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REVIEWS

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The gut microbial influence on cholestatic liver disease

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Abstract

Patients with cholestatic liver diseases like primary sclerosing cholangitis (PSC) and primary biliary cholangitis (PBC) have a different gut microbiome composition than healthy controls. In contrast with PBC, PSC has a strong association with inflammatory bowel disease and is the prototypical disease of the gut-liver axis. Still, there are some distinct overlapping microbial features in the microbiome of patients with PSC and PBC suggesting similarities in cholestatic diseases, although the possible pathogenetic involvement of these shared microbial changes is unknown. Herein, we present an overview of the available data and discuss the relevance for potential disease relevant host-microbiota interactions. In general, the microbiome interacts with the host via the *immunobiome* (interactions between the host immune system and the gut microbiome), the *endobiome* (where the gut microbiome contributes to host physiology by producing or metabolizing endogenous molecules) and the *xenobiome* (gut microbial transformation of exogenous compounds, including nutrients and drugs). Experimental and human observational evidence suggest that the presence and functions of gut microbes are relevant for the severity and progression of cholestatic liver disease. Interestingly, the majority of new drugs that are currently being tested in PBC and PSC in clinical trials act on bile acid homeostasis, where the *endobiome* is important. In the future, it will be paramount to perform longitudinal studies, through which we can identify new intervention targets, biomarkers or treatment-stratifiers. In this way, gut microbiome-based clinical care and therapy may become relevant in cholestatic liver disease within the foreseeable future.

KEYWORDS
cholestasis, microbiome, primary biliary cholangitis, primary sclerosing cholangitis

1 | INTRODUCTION

The gut microbes are an integrated part of human metabolism and the immune system, essential for human well-being. The gut microbial metabolism involve a wide range of molecules ranging from short-chain fatty acids and vitamins to secondary bile acids and neurotransmitters.[1,2] Blood from the gut enters the systemic circulation through the liver via the portal vein, meaning that the liver is

Abbreviations: AIH, autoimmune hepatitis; ATRA, all-trans retinoic acid; FGF19, fibroblast growth factor 19; FMT, faecal microbiota transplantation; FXR, farnesoid X-receptor; HCC, hepatocellular carcinoma; IBD, inflammatory bowel disease; LPS, lipopolysaccharide; NLRP3, nucleotide-binding domain, leucine-rich repeat,pyrin domain-containing 3; *nor*-UDCA, *nor*-ursodeoxycholic acid; OCA, obeticholic acid; p-ANCA, perinuclar anti-neutrophil cytoplasmic antibodies; PBC, primary biliary cholangitis; PPAR, peroxisome proliferator-activated receptor; PSC, primary sclerosing cholangitis; SASP, senescence-associated secretory phenotype; SCFA, short-chain fatty acids; TMA, trimethylamine; TMAO, trimethylamine-N-oxide; UDCA, ursodeoxycholic acid; VAP-1, vascular adhesion protein 1.

at the centre of gut microbiome-host interaction.[3,4] Microbiota-derived molecules have the potential to activate the immune system and trigger inflammation,[4] providing the liver with the challenging task of balancing tolerance to beneficial and unharmful molecules and metabolites with the need to act as a firewall against pathogens and harmful microbe-derived molecules.[5] Disturbances of the gut-liver axis are therefore potentially the key players in several liver diseases, and maybe biliary diseases especially as the gut microbiome is central to bile acid homeostasis.

Cholestatic disease is a diverse collection of conditions ranging from monogenic paediatric diseases and biliary atresia to adult polygenic phenotypes strongly influenced by environmental factors, e.g. primary sclerosing cholangitis (PSC) and primary biliary cholangitis (PBC). PSC and PBC are chronic, progressive and inflammatory diseases, with PSC characterized by fibro-obliterative changes in both the large and small bile ducts, while PBC only affects the small ducts. The majority of PSC patients are male, in contrast with PBC, which has an overwhelming female predominance. PSC is the prototypical disease of the gut-liver axis, often considered an extra-intestinal manifestation of inflammatory bowel disease (IBD). Up to 80% of PSC patients are concurrently affected by IBD of the colon, an association not seen in PBC.[6,7]

In this review, we will provide an overview of the gut microbial alterations reported in human cholestatic conditions, focusing on PSC and PBC. Then we will introduce the three *biomes* of microbiome-host interaction: the *immunobiome*, *endobiome and xenobiome*, and discuss the evidence for their involvement in cholestatic liver disease. Finally, we will point out clinical opportunities and future perspectives.

Key points

- Cross-sectional studies of the gut microbiome show large differences in both primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) compared with controls.
- Multiple experimental studies implicate the gut microbiome in cholestatic diseases, suggesting that the gut microbiome acts differently depending on disease mechanisms.
- The gut microbiome could affect cholestatic liver disease via endogenous molecules produced by the microbiota (*endobiome*), bacterial processing of pharmacological agents or dietary compounds (*xenobiome*) and specific bacterial molecules or metabolites driving the immune process (*immunobiome*).
- Many new drugs in clinical testing in these conditions affect bile acid homeostasis to which the gut *endobiome* is an integrated part.
- Future research should prioritize longitudinal studies of the microbiome in order to identify its role in disease progression, possibly new biomarkers, markers to help in stratifying treatment, and possibly also new targets for treatment interventions.

