;2:361–367.
- [24] Chieppa M, Rescigno M, Huang AY, Germain RN. Dynamic imaging of dendritic cell extension into the small bowel lumen in response to epithelial cell TLR engagement. *J Exp Med* 2006;203:2841–2852.
- [25] Niess JH, Brand S, Gu X, Landsman L, Jung S, McCormick BA, et al. CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. *Science* 2005;307:254–258.
- [26] Mazzini E, Massimiliano L, Penna G, Rescigno M. Oral tolerance can be established via gap junction transfer of fed antigens from CX3CR1 macrophages to CD103 dendritic cells. *Immunity* 2014;40:248–261.
- [27] Brennan PJ, Brigl M, Brenner MB. Invariant natural killer T cells: an innate activation scheme linked to diverse effector functions. *Nat Rev Immunol* 2013;13:101–117.
- [28] Corbett AJ, Eckle SB, Birkinshaw RW, Liu L, Patel O, Mahony J, et al. T-cell activation by transitory neo-antigens derived from distinct microbial pathways. *Nature* 2014;509:361–365.
- [29] Kjer-Nielsen L, Patel O, Corbett AJ, Le Nours J, Meehan B, Liu L, et al. MR1 presents microbial vitamin B metabolites to MAIT cells. *Nature* 2012;491:717–723.
- [30] Dias J, Leansyah E, Sandberg JK. Multiple layers of heterogeneity and subset diversity in human MAIT cell responses to distinct microorganisms and to innate cytokines. *Proc Natl Acad Sci U S A* 2017;114:E5434–E5443.
- [31] Sandquist I, Kolls J. Update on regulation and effector functions of Th17 cells. *F1000Res* 2018;7:205.
- [32] Gaboriau-Routhiau V, Rakotobe S, Lecuyer E, Mulder I, Lan A, Bridonneau C, et al. The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. *Immunity* 2009;31:677–689.
- [33] Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* 2009;139:485–498.
- [34] Hirota K, Turner JE, Villa M, Duarte JH, Demengeot J, Steinmetz OM, et al. Plasticity of Th17 cells in Peyer's patches is responsible for the induction of T cell-dependent IgA responses. *Nat Immunol* 2013;14:372–379.
- [35] Sharma A, Rudra D. Emerging functions of regulatory T cells in tissue homeostasis. *Front Immunol* 2018;9:883.
- [36] Park JH, Eberl G. Type 3 regulatory T cells at the interface of symbiosis. *J Microbiol* 2018;56:163–171.
- [37] Tait Wojno ED, Artis D. Emerging concepts and future challenges in innate lymphoid cell biology. *J Exp Med* 2016;213:2229–2248.
- [38] Gopalakrishnan V, Spencer CN, Nezi L, Reuben A, Andrews MC, Karpnits TV, et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science* 2018;359:97–103.
- [39] Matson V, Fessler J, Bao R, Chongsuwan T, Zha Y, Alegre ML, et al. The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. *Science* 2018;359:104–108.
- [40] Ismail AS, Behrendt CL, Hooper LV. Reciprocal interactions between commensal bacteria and gamma delta intraepithelial lymphocytes during mucosal injury. *J Immunol* 2009;182:3047–3054.

- [41] Ismail AS, Severson KM, Vaishnava S, Behrendt CL, Yu X, Benjamin JL, et al. Gammadelta intraepithelial lymphocytes are essential mediators of host-microbial homeostasis at the intestinal mucosal surface. *PNAS* 2011;108:8743–8748.
- [42] Hansson GC, Johansson ME. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Gut Microbes* 2010;1:51–54.
- [43] Mosca F, Gianni ML, Rescigno M. Can postbiotics represent a new strategy for NEC? *Adv Exp Med Biol* 2019;1125:37–45.
- [44] Schulthess J, Pandey S, Capitani M, Rue-Albrecht KC, Arnold I, Franchini F, et al. The short chain fatty acid butyrate imprints an antimicrobial program in macrophages. *Immunity* 2019;50:432–445 e437.
- [45] Belkaid Y, Harrison OJ. Homeostatic immunity and the microbiota. *Immunity* 2017;46:562–576.
- [46] Reynes B, Palou M, Rodriguez AM, Palou A. Regulation of adaptive thermogenesis and browning by prebiotics and postbiotics. *Front Physiol* 2018;9:1908.
