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Review

Crosstalk between bile acid-activated receptors and microbiome in entero-hepatic inflammation

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Bile acids are potent signaling molecules exerting diverse actions through bile acid-activated receptors. Among them, the Farnesoid X receptor (FXR) and the Takeda G protein-coupled receptor 5 (TGR5; GPBAR1), modulate the inflammation occurring in chronic/acute hepatitis, cholestasis, and inflammatory bowel disease. A role for other bile acid-responsive receptors in this context is emerging. This review aims to summarize recent advances on the immune-modulatory actions of the bile acid-responsive receptors Shingosine-1-phosphate receptor 2 (S1PR2), pregnane X receptor (PXR), constitutive androstane receptor (CAR), vitamin D receptor (VDR), and retinoic acid-related orphan receptor γt (RORγt). How microbiota-derived bile acids contribute to intestinal and hepatic inflammation, potentially through these receptors, is also discussed. These concepts pave the way to novel and innovative strategies aiming at modulating the gut microbiota to tackle inflammatory syndromes.

Highlights

Bile acids modulate metabolism and immunity through the Farnesoid X receptor (FXR) and the Takeda G protein-coupled receptor 5 (TGR5).

Other receptors may also drive the immunomodulatory action of bile acids in the liver and intestine, such as the Shingosine-1-phosphate receptor 2 (S1PR2), the pregnane X receptor (PXR), the constitutive androstane receptor (CAR), the vitamin D receptor (VDR), and the retinoic acid-related orphan receptor γt (RORγt).

The gut microbiota contributes to the diversity of the bile acid pool by metabolizing the bile acids.

Some of these microbially derived bile acids reduce hepatic and/or intestinal inflammation in preclinical models, revealing the therapeutic potential of microbial metabolism in modulating host inflammation. The receptor involved in these effects has been identified for a few metabolites (e.g., 3-oxo-lithocholic acid and ROR t).

Bile acids and associated responsive receptors

Synthesis of **primary bile acids** (see Glossary) from hydrophobic cholesterol occurs in hepatocytes; they are converted into amphipathic compounds via several enzymatic reactions (Box 1) [1]. This conversion confers the first biological function to bile acids, namely, the elimination of cholesterol from the body. Primary bile acids are then conjugated predominantly with glycine (in humans) or taurine (in mice), increasing their hydrophilicity to foster their transition into the bile canalicular lumen [1,2]. This transition is key to the major osmotic force driving the bile salt-dependent bile flow, the second function of bile acids. The bile is then stored in the gallbladder and released in the intestine through gallbladder contraction during a meal. The third classical function of conjugated bile acids is their contribution to the emulsification of dietary lipids during digestion and absorption in the intestine. Most of these conjugated bile acids are then reabsorbed through specific transporters in the terminal ileum and recycled to the liver to complete the enterohepatic cycle. A small proportion of bile acids can be deconjugated and metabolized into **secondary bile acids** by the gut **microbiota**, which can be passively reabsorbed and reach the liver [1,2].

Importantly, beside their digestive functions, bile acids are also powerful signaling molecules and exert diverse actions on host metabolism and immunity through bile acid-activated receptors. Several of these receptors have been attributed inflammation-modulating properties in specific pathological contexts. In this review, we will focus on hepatic inflammation, occurring in chronic/acute hepatitis or in cholestasis, and on intestinal inflammation, in the context of inflammatory bowel disease (IBD, Box 2).

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Box 1. Primary bile acid synthesis

Bile acid synthesis is a complex metabolic process occurring mainly in the liver and for which 16 enzymes and 17 reactions have been identified [1]. The bile acid synthesis is divided into two pathways: the classical pathway, which synthesizes most bile acids (up to 75%), and the alternative pathway [91]. The classical pathway is characterized by the rate-limiting enzyme cytochrome P450 family 7 subfamily A member 1 (CYP7A1), catalyzing the 7α-hydroxylation of cholesterol and leading to the production of CA and CDCA. The alternative pathway begins with the cytochrome P450 family 27 subfamily A member 1 (CYP27A1) and mainly generates CDCA. Oxysterols from the peripheral tissues can also reach the liver and enter the alternative pathway. Rodents generate α/β-MCA and UDCA from CDCA through the cytochrome P450 family 2 subfamily C member 70 (Cyp2c70) [92]. Bile acids are then conjugated through the bile acid-CoA ligase (BAL) and the bile acid-CoA:amino acid N-acyltransferase (BAAT) to foster their entero-hepatic circulation.

First, we will briefly introduce the immune-modulatory properties of the two most studied bile acid receptors, namely the Farnesoid X receptor (FXR) and the Takeda G protein-coupled receptor 5 [TGR5; also known as G protein-coupled bile acid receptor 1 (GPBAR1)]. We will then focus on other receptors that can also be directly or indirectly activated by bile acids, including the Shingosine-1-phosphate receptor 2 (S1PR2), the pregnane X receptor (PXR), the constitutive androstane receptor (CAR), the vitamin D receptor (VDR), and the retinoic acid-related orphan receptor γt (RORγt). Whether and how these last receptors can modulate inflammation and immunity through bile acids remains largely unclear and is one of the key knowledge gaps in the field. Filling this knowledge gap will allow the identification of new pharmacological targets and the design of novel pharmacological tools to tackle inflammatory disorders.

FXR and TGR5

FXR is a **nuclear receptor** found in hepatocytes, hepatic stellate cells, and intestinal epithelial cells. FXR is also expressed to some extent in monocytes and lymphocytes, with an expression

Box 2. Pathophysiology of cholestasis and inflammatory bowel disease (IBD)