1.1 | The gut microbial composition in cholestatic liver diseases

Multiple cross-sectional descriptive studies of the composition of the microbiome in mucosal biopsies[8-11] and faecal samples[12-16] from patients with PSC have been published in the last few years. These studies have been reviewed extensively in recent publications.[6,17,18] Overall, the main and undisputed observation is that the microbiome is different in patients with PSC compared with patients with IBD without liver disease and healthy controls. Considering the studies of the mucosal microbiome in PSC, these may be difficult to interpret. The study-size in general is quite small (*n* min–max: 11-20) and few results overlap between studies, which could in part relate to differences in the control groups used.[18] The studies of the faecal microbiome in adult PSC patients published in full-length, peer-reviewed articles that have included healthy controls (Table 1) are larger (*n* min–max: 43-85), but there are methodological differences between the studies related to study design and inclusion and exclusion criteria, choice of 16S ribosomal RNA (rRNA) gene-region and database used for taxonomic assignment. Still, several observations are consistent across large geographical areas and age-groups, and then probably also very different dietary habits, although no dietary data is reported: (a) The overall bacterial community is different in patients with PSC compared with controls. (b) The intra-individual gut bacterial diversity (*alpha* diversity) is reduced in PSC patients, with diminished bacterial *richness* and *evenness*. (c) There is an enrichment of some bacterial genera in the gut of PSC patients, i.e. *Streptococcus* and *Veillonella*. In addition, all but one study reported an enrichment of *Enterococcus*, which also correlates with serum levels of alkaline phosphatase (ALP), a clinical marker for cholestasis.[12] Finally, (d) IBD-status has a negligible effect on the composition of the microbiome in patients with PSC, in contrast with what is seen in patients with IBD without liver disease.

More recently, the first data on the salivary microbiome in patients with PSC was reported,[19] based on a cohort of newly diagnosed, paediatric PSC patients from Japan, where faecal microbiota composition was in line with the description above.[16] The salivary microbiome was characterized by changes in bacterial taxa that mirror the findings from faecal microbiota, with *Haemophilus spp*, *Veillonella spp* and *Streptococcus spp* being different between paediatric PSC patients and age-matched controls. Another novel data type, faecal bile acid profile, was recently reported in 15 adult PSC patients with IBD, compared to 15 IBD patients without PSC from Portugal,[20] and a smaller pilot-study from the United States.[21] There was limited overlap with the conclusions listed above and also between the two studies, and it thus seems important to increase sample-size to reach firm conclusions, especially regarding correlations between microbiota and bile acids. Still, taken together, thes studies

TABLE 1 Studies of the faecal gut microbiome in primary sclerosing cholangitis (PSC) published in full-length, peer-reviewed articles

	Kummen et al[13]		Sabino et al[12]		Bajer et al[15]		Lemoinne et al[23]			
	n/median	(%/min-max)	n/median	(%/IQR)	n/median	(%/min-max)	n/median	(%/min-max)		
PSC patients	85	(100)	66	(100)	43	(100)	49	(100)		
Gender, males	53	(62)	48	(73)	34	(79)	34	(69)		
Age, years	49	(21-82)	49/43/49[a]	(15/14/17)[a]	35/45[a]	(18-60/18-69)[a]	41	(21-68)		
BMI, kg/m^2	25	(18-38)	23/24/24[a]	(8/6/5)[a]	('normal', no statistics)		22	(16-32)		
Smoking, yes	2	(2)	9	(14)	–		1	(2)		
PSC-IBD, yes	55	(65)	48	(73)	32	(74)	27	(55)[g]		
PSC-UC, yes	44	(52)	27	(41)	–		12	(25)		
PSC–CD, yes	11	(13)	21	(32)	–		11	(22.4)		
Small duct PSC, yes	3	(4)	–		–		3	(6)		
Disease duration PSC, years	9	(1-32)	–		–		6	(0-40)		
Age at diagnosis, years	–		38/32/35[a]	(15/9/21)[a]	–		–			
Cirrhosis, yes	–		13	(20)	–		6	(12)		
Liver transplanted, yes	0	(0)	15	(23)	0	(0)	0	(0)		
Antibiotics < 4 weeks, yes	0	(0)	11	(17)	0	(0)	0	(0)		
UDCA treatment, yes	25	(29)	47	(71)	43	(100)	46	(94)		
5-ASA treatment, yes	35	(41)	22	(33)	26	(60)	22	(45)		
IBD controls	36	(100)	43	(100)	32	(100)	33	(100)[g]		
Gender, males	16	(44)	19	(44)	17	(53)	17	(52)		
Age, years	40	(22-69)	50/52[b]	(28/14)[b]	40	(20-71)	36	(19-68)		
BMI, kg/m^2	24	(18-34)	26/25[b]	(5/5)[b]	('normal', no statistics)		24	(17-37)		
Smoking, yes	0	(0)	13	(30)	–		3	(9)		
Ulcerative colitis, yes	36	(100)	13	(30)	32	(100)	14	(42)		
Crohn's disease, yes	0	(0)	30	(70)	0	(0)	14	(42)		
Healthy controls (HC)	263	(100)	66	(100)	31	(100)	30	(100)		
Gender, males	108	(41)	49	(74)	13	(42)	9	(35)		
Age, years	46	(30-61)	52	(17)	44	(22-72)	31	(22-62)		
BMI, kg/m^2	26	(18-43)	24	(5)	('normal', no statistics)		22	(17-27)		
Smoking, yes	30	(11)	4	(6)	–		2	(7)		
Methods							Bacteria	Fungi		
Gene-region amplified	V3-V4 (16S rRNA)		V4 (16S rRNA)		V3-V4 (16S rRNA)		V3-V4	ITS2		
Sequencing method	Illumina MiSeq		Illumina MiSeq		Illumina MiSeq		Illumina MiSeq			
Collection	Preservative (PSP tube)		Fresh frozen		Fresh frozen		Fresh frozen			
Extraction kit	PSP spin stool DNA		MoBio		Masterpure		In house protocol			
Bead beating during extraction	yes		yes		yes		Yes			
Alpha-diversity in PSC	**vs. HC**	**vs. IBD**	**vs. HC**	**vs. IBD**	**vs. HC[f]**	**vs. IBD[f]**	**vs. HC**	**vs. IBD**	**vs. HC**	**vs. IBD**
Shannon index	↓	↔			↔	↔	↓[h]	↓[h]	↔[i]	↑
Species (OUT) richness			↓							
Chao1	↓	↔			↔	↔	↓[h]	↓[h]		
Phylogenetic diversity	↓	↔								