- [47] Wahlstrom A, Sayin SI, Marschall HU, Backhed F. Intestinal crosstalk between bile acids and microbiota and its impact on host metabolism. *Cell Metab* 2016;24:41–50.
- [48] de Aguiar Vallim TQ, Tarling EJ, Edwards PA. Pleiotropic roles of bile acids in metabolism. *Cell Metab* 2013;17:657–669.
- [49] Sayin SI, Wahlstrom A, Felin J, Jantti S, Marschall HU, Bamberg K, et al. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. *Cell Metab* 2013;17:225–235.
- [50] Gadaleta RM, van Erpecum KJ, Oldenburg B, Willemsen EC, Renooij W, Murzilli S, et al. Farnesoid X receptor activation inhibits inflammation and preserves the intestinal barrier in inflammatory bowel disease. *Gut* 2011;60:463–472.
- [51] Gonzalez FJ, Jiang C, Xie C, Patterson AD. Intestinal farnesoid X receptor signaling modulates metabolic disease. *Dig Dis* 2017;35:178–184.
- [52] Li F, Jiang C, Krausz KW, Li Y, Albert I, Hao H, et al. Microbiome remodelling leads to inhibition of intestinal farnesoid X receptor signalling and decreased obesity. *Nat Commun* 2013;4:2384.
- [53] Jiang C, Xie C, Li F, Zhang L, Nichols RG, Krausz KW, et al. Intestinal farnesoid X receptor signaling promotes nonalcoholic fatty liver disease. *J Clin Invest* 2015;125:386–402.
- [54] Parseus A, Sommer N, Sommer F, Caesar R, Molinaro A, Stahlman M, et al. Microbiota-induced obesity requires farnesoid X receptor. *Gut* 2017;66:429–437.
- [55] Tilg H, Moschen AR, Szabo G. Interleukin-1 and inflammasomes in alcoholic liver disease/acute alcoholic hepatitis and nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *Hepatology* 2016;64:955–965.
- [56] Bull-Otterson L, Feng W, Kirpich I, Wang Y, Qin X, Liu Y, et al. Metagenomic analyses of alcohol induced pathogenic alterations in the intestinal microbiome and the effect of *Lactobacillus rhamnosus* GG treatment. *PLoS ONE* 2013;8:e53028.
- [57] Bode JC, Bode C, Heidebach R, Durr HK, Martini GA. Jejunal microflora in patients with chronic alcohol abuse. *Hepatogastroenterology* 1984;31:30–34.
- [58] Chen Y, Yang F, Lu H, Wang B, Chen Y, Lei D, et al. Characterization of fecal microbial communities in patients with liver cirrhosis. *Hepatology* 2011;54:562–572.
- [59] Yan AW, Fouts DE, Brandl J, Starkel P, Torralba M, Schott E, et al. Enteric dysbiosis associated with a mouse model of alcoholic liver disease. *Hepatology* 2011;53:96–105.
- [60] Mutlu EA, Gillevet PM, Rangwala H, Sikaroodi M, Naqvi A, Engen PA, et al. Colonic microbiome is altered in alcoholism. *Am J Physiol Gastrointest Liver Physiol* 2012;302:G966–G978.
- [61] Llopis M, Cassard AM, Wrzosek L, Bosch L, Bruneau A, Ferrere G, et al. Intestinal microbiota contributes to individual susceptibility to alcoholic liver disease. *Gut* 2016;65:830–839.
- [62] Yang AM, Inamine T, Hochrath K, Chen P, Wang L, Llorente C, et al. Intestinal fungi contribute to development of alcoholic liver disease. *J Clin Invest* 2017;127:2829–2841.
- [63] Rao RK. Acetaldehyde-induced barrier disruption and paracellular permeability in Caco-2 cell monolayer. *Methods Mol Biol* 2008;447:171–183.
- [64] Wood S, Pithadia R, Rehman T, Zhang L, Plichta J, Radek KA, et al. Chronic alcohol exposure renders epithelial cells vulnerable to bacterial infection. *PLoS ONE* 2013;8:e54646.
