Regulation of Immune Cell Functions by Bile Acid-Activated Receptors

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November 21, 2022

Abstract

Bile Acid-Activated Receptors (BARs) such as a G-protein-coupled receptor (TGR5) and the farnesol X receptor (FXR) activated by bile acids (BAs) are implicated in the regulation of microbiota-host immunity in the intestine, as well as in dendritic cells, macrophages and T cells. The mechanistic roles of these receptors in immune signaling suggest they may also influence the development of metabolic disorders. In this perspective, we provide a summary of recent literature reports describing the main regulatory pathways and mechanisms of BARs and how they affect immune cell proliferation, activation, and signaling in the context of inflammatory diseases.

Regulation of Immune Cell Functions

by Bile Acid-Activated Receptors

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Abstract

Bile Acid-Activated Receptors (BARs) such as a G-protein-coupled receptor (TGR5) and the farnesol X receptor (FXR) activated by bile acids (BAs) are implicated in the regulation of microbiota-host immunity in the intestine, as well as in dendritic cells, macrophages and T cells. The mechanistic roles of these receptors in immune signaling suggest they may also influence the development of metabolic disorders. In this perspective, we provide a summary of recent literature reports describing the main regulatory pathways and mechanisms of BARs and how they affect immune cell proliferation, activation, and signaling in the context of inflammatory diseases.

Keywords: bile acid, intestinal macrophage, TGR5, FXR, RORγ

Abbreviations: BAs, bile acids; ASBT (IBAT), apical sodium dependent bile acid transporter; CA, cholic acid; MCAs, muricholic acids; βMCA, beta-MCA; CDCA, chenodeoxycholic acid; CYP7A1, cholesterol-7α-hydroxylase; CYP8B1, sterol 12α-hydroxylase; CYP27A1, cholesterol to 27-hydroxy-cholesterol by sterol 27-hydroxylase; CYP7B1, oxysterol 7α-hydroxylase; BAAT, bile acid-CoA amino acid N-acyltransferase; TCA, tauro-CA; TCDCA, tauro-CDCA; GCA, glyco-CA; GCDCA, glycolyl-CDCA; IBD, inflammatory bowel disease; DCA, deoxycholic acid; LCA, lithocholic acid; GF, germ free; SPF, specific pathogen-free; BARs, bile acids activated receptors; S1PR2, sphingosine-1-phosphate receptor; DC, dendritic cell; ILC3s, innate lymphoid 3 cells; GM, gut Microbiota; Th17, T helper 17 cells; IFN, interferon; IL, interleukin; TNF, tumour necrosis factor; CREB, cAMP response element binding protein; SHP, small heterodimer partner; NCor1, nuclear receptor corepressor 1; TLR-4, toll-like receptor-4; OCA, obeticholic acid; UDCA, ursodeoxycholic acid; norUDCA, 24-norursodeoxycholic acid, T-MCA, tauro-muricholic acid; Gly-MCA, glycine-muricholic acid; SCFAs, short-chain fatty acids.

Introduction

Bile acids (BAs), as 24-carbon steroids, are natural surfactants that are generated from cholesterol in the liver. Then they are stored in the gall bladder and secreted into the duodenum (1). They have a crucial role in lipid digestion, lipid absorption, bacterial defense, and cholesterol homeostasis (2, 3). The apical sodium dependent bile acid transporter (ASBT, also known as IBAT), which is found in the distal ileum, reabsorbs around 95% of the biliary produced BAs from the intestine and circulates them to the liver where they are then released once more (4). This process, known as enterohepatic circulation, takes place around six times every day in human systems (4). The remaining small proportion of BAs is deconjugated and metabolized into secondary BAs by gut microbiota. BAs can affect gut bacterial homeostasis whilst the secondary BAs can participate in the enterohepatic circulation to regulate the BA pool (5).

BAs are also signaling molecules that promote G protein-coupled receptors and intracellular ligand-activated nuclear receptors interaction, playing regulatory roles in different processes from lipid and glucose metabolism to immunity (6). Dysregulation of BA-immune cell interaction, caused by both genetic and environmental factors, may promote response or predispose to disease, such as cholestasis, in which increased levels of BAs in the liver lead to hepatic diseases (7). As well, increasing evidence declares the importance of BAs in maintaining innate immune response via receptors, such as autoimmune uveitis (8-12). These observations identify the BA pathway as an attractive target for intervention in inflammatory disease.