(Continues)

TABLE 1 (Continued)

	Kummen et al[13]	Sabino et al[12]	Bajer et al[15]	Lemoinne et al[23]	
	n/median (%/min-max)	n/median (%/IQR)	n/median (%/min-max)	n/median	(%/min-max)
Beta-diversity				Bacteria	Fungi
	Unweighted UniFrac	Bray-Curtis	Unweighted UniFrac	Bray–Curtis	
PSC vs HC	Different	Different	Different	Different (healthy vs PSC-IBD)	Different (healthy vs PSC without IBD)
PSC vs IBD	Different	Different (only PSC-UC vs UC)	Different (PSC-IBD vs IBD)	Different (PSC-IBD vs IBD)	Different (PSC-IBD vs IBD)
PSC (no IBD) vs PSC-IBD	Similar	Similar	Similar	Similar	Different
Taxa ↑ in PSC vs healthy controls (at genus level)				Bacteria	Fungi
	Veillonella	*Veillonella*[c]	*Veillonella*	*Veillonella*	*Exophiala*
		Streptococcus[d]	*Streptococcus*		
		Enterococcus	*Enterococcus*		
		Lactobacillus	*Clostridium*		
		Fusobacterium[e]	*Haemophilus*		
			Rothia		
Taxa ↓ in PSC vs healthy controls (at genus level)					
	Coprococcus[e]		*Coprococcus*	*Ruminococcus*	*Saccharomyces cerevisiae*
	Unknown genus (Lachnospiraceae family)[e]		Unknown genus (Lachnospiraceae family)	*Ruminiclostridium*	
	Phascolarctobacterium[e]			*Faecalibacterium*	
	Unknown genus (Christensenellaceae family)[e]			*Lachnoclostridium*	
	Unknown genus (S24.7 order)[e]			*Blautia*	
	Unknown genus (RF32 order)[e]				
	Unknown genus (YS2 order)[e]				
Taxa differing between PSC non-IBD and PSC-IBD	None	None	*Coprobacillus*, *Escherichia*, *Corynebacterium*, *Lactobacillus (all enriched in PSC-IBD)*	Not stated	Not stated

Letters, pilot studies, studies without healthy controls and other communications are reviewed in the text.

Abbreviations: 5-ASA, 5-Aminosalicylic acid; CD, Crohn´s disease; HC, healthy controls; IBD, inflammatory bowel disease; PSC, primary sclerosing cholangitis; PSC-IBD, primary sclerosing cholangitis with concomitant inflammatory bowel disease; UC, ulcerative colitis; UDCA, ursodeoxycholic acid; vs, versus; –, not stated.

[a]PSC only/PSC-UC/PSC-CD in Sabino et al, PSC only/PSC-IBD in Bajer et al.

[b]UC/CD.

[c]Not significant when excluding patients with cirrhosis.

[d]Not significant after excluding patients on antibiotics < 4 weeks.

[e]Similar in PSC vs IBD.

[f]PSC without IBD and PSC-IBD were analysed separately: IBD significantly reduced vs HC, PSC/PSC-IBD in between.

[g]In PSC and IBD: n = 4 and n = 5 with unclassified colitis respectively.

[h]Only significant for PSC-IBD.

[i]Trend towards increased Shannon diversity in PSC vs healthy controls, $P = 0.08$.

point to the potential in further studies of the intestinal microbiome from the upper gastro-intestinal tract in both paediatric and adult cholestatic patients, in addition to measuring a functional read-out (e.g. bile acid profiles).[2]

Importantly, bacteria are not the only microbes in the gut. Could fungi and bacterial-fungal interactions be relevant, as observed in IBD?[22] A very recent publication by Lemoinne et al is the first to explores this topic in PSC, and describe both bacterial and fungal changes in PSC patients compared to healthy controls.[23] The study does not include a validation cohort similar to most of the other large microbiome studies in PSC, but still replicate several of the bacterial changes described above, and report an increase of the fungal Exophiala genus and Sordariomycetes class, and a depletion of the Saccharomycetaceae family, in addition to signs of disruptions in fungal-bacterial networks in the gut microbiome of patients with PSC. In addition, IBD status in PSC does not seem to have an impact on the fungal composition.[23]

Less is known about the gut microbiome in patients with PBC than in PSC, but a few studies are worth reviewing. Similar to the studies of PSC patients, there are methodological differences, but some overlapping results (Table 2). In an excellent study by Tang et al, data from two cross-sectional cohorts of 60 and 19 PBC patients without previous ursodeoxycholic acid (UDCA) treatment were compared with 80 and 34 healthy controls, respectively, using an exploration-/validation-panel design.[24] Subsequently, 37 patients were followed over time to investigate the effect of UDCA-treatment on the microbiome. Similar to PSC, the gut microbiome in PBC patients is characterized by reduced bacterial alpha diversity, and large differences in beta diversity and several genera, including *Haemophilus*, *Veillonella* and *Streptococcus*. Interestingly, several genera, including *Haemophilus* and *Streptococcus*, were affected by UDCA treatment in the follow-up cohort. In addition, *Veillonella* abundance was reduced after treatment in patients with an adequate UDCA-response, while *Veillonella* increased during UDCA treatment in patients with an inadequate response. This could suggest that microbial markers (like e.g., *Veillonella* abundance) could be used as potential therapeutic or prognostic biomarkers, but this would have to be investigated in dedicated trials and validated in the future.