Cholestasis is an impairment of bile secretion and flow that can be generally classified in several categories according to the origin of the defect. An impeded bile flow can occur due to mechanical blocking observed in clinical obstructive cholestasis, often termed as extrahepatic cholestasis, such as gallstones, cholangiocarcinoma, or pancreatic cancer. Intrahepatic cholestasis can also be due to defects in bile formation and secretion observed in various contexts, such as hepatocellular conditions (e.g., viral hepatitis, acute alcoholic hepatitis), genetic defects in bile transporters (e.g., progressive familial intrahepatic cholestasis), or bile duct diseases (e.g., biliary cholangitis or PSC) [93]. Regardless of the source, cholestasis is marked by increased hepatic bile acid levels, including toxic bile acids. In the case of hepatic bile acid accumulation, adaptive mechanisms can be initiated, including the activation of an alternative urinary excretion pathway. Excessive accumulation of toxic bile acid leads to bile duct epithelial cell and hepatocyte damages, liver injury, and inflammation. This hepatic inflammation is often characterized by increased proinflammatory cytokines and chemokines, neutrophil recruitment, and activation of Kupffer cells [93]. It has also been proposed that bile acids themselves can directly induce the production of inflammatory cytokines in hepatocytes [94]. Conversely, inflammation can induce cholestasis. The term 'inflammation-induced cholestasis' has been coined to describe the hepatocellular cholestasis that results from activation of the inflammatory cytokine system. Inflammation-induced cholestasis occurs in a large set of diseases, including intra- and extrahepatic infections, drug and alcohol-induced liver injury, cancer cachexia, or nonmetastatic neoplastic conditions [2,95]. In this process, common mediators, including systemic cytokines or endotoxins, reach the liver and induce the production of local inflammatory cytokines (mainly by Kupffer cells). These latter may alter the expression and function of hepatobiliary transporters, resulting in bile acid accumulation, neutrophil recruitment, and liver injury. Nevertheless, liver damages and inflammation are intrinsically linked and the exact mechanisms are not yet fully elucidated. Understanding how bile acids modulate these processes through dedicated receptors will pave the way for new therapeutic strategies.

IBD combines two major forms of chronic inflammatory bowel disorders: Crohn's disease and ulcerative colitis. Crohn's disease can affect any part of the intestine (usually ileum and colon) often discontinuously and is described as transmural (meaning that it can affect all the intestinal layers) with intestinal granulomas, strictures, and fistulas. On the contrary, ulcerative colitis is restricted to the colon and characterized by mucosal inflammation [96]. IBD is basically defined by an inappropriate and aberrant immune response in the intestinal gut, but the etiology is still not fully understood. Recent advances highlight a complex interaction between the host genetic background, environmental factors (such as smoking, diet, or stress), the gut microbiota, and dysfunctions of innate and adaptive immune pathways [62,63]. Interestingly, advances in the field of the gut microbiota have brought forward the crucial role of microbiota-derived metabolites, including bile acids, short-chain fatty acids, and tryptophan metabolites in the pathogenesis of IBD [79].

Glossary

BDL mice: bile duct-ligated mice are models of obstructive cholestasis, performed by surgical ligation of the common bile duct and leading to hepatic inflammation and fibrosis.

DSS-induced colitis: this is a mouse model of inflammatory bowel disease. Mice are treated through their drinking water with dextran sulfate sodium (DSS), a chemical to induce epithelial damage and colitis. Acute, chronic, and relapsing models of intestinal inflammation can be achieved by modifying the concentration and the type of DSS as well as the frequency of administration.

Gut dysbiosis: the term 'dysbiosis' usually refers to a change or difference from normal with respect to the microbiota. This definition remains a debate as it implies an understanding of the normal microbiome and an abnormality when, in some cases, the difference or change might be appropriate and expected [104].

Inverse agonists: some receptors own a constitutive activity and binding with inverse agonists leads to an opposite effect as compared with an agonist of that receptor. Both agonists and inverse agonists can be blocked by antagonists.

Microbiome: the term 'microbiome' combines both the microbiota (community of microorganisms) and their 'theatre of activity', involving the whole spectrum of molecules produced by the microorganisms, including their structural elements (nucleic acids, proteins, lipids, polysaccharides), metabolites (signaling molecules, toxins, organic, and inorganic molecules), and molecules produced by coexisting hosts and structured by the surrounding environmental conditions [105].

Microbiota: the microbiota comprises all living members forming the microbiome. Bacteria, archaea, fungi, algae, and small protists should be considered members of the microbiome [105].

Nuclear receptors: a family of highly conserved transcription factors regulated in response to ligands, often lipophilic compounds such as steroid hormones or various lipid-soluble signals. Unlike other signaling pathways, ligands activate the nuclear receptor by directly interacting with it and the complex then enters the nucleus to modulate gene transcription. Nuclear receptors that are considered activated

level around ten times lower than in hepatocytes [3–5] (Box 3). Its main natural ligands, in order of potency, are chenodeoxycholic acid (CDCA), lithocholic acid (LCA), deoxycholic acid (DCA), and cholic acid (CA) [6,7]. Ursodeoxycholic acid (UDCA) has a negligible effect on FXR [6,7], while tauro-α/β-muricholic acid (Tα/β-MCA) antagonizes FXR [8]. FXR is widely known for its ability to maintain bile acid homeostasis and as a major regulator of glucose and lipid metabolism [1,3]. FXR influences the progression of many diseases, including metabolic diseases, gastrointestinal diseases, nonalcoholic steatohepatitis, and liver cancer [9,10]. It has been proposed that FXR could reduce the inflammatory response through direct and indirect mechanisms. Direct mechanisms involve a control of the activation and proliferation of immune cells by FXR, while indirect mechanisms include the modulation of liver metabolism and the strengthening of the intestinal barrier function [1,4,11–14]. Which mechanisms are preponderant remains to be elucidated.

TGR5 acts on glucose and energy homeostasis and has potent immune-regulatory properties [15]. The main natural ligands of this G protein-coupled receptor, in order of potency, are LCA, DCA, CDCA, CA, and UDCA [16,17]. In contrast to FXR, TGR5 is more expressed in nonparenchymal liver cells, including endothelial cells, Kupffer cells, and cholangiocytes, than in hepatocytes, where its expression is often considered negligible [16]. TGR5 is highly expressed by several innate immune cells, including macrophages, monocytes, dendritic cells, NK cells, and NKT cells, and negatively modulates inflammation in several pathological contexts, including liver diseases, nonalcoholic steatohepatitis, type 2 diabetes, and atherosclerosis [11,15,16].

by bile acids are FXR, PXR, VDR and the recently discovered RORγt.

Primary bile acids: bile acids that are produced in hepatocytes (Box 1). The main primary bile acids are cholic acid (CA) and chenodeoxycholic acid (CDCA).

Secondary bile acids: include all bile acids that are produced through the microbial metabolism of primary bile acids (Figure 2). The main secondary bile acids are deoxycholic acid (DCA) and lithocholic acid (LCA).

Box 3. Bile acids and immune cells

Immunity is separated into two main components, namely, innate immunity and adaptive immunity, according to the speed and specificity of the response [97]. Innate immunity is defined as the immediate host defense at the site of infection or tissue damage. Cells involved in the innate immune system are mainly dendritic cells, macrophages, monocytes, neutrophils, and epithelial cells, as well as innate lymphoid cells, mast cells, eosinophils, basophils, and natural killer (NK) cells. In contrast, adaptive immunity is characterized by an antigen-specific response through T and B lymphocytes. Natural killer T (NKT) cells combine characteristics of both T cells and NK cells.