BA synthesis

The main mechanism for cholesterol catabolism is the BA production from cholesterol. At least 17 distinct enzymes catalyze the conversion of cholesterol into BA in the liver (13). The BA synthesis is divided into two pathways, known as “classic/neutral” and “alternative/acidic”. Cholesterol-7α-hydroxylase (CYP7A1) is the rate-limiting enzyme for the classic pathway (over than 75% of total BA pool). Cholesterol is converted to 7α-hydroxy-cholesterol by CYP7A1. Then it can be metabolized to 7α-hydroxy-4-cholesten-3-one (C4) by a specific steroid dehydrogenase. The activity of the enzyme sterol 12-hydroxylase (CYP8B1), which is essential for the production of CA, provides access to the dispersion of these two BAs. As a branching enzyme, the sterol 12α-hydroxylase (CYP8B1) can hydroxylate C4 and produce cholic acid (CA). Otherwise, C4 would be metabolized to chenodeoxycholic acid (CDCA) with CYP8B1 (14). The alternative pathway is a conversion of cholesterol to a component of 27-hydroxy-cholesterol by steroid 27-hydroxylase (CYP27A1), followed by hydroxylation on ring B, metabolized by oxysterol 7α-hydroxylase (CYP7B1), and side chain modification for the synthesis of CDCA. In addition, this pathway produces only CDCA, with less than 25% of total BA pool (15). Primary BAs are bound to glycine or taurine at position C-24 via bile acyl synthetase and BA-CoA amino acid N-acyltransferase (BAAT) to produce tauro-CA (TCA), tauro-CDCA (TCDCA), glyco-CA (GCA) and glycolyl-CDCA (GCDCA), respectively, which are then secreted into the bile canaliculi. CA and CDCA contain glycine and taurine in the human liver in a ratio of 3:1. In mice, about 95% of primary BAs are tauro-conjugated. Bile salts are intermediate derivatives of primary BAs, which are released into the bile ducts and sent to the intestine for microbial modification (**Figure 1**) (**Table 1**). Notably, the secondary BAs concentrations are in the hundreds of micromolar range in the healthy human

gut (4). Rats create cholic acid (CA) and muricholic acids (MCAs), primarily beta-MCA (βMCA), while humans primarily make chenodeoxy-cholic acid (CDCA) and CA (16).

Gut Microbiome and BAs

The gut microbiome, as the most densely populated natural ecosystem, is comprised of over 10^{14} bacterial cells (17). It is a community of commensal, symbiotic, and pathogenic microorganisms consisting of bacteria, archaea, fungi, and viruses that can inhabit the gastrointestinal tract contributing both to human health and disease (18, 19). BAs play protection roles in the gut epithelium health and defense against pathogens such as Clostridium difficile (20). In addition, a decreased amount of *Ruminococcaceae* and*Lachnospiraceae* is described as a potential cause of different autoimmune diseases such as inflammatory uveitis (21), inflammatory bowel disease (IBD) (22), enthesitis-related arthritis (23), pediatric multiple sclerosis (10) and type 1 diabetes (24). *Ruminococcaceae*and *Lachnospiraceae* stimulate 7α-dehydroxylation, which converts the primary BA (CA and CDCA) into secondary BAs, such as deoxycholic acid (DCA) and lithocholic acid (LCA) (25). It's interesting to note that the intestinal microbiota also produces secondary BAs, the GP-BAR1 ligands, and derivatives of oxo-BAs, the RORt ligands, from the breakdown of cholesterol. Recent studies have also found a reduced amount of fecal secondary BAs in germ-free (GF) mice in comparison with specific pathogen-free (SPF) mice (26), providing more evidence showing the importance of microbiota on BA metabolism.

A body of evidence has shown that the influence of diet and GMs, as well as BAs may play an essential role in modulating colonic Tregs *in vivo* . In particular, a study constructed by Ramanan *et al.*demonstrated that a deficiency in BAs causes a reduction in Tregs, contributing to inflammatory colitis progression (27). As transgene-derived BAs are involved in intestinal immunity and inflammation, Ramanan *et al.* first silenced the BA conversion gene in various GMs. Then the modified and non-modified microbes were placed into the specially bred mice with no microorganisms in their gut. The result demonstrated that the Treg cell population was significantly lower in mice intestines lacking the GMs and the BA conversion gene (27). The data also suggests that both GMs and food-derived BAs are important factors in immune modulation, as germ-free mice with food intake also had low levels of Tregs. Importantly, Eberl and colleagues have recently shown that the microbiota balances type 2 responses through the local induction of Treg cells that express the transcription factor RORγt (28). Also, another finding has illustrated that the non-genetic transfer of an important immunoregulatory trait by immunologic means, for which the entero-mammary axis provides the mechanistic underpinning of multi-generational matrilineal transmission (27).

The composition of human GM can be changed by age, diet, lifestyle, disease and antibiotics, leading to alternations of BA pools which affect host metabolism and homeostasis. Indeed, BAs have been demonstrated to exert both direct antimicrobial effects and indirect antimicrobial effects on gut microbes (26). For such reason BAs are currently regarded as a major regulator of the GM.