Several of the findings by Tang et al replicate results from an earlier study of the microbiome in 42 early-stage PBC patients, which also showed that several genera depleted in the gut of PBC patients were negatively associated with markers of liver injury and inflammation.[25] In addition, *Veillonella* has also been reported to be increased in the salivary microbiome of both PBC (n = 39) and autoimmune hepatitis (AIH, n = 17) patients, and correlated positively with IL-1β, IL-8 and immunoglobulin A.[26]

Overall, it is probably reasonable to conclude that there are major alterations of the gut microbiome of patients with cholestatic liver diseases. Patients with PSC show a broad depletion of bacterial diversity, which is also evident but perhaps less pronounced in PBC, but both conditions show enrichment of specific taxa, e.g. *Streptococcus*, *Haemophilus* and *Veillonella*. The latter, and other described changes, could be related to development of cirrhosis, but the increased abundance of *Veillonella* in e.g. paediatric PSC patients and non-cirrhotic PBC patients suggests otherwise. Also, *Veillonella* has been associated with other non-cirrhotic inflammatory and fibrotic diseases e.g., idiopathic pulmonary fibrosis, systemic sclerosis, rheumatoid arthritis, pulmonary cystic fibrosis and Crohn's disease with ileal involvement, which is associated with fibrotic stenosis in these patients.[13] How the described alterations relate to disease progression, including progression of liver fibrosis and cirrhosis, and disease prognosis, is so far not known, but the observation that disease-associated microbes change during treatment in PBC is of great interest. Given these broad range of microbiome alterations, the critical follow-up questions are then (a) Are the changes driven by the gut or liver or both? (b) Are the changes relevant for disease activity and severity? and c) what could the mechanisms be?

1.2 | The three *biomes* of microbiome-host interaction: The *immunobiome*, *endobiome and xenobiome*

In what ways could the gut microbes affect cholestatic liver disease? Didactically, we have recently presented a conceptual framework, suggesting to categorize the disease-related host-microbiome interactions into three main "*biomes*",[17] as illustrated in Figure 1: (a) The *immunobiome*, comprising the complex interactions between the host immune system and the gut microbiome, balancing immune tolerance to commensal bacteria on one hand and protection against pathogens and dysregulation of immune-responses on the other. (b) The *endobiome*, with all the biochemical pathways in the gut microbiome contributing to host physiology either by synthesis of crucial compounds or by co-metabolizing molecules produced by the host (e.g. short-chain fatty acids [SCFA] or secondary bile acids).[2,27] (c) The *xenobiome*: where the gut microbiome are involved in the transformation of exogenous compounds, including nutrients, drugs and a broad array of poorly defined environmental exposures resulting in the production of a broad range of metabolites detectable in the blood of the host (e.g. trimethylamine, digoxin and 5-Aminosalicylic acid).[2,27] In the following, we apply these categories on the available data.

1.3 | The three *biomes* in cholestatic liver disease

1.3.1 | The endobiome

One important question is whether changes in the gut microbiome influence models of cholestatic liver disease. Mice with a defect in the multidrug resistance 2 gene (*Mdr2*$^{-/-}$) develop sclerosing cholangitis resembling human PSC due to a lack of phospholipids in bile.[17] In a study by Tabibian et al, *Mdr2*$^{-/-}$ mice raised in a germ-free environment showed an exacerbated fibrotic biliary disease and increased cholangiocyte senescence.[28] The potential transition of senescent cells into a senescence-associated secretory phenotype (SASP) where they can induce senescence in neighboring cells, stimulate fibrogenesis and initiate proinflammatory responses is thought

TABLE 2 Studies of the gut microbiome in primary biliary cholangitis (PBC)