Briefly, naïve mature B cells exposed to specific antigen are activated to produce antibodies in a T cell-dependent response or in a T cell-independent response [97]. $CD8^+$ T cells proliferate and differentiate into CD8 cytotoxic cells. Naïve $CD4^+$ T cells exposed to specific antigen proliferate and differentiate into T effector cells that have different functions [98]. T helper 1 (Th1) cells stimulate classical macrophages. Th2 cells stimulate mast cells, eosinophils, and basophils and mediate immune responses to helminths. Th17 cells stimulate many cell types to recruit neutrophils to sites of infection and mediate immune responses against extracellular bacteria and fungi. T follicular helper (Tfh) cells stimulate B cell maturation at germinal centers [98]. Regulatory T (Treg) cells inhibit immune responses to maintain immune homeostasis. $ROR\gamma t^+$ Treg cells are a distinct Treg population induced in the colon that contribute to the response to commensal microbes [65]. A dysregulation of the balance of Th17/Treg is found in autoimmune disease, including IBD [98].

The profile of expression of bile acid receptors varies among various immune cells. FXR is expressed to some extent in monocytes and lymphocytes, with an expression level around ten times lower than in hepatocytes [5]. TGR5 is highly expressed in macrophages, monocytes, dendritic cells, NK cells, and NKT cells [16]. S1PR2 is expressed in macrophages and in liver sinusoidal endothelial cells [16]. CAR is expressed in $CD4^+$ effector T cells [35]. VDR is also expressed in immune cells, mainly in B cells, T cells, Treg cells, monocytes, dendritic cells, and, to a lower extent, in macrophages [49,58]. RORγt is expressed only in a few specific immune cell populations, including Th17 cells, lymphoid tissue inducer cells, innate lymphoid 3 cells and γδ T cells, and $ROR\gamma t^+$ Treg cells. The binding of bile acids to these receptors depends on whether it is a cell surface or nuclear receptor. For cell surface receptors, including TGR5 and S1PR2, ligands can directly activate the receptor without having to enter the cell. The activation of nuclear receptors, including FXR, PXR, VDR, or RORγt, requires the entry of bile acids into the cell, either through active transporters, for hydrophilic species, or by passive diffusion, for more hydrophobic species [99]. So far, the bile acids that impact the function of immune cells through PXR, VDR, and RORγt are mainly LCA and derivates, which are hydrophobic species, suggesting a passive diffusion within the cells and questioning the presence of functional bile acid transporters at the surface of these cells. Which bile acid receptor would be dominant in case of coexpression in a specific immune cell population remains unclear and might be dependent on the pathological context.

FXR and TGR5 are receptors dedicated to bile acids, of which they are natural ligands. They are activated at nonpathological concentrations and play a critical role in the maintenance of bile acid homeostasis [15,18]. Other receptors, including S1PR2, VDR, and PXR, also respond to bile acid activation, but at higher concentrations, such as those found in pathological conditions [15,18,19]. The next part of this review will focus on these less explored bile acid-responsive receptors.

S1PR2

S1PR2 is a G protein-coupled receptor, with the natural ligand sphingosine 1-phosphate (S1P), a potent bioactive lipid [20]. S1PR2 activation by S1P is involved in numerous functions, including metabolic, renal, neurovascular, and muscular functions as well as vascular integrity and immune cell trafficking [20]. S1PR2 can also be activated by bile acids but only in their conjugated form, taurine- or glycine-conjugated [19] (Figure 1, Key figure). In the liver, S1PR2 is expressed in hepatocytes, cholangiocytes, hepatic stellate cells, liver sinusoidal endothelial cells, and macrophages [16].

In rat hepatocytes, the exposition to conjugated bile acids activates phosphorylation of the extracellular signal-regulated kinases ERK1/2 and AKT, with a much lower potency than S1P. The role of S1PR2 in the TCA-induced ERK1/2 and AKT activation was demonstrated using primary rat hepatocytes isolated from S1PR2$^{-/-}$ mice, as well as JTE-013, a S1PR2 antagonist [19]. Hepatocytes isolated from S1PR2$^{-/-}$ mice also displayed decreased nuclear levels of S1P, leading to a reduced acetylation of histone H3K9, H4K5, and H2BK12 and a lower expression of key genes involved in sterol and lipid metabolism. These data are consistent with previous data from the same team showing the ability of S1P to regulate histone acetylation in the nucleus [21].

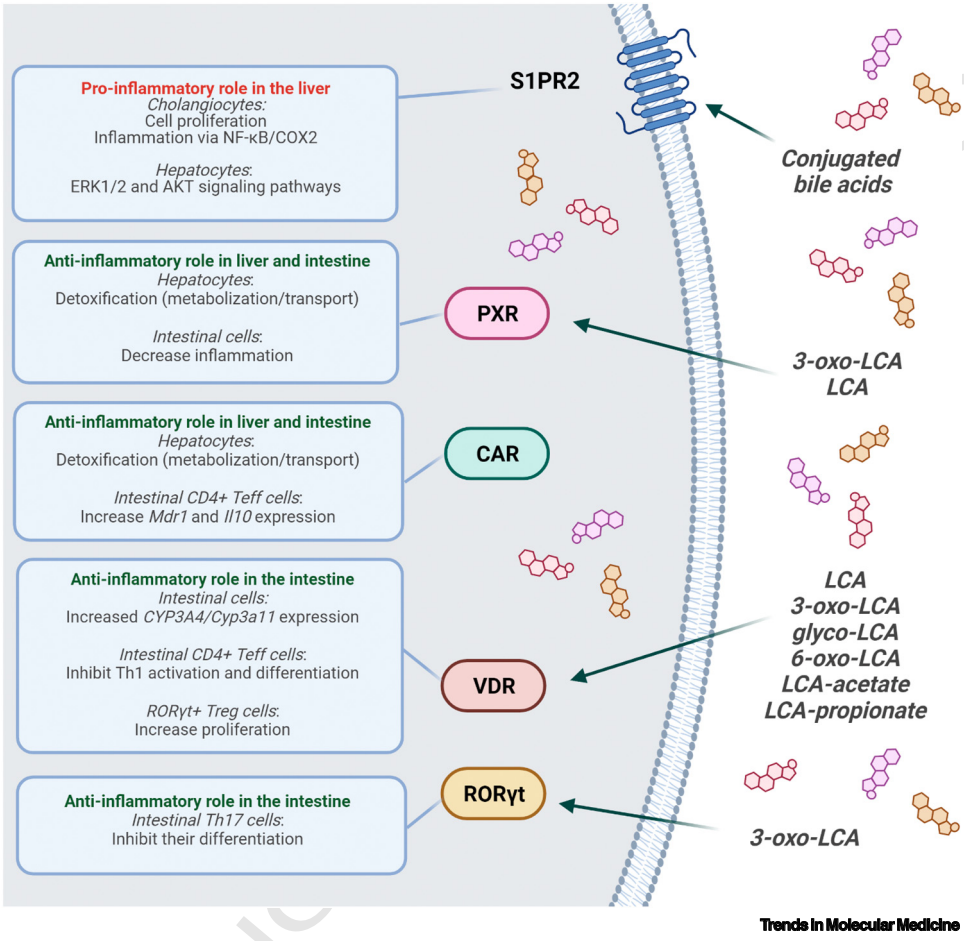
S1PR2 activation by TCA induces cholangiocyte proliferation and cyclooxygenase 2 (*COX2*) expression through ERK1/2/nuclear factor kappa B (NF-κB) and AKT signaling pathways in a human cholangiocarcinoma cell line [22]. Reciprocally, S1PR2 gene ablation reduces cholangiocyte proliferation and liver injury in bile duct-ligated mice (**BDL mice**) and decreases *Cox2* expression in isolated cholangiocytes, supporting the idea that S1PR2 could be implicated in cholangiocyte inflammation induced by conjugated bile acids [23].