Regarding the BA's direct effect, BAs could increase cell membrane permeability to suppress the proliferation of bacteria, which could lead to cell damage (29-31). BAs could also destroy macromolecule stability by damaging DNA, interfering with RNA secondary structures, and promoting protein misfolding (17, 30, 31). Furthermore, BAs could induce oxidative stress by chelating critical cellular ions (32). As one of the most effective antimicrobial BAs, the bactericidal activity of DCA is ten times higher than that of CA, which could positively prohibit GM proliferation (33). The indirect effect of BAs on farnesoid X receptor (FXR) and vitamin D receptor (VDR) could act as an antimicrobial (29, 34, 35). Inagaki *et al.* (35) revealed that the FXR agonist GW4064 prohibited the overgrowth of aerobic and anaerobic bacteria in the ileum and cecum. Moreover, FXR activation could induce antimicrobial peptide production and regulate the host immune response. Further studies have shown that BAs (CDCA and UDCA) act through FXR and VDR to induce the expression of cathelicidin, an antimicrobial peptide stored in the lysosomes of immune cells (29, 36, 37).

BAs and Bile Acids-Activated Receptors (BARs)

BAs are natural ligands of various receptors, and they are subjected to biotransformation to their unconju-

gated forms by the resident microbial community, a process which is critical to metabolic homeostasis (29, 32, 38). The unconjugated BAs can activate intracellular signaling by binding in BA receptors, namely FXR, pregnane X receptor (PXR), constitutive androstane receptor (CAR), VDR, and G-protein coupled receptor TGR5 (14, 38, 39). In humans, CDCA (CA in mice) is regarded as the most potent FXR ligand, whilst secondary BAs DCA and LCA are ligands for TGR5, PXR, and VDR. In addition, the remaining BAs, such as HCA, can bind to liver-X-receptor α and β (LXRα/β) (40). These receptors are highly expressed by innate and adaptive immune cells, including dendritic cells (DC), macrophages, innate lymphoid 3 cells (ILC3s), and T helper 17 cells (41). These features point to the possibility that different BA species may regulate intestinal immunity by influencing the intestinal T cell response to intestinal microbial antigens. The hematopoietic and non-hematopoietic cells of the innate immune system are located at the interface of the host microbiome. They can recognize microorganisms and their metabolic products to regulate the host's physiological responses and microecology (42). Therefore, understanding the mechanisms that control dysbiosis, changes in the GM, and the composition of the BA is essential to comprehend the emergence of metabolic disorders (43-45).

TGR5 (GPBAR1)

TGR5 is a cell membrane receptor for secondary BAs and it binds with both DCA and LCA and their T and G derivatives (46). TGR5 is regarded as a metabolic regulator in BA synthesis, glucose metabolism, and energy homeostasis, and a potent immune regulator. Interestingly, some evidence confirmed that the total BA pool size in TGR-/- mice decreased compared with that of wild-type mice, although the reason is still unknown (47). TGR5 is highly expressed by several innate immune cells, including macrophages, monocytes, dendritic cells, NK cells, and NKT cells (47-49). As reported, the expression of TGR5 is negatively related to modulating inflammation in liver diseases, nonalcoholic steatohepatitis, type 2 diabetes, and atherosclerosis (3, 5, 40, 50)

Monocytes are the "sentinel cells" of the innate immune system and can differentiate into populations of dendritic cells and macrophages, which have implications for the homeostasis of cells. Interestingly, Kawamata *et al.* selectively addressed the inhibitory role of BA: TGR5 signaling on $CD14^+$ peripheral blood monocytes and observed that the expression of TGR5 was dramatically reduced during dendritic cell differentiation from monocytes (51). Macrophages are major regulators of cytokine production in the gastrointestinal tract, and a body of evidence has suggested that TGR5 activation by BAs plays a key role in modulating macrophage phenotype. In the M1-like pro-inflammatory phenotype, TGR5-dependent transactivation of EGFR is essential for triggering SRC activation and its downstream cascades (ERK1/2 and PKC) (52, 53). This transactivation occurs when EGFR and TGR5 are co-localized on caveolae, lipid rafts enriched with cholesterol and sphingolipid, which plays a key role in modulating intracellular signaling. In this regard, metalloproteinase-dependent cleavage of the EGFR ligand HB-EGF occurs following TGR5 activation (54). TGR5–dependent PKC activation further stimulates the NF-ϰB pathway and the autocrine activation of ERK1/2, causing the release of pro-inflammatory cytokines such as IL-1β, IL-6, and TNFα. SRC-dependent activation of ERK1/2 in the M1-like phenotype further promotes the c-MYC upregulation and results in cell death (55).

Innate immunity is also triggered by pro-inflammatory M1-like signaling by stimulating the conversion of naïve T cells in T helper 17 (Th17) cells (increased IL-1β, IL-6) and Th1 cells (increased IL-12, IFNγ), with concurrent suppression of immunosuppressive Treg cells (decreased IL-10) via production of pro-inflammatory cytokines (56). During BA-dependent activation of TGR5, the pro-inflammatory M1-like phenotype is inhibited. At the same time, STAT3 is activated through SRC-dependent signaling, which upregulates the anti-inflammatory effects. In this respect, the level of Th17 and Th1-cells, TNFα, IFNβ, IL-6, and IL-12 was decreased, while the level of IL-10 and TGFβ was increased (57).