	Tang et al (feces)[24]		Lv et al (feces)[25]		Abe et al (feces & saliva)[26]	
	n/median	(% or min-max)	n/mean	(%/±sd.)	n/mean	(% or ± sd.)
PBC patients	79	(100)	42	(100)	39	(100)
Gender, female	67	(85)	41	(98)	34	(87)
Age, years	52	(22-78)	50	(±1.3)	63	(±12)
BMI, kg/m^2	22.6	(17.6-29.0)	–		23.1	(±2.8)
Duration of disease, years	–		1.1 (median)		7.5	(±5)
AMA, positive	77	(97)	–		36	(92)
ALT, U/L	66	(8-761)	36	(±4)	27	(±17)
AST, U/L	58	(9-510)	47	(±5)	32	(±14)
ALP, U/L	197	(47-1416)	152	(±17)	321	(±112)
GGT, U/L	189	(13-1560)	143	(±30)	59	(±44)
Total bilirubin, μmol/L	14	(5-250)	–		14	(±5)
UDCA treatment, yes	0	(0)	–[a]		37	(95%)
Scheuer 1/2/3/4	–		–		22/3/2/2	
Disease controls (AIH)	Not recruited		Not recruited		17	(100)
Gender, female					15	(88)
Age, years					60	(±11)
BMI, kg/m^2					22.7	(±3.5)
UDCA treatment, yes					14	(82)
AMA, positive					3	(18)
ANA, positive					16	(94)
Healthy controls (HC)	114	(100)	30	(100)	15	(100)
Gender, female	91	(80)	–		13	(87)
Age, years	47.5	(25-65)	51.5	(±1.3)	58	(±10)
BMI, kg/m^2	22.4	(16.2-29.4)	–		23.2	(±1.6)
Methods	***Feces***		***Feces***		***Feces***	***Saliva***
Technique	16s rRNA sequencing		16s rRNA sequencing		Terminal restriction fragment length polymorphism	
16S rRNA region amplified	V3-V4		V3-V4			
Sequencing method	Illumina MiSeq		Illumina MiSeq			
Collection	Fresh frozen		Fresh frozen		Fresh frozen	Fresh frozen
Extraction kit	QIAamp Fast DNA Stool		QIAamp DNA Stool		MagDEA DNA 200 (automatic)	MORA-EXTRACT DNA
Bead beating during extraction	No		Not specified		Yes	Yes
Alpha-diversity						
Shannon index	↔ in PBC vs HC		↔ in PBC vs HC		↔ in PBC vs HC	↔ in PBC vs HC
Species (OTU) richness	↓ in PBC vs HC					
Chao1			↔ in PBC vs HC			
Phylogenetic diversity						
Beta-diversity	*Unweighted UniFrac*		*Unweighted UniFrac*		*Hierarchical cluster analysis*	
PBC vs HC	Different		No difference		No difference	No difference

(Continues)

TABLE 2 (Continued)

	Tang et al (feces)[24]		Lv et al (feces)[25]		Abe et al (feces & saliva)[26]	
	n/median	(% or min-max)	n/mean	(%/±sd.)	n/mean	(% or ± sd.)
Taxa ↑ in PBC vs healthy controls (at genus level)						
	Haemophilus		*Bifidobacterium*		*Lactobacillales*	*Eubacterium*
	Veillonella		*Veillonella*			*Veillonella*
	Clostridium		*Neisseria*			
	Lactobacillus		*Klebsiella*			
	Streptococcus					
	Pseudomonas					
	Klebsiella					
	Unknown genus (Enterobacteriaceae)					
Taxa ↓ in PBC vs healthy controls (at genus level)						
	Bacteroidetes spp		*Desulfovibrio*		*Clostridium sub-cluster XIVa*	*Fusobacterium*
	Sutterella		*Megamonas*			
	Oscillospira					
	Faecalibacterium					

Abbreviations: AIH, autoimmune hepatitis; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMA, anti-mitochondrial antibody; ANA, anti-nuclear antibody; AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma-glutaryltransferase; HC, healthy controls; OTU, operational taxonomic unit; PBC, primary biliary cholangitis; sd., standard deviation; UC, ulcerative colitis; UDCA, ursodeoxycholic acid; vs, versus; –, not stated.
[a]The frequency of UDCA-usage is not stated, but mean/sd. of "grams UDCA/year" is given.

to be important.[28]Alterations in bile acid homeostasis due to lack of microbes and the *endobiome* production of secondary bile acids was suggested to be driving the disease in this study. Interestingly, UDCA (a secondary bile acid) abrogated senescence in vitro in this study.[28] However, the benefit from UDCA in PSC is still questionable, mainly due to the lack of adequately powered clinical trials, and so far, few studies have been unable to identify differences in the microbiome between PSC patients that use UDCA and those who do not.[17]

The NOD.c3c4 mice develop immune-driven cholangitis affecting both the intra- and extrahepatic biliary tree.[29] The condition has similarities to both PBC (develops antimitochondrial antibodies) and human PSC (distribution of inflammation and presence of biliary dilatation).[29] Interestingly, when NOD.c3c4 mice are raised in a germ-free environment, they show an ameliorated liver phenotype, with less extra-hepatic bile duct dilatation, less biliary inflammation and less CD3-positive cell infiltrates around the intra-hepatic bile ducts, with a similar trend seen in conventionally raised mice treated with antibiotics.[29] This indicates that the gut microbiome in the normal setting contributes to a worsening of disease, in contrast with the observation in *Mdr2*$^{-/-}$ mice,[28,29] suggesting that the gut microbiome acts differently depending on disease mechanisms. Whether the attenuation of disease in germ-free NOD.c3c4 mice is caused by a general hypomorphic immune system or more specific effects of the *immunobiome* is therefore not known.