- [65] Chen P, Starkel P, Turner JR, Ho SB, Schnabl B. Dysbiosis-induced intestinal inflammation activates tumor necrosis factor receptor 1 and mediates alcoholic liver disease in mice. *Hepatology* 2015;61:883–894.
- [66] Bode C, Kugler V, Bode JC. Endotoxemia in patients with alcoholic and non-alcoholic cirrhosis and in subjects with no evidence of chronic liver disease following acute alcohol excess. *J Hepatol* 1987;4:8–14.
- [67] Bala S, Marcos M, Gattu A, Catalano D, Szabo G. Acute binge drinking increases serum endotoxin and bacterial DNA levels in healthy individuals. *PLoS ONE* 2014;9:e96864.
- [68] Hartmann P, Chen P, Wang HJ, Wang L, McCole DF, Brandl K, et al. Deficiency of intestinal mucin-2 ameliorates experimental alcoholic liver disease in mice. *Hepatology* 2013;58:108–119.
- [69] Wang L, Fouts DE, Starkel P, Hartmann P, Chen P, Llorente C, et al. Intestinal REG3 lectins protect against alcoholic steatohepatitis by reducing mucosa-associated microbiota and preventing bacterial translocation. *Cell Host Microbe* 2016;19:227–239.
- [70] 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;13:1324–1332.
- [71] Hartmann P, Hochrath K, Horvath A, Chen P, Seebauer CT, Llorente C, et al. Modulation of the intestinal bile acid/farnesoid X receptor/fibroblast growth factor 15 axis improves alcoholic liver disease in mice. *Hepatology* 2018;67:2150–2166.
- [72] 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;49:1877–1887.
- [73] Kapil S, Duseja A, Sharma BK, Singla B, Chakraborti A, Das A, et al. Small intestinal bacterial overgrowth and toll-like receptor signaling in patients with non-alcoholic fatty liver disease. *J Gastroenterol Hepatol* 2016;31:213–221.
- [74] Boursier J, Mueller O, Barret M, Machado M, Fizzanne L, Araujo-Perez F, et al. The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. *Hepatology* 2016;63:764–775.
- [75] Loomba R, Seguritan V, Li W, Long T, Klitgord N, Bhatt A, et al. Gut microbiome-based metagenomic signature for non-invasive detection of advanced fibrosis in human nonalcoholic fatty liver disease. *Cell Metab* 2017;25:1054–1062.
- [76] Zhu L, Baker SS, Gill C, Liu W, Alkhouri R, Baker RD, et al. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. *Hepatology* 2013;57:601–609.
- [77] Verdam FJ, Rensen SS, Driessen A, Greve JW, Buurman WA. Novel evidence for chronic exposure to endotoxin in human nonalcoholic steatohepatitis. *J Clin Gastroenterol* 2011;45:149–152.
- [78] Luck H, Tsai S, Chung J, Clemente-Casares X, Ghazarian M, Revelo XS, et al. Regulation of obesity-related insulin resistance with gut anti-inflammatory agents. *Cell Metab* 2015;21:527–542.
- [79] Schroeder BO, Birchenough GMH, Stahlman M, Arike L, Johansson MEV, Hansson GC, et al. Bifidobacteria or fiber protects against diet-induced microbiota-mediated colonic mucus deterioration. *Cell Host Microbe* 2018;23:27–40.
- [80] Rahman K, Desai C, Iyer SS, Thorn NE, Kumar P, Liu Y, et al. Loss of junctional adhesion molecule a promotes severe steatohepatitis in mice on a diet high in saturated fat, fructose, and cholesterol. *Gastroenterology* 2016;151:733–746.
- [81] Bluemel S, Wang L, Martino C, Lee S, Wang Y, Williams B, et al. The role of intestinal C-type regenerating islet derived-3 lectins for nonalcoholic steatohepatitis. *Hepatology Commun* 2018;2:393–406.
- [82] Rivera CA, Adegboyega P, van Rooijen N, Tagalicud A, Allman M, Wallace M. Toll-like receptor-4 signaling and Kupffer cells play pivotal roles in the pathogenesis of non-alcoholic steatohepatitis. *J Hepatol* 2007;47:571–579.
- [83] Saberi M, Woods NB, de Luca C, Schenk S, Lu JC, Bandyopadhyay G, et al. Hematopoietic cell-specific deletion of toll-like receptor 4 ameliorates hepatic and adipose tissue insulin resistance in high-fat-fed mice. *Cell Metab* 2009;10:419–429.