Moreover, the activation of TGR5 by BAs triggers the cAMP pathway signaling independently of EGFR transactivation. The activation of PKA by cAMP exhibits increased cAMP response element binding protein (CREB) expression and activity**(Figure 2)** . cFos, a key target gene of the CREB pathway, can bind to the p65 subunit of activated NF-ϰB, inhibiting its translocation into the nucleus and eliciting an anti-

inflammatory response mediated by TGR5 activation. Besides, BAs can also induce the anti-inflammatory capability of human macrophages by increasing the IL-10/IL-12 ratio in response to PKA activation (57, 58).

FXR

FXR activation on monocytes and macrophages has direct and indirect actions on the atypical nuclear receptor small heterodimer partner (SHP) (44, 59, 60). The FXR activation and induction of its target genes depend on SHP binding via protein–protein interactions (61). Yang*et al.* showed that SHP occupation stabilizes the inhibitory complex binding on the promoter of the chemokine (C-C motif) ligand CCL 2 by inhibiting the recruitment of the NF-kB p65 subunit, which led to a decrease in CCL2 expression in macrophages followed by inhibition of cell migration and invasion (62).

The primary BAs that activate FXR may trigger activation of iNOS, TNF- α and IL1 β to the promoters of metabolism which involves the binding to the nuclear receptor corepressor 1 (NCor1) complex. The NCor1 complex binds to the promoters of these genes, repressing NF-kB at the basal level and maintaining a state of transcriptional inactivity (63). The release of NCor1 from the promoters upregulates the transcription of these genes and causes an upregulation of toll-like receptor-4 (TLR-4) (44). However, treatment with obeticholic acid (OCA), also called INT-747, stabilized the NCoR1 complex on the iNOS and IL-1β promoters, leading to transrepression (45). Additionally, Massafr*et al.* also found that OCA treatment induced an anti-inflammatory immune state in an experimental model of inflammatory bowel disease. The state included retention of DCs in the spleen, which was associated with a reduction in colonic inflammation. Therefore, these findings support the hypothesis that pharmacological FXR activation is an attractive new drug target for the treatment of IBD (64).

Intrestingly, definitive evidence for the potential use of OCA in acute hepatitis has recently been provided by studies in murine models. Treatment of OCA led to an inhibition of the infiltration of NKT cells. Thus, it was shown that the gut microbiome uses BAs as messengers to control the mechanism of chemokine-dependent accumulation of hepatic NKT cells and hepatic anti-tumour immunity (65). Besides, Willart *et al* . demonstrated that ursodeoxycholic acid (UDCA) promotes DC-driven Th1 development by acting on the FXR (66). Further findings also suggest that activation of the bile salt nuclear receptor FXR is inhibited by pro-inflammatory cytokines through activation of NF-ϰB signaling in the gut (67).

Recent research has also investigated the effects of FXR in AIM2, NLRP1, NLRP3, and NLRC4 inflammasomes. They are a class of cytoplasmic multiprotein complexes that could sense endogenous and exogenous pathogen-associated or damage-associated molecular patterns (PAMP and DAMP, respectively). The typical inflammasome consists of nucleotide-binding structural domains, leucine-rich repeat containing proteins (NLRs) and is absent in melanoma 2-like receptors (AIM2), which are in complex with the adapter protein ASC and caspase-1 (68). Caspase-1 is a protease that triggers the cleavage of the cytokine precursors interleukin-1β (IL-1β) and IL-18, which help the host to defend against infection (69). NLRP3 is a known inflammasome that has been recognized in several diseases. Consistent with this idea, recent studies have shown that FXR function as a negative modulator of NLRP3 assembly through an interaction with NLRP3 and caspase 1. Furthermore, SHP has been shown to suppress the formation of NLRP3 (70). Notably, increased BA concentrations, which are often seen in patients or models of obstructive cholestasis, mediated the activation of the inflammasome (71). A summary of BARs expression on immune cells and their mode of action are shown in **Table 2** .

The retinoid-related orphan receptors (RORs)

RORs are members of the nuclear receptor family of intracellular transcription factors. Three forms of ROR (ROR-α, -β and –γ) are available and mapped to human chromosomes 15q22.2, 9q21.13 and 1q21.3, respectively (72). Two different isoforms of RORγ are produced: RORγ1 and RORγt (or γ2), encoded by the RORC gene. RORγ shows a regulatory role in the circadian expression of clock genes and downstream targets in adipose tissues and the liver (73).

Interestingly, the major breakthrough, led by immunologists Saiyu Hang and Xinyang Song has demonstrated the role of microbial-derived BA metabolites in the modulation of gut RORγ$^+$ regulatory T cell homeostasis (26, 74). It has been shown that RORγt can bind to some oxygen derivatives of BAs, such as 3-oxo-LCA, as an inverse agonist. Indeed, RORγt is an essential transcription factor for thymic T cell development, secondary lymphoid tissue organogenesis, and peripheral immune cell (75, 76). They are expressed by Th17 cells, ILC3s, and γδ T cells (77, 78). In CD4$^+$ T cells, RORγt is important for the differentiation and production of Th17 cells and for IL-17 and by ILC3 (79) (**Table 2**). Although RORs are thought to be orphan nuclear receptors, various oxygen steroids can activate RORγt, particularly the cholesterol precursor and the cholesterol metabolite 25-hydroxycholesterol (80).