1.3.2 | The immunobiome

It is reasonable to suggest that multiple disease mechanisms likely operate at the same time. In a recent study by Tedesco et al, conventionally raised *Mdr2*$^{-/-}$ mice showed enrichment of *Lactobacillus*, which is also enriched in PSC patients, compared to wild-type mice.[7] The *Mdr2*$^{-/-}$ mice also showed increased gut leakage, and translocation of *Lactobacillus* to the liver, where it activated and induced the expansion of unconventional T cells (γδ T cells) that are highly prevalent in the liver. These microbiome-induced γδ T cells produced IL-17, which could be a driver of the chronic inflammation in liver diseases like PSC, suggesting that the gut *immunobiome* in the *Mdr*$^{-/-}$-model modifies the disease phenotype. Importantly, the study also found that γδ T cells from the livers of PSC patients produced IL-17, in contrast with γδ T cells from patients with other liver diseases, and treatment with γδ T-cell receptor blockers attenuated liver fibrosis and inflammation.[7] Furthermore, a recent publication by Nakamoto et al showed how "humanizing" a mouse microbiota by inoculating germ-free mice with faeces from PSC patients induced T helper 17 (Th17) cell responses in the liver and increased susceptibility to hepatobiliary injury, which could be ameliorated with antibiotics.[30] They also isolated *Klebsiella pneumoniae*, *Proteus mirabilis* and *Enterococcus gallinarum* from mesenteric lymph nodes in these mice using bacterial culture, and showed that these were associated with bacterial translocation and epithelial damage.[30] If reproduced, this is a

promising avenue of research. The bacterial translocation and epithelial damage were attributed only to certain strains of *Klebsiella*, reminding us about the need to increase resolution in microbiome studies. So far data are only reported at the genus level in the human studies, where e.g. *Enterococcus* has been frequently reported as enriched in PSC patients.[30]

The concept of intestinal translocation of bacterial products as a driver of chronic inflammation is of great interest in many systemic and organ specific conditions. Could changes in the gut microbiome or bacterial translocation lead to bile duct disease in vulnerable hosts without established liver disease? Liao et al investigated this in a very recent publication, which also highlights the importance of the innate immune system in the *Mdr2*$^{-/-}$ model.[31] They replicate results from the study by Tedesco et al described above with increased gut leakage in *Mdr2*$^{-/-}$ mice compared to wild-type mice. They further reported increased levels of *Enterococcus* in the liver of *Mdr2*$^{-/-}$ mice, and showed that the nucleotide-binding domain, leucine-rich repeat, pyrin domain-containing 3 (NLRP3) inflammasome activation of caspase-1 in the gut and in the liver plays an essential role in the progression of liver disease in this model, and were also able to ameliorate some of these changes with pharmacological inhibition of the caspase system, resulting in less periportal inflammation, less bile duct proliferation, alterations in the gut microbiome and a significant decrease in serum bile acid concentration (indication potential *endobiome* involvement) in treated *Mdr2*$^{-/-}$ mice. Lastly, and maybe most importantly, they show how faecal microbiota transplantation (FMT) from *Mdr2*$^{-/-}$ donor mice into wild-type mice resulted in increased gut leakage, liver injury and a pronounced NLRP3 inflammasome activation both in gut and liver of previously healthy recipient wild-type mice, demonstrating that the phenotype of *Mdr2*$^{-/-}$ is transmissible, at least in part, indicating a casual role of the gut microbiota in the pathogenies of cholestatic liver disease.[31]

In a series of classical studies from the early 1990s, Lichtman et al showed how disturbing the normal intestinal microbiome by inducing small bowel bacterial overgrowth in rats, resulted in both extra- and intra-hepatic bile duct dilatation, irregularity and beading, resembling human PSC.[32,33] Interestingly, this effect was only observable in certain rat strains, and was reversed by antibiotics, but not with UDCA- or prednisone-treatment.[32] The phenotype was driven by translocation of bacterial wall peptidoglycans, in line with a role of the *immunobiome* and pro-inflammatory microbial peptides (Figure 1). IBD may also induce biliary disease in vulnerable hosts. Cystic fibrosis is known to associate with biliary disease. In mice lacking a functional cystic fibrosis gene (CFTR), which alters bile composition, induction of colitis has been shown to cause biliary inflammation that could be attenuated using antibiotics, likely caused by pro-inflammatory lipopolysaccharide (LPS).[34] Interestingly, the cholangiopathy observed in these mice can be attenuated by diet.[35] These are intriguing observations, but the circulating levels of LPS in PSC patients was not elevated compared with controls.[4] However, LPS-binding protein and soluble CD14 are elevated in PSC and associated with reduced liver transplantation-free survival, suggesting that bacterial translocation and associated activated monocytes could potentially be relevant for disease activity.[4,17] Similar observations have been made for soluble CD14 in PBC.[36]

Data from both PSC and PBC patients suggest that immune-reactivity towards bacterial antigens i.e. the *immunobiome*, could be implicated in disease pathogenesis. In PBC, antimitochondrial antibodies play a key role.[3] These antibodies, and others, cross-react with bacterial proteins from bacteria, suggesting an immune response against the microbiome.[3] A similar observation has been made in PSC, where perinuclear anti-neutrophil cytoplasmic antibodies (p-ANCA), cross-react with the microbial cell division protein FtsZ, which has a similar structure as the human autoantigen β-tubulin isotype 5.[37] This could thus reflect an abnormal immune response in to commensal bacteria in susceptible patients. Unfortunately, these observations have not been validated in other studies. In light of the large, but opposite, gender predominance in PSC and PBC (female:male ratio of 1:2 and 9:1, respectively),[38,39] it is also interesting to note that studies have demonstrated how early-life microbial exposures have a significant impact on sex hormone levels and modify the progression of autoimmunity,[40] but so far we have little knowledge on the effect of sex hormones and potential interaction with the microbiome in cholestatic conditions.