- [84] Miura K, Kodama Y, Inokuchi S, Schnabl B, Aoyama T, Ohnishi H, et al. Toll-like receptor 9 promotes steatohepatitis by induction of interleukin-1beta in mice. *Gastroenterology* 2010;139:323–334.
- [85] Henaoui-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;482:179–185.
- [86] Dumas ME, Barton RH, Toye A, Cloarec O, Blancher C, Rothwell A, et al. Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice. *Proc Natl Acad Sci U S A* 2006;103:12511–12516.
- [87] Chen YM, Liu Y, Zhou RF, Chen XL, Wang C, Tan XY, et al. Associations of gut-flora-dependent metabolite trimethylamine-N-oxide, betaine and

- choline with non-alcoholic fatty liver disease in adults. *Sci Rep* 2016;6:19076.
- [88] Engstler AJ, Aumiller T, Degen C, Durr M, Weiss E, Maier IB, et al. Insulin resistance alters hepatic ethanol metabolism: studies in mice and children with non-alcoholic fatty liver disease. *Gut* 2016;65:1564–1571.
 - [89] Hoyle L, Fernandez-Real JM, Federici M, Serino M, Abbott J, Charpentier J, et al. Molecular phenomics and metagenomics of hepatic steatosis in non-diabetic obese women. *Nat Med* 2018;24:1070–1080.
 - [90] Koh A, De Vadder F, Kovatcheva-Datchary P, Backhed F. From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. *Cell* 2016;165:1332–1345.
 - [91] Sawicki CM, Livingston KA, Obin M, Roberts SB, Chung M, McKeown NM. Dietary fiber and the human gut microbiota: application of evidence mapping methodology. *Nutrients* 2017;9(2), pii: E125.
 - [92] 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.
 - [93] Zhao L, Zhang F, Ding X, Wu G, Lam YY, Wang X, et al. Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes. *Science* 2018;359:1151–1156.
 - [94] Jiao N, Baker SS, Chapa-Rodriguez A, Liu W, Nugent CA, Tsompana M, et al. Suppressed hepatic bile acid signalling despite elevated production of primary and secondary bile acids in NAFLD. *Gut* 2018;67:1881–1891.
 - [95] Ferslew BC, Xie G, Johnston CK, Su M, Stewart PW, Jia W, et al. Altered bile acid metabolome in patients with nonalcoholic steatohepatitis. *Dig Dis Sci* 2015;60:3318–3328.
 - [96] Shah A, Shanahan E, Macdonald GA, Fletcher L, Ghasemi P, Morrison M, et al. Systematic review and meta-analysis: prevalence of small intestinal bacterial overgrowth in chronic liver disease. *Semin Liver Dis* 2017;37:388–400.
 - [97] Qin N, Yang F, Li A, Prifti E, Chen Y, Shao L, et al. Alterations of the human gut microbiome in liver cirrhosis. *Nature* 2014;513:59–64.
 - [98] Bajaj JS, Heuman DM, Hylemon PB, Sanyal AJ, White MB, Monteith P, et al. Altered profile of human gut microbiome is associated with cirrhosis and its complications. *J Hepatol* 2014;60:940–947.
 - [99] Bajaj JS, Idilman R, Mabudian L, Hood M, Fagan A, Turan D, et al. Diet affects gut microbiota and modulates hospitalization risk differentially in an international cirrhosis cohort. *Hepatology* 2018;68:234–247.
 - [100] Chesta J, Defilippi C, Defilippi C. Abnormalities in proximal small bowel motility in patients with cirrhosis. *Hepatology* 1993;17:828–832.
 - [101] Sadik R, Abrahamsson H, Björnsson E, Gunnarsdóttir A, Stotzer PO. Etiology of portal hypertension may influence gastrointestinal transit. *Scand J Gastroenterol* 2003;38:1039–1044.
 - [102] Gunnarsdóttir SA, Sadik R, Shev S, Simren M, Sjövall H, Stotzer PO, et al. Small intestinal motility disturbances and bacterial overgrowth in patients with liver cirrhosis and portal hypertension. *Am J Gastroenterol* 2003;98:1362–1370.