Additional reports suggest that S1PR2 is involved in hepatic inflammation and immune cell recruitment in cholestatic liver mice. Indeed, S1PR2 promotes the recruitment and retention of macrophages at the site of inflammation by inhibiting their chemokine-induced cellular motility [20]. In BDL mice, administration of JTE-013 blocks the recruitment of bone marrow-derived monocytes/macrophages, decreases the expression of inflammatory cytokines and chemokines, including *Il6*, *Mcp1*, and *Tnfa*, and thereby reduces hepatic fibrosis [24]. Administration of JTE-013 in BDL mice also reduces the number of recruited neutrophils [25]. In addition, JTE-013 administration in BDL mice reduces the priming and activation of the NOD-, LRR-, and pyrin domain-containing protein 3 (NLRP3) in the liver, an effect that was shown using *in vitro* blockage of S1PR2/G$\alpha_{(12/13)}$/MAPK signaling in bone marrow-derived macrophages [26].

S1PR2 is also emerging as a target of interest in IBD due to its role in the regulation of vascular permeability and immune cell trafficking, as well as in the maintenance of the intestinal epithelial barrier integrity [27–29]. S1PR2$^{-/-}$ mice display increased sensitivity to dextran sodium sulfate-induced colitis (**DSS-induced colitis**), alongside increased intestinal permeability and CD4^{+} T cell proliferation [30]. Paradoxically, administration of DCA exacerbates DSS-induced colitis and treating these mice with JTE-013 may repress the exacerbation of mucosal inflammatory cell infiltration induced by DCA [31].

Key figure

Regulation of hepatic and intestinal inflammation by the Shingosine-1-phosphate receptor 2 (S1PR2), the pregnane X receptor (PXR), the constitutive androstane receptor (CAR), the vitamin D receptor (VDR), and the retinoic acid-related orphan receptor γt (RORγt) in response to bile acids



Q1 Figure 1. Bile acids reported to activate these receptors include conjugated bile acids, lithocholic acid (LCA), and derivatives (3-oxo-LCA, 6-oxo-LCA, glycol-LCA, LCA-acetate, and LCA-propionate). S1PR2 plays a proinflammatory role in the liver. In response to bile acids, S1PR2 fosters proliferation and inflammation in cholangiocytes, the later through the nuclear factor kappa B (NF-κB) and cyclooxygenase 2 (COX2) pathways. In hepatocytes, S1PR2 activates the extracellular signal-regulated kinases (ERK1/2) and AKT signaling. PXR and CAR exhibit anti-inflammatory effects both in the liver and in the intestine, among others, through the detoxification of xenobiotics and endogenous compounds in hepatocytes. In effector T ($CD4^+$ Teff) cells, CAR promotes the expression of *interleukin 10* (*Il10*) and the *multidrug resistance protein 1* (*Mdr1*), a xenobiotic transporter essential to maintain intestinal homeostasis. VDR is essential for induction of the expression of cytochrome *CYP3A4* (the human ortholog to the murine isoform *Cyp3a11*) involved in LCA detoxification; it inhibits the activation and differentiation of T helper 1 (Th1) cells and it promotes the proliferation of $ROR\gamma t^+$ regulatory T (Treg) cells. Finally, 3-oxo-LCA inhibits Th17 cell differentiation through its interaction with RORγt. This figure was created with BioRender.

Altogether, these reports suggest that S1PR2 plays a proinflammatory role in the liver, while its intestinal function in response to bile acids remains unclear. Whether S1PR2 mediates the immune-modulatory effect of bile acids in these organs remains largely unexplored and clearly deserves further investigation. For instance, as S1PR2 has been involved in immune cell trafficking, it would be interesting to determine whether bile acids impact this cell trafficking through S1PR2 in pathological conditions characterized by high levels of bile acids.

PXR and CAR

PXR and CAR are two nuclear receptors that act as sensors for toxic products (derived from endogenous metabolism or exogenous chemicals), to promote their metabolism and elimination. PXR and CAR are both predominantly expressed in the liver. PXR (and CAR to a lower extent) is also well expressed in the intestine, while other sites of expression include the kidney and lung [32]. PXR is activated by LCA derivatives, mainly 3-oxo-LCA. LCA itself is presented in some studies as a PXR activator [33,34]. CAR is considered as an indirect sensor of bile acids, as none of the major bile acids directly bind to this nuclear receptor [35]. One of the challenges in the field is to delineate the therapeutic potential of each of these two receptors, as their biological functions are very similar and many agonists are targeting both receptors.

In hepatocytes, numerous studies support the protective effect of PXR against LCA toxicity, mainly in LCA-administered or BDL mice, by fostering the expression of enzymes involved in bile acid metabolization [cytochrome P450 3A11 (CYP3A11), UDP-glucuronosyltransferases, sulfotransferases] and transport [multidrug resistance protein 3 (MRP3) and organic anion-transporting polypeptide 2 (OATP2)] [3]. Interestingly, a downregulation of the NF-κB signaling pathway was shown to be involved in the anti-inflammatory response ensuing from PXR activation in primary cultures of hepatocytes and HepG2 cell line [36,37]. However, the direct implication of PXR on chemokine expression and neutrophil infiltration in mouse models of chronic liver injury remains controversial [38,39], and whether LCA and their derivatives can reduce hepatic inflammation *in vivo* through PXR has not been investigated so far.