4.4 PXR

The progesterone X receptor (PXR) is a member of the nuclear receptor superfamily and is expressed in the liver and intestine as well as in other tissues and cells. The PXR is activated by a variety of endogens, dietary compounds and drugs (81). This nuclear receptor is a major transcriptional regulator of CYP3A isozymes and also regulates a large number of enzymes and transporters involved in the pharmacokinetics of endogenous and exogenous drugs (82). PXR affects energy balance by regulating glucolipid metabolism. Activation of the PXR also protects the liver from toxic BA. A novel role for the PXR in intrahepatic homeostasis, inflammatory bowel disease, hepatic steatosis and fibrous formation has been demonstrated. PXR directly regulates the expression of multidrug resistance protein 1 (MDR1) and other important proteins involved in drug metabolism (83).

Drugs and Agents: Profiling of BARs

Considering all research highlighting the anti-inflammatory activity of BA over the last recent years, the interest of the pharmaceutical industry has driven the development of several specific agonists for BARs. **Table 3** summarizes clinical projects on bile acids for the treatment of diseases. In parallel, some drugs classically used for other therapeutic purposes (e.g., rifaximin) which demonstrated BAR activity has been recently proposed as a regulator of immune cells phenotype. Another strategy consists in using specific strains of gut bacteria to regulate BA production in the intestine. Additionally, dietary supplements might be adapted to the target treatments to optimize BA production and BA metabolites.

Synthetic agonists of BARs

Given that several studies have pinpointed a potential beneficial effect of BA for the treatment of inflammatory diseases, recent research has focused on developing synthetic agonists of BARs to improve their bioavailability and modulate immunological response to improve the therapeutic outcome of inflammatory diseases.

FXR

Among the synthetic FXR agonist, OCA, INT-767, and GW4064 have shown the best pharmacological activity and therapeutic outcomes in pre-clinical and translational studies. For instance, oral administration of OCA promoted a good therapeutic response in experimental models of colitis (84, 85). Such an effect was accompanied by decreased leukocyte infiltration and reduction of pro-inflammatory cytokines in the colon, such as IL1β, IL6, and MCP1 (84). *In vitro* analysis demonstrated that OCA prevented TNFα secretion by peripheral blood mononuclear cells (PBMCs), monocytes, dendritic cells (84), and mucosal-associated invariant T cells (86). Further, OCA inhibited the secretion of IFNγ, IL17, TNFα, and IL1β by lamina propria-derived mononuclear cells (84, 85). Similarly, INT-767 improved disease progression in experimental models of non-alcoholic liver diseases (87, 88), such effects have been associated with inhibition of NFκB activation, reduced TNFα production (87), and inhibition of macrophage recruitment to the liver (88). Further, *in vitro* INT-767 treatment inhibited the production of pro-inflammatory cytokines (TNFα, IL1β, and MCP-1) and increased IL10 by bone marrow-derived macrophages (88).

The synthetic FXR agonist GW064 has also shown promising results in pre-clinical studies. For instance, oral administration of GW4064 (twice/daily) increased fasting plasma corticosteroid levels in C57BL/6 mice

(89). Treatment with GW4064 prevented exacerbated macrophage infiltration on the liver of a murine model of endotoxin-induced hepatic inflammation in non-alcoholic fatty liver disease (90). Further, the authors have shown that LPS-induced mRNA expression of TNFα, MCP-1, IL1β, and IL6 in high-fat diet fed mice was attenuated by GW4064 treatment (90). In addition, by studying a murine model of diet-induced obesity and hepatic steatosis, GW4064 treatment significantly suppressed the diet-induced elevation of macrophage markers in the liver, such as F4/80, CD68, CD11c and CD11b (91). The protective effect of GW4064 on liver diseases was further confirmed by demonstrating that GW4064 attenuated LPS-induced expression of TNFα and increased the expression of IL10 in murine Kupffer cells, resident macrophages of the liver (92). Remarkably, the FXR agonist OCA showed a beneficial effect in mice subjected to experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (93). In part, this effect was mediated by inhibition of lymphocyte activation, in particular a decrease in $CD4^+$ T cells and $CD19^+$ B cells associated with downregulation of VLA4 (α4β1 integrin), which is necessary for lymphocyte extravasation across the blood-brain barrier (93).