 - [103] Perez-Paramo M, Munoz J, Albillos A, Freile I, Portero F, Santos M, et al. Effect of propranolol on the factors promoting bacterial translocation in cirrhotic rats with ascites. *Hepatology* 2000;31:43–48.
 - [104] Lorenzo-Zuniga V, Bartoli R, Planas R, Hofmann AF, Vinado B, Hagey LR, et al. Oral bile acids reduce bacterial overgrowth, bacterial translocation, and endotoxemia in cirrhotic rats. *Hepatology* 2003;37:551–557.
 - [105] Kakiyama G, Hylemon PB, Zhou H, Pandak WM, Heuman DM, Kang DJ, et al. Colonic inflammation and secondary bile acids in alcoholic cirrhosis. *Am J Physiol Gastrointest Liver Physiol* 2014;306:G929–G937.
 - [106] Kakiyama G, Pandak WM, Gillevet PM, Hylemon PB, Heuman DM, Daita K, et al. Modulation of the fecal bile acid profile by gut microbiota in cirrhosis. *J Hepatol* 2013;58:949–955.
 - [107] Teltschik Z, Wiest R, Beisner J, Nuding S, Hofmann C, Schoelmerich J, et al. Intestinal bacterial translocation in rats with cirrhosis is related to compromised Paneth cell antimicrobial host defense. *Hepatology* 2012;55:1154–1163.
 - [108] Ubeda M, Lario M, Munoz L, Borrero MJ, Rodriguez-Serrano M, Sanchez-Diaz AM, et al. Obeticholic acid reduces bacterial translocation and inhibits intestinal inflammation in cirrhotic rats. *J Hepatol* 2016;64:1049–1057.
 - [109] Albillos A, Abreu L, Alvarez-Mon M, Gea F, Gonzalo MA, Rossi I, et al. Study of the secretion of pepsinogen I in cirrhotic humans with and without portacaval shunt. *Am J Gastroenterol* 1988;83:37–41.
 - [110] Shindo K, Machida M, Miyakawa K, Fukumura M. A syndrome of cirrhosis, achlorhydria, small intestinal bacterial overgrowth, and fat malabsorption. *Am J Gastroenterol* 1993;88:2084–2091.
 - [111] Llorente C, Jepsen P, Inamine T, Wang L, Bluemel S, Wang HJ, et al. Gastric acid suppression promotes alcoholic liver disease by inducing overgrowth of intestinal *Enterococcus*. *Nat Commun* 2017;8:837.
 - [112] Bajaj JS, Betrapally NS, Hylemon PB, Heuman DM, Daita K, White MB, et al. Salivary microbiota reflects changes in gut microbiota in cirrhosis with hepatic encephalopathy. *Hepatology* 2015;62:1260–1271.
 - [113] Bajaj JS, Hylemon PB, Ridlon JM, Heuman DM, Daita K, White MB, et al. Colonic mucosal microbiome differs from stool microbiome in cirrhosis and hepatic encephalopathy and is linked to cognition and inflammation. *Am J Physiol Gastrointest Liver Physiol* 2012;303:G675–G685.
 - [114] Pijls KE, Jonkers DM, Elamin EE, Masclee AA, Koek GH. Intestinal epithelial barrier function in liver cirrhosis: an extensive review of the literature. *Liver Int* 2013;33:1457–1469.
 - [115] Albillos A, Lario M, Alvarez-Mon M. Cirrhosis-associated immune dysfunction: distinctive features and clinical relevance. *J Hepatol* 2014;61:1385–1396.
 - [116] Muñoz L, Borrero MJ, Ubeda M, Conde E, Del Campo R, Rodriguez-Serrano M, et al. Intestinal immune dysregulation driven by dysbiosis promotes barrier disruption and bacterial translocation in rats with cirrhosis. *Hepatology* 2019;70:925–938.
 - [117] Muñoz L, Jose Borrero M, Ubeda M, Lario M, Diaz D, Frances R, et al. Interaction between intestinal dendritic cells and bacteria translocated from the gut in rats with cirrhosis. *Hepatology* 2012;56:1861–1869.
 - [118] Garcia-Tsao G, Lee FY, Barden GE, Cartun R, West AB. Bacterial translocation to mesenteric lymph nodes is increased in cirrhotic rats with ascites. *Gastroenterology* 1995;108:1835–1841.