PXR is expressed all along the intestinal tract [40] and represents an interesting target in the context of IBD. Several studies report an anti-inflammatory role of PXR agonists in DSS-induced colitis mice, through inhibition of NF-κB and reduced expression of cytokines and chemokines [40,41]. Recently, a few reports have suggested a potential role for bile acids in the modulation of intestinal inflammation through PXR. In a mouse model of necrotizing enterocolitis, administration of low doses of LCA reduced the intestinal expression of *Il6* in a PXR-dependent fashion [42]. Furthermore, recapitulating the plasma bile acid profile found in Crohn's disease patients led to a decreased PXR activity in a cell reporter assay [43].

CAR holds a constitutive transcriptional activity. In the liver, CAR acts as a sensor that protects against bile acid liver toxicity. CAR activation using phenobarbital and TCPOBOP helps the detoxification of bile acids in CA-supplemented mice lacking FXR and PXR [44]. However, CAR and PXR seem to have distinct protective properties against bile acid toxicity. A comparison of mice administered with LCA and lacking CAR, PXR, or both, evidenced that $CAR^{-/-}$ mice have more severe liver toxicity and a lower survival rate and that PXR and CAR do not regulate the same bile acid detoxification enzymes and transporters [45]. Similarly, in the BDL model, PXR and CAR appear to have distinct target genes contributing to the same goal of detoxification. Theirs effects depend on the disease stage, as both appear to exert a protective effect only at the early stage of cholestatic injury [46].

CAR also appears important in the control of intestinal immunity. CAR is necessary for the expression of the multiple resistance protein 1 (MDR1) in $CD4^+$ effector T (Teff) cells [35]. This xenobiotic transporter MDR1 in Teff cells is essential to maintain intestinal homeostasis in presence of conjugated bile acids [47]. Accordingly, deletion of CAR in Teff cells worsens bile acid-induced ileitis in mice, while the disease is reduced upon CAR activation. This anti-inflammatory effect of CAR relies on the ability of CAR to induce the expression of *Il10* in Teff cells in response to bile acids [35]. These results point to CAR as a novel therapeutic target in chronic inflammatory diseases.

VDR

VDR is a nuclear receptor whose natural ligand is the active form of vitamin D, namely, calcitriol [48,49]. Calcitriol activates VDR to maintain calcium and phosphate levels essential to bone homeostasis. VDR activation leads to its heterodimerization with the retinoid X receptor (RXR) and acts as a transcriptional factor, positively or negatively regulating the transcription of its target genes. Furthermore, VDR is implicated in other physiological processes, including cell proliferation, cell differentiation, and immunity [48]. VDR is activated by LCA and its metabolites 3-oxo-LCA, glyco-LCA, and 6-oxo-LCA [50]. VDR can also be activated by two synthetic derivatives, LCA-acetate and LCA-propionate [51,52].

In hepatocytes, activation of VDR impacts on the transcription of bile acid metabolism enzymes by decreasing the small heterodimer partner *Shp*, thereby leading to increased *Cyp7a1* expression [53]. VDR is also expressed in Kupffer cells, where its activation decreases the inflammatory response in diet-induced obese mice [54]. However, the direct links between bile acids levels, VDR activation, and hepatic inflammation remains largely unexplored.

VDR is highly expressed throughout the gastrointestinal tract [32] and it is in the intestine that VDR has been mainly explored as a bile acid sensor. Indeed, LCA administration in mice increases the ileal expression of *Cyp24a1*, a VDR target gene involved in the metabolism of calcitriol [51,52,55]. LCA administration also induces the ileal expression of *Cyp3a11* (a gene that is also controlled by intestinal VDR), regulating the detoxification of LCA [50,51]. Interestingly, VDR deficiency in the intestine also exacerbates LCA-induced hepatotoxicity. The intestinal transgenic expression of *CYP3A4* (human ortholog to mouse *Cyp3a11*) protects mice lacking VDR, specifically in the intestine, from LCA-induced hepatotoxicity [56]. Similarly, mice lacking VDR specifically in the intestine display a higher sensitivity to DSS-induced colitis [57]. Altogether, these data highlight the role of VDR in the control of the inflammatory tone in the intestine and in the elimination of toxic bile acids through CYP3A4/Cyp3a11.

VDR is also expressed in immune cells, mainly in B cells, T cells, regulatory T (Treg) cells, monocytes, dendritic cells, and, to a lower extent, in macrophages [49,58]. Calcitriol decreases T and B cell proliferation and inhibits the differentiation of dendritic cells. VDR activation in $CD4^+$ T cells promotes the shift toward Th2 cells, induces the $CD4^+$ $Foxp3^+$ Treg cells, and inhibits Th17 response [49]. More recently, an *in vitro* study has shown that LCA participates in the inhibition of Th1 activation and differentiation through VDR by reducing ERK1/2 phosphorylation in primary human and mouse $CD4^+$ Th cells [59].

Consistent with these findings, epidemiological data suggest an implication of VDR in IBD patients. For instance, low serum levels of vitamin D are associated with a worsening of disease in patients with Crohn's disease [60] and a higher risk of clinical relapse in patients with ulcerative colitis [61]. Patients with IBD also exhibit strong alterations in bile acid composition, characterized by increased levels of primary conjugated bile acids along with decreased levels of secondary bile

acids, associated with changes in gut microbiota composition [62,63]. These observations, alongside many others, justify the emerging interest in better understanding the triad between bile acid-responsive receptors (including VDR), the gut microbiota, and inflammation in the context of IBD.

RORγt

RORγt is a nuclear receptor involved in several inflammatory and autoimmune diseases [64,65]. This receptor is expressed only in a few specific immune cell populations, including Th17 cells, lymphoid tissue inducer cells, innate lymphoid 3 cells, and γδ T cells [66]. Several **inverse agonists** have been identified, including cholesterol intermediates and oxysterols. These inverse agonists can reduce the transcriptional binding activity of RORγt and reduce the expression of proinflammatory cytokines in inflammatory and autoimmune diseases [66].