1.3.3 | The xenobiome

Diet is one of the exogenous factors that has the strongest impact on the gut microbiome composition and function, and the effect of carbohydrates are probably best understood among the macronutrients.[41] The collective bacterial genome encode several hundred-fold more carbohydrate-degrading enzymes than the human genome, and this *xenobiome* allows the use of carbohydrates that are indigestible to humans as an energy source.[41] These "microbiota-accessible carbohydrates" are fermented to SCFAs by bacteria in the colon.[41] SCFAs like butyrate are important nutrients for the intestinal epithelium, act as signalling molecules modifying immune responses and are generally considered beneficial. However, recently, Singh et al showed that feeding of microbiota-accessible carbohydrates (in this case inulin, a prebiotic) to mice induced a microbiota-dependent cholestasis and hepatocellular carcinoma (HCC), in contrast with a diet with carbohydrates not metabolized by the microbiome.[42] The condition was shown to depend on dysbiosis, and with the use of the current vocabulary; both the *xenobiome and endobiome* (increased microbial production of SCFAs and bile acids respectively). Blocking bile reabsorption with cholestyramine protected against the development of cholestasis. While these are intriguing observations, the overall effect of dietary fiber in humans seems to be beneficial,[43] and additional data are needed to establish this model as relevant in humans. Less controversial is that microbiota-dependent bile acid metabolism has been shown to regulate experimental HCC development.[44] In contrast, there is little data on the role of the gut microbiome in the development of cholangiocarcinoma, a common and devastating complication to PSC.[17]

An important hypothesis also suggests that the biliary inflammation in PSC could be caused by T cells primed in the gut,

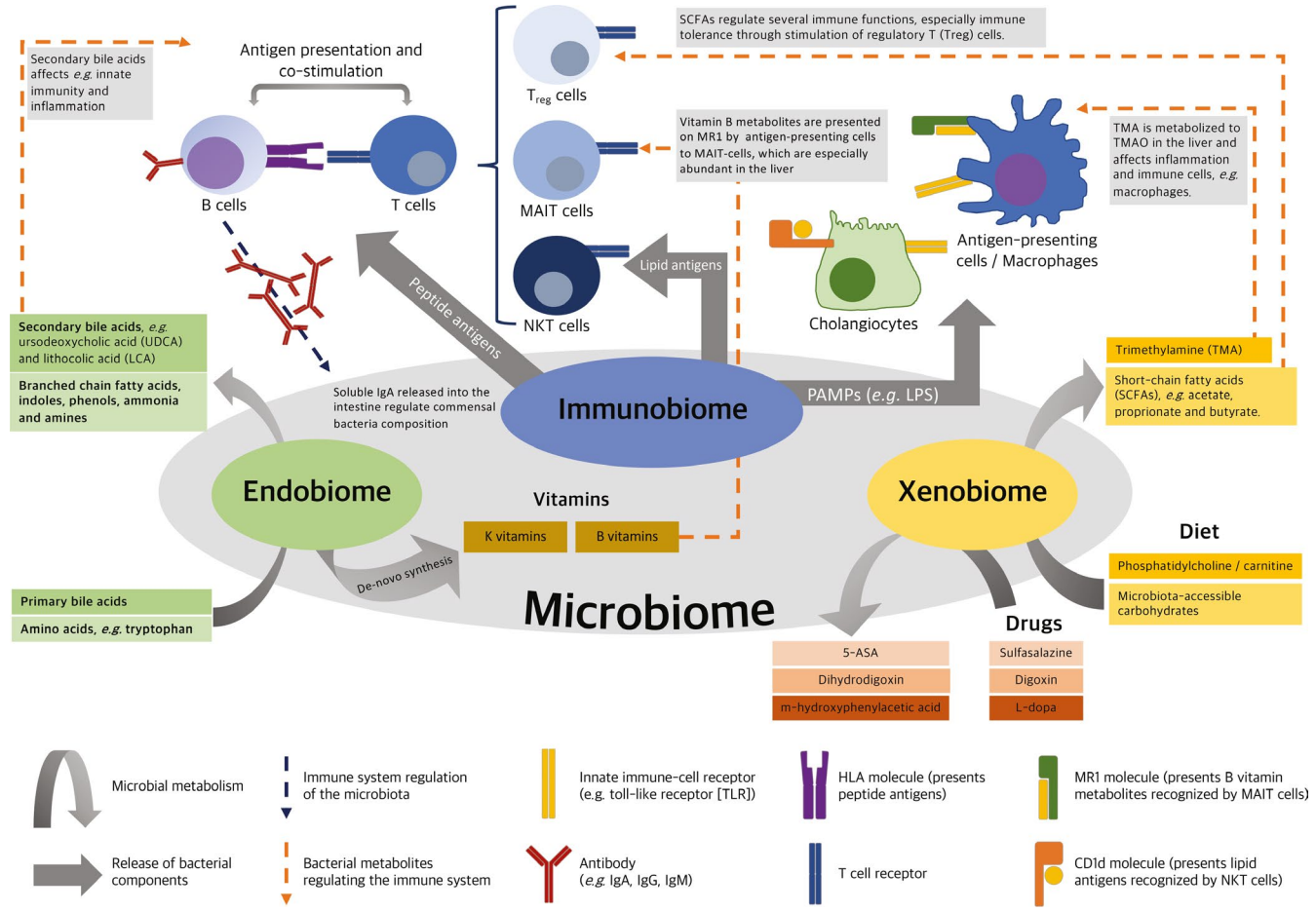

FIGURE 1 The three-*biomes* of microbiome-host interaction: The *immunobiome*, *endobiome and xenobiome*. Experimental and human observational data suggest that a diverse set of microbial functions may be relevant for microbiome-host interaction, including endogenous molecules produced by the microbiota (the *endobiome*), bacterial processing of pharmacological agents or dietary compounds (the *xenobiome*) and specific bacterial molecules or metabolites driving the immune process (the *immunobiome*). ASA, aminosalicylic acid; HLA, human leukocyte antigen; Ig, immunoglobulin; MAIT, mucosal-associated invariant T; MR1, MHC-related protein 1; NKT; natural killer T; SCFA, short-chain fatty acid; TMA, trimethylamine; TMAO, trimethylamine-N-oxide