 - [119] Wiest R, Lawson M, Geuking M. Pathological bacterial translocation in liver cirrhosis. *J Hepatol* 2014;60:197–209.
 - [120] Sorribas M, Jakob MO, Yilmaz B, Li H, Stutz D, Noser Y, et al. FxR-modulates the gut-vascular barrier by regulating the entry sites for bacterial translocation in experimental cirrhosis. *J Hepatol* 2019;71:1126–1140.
 - [121] Schwabl P, Hambruch E, Seeland BA, Hayden H, Wagner M, Garnys L, et al. The FXR agonist PX20606 ameliorates portal hypertension by targeting vascular remodelling and sinusoidal dysfunction. *J Hepatol* 2017;66:724–733.
 - [122] Verbeke L, Farre R, Verbinen B, Covens K, Vanuytsel T, Verhaegen J, et al. The FXR agonist obeticholic acid prevents gut barrier dysfunction and bacterial translocation in cholestatic rats. *Am J Pathol* 2015;185:409–419.
 - [123] Inagaki T, Moschetta A, Lee YK, Peng L, Zhao G, Downes M, et al. Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. *Proc Natl Acad Sci U S A* 2006;103:3920–3925.
 - [124] Llovet JM, Bartoli R, Planas R, Cabre E, Jimenez M, Urban A, et al. Bacterial translocation in cirrhotic rats. Its role in the development of spontaneous bacterial peritonitis. *Gut* 1994;35:1648–1652.
 - [125] Du Plessis J, Vanheel H, Janssen CE, Roos L, Slavik T, Stivaktas PI, et al. Activated intestinal macrophages in patients with cirrhosis release NO and IL-6 that may disrupt intestinal barrier function. *J Hepatol* 2013;58:1125–1132.
 - [126] Shi H, Lv L, Cao H, Lu H, Zhou N, Yang J, et al. Bacterial translocation aggravates CCl₄-induced liver cirrhosis by regulating CD4(+) T cells in rats. *Sci Rep* 2017;7:40516.
 - [127] Stiehl A, Raedtsch R, Rudolph G, Gundert-Remy U, Senn M. Biliary and urinary excretion of sulfated, glucuronidated and tetrahydroxylated bile acids in cirrhotic patients. *Hepatology* 1985;5:492–495.
 - [128] Santiago A, Pozuelo M, Poca M, Gely C, Nieto JC, Torras X, et al. Alteration of the serum microbiome composition in cirrhotic patients with ascites. *Sci Rep* 2016;6:25001.
 - [129] Schierwagen R, Alvarez-Silva C, Madsen MSA, Kolbe CC, Meyer C, Thomas D, et al. Circulating microbiome in blood of different circulatory compartments. *Gut* 2018, Mar 26. pii: gutjnl-2018-316227.
 - [130] Alvarez-Silva C, Schierwagen R, Pohlmann A, Magdaleno F, Uschner FE, Ryan P, et al. Compartmentalization of immune response and microbial translocation in decompensated cirrhosis. *Front Immunol* 2019;10:69.
 - [131] Wiest R, Albillos A, Trauner M, Bajaj JS, Jalan R. Targeting the gut-liver axis in liver disease. *J Hepatol* 2017;67:1084–1103.
 - [132] Greenway FL, Aronne LJ, Raben A, Astrup A, Apovian CM, Hill JO, et al. A randomized, double-blind, placebo-controlled study of gelesis100: a novel nonsystemic oral hydrogel for weight loss. *Obesity (Silver Spring)* 2019;27:205–216.
 - [133] Silvestri ASA, Vitale M, Mouriès J, Spadoni I, Demitri C, Chiquette E, et al. LBP-33-Gelesis superabsorbent hydrogel prevents hepatic steatosis in a high fat diet-induced NAFLD pre-clinical model. *J Hepatol* 2019;70:e157–e158.

- [134] Connor EFLI, Maclean D. Polymers as drugs—Advances in therapeutic applications of polymer binding agents. *J Polym Sci Part Polym Chem* 2017;55:3146–3157.
- [135] Nerild HH, Christensen MB, Knop FK, Bronden A. Preclinical discovery and development of colesevelam for the treatment of type 2 diabetes. *Expert Opin Drug Discov* 2018;13:1161–1167.