TGR5

Several pre-clinical studies have shown the beneficial effects of synthetic TGR5 agonists for modulating immune response and improving therapeutic outcomes. In particular, INT-777 has been extensively used due its anti-inflammatory activity in immune cells. For instance,*in vitro* pre-treatment of bone marrow-derived macrophages with INT-777 prevented LPS-induced expression of TNFα, IL6, CXCL-10 and MCP-1, while it increased expression of the anti-inflammatory cytokine IL10 (93, 94). *In vitro* pre-treatment of mouse peritoneal macrophages (PEMs) with INT-777 inhibited vesicular stomatitis virus (VSV) infection in a concentration dependent manner. Besides, INT-777 treatment induced IFNβ expression in PEMs exposed to VSV and herpes simplex virus type 1 (HSV-1) via AKT-mediated IRF3 activation (94, 95).*In vitro* treatment of primary macrophages with INT-777 reduced the ability of macrophage recruitment towards the chemokine CCL2. Further, INT-777 treatment attenuated the LPS-dependent genic upregulation of CCL2, CC3 and CCL4 in bone marrow-derived macrophages which was mediated by AKT-dependent activation of mTOR complex 1 (96). Besides its protective effect on *in vitro* studies, INT-777 also has protective effects on experimental disease models. In a mouse model of acute pancreatitis (AP) induced by caerulein, INT-777 reduced serum levels of pro-inflammatory cytokines (TNFα, IL6, and IL1β) and inhibited the activation of the NLRP3 inflammasome pathway (97). By using a murine model of Parkinson's disease (PD), intranasal administration of INT-777 ameliorated motor deficits and cognitive impairment. Further, the authors have found that INT-777 prevented PD-induced microglial activation, which was associated with the downregulation of TNFα and chemokines CCL3 and CCL6 in the brain, preventing leukocyte infiltration into the brain (98). In a rat model of subarachnoid hemorrhage (SAH), intranasal administration of INT-777 prevented microglial activation and neuroinflammation, as evidenced by reduced Iba-1 and CD68 positive microglia. Additionally, the authors observed that INT-777 treatment inhibited NLRP3 activation and expression of IL-6, IL1β and TNFα, resulting in reduced neutrophil infiltration in the brain (99). Lastly, intranasal administration of INT-777 to rats subjected to middle cerebral artery occlusion (MCAO), an experimental model of stroke, reduced infarct volume and improved neurological scores. Further analysis of brain homogenates has shown that INT-777 treatment prevented MCAO-induced NLRP3 activation and production of TNFα, IL1β and IL18 (100).

PXR

Although the synthetic PXR agonists have not been as extensively described as FXR and TGR5 agonists, recent studies showed the promising effects of such drugs. Rifaximin, a poorly absorbed oral antimicrobial agent, has been recently used in the treatment of inflammatory bowel diseases due to its agonistic activity on PXR in the gut. Its anti-inflammatory activity was confirmed by *in vitro* studies showing that pretreatment with rifaximin prevented LPS-induced production of TNFα, IL8, PGE2, and RANTES in supernatants of human colon epithelial cells. Such effect was associated with the suppression of LPS-induced NFκB DNA binding activity, which possibly was mediated through the increased association between PXR and NFκB p65 (101). In a murine model of DSS-induced colitis, treatment with the synthetic PXR agonist PCN

attenuated body weight loss, diarrhoea, rectal bleeding, and reduction of colon length (102). Further mRNA analysis of colonic tissue has shown that PCN treatment downregulated DSS-induced genic expression of several inflammatory markers, such as IL1β, TNFα, IL6, iNOS, and MCP-1. *In vitro* studies using colon cells (HCT116 cells) suggested that such effects were possibly mediated by inhibition of NFKB activation (102). The relevance of PXR agonists on the regulation of immune response was confirmed by a study showing that synthetic PXR agonists PCN and RU-486 decreased the expression of the cell surface activation marker CD25 in murine T lymphocytes, while rifampicin and RU-486 reduced the expression of CD25 in Jurkat T cells (103). Besides, RU486 treatment decreased T lymphocyte-derived IFN in a PXR-dependent manner, suggesting that PXR ligand compromised T lymphocyte function by suppressing CD25 expression and IFNγ. Besides, PCN treatment reduced the level of phosphorylated p65 NFKB and MEK1/2 in activated lymphocytes, which are important signals for lymphocyte proliferation (103).

VDR

The anti-inflammatory activity of VDR has stimulated the development of specific and stable agonists. For instance, EB1089 favors the conversion of Th2 cells in Tregs, which possess immune suppressive functions. In this regard, *in vitro* treatment of $CD4^+$, $CD25^+$, $CD127^+$ cells from ulcerative colitis with EB1089 promoted its conversion into $FoxP3^+$ Tregs, and such cells have the ability to suppress the effector T cell proliferation (104). Synthetic VDR ligands LY2108491 and LY2109866 were efficient in preventing the TPA and PHA-induced genic upregulation of IL2 and IL4, while they increased anti-inflammatory IL10 in PBMCs. Further, VDR agonists decreased Th1 cytokine response *in vivo* , as evidenced by decreased IFNγ and IL2 production by splenocytes from mice treated with LY2109866 (105).