subsequently homing to the liver.[32] Vascular adhesion protein-1 (VAP-1) is thought to play a critical role in intestinal-hepatic lymphocyte-trafficking which is thought to constitute an important step in the pathogenesis of PSC and IBD.[6,8] Intriguingly, *Veillonella* and other PSC-associated genera have genes encoding for amine oxidases which produce primary amines, e.g. methylamines, that act as substrates for VAP-1.[8] Trimethylamine (TMA), a fully dietary- and bacteria-dependent metabolite, has also been shown to cause cholestasis, cholangiocyte proliferation and cholangiofibrosis in rats.[45] TMA is converted to trimethylamine-N-oxide (TMAO) by the liver, and TMAO has been associated with shorter transplantation-free survival in PSC.[45] Furthermore, serum autoantibodies against pancreatic glycoprotein 2 (GP2), a protein that facilitates immune responses against bacteria and binding of bacterial proteins, are identified in a majority of PSC patients and has been associated with and increased risk of cholangiocarcinoma.[17] Taken together, these data show how intertwined the *endobiome*, *xenobiome* and *immunobiome* are, and underscore the importance of longitudinal studies that incorporate data on all these -biomes to further elucidate the role of gut microbes, function and metabolites as drivers of disease in cholestatic conditions.

1.4 | Clinical opportunities and perspectives for the future

Some of the first treatment trials in PSC utilized antibiotics, with the first case series emerging in the late 1950s.[6,7,46] Both vancomycin and metronidazole have later been used in subsequent treatment trials in PSC with effect on e.g. ALP and gamma-glutamyl transferase (GGT) levels, but so far without effect on harder end-points like liver transplantation-free survival.[6,7] Vancomycin is still considered promising,[46] and more trials will be performed in PSC before and after transplantation with this drug. Similar studies in PBC are scarce, except that the antibiotic rifampicin could be used to alleviate pruritus in PBC, although the exact mechanisms involved here are not clear. Changing the microbiome with more specific pro- or pre-biotics than what is used today could also be a possibility. Current bacterial strains in use are of limited value, and in line with this, no effect has been observed

in small trials in PSC.[47] Another possibility is to perform interventional proof-of-concept trials targeting the microbiome. One example of this is FMT, which has attracted interest also in PSC.[48] Allegretti et al recently published promising data from an open-label pilot study of FMT in 10 PSC patients, all receiving material from a single donor.[49] They reported an increase in bacterial diversity, and the data also suggested that engraftment correlated with improvement in ALP levels, and importantly, they observed no adverse events. If found effective, such studies may be used to identify specifically which microbes or microbial metabolites that are involved in the disease process, or help us to stratify which FMT-donor is best suited for which recipient. The latter is maybe the most important among the methodological hurdles that need to be addressed in FMT-treatment.

As we enter the era of personalized medicine it will be important to consider the role of microbiota composition in determining individual efficacy and safety of several drugs. Interestingly, the majority of new drugs currently in clinical testing in both PBC and PSC that show promising results act on bile acid homeostasis,[48] where the *endobiome* is important; including pharmacological compounds affecting the farnesoid X-receptor (FXR) e.g. obeticholic acid (OCA) and all-trans retinoic acid (ATRA), fibroblast growth factor 19 (FGF19) analogs, *nor*-ursodeoxycholic acid (*nor*-UDCA), activators of the peroxisome proliferator-activated receptor (PPAR), e.g. fibrates like bezafibrate and fenofibrate, and selective α- and/or δ-receptor isoform activators *e.g.* seladelpar.[48] How these different agents affect the microbiome, or *vice versa* (involving the *xenobiome*), is not well-established.[2] One important example illustrating basic principles is metformin, a common drug that has been used for a long time in diabetes type 2. Metformin has recently in been shown to act through the *endobiome* (also here the bile acid–FXR axis is involved).[50]

There is also a potential role of the microbiome in the clinic as biomarker of disease, disease severity and activity, and as predictor of treatment efficacy. Longitudinal studies of the microbiome and the related-*biomes* are needed if we are to understand how they relate to disease progression. Microbial profiles could help in diagnosing biliary disease or relevant subgroups or complications in a biliary disease (e.g. cholangiocarcinoma in PSC).[13,14,17] Finally, as shown by a series of publications from oncology, the microbiome can be used to identify patients responding to treatment,[51] or who are at risk of severe side effects.[51]

2 | CONCLUSIONS

The gut microbiome and the liver are part of an integrated metabolic machinery, which is altered in patients with cholestatic diseases like PSC and PBC, but how and in what way microbes and related molecules are involved in disease initiation and/or progression of disease is so far not well-established. Experimental and human observational data suggest that a diverse set of microbial functions may be relevant, including endogenous molecules produced by the microbiota (*endobiome*), bacterial processing of pharmacological agents or dietary compounds (*xenobiome*) and specific bacterial molecules or metabolites driving the immune process (*immunobiome*). A better understanding of the host-microbial interactions in cholestatic diseases may therefore greatly improve clinical care in these conditions.

CONFLICT OF INTEREST

There was no conflict of interest.

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