- [136] Johnson S, Gerding DN, Louie TJ, Ruiz NM, Gorbach SL. Sustained clinical response as an endpoint in treatment trials of *Clostridium difficile*-associated diarrhea. *Antimicrob Agents Chemother* 2012;56:4043–4045.
- [137] McCarville JL, Nisemblat Y, Galipeau HJ, Jury J, Tabakman R, Cohen A, et al. BL-7010 demonstrates specific binding to gliadin and reduces gluten-associated pathology in a chronic mouse model of gliadin sensitivity. *PLoS ONE* 2014;9:e109972.
- [138] Macnaughtan JRI, Soeda J, Sawhney R, Oben J, Davies N, et al. Oral therapy with non-absorbable carbons of controlled porosity (YAQ-001) selectively modulates stool microbiome and its function and this is associated with restoration of immune function and inflammasome activation. *J Hepatol* 2015;62:S240.
- [139] Chan BK, Turner PE, Kim S, Mojibian HR, Eleftheriades JA, Narayan D. Phage treatment of an aortic graft infected with *Pseudomonas aeruginosa*. *Evol Med Public Health* 2018;2018:60–66.
- [140] Schooley RT, Biswas B, Gill JJ, Hernandez-Morales A, Lancaster J, Lessor L, et al. Development and use of personalized bacteriophage-based therapeutic cocktails to treat a patient with a disseminated resistant *Acinetobacter baumannii* infection. *Antimicrob Agents Chemother* 2017;61(10).
- [141] Kortright KE, Chan BK, Koff JL, Turner PE. Phage therapy: a renewed approach to combat antibiotic-resistant bacteria. *Cell Host Microbe* 2019;25:219–232.
- [142] Nakamoto N, Sasaki N, Aoki R, Miyamoto K, Suda W, Teratani T, et al. Gut pathobionts underlie intestinal barrier dysfunction and liver T helper 17 cell immune response in primary sclerosing cholangitis. *Nat Microbiol* 2019;4:492–503.
- [143] Kurtz CB, Millet YA, Puurunen MK, Perreault M, Charbonneau MR, Isabella VM, et al. An engineered *E. coli* Nissle improves hyperammonemia and survival in mice and shows dose-dependent exposure in healthy humans. *Sci Transl Med* 2019;11(475).
- [144] Isabella VM, Ha BN, Castillo MJ, Lubkowitz DJ, Rowe SE, Millet YA, et al. Development of a synthetic live bacterial therapeutic for the human metabolic disease phenylketonuria. *Nat Biotechnol* 2018;36:857–864.
- [145] Braat H, Rottiers P, Hommes DW, Huyghebaert N, Remaut E, Remon JP, et al. A phase I trial with transgenic bacteria expressing interleukin-10 in Crohn's disease. *Clin Gastroenterol Hepatol* 2006;4:754–759.
- [146] Bajaj JS, Salzman NH, Acharya C, Sterling RK, White MB, Gavis EA, et al. Fecal microbial transplant capsules are safe in hepatic encephalopathy: a phase 1, randomized, placebo-controlled trial. *Hepatology* 2019;70:1690–1703.
- [147] Bajaj JS, Kakiyama G, Savidge T, Takei H, Kassam ZA, Fagan A, et al. Antibiotic-associated disruption of microbiota composition and function in cirrhosis is restored by fecal transplant. *Hepatology* 2018;68:1549–1558.
- [148] Bajaj JS, Fagan A, Gavis EA, Kassam Z, Sikaroodi M, Gillevet PM. Long-term outcomes of fecal microbiota transplantation in patients with cirrhosis. *Gastroenterology* 2019;156:1921–1923.
- [149] Liu R, Kang JD, Sartor RB, Sikaroodi M, Fagan A, Gavis EA, et al. Neuroinflammation in murine cirrhosis is dependent on the gut microbiome and is attenuated by fecal transplant. *Hepatology* 2019. <https://doi.org/10.1002/hep.30827>. [Epub ahead of print].
- [150] Garcia-Lezana T, Raurell I, Bravo M, Torres-Arauz M, Salcedo MT, Santiago A, et al. Restoration of a healthy intestinal microbiota normalizes portal hypertension in a rat model of nonalcoholic steatohepatitis. *Hepatology* 2018;67:1485–1498.