Strategies modulating BA metabolism

Recently, a body of evidence has proposed that the treatment for some diseases such as cholestatic liver disease, fatty liver disease, diabetes mellitus, gallstones, obesity, and metabolic syndrome may benefit from modulating the FXR, PXR, CAR, and VDR targets (106). They make excellent therapeutic targets because they participate in the transport and metabolism of BAs, which are connected to BA signaling and BA pool size and composition. For instance, phase II and phase III clinical trials are currently evaluating the therapeutic effect of UDCA treatment and the synthetic FXR agonist PX-102 (107). The FDA has approved the BA derivative OCA for the treatment of Primary Biliary Cholangitis (PBC) in adults who have an unsatisfactory response to UDCA or as a monotherapy in individuals who are unable to tolerate UDCA (108).

Further studies have designed the 24-norursodeoxycholic acid (norUDCA), a UDCA derivative produced by removing a methylene group, which is more hydrophilic and less toxic than UDCA, and it can be passively absorbed by cholangiocytes (109). As a consequence of norUDCA-induced secretion of bicarbonate (HCO_3^-) from biliary cells, an alkaline "umbrella" is formed on the apical surface of cholangiocytes, which inhibits the entry of apolar hydrophobic BAs into cholangiocytes and hepatocytes (109).

Noticeably, numerous studies have demonstrated the GM's potential therapeutic advantages in the management of hyperammonemia. For instance, the administration of *Lactobacillus plantarum* , which has been genetically modified to consume ammonia, showed benefits in comparison to wild-type bacteria (110). In this regard, mice treated with the ammonia-hyperconsuming *L. plantarum* strain had reduced ammonia levels in the blood and feces and increased survival rates (110). Further, the microencapsulated *L. plantarum* 80 (pCBH1) was demonstrated to effectively degrade and eliminate BAs (GDCA and TDCA)*in vitro* , supporting this theory (111). Such techniques could be more advantageous than modulating the gut microbiome. Additionally, genetically modified strains are more productive and strive for certain objectives rather than the whole microbial community.

The diverse BA compositions in mice and humans, however, encourage various physical challenges and intervention effects. For instance, mice produce tauro-muricholic acid (T-MCA), a powerful FXR antagonist that can preserve intestinal integrity and act as an intestinal FXR antagonist because it is resistant to bacterial bile salt hydrolase (112). As a result, oral administration of the T-MCA derivative glycine-muricholic acid

(Gly-MCA) ameliorated insulin resistance, hepatic steatosis, and obesity linked to lower blood ceramide levels (112). In addition, patients may not translate mouse-based data prompt BA control in glycemic reactions, in the same way, therefore such findings should be carefully analyzed before clinical trials.

Last but not least, SCFA is recognized as the primary byproduct of saccharide microbe fermentation in the gut (113). Short-chain fatty acids (SCFAs) have the potential to reduce the severity of immune-mediated and endotoxin-induced uveitis (114). Recent investigations have also shown that people with bowel disease had high amounts of fecal acetate and low levels of fecal butyrate (115). Additionally, people with bowel diseases' feces showed decreased levels of bacteria that produce butyrate (11).

Conclusions and Perspectives

Communication between the intestinal immune system and the GM is facilitated by the signaling molecules known as BAs. Leukocytes play a crucial role in the formation of immune cells and express FXR and GPBAR1. These receptors are essential for intestinal macrophage immunological tolerance by gene knockout studies. Recent research demonstrates that various BA oxo-derivatives regulate the immune system via influencing RORγt activity. These BA oxo-derivatives function as RORγt inverse agonists, specifically inhibiting Th17 cell-specific inflammation.

Further understanding of BAs and their receptors, FXR, GPBAR1, and RORt, enables one to comprehend the communication between the immune system and the intestinal microbiota. In addition, studies have shown that using diet supplements like SCFA to manipulate the GM or sharing healthy people's microorganisms with others may have advantages.

Acknowledgement

Compliance with ethics guidelines

No conflict of interest.

Reference

1. Sahin M, Kayadibi H. Importance of the bile acid receptors in different metabolisms. Integrative Obesity and Diabetes. 2017;3(6).

2. Parks DJ, Blanchard SG, Bledsoe RK, Chandra G, Consler TG, Kliewer SA, et al. Bile Acids: Natural Ligands for an Orphan Nuclear Receptor. Science. 1999;284:1365-8.

3. Keitel V, Stindt J, Haussinger D. Bile Acid-Activated Receptors: GPBAR1 (TGR5) and Other G Protein-Coupled Receptors. Handb Exp Pharmacol. 2019;256:19-49.

4. Wahlstrom A, Sayin SI, Marschall HU, Backhed F. Intestinal Crosstalk between Bile Acids and Microbiota and Its Impact on Host Metabolism. Cell Metab. 2016;24(1):41-50.

5. Thibaut MM, Bindels LB. Crosstalk between bile acid-activated receptors and microbiome in entero-hepatic inflammation. Trends Mol Med. 2022;28(3):223-36.