Hang and colleagues reported that 3-oxo-LCA inhibits Th17 cell differentiation by physically interacting with RORγt, inhibiting its transcriptional activity, and reducing *Il-17a* expression [67]. This result put forward that RORγt could act as a bile acid-activated receptor with immune-modulatory properties. Paradoxically, the expression of RORγt is induced in colonic Treg cells in response to commensal microbes, leading to the generation of a specific subset of Treg, namely, RORγt$^+$ Treg cells, with anti-inflammatory properties [65]. Song and colleagues noted that microbial bile acids, including a combination of LCA/3-oxo-LCA, specifically increase the RORγt$^+$ Treg cell population. In this case, they showed that the proliferative effect on RORγt$^+$ Treg cells is dependent on VDR [68]. Consistently, Campbell and colleagues documented increased colonic levels of RORγt$^+$ Treg cells after administration of bacterial strains engineered to produce isoDCA. Here, the authors suggest that FXR activity in dendritic cells is involved in the stimulation of RORγt$^+$ Treg proliferation under isoDCA treatment [69]. These leading studies bring forward the role of microbial bile acid derivatives in the modulation of T cells expressing RORγt. Further investigation needs to be carried out to fully clarify the underlying mechanisms. On a broader level, these studies reinforce interest in considering the gut microbiota as a fully-fledged component, regulating bile acid homeostasis and function. In the last part of the review, we will focus on the crosstalk between the gut microbiota and bile acids and how the gut microbiota can shape the bile acid profile to modulate the activation of bile acid-responsive receptors.

Bidirectional interactions between gut microbiota and bile acids

Bile acids and the gut microbiota maintain a complex and bidirectional relationship

On the one hand, bile acids affect the gut bacterial homeostasis due to their antimicrobial properties. Because of their detergent activity, bile acids induce cell membrane damage and intracellular instability of molecules, leading to DNA damage and protein misfolding. Bile acids are also able to chelate iron and calcium, which are necessary for essential bacterial cellular processes, and therefore limit their proliferation. Some bile acids can inhibit the growth of specific bacteria and therefore alter the bacterial community structure. Recently, a systematic study has analyzed the responses of bacteria to the exposure to several bile acids *in vivo* and *in vitro*. The authors confirmed that unconjugated bile acids induce more inhibition of bacterial growth compared with conjugated ones (in line with their higher hydrophobicity) and that Gram-negative bacteria are more sensitive to bile acid exposure than Gram-positive [70].

On the other hand, the gut microbiota metabolizes bile acids, leading to the production of secondary bile acids. These secondary bile acids can reach the enterohepatic circulation and modulate the bile acid pool, thereby affecting the host's metabolism and immunity. The first step in the microbial metabolism of bile acids is the hydrolysis of glycine- and taurine-conjugated bile acids into free bile acids [catalyzed by the bile salt hydrolase (BSH)]. These free bile acids then

undergo dehydroxylation [mainly a 7-α-dehydroxylation (7αDH)], oxidation (dehydrogenation) to generate oxo-bile acids, and subsequent reduction (epimerization) into iso-bile acids, or microbial conjugation with phenylalanine, tyrosine, and leucine (Figure 2) [71]. Microbially conjugated bile acids (namely phenylalanocholic acid, tyrosocholic acid, and leucocholic acid), can activate FXR *in vitro* and may be involved in severe IBD [72].

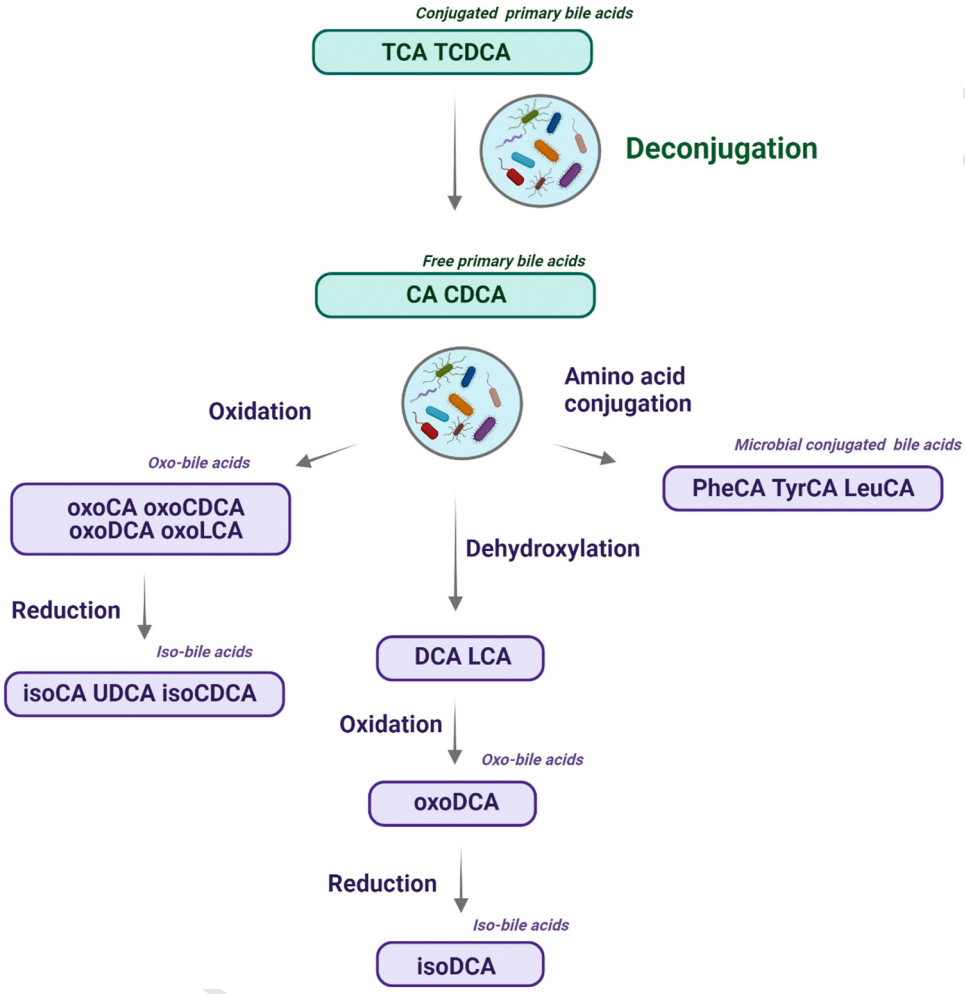


Figure 2. Summary of the microbial metabolism pathways of bile acids. Conjugated primary bile acids are first deconjugated in free primary bile acids through the action of the bile salt hydrolase (BSH). This step is essential to further bacterial modification. Free primary bile acids then undergo dehydroxylation, oxidation, or amino acid conjugation. Dehydroxylation occurs mainly in the form of 7-α-dehydroxylation, which transforms cholic acid (CA) in deoxycholic acid (DCA) and chenodeoxycholic acid (CDCA) in lithocholic acid (LCA). Oxidation of CA leads to the production of oxoCA, oxoCDCA, and oxoLCA, while the oxidation of CDCA leads to the formation of oxoCDCA and oxoLCA. Reduction of these products leads to the production of epimerized iso-bile acids [isoCA, isoCDCA, ursodeoxycholic acid (UDCA)]. OxoDCA can also be produced from the oxidation of DCA and transformed into isoDCA through reduction. Finally, CA can be conjugated to the amino acids phenylalanine, tyrosine, and leucine to produce, respectively, phenylalanocholic acid (PheCA), tyrosocholic acid (TyrCAl), and leucocholic acid (LeuCA). Of note, UDCA is a secondary bile acid in humans, but a primary bile acid in rodents. Oxo-bile acids are also referred to as keto-bile acids. Primary bile acids are denoted in green while secondary bile acids are denoted in purple. For sake of clarity, reactions have been selected to display the production of secondary bile acids discussed in this review. This figure was created with BioRender.