- [151] Neuschwander-Tetri BA, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF, et al. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. *Lancet* 2015;385:956–965.
- [152] Hirschfield GM, Chazouilleres O, Drenth JP, Thorburn D, Harrison SA, Landis CS, et al. Effect of NGM282, an FGF19 analogue, in primary sclerosing cholangitis: a multicenter, randomized, double-blind, placebo-controlled phase II trial. *J Hepatol* 2019;70:483–493.
- [153] Mayo MJ, Wigg AJ, Leggett BA, Arnold H, Thompson AJ, Weltman M, et al. NGM282 for treatment of patients with primary biliary cholangitis: a multicenter, randomized, double-blind, placebo-controlled trial. *Hepatol Commun* 2018;2:1037–1050.
- [154] Joshi JJ, Coffey H, Corcoran E, Tsai J, Huang CL, Ichikawa K, et al. H3B-6527 is a potent and selective inhibitor of FGFR4 in FGF19-driven hepatocellular carcinoma. *Cancer Res* 2017;77:6999–7013.
- [155] Lin ZZ, Hsu C, Jeng YM, Hu FC, Pan HW, Wu YM, et al. Klotho-beta and fibroblast growth factor 19 expression correlates with early recurrence of resectable hepatocellular carcinoma. *Liver Int* 2019;39:1682–1691.
- [156] Gnuli D, Calcagno A, Dalla Libera L, Calvani R, Leccesi L, Caristo ME, et al. High-fat feeding stimulates endocrine, glucose-dependent insulinotropic polypeptide (GIP)-expressing cell hyperplasia in the duodenum of Wistar rats. *Diabetologia* 2010;53:2233–2240.
- [157] Theodorakis MJ, Carlson O, Michopoulos S, Doyle ME, Juhaszova M, Petraki K, et al. Human duodenal enteroendocrine cells: source of both incretin peptides, GLP-1 and GIP. *Am J Physiol Endocrinol Metab* 2006;290:E550–E559.
- [158] Haidry RJ, van Baar AC, Galvao Neto MP, Rajagopalan H, Caplan J, Levin PS, et al. Duodenal mucosal resurfacing: proof-of-concept, procedural development, and initial implementation in the clinical setting. *Gastrointest Endosc* 2019;90:673–681.
- [159] Gao J, Li Y, Wan Y, Hu T, Liu L, Yang S, et al. A novel postbiotic from *Lactobacillus rhamnosus* GG with a beneficial effect on intestinal barrier function. *Front Microbiol* 2019;10:477.
- [160] Compare D, Rocco A, Coccoli P, Angrisani D, Sgamato C, Iovine B, et al. *Lactobacillus casei* DG and its postbiotic reduce the inflammatory mucosal response: an ex-vivo organ culture model of post-infectious irritable bowel syndrome. *BMC Gastroenterol* 2017;17:53.
- [161] Mileti E, Matteoli G, Iliev ID, Rescigno M. Comparison of the immunomodulatory properties of three probiotic strains of *Lactobacilli* using complex culture systems: prediction for in vivo efficacy. *PLoS ONE* 2009;4:e7056.
- [162] Tsilingiri K, Barbosa T, Penna G, Caprioli F, Sonzogni A, Viale G, et al. Probiotic and postbiotic activity in health and disease: comparison on a novel polarised ex-vivo organ culture model. *Gut* 2012;61:1007–1015.
- [163] Fanning S, Hall LJ, Cronin M, Zomer A, MacSharry J, Goulding D, et al. Bifidobacterial surface-exopolysaccharide facilitates commensal-host interaction through immune modulation and pathogen protection. *Proc Natl Acad Sci U S A* 2012;109:2108–2113.
- [164] Forster V, Signorell RD, Roveri M, Leroux JC. Liposome-supported peritoneal dialysis for detoxification of drugs and endogenous metabolites. *Sci Transl Med* 2014;6:258ra141.
- [165] Giacalone G, Matoori S, Agostoni V, Forster V, Kabbaj M, Eggenschwiler S, et al. Liposome-supported peritoneal dialysis in the treatment of severe hyperammonemia: an investigation on potential interactions. *J Control Release* 2018;278:57–65.