BSH activity is a widespread and highly conserved function in the gut bacterial community [73]. The BSH exists in different isoforms, with some of them targeting some subsets of bile acids more specifically. Indeed, mutation of a specific BSH isoform leads to a selective modulation in the levels of Tβ-MCA and to transcriptional changes in immune pathways in the gut and the liver [74]. Along these lines, a large-scale metagenomic analysis revealed associations between specific BSH clusters and several pathologies, including gastrointestinal, liver, and cardiovascular diseases, underlying the importance of BSH activity for host health [73].

The second most-characterized bile acid biotransformation is the 7αDH occurring in the distal part of the gut. The *Bai* operon coding for the proteins required for this metabolic reaction was characterized in *Clostridium scindens* [75,76]. Unlike BSH activity, only few bacteria possess the *Bai* operon. To date, most of them are anaerobic Firmicutes belonging to the *Clostridium* clusters IV, XI, and XIVa [68,72]. Genetic manipulation of *Clostridium* strains is highly challenging [77]. Generation of *C. scindens* mutants deficient for their 7αDH activity has been proven unsuccessful so far [76]. However, the opposite approach, namely, overexpression of the *Bai* operon in a strain without 7αDH activity, has been achieved with some success [76]. A mouse isolate with 7αDH activity has been recently identified, *Extibacter muris*. *E. muris* is a member of the Lachnospiraceae family. Its impact *in vivo* has been explored through the colonization of mice with a minimal gut microbiota characterized by the absence of 7αDH activity. *E. muris* modulates amino acid, glucose, lipid, and drug metabolism pathways in the liver of these mice, without any impact on hepatic inflammatory parameters [78]. These new tools (e.g., isogenic wild type and 7αDH-overexpressing strains, mouse isolate with 7αDH activity, minimal gut microbiota) represent exciting opportunities to evaluate the contribution of 7αDH activity to gut and liver inflammatory conditions, a significant knowledge gap in the field.

Interaction between the gut microbiota and bile acid-activated receptors

With rising progress in the field of the gut **microbiome** and knowledge of its complex bidirectional interaction with bile acids, investigating how gut microbes impact the activity of bile acid-activated receptors opens the door to innovative and exciting therapeutic targets. Indeed, several pieces of evidence showed that shaping the gut microbiota could have beneficial effects on immune and metabolic disorders through bile acid-activated receptors.

Alterations in the gut microbiota composition have been well described in the context of IBD, mainly characterized by low bacterial diversity, with reduced Firmicutes and enriched Proteobacteria abundance [79]. A distinction can be made between microbial alterations in patients with Crohn's disease and ulcerative colitis, with a more severe **gut dysbiosis** in patients with Crohn's disease [62]. Patients with Crohn's disease display decreased levels of Clostridiales, Ruminococcaceae, and Lachnospiraceae, as well as *Faecalibacterium prausnitzii*, and increased levels of Enterobacteriaceae, including *Escherichia coli* [62,63]. Among other changes, the microbial signature in patients with ulcerative colitis is characterized by reduced abundances of *Roseburia hominis* and *F. prausnitzii* [80,81].

Interestingly, some studies have pointed to the association between gut dysbiosis and alterations in bile acid profile in IBD patients. For instance, a low abundance of *F. prausnitzii* and high levels of *E. coli* were associated with decreased proportions of secondary bile acids and increased 3-OH-sulphated bile acids in fecal samples of IBD patients [82]. Recently, two large-scale studies analyzed the molecular profile and the gut microbiome in samples from IBD patients, revealing the association between several bacterial taxa and increased primary conjugated bile acids along with decreased secondary bile acids, namely, DCA and LCA [62,63]. DCA and LCA are strong TGR5 agonists. Knowing the TGR5 anti-inflammatory properties, one could postulate

Clinician's corner

Within the therapeutic options to manage cholestatic liver diseases, a few molecules target bile acid-activated receptors. Ursodeoxycholic acid (UDCA) is the most widely used of these molecules and is a poor agonist of the Farnesoid X receptor (FXR) and Takeda G protein-coupled receptor 5 (TGR5; GPBAR1). Obeticholic acid, a FXR agonist, is the second-line therapy for patients with primary biliary cholangitis who do not respond to UDCA treatment. Other FXR agonists are in clinical trials. TGR5 agonists have failed so far to reach the clinics due to severe side effects (e.g., pruritus, gallbladder distention).

Research on other bile acid-activated receptors is expected to lead to the identification of new pharmacological targets and the design of novel pharmacological tools to tackle cholestatic and other inflammatory disorders.

Our knowledge about the complex interaction between gut microbiota and bile acids and the contribution of microbiota-derived bile acids to intestinal inflammation is rising. These discoveries will contribute to the definition of innovative therapeutic strategies aiming at modulating the gut microbiome to tackle inflammatory syndromes.

The fact that bile acids can act on such a broad set of receptors, each with specific functions, underlines the importance of maintaining bile acid homeostasis beyond the pathological inflammatory contexts directly involving the bile acid pathway, such as cholestatic diseases. Inflammatory bowel disease is a good illustration of the importance of bile acid homeostasis, as recent studies support the idea that the inflammatory tone could result from gut dysbiosis decreasing the production of secondary bile acid, the latter being known for their anti-inflammatory properties.

that stimulating the microbial 7αDH pathway may promote the production of these secondary bile acids and thereby dampen intestinal inflammation. In accordance with this hypothesis, patients with ulcerative colitis display decreased expression of the *Bai* genes and reduced microbial production of LCA and DCA in cultured stools [83]. Moreover, LCA administration in DSS-induced colitis mice improves the inflammatory phenotype through TGR5 [83]. Altogether, these studies support a scenario where the gut dysbiosis present in IBD leads to reduced microbial bile acid biotransformation, inducing higher levels of conjugated primary bile acids and decreased secondary bile acids.

Intriguingly, several reports suggest a close association between primary sclerosing cholangitis (PSC) and IBD: up to 80% of patients with PSC also suffer from IBD, resulting in a specific phenotype named PSC-IBD [84]. Patients with PSC-IBD display gut dysbiosis that is different from patients with PSC, IBD, and healthy controls and is marked by an increase in *Veillonella*, *Escherichia*, Lachnospiraceae, and *Megasphaera* and a decrease in *Prevotella* and *Roseburia* [84,85]. Only a few reports analyzed the fecal bile acid profile in PSC-IBD patients, revealing less total and secondary bile acids and a significant proportion of bacterial taxa that correlated with fecal bile acids [86,87]. Patients with PSC-IBD also exhibit an upregulation of the bile acid signaling pathways in the colon compared with patients with ulcerative colitis (e.g., upregulation of *Fxr*, *Pxr*, and *Mrp3*), which is associated with gut dysbiosis [88]. These patients display significantly higher Th17 cell and IL-17-producing $CD4^+$ T cell populations [88], two cell populations that can be modulated by microbial bile acids [67].

Lately, the contribution of gut microbiota to the hepatic alterations associated with cholestasis itself, independently of an underlying IBD, have been investigated in two preclinical studies. In the first study, chemically induced accumulation of bile acids failed to promote liver injury in germ-free mice. In this case, the authors put forward that endotoxin, levels of which are regulated by gut permeability, sensitizes hepatocytes to bile acid-induced cell death [89]. In the second study, reduction of the microbial load using broad-spectrum antibiotics in a genetic mouse model of PSC exacerbated cholestatic liver injury, likely through the loss of the FXR-mediated negative feedback on bile acid synthesis and the disruption of the bile duct barrier function [90]. These studies put forward two mechanisms linking gut dysbiosis to hepatic injury ensuing from cholestasis, with opposite impact on the disease.

Altogether, these studies bring forward the possibility of controlling intestinal and hepatic inflammation by promoting the microbial transformation of primary bile acids into secondary bile acids, an objective that could be reached through, for instance, the use of bacterial strains with a high 7αDH activity.

Concluding remarks

Despite the important progress made over the last years, numerous important questions remain (see Outstanding questions) and many of these receptors will need extensive complementary studies to raise them to the rank of bile acid-activated molecular therapeutic targets in hepatic and intestinal inflammatory syndromes. In view of the redundancy between bile acids and their receptors and the pleiotropic contribution of bile acids to metabolic and immune physiological processes, one of the key challenges will be to exploit, selectively and without severe side effects, one bile acid receptor. This difficulty is one of the reasons why TGR5 agonists have currently reached a dead end in clinical trials (see Clinician's corner). The bile acid system appears now as a complex physiological system, regulated at many levels, and whose homeostasis needs to be protected to maintain health and to avoid the evolution of pathological conditions. Targeting the gut microbiota (Box 4) may be one of the options to maintain or restore the bile acid homeostasis.

Outstanding questions

Many bile acids bind multiple bile acid receptors and display immunomodulatory properties. However, a direct causal role between their binding to one receptor and their immune impact has been formally demonstrated only for a handful of bile acid–receptor pairs and is lacking in many cases.

The Shingosine-1-phosphate receptor 2 (S1PR2) plays a crucial role in vascular integrity and immune cell trafficking. Do bile acids modulate inflammatory syndromes through a S1PR2-dependent impact on immune cell adhesion?

Administration of lithocholic acid was shown to improve the inflammatory phenotype in a mouse model of colitis. Considering that lithocholic acid is a controversial ligand of the pregnane X receptor (PXR), can lithocholic acid and its derivatives reduce intestinal and/or hepatic inflammation through PXR?

The vitamin D receptor (VDR) was mainly explored in the intestine as a bile acid sensor modulating inflammation. Studies investigating the impact of bile acids through VDR on hepatic inflammation are severely lacking. Do bile acids modulate hepatic inflammation through VDR?

A recent report shows the direct binding of bile acids on the retinoic acid-related orphan receptor γt (RORγt). Moreover, microbial bile acids modulate $ROR\gamma t^+$ Treg cell proliferation through other bile acid receptors. Do bile acids modulate $ROR\gamma t^+$ Treg cell proliferation through direct binding of RORγt?

Our knowledge about many microbially derived bile acids is scarce. Which receptors are they activating? What are their biological functions? Which bacteria contribute to their production?

Can we harness the microbial metabolism of bile acids to create new therapeutic options in the treatment of inflammatory disorders? For instance, what is the contribution of the microbial 7α-dehydroxylation activity to gut/liver inflammation in patients with inflammatory bowel disease or cholestatic liver?

Box 4. Targeting the gut microbiota

Targeting the gut microbiota can be achieved through multiple ways. Prebiotics and probiotics were historically the first tools used to harness the therapeutic potential of the gut microbiota. Recently, alternative tools to target the gut microbiota have also been investigated, such as specific diets, fecal material transplant, and bacteriocins. Probiotics are 'live microorganisms which when administered in adequate amounts confer a health benefit on the host' [100]. Probiotics have been traditionally limited to *Bifidobacterium* and *Lactobacillus* strains, but strains from other genera are now emerging as probiotics. Prebiotics have been consensually defined as 'a substrate that is selectively utilized by host microorganisms conferring a health benefit' [100]. The most established prebiotics are inulin-type fructans and galacto-oligosaccharides. Many mechanisms of action have been pinpointed to explain the health benefits conferred by probiotics and prebiotics [100] and modulation of the bile acid profile is emerging as one potential mechanism that may underlie the impact of probiotics and prebiotics. In a preclinical model, inulin-type fructans restore the endothelial dysfunction and this improvement is linked to changes in bile acid composition. Indeed, inulin-type fructans increased primary bile acids and reduced secondary bile acids in multiple compartments in this model [101]. Increasing the BSH activity has also shown promise. Administration of a BSH-negative strain of *E. coli* expressing, or not, the *bsh* allele gene of *Lactobacillus salivarius* JCM1046 reduces weight gain, serum cholesterol, and liver triglycerides in mice [102]. Along these lines, restoration of gut BSH functionality appears to contribute to the efficacy of fecal material transplant in treating recurrent *Clostridioides difficile* infection [103]. Altogether, these studies pave the way for a rational selection and optimization of probiotic strains based on their functional metabolic properties.

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Declaration of interests

No interests are declared.

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