6. Li T, Chiang JY. Bile acid signaling in metabolic disease and drug therapy. Pharmacological reviews. 2014;66(4):948-83.

7. Zollner G, Trauner M. Mechanisms of cholestasis. Clin Liver Dis. 2008;12(1):1-26, vii.

8. Huang X, Ye Z, Cao Q, Su G, Wang Q, Deng J, et al. Gut Microbiota Composition and Fecal Metabolic Phenotype in Patients With Acute Anterior Uveitis. Invest Ophthalmol Vis Sci. 2018;59(3):1523-31.

9. Nakamura YK, Metea C, Karstens L, Asquith M, Gruner H, Moscibrocki C, et al. Gut Microbial Alterations Associated With Protection From Autoimmune Uveitis. Invest Ophthalmol Vis Sci. 2016;57(8):3747-58.

10. Tremlett H, Fadrosh DW, Faruqi AA, Zhu F, Hart J, Roalstad S, et al. Gut microbiota in early pediatric multiple sclerosis: a case-control study. Eur J Neurol. 2016;23(8):1308-21.

11. Ye Z, Zhang N, Wu C, Zhang X, Wang Q, Huang X, et al. A metagenomic study of the gut microbiome in Behcet's disease. Microbiome. 2018;6(1):135.

12. Hu J, Wang C, Huang X, Yi S, Pan S, Zhang Y, et al. Gut microbiota-mediated secondary bile acids regulate dendritic cells to attenuate autoimmune uveitis through TGR5 signaling. Cell Rep. 2021;36(12):109726.

13. de Aguiar Vallim TQ, Tarling EJ, Edwards PA. Pleiotropic roles of bile acids in metabolism. Cell Metab. 2013;17(5):657-69.

14. Thomas C, Pellicciari R, Pruzanski M, Auwerx J, Schoonjans K. Targeting bile-acid signalling for metabolic diseases. Nat Rev Drug Discov. 2008;7(8):678-93.

15. Li T, Chiang JY. Bile acid signaling in metabolic disease and drug therapy. Pharmacol Rev. 2014;66(4):948-83.

16. Fiorucci S, Distrutti E, Biagioli M. Special FX: Harnessing the Farnesoid-X-Receptor to Control Bile Acid Synthesis. Dig Dis Sci. 2021;66(11):3668-71.

17. Winston JA, Theriot CM. Diversification of host bile acids by members of the gut microbiota. Gut Microbes. 2020;11(2):158-71.

18. Wu L, Feng J, Li J, Yu Q, Ji J, Wu J, et al. The gut microbiome-bile acid axis in hepatocarcinogenesis. Biomed Pharmacother. 2021;133:111036.

19. <oncotarget-08-115736.pdf>.

20. Vitek L. Bile acid malabsorption in inflammatory bowel disease. Inflamm Bowel Dis. 2015;21(2):476-83.

21. Kalyana Chakravarthy S, Jayasudha R, Sai Prashanthi G, Ali MH, Sharma S, Tyagi M, et al. Dysbiosis in the Gut Bacterial Microbiome of Patients with Uveitis, an Inflammatory Disease of the Eye. Indian J Microbiol. 2018;58(4):457-69.

22. Maukonen J, Kolho KL, Paasela M, Honkanen J, Klemetti P, Vaarala O, et al. Altered Fecal Microbiota in Paediatric Inflammatory Bowel Disease. J Crohns Colitis. 2015;9(12):1088-95.

23. Stoll ML, Kumar R, Morrow CD, Lefkowitz EJ, Cui X, Genin A, et al. Altered microbiota associated with abnormal humoral immune responses to commensal organisms in enthesitis-related arthritis. Arthritis Res Ther. 2014;16(6):486.

24. Candon S, Perez-Arroyo A, Marquet C, Valette F, Foray AP, Pelletier B, et al. Antibiotics in early life alter the gut microbiome and increase disease incidence in a spontaneous mouse model of autoimmune insulin-dependent diabetes. PLoS One. 2015;10(5):e0125448.

25. Just S, Mondot S, Ecker J, Wegner K, Rath E, Gau L, et al. The gut microbiota drives the impact of bile acids and fat source in diet on mouse metabolism. Microbiome. 2018;6(1):134.

26. Song X, Sun X, Oh SF, Wu M, Zhang Y, Zheng W, et al. Microbial bile acid metabolites modulate gut RORgamma(+) regulatory T cell homeostasis. Nature. 2020;577(7790):410-5.

27. Ramanan D, Sefik E, Galvan-Pena S, Wu M, Yang L, Yang Z, et al. An Immunologic Mode of Multigenerational Transmission Governs a Gut Treg Setpoint. Cell. 2020;181(6):1276-90 e13.

28. Visan I. RORγt+ Treg cells. Nature Immunology. 2015;16(9):906-.

29. Yang M, Gu Y, Li L, Liu T, Song X, Sun Y, et al. Bile Acid–Gut Microbiota Axis in Inflammatory Bowel Disease: From Bench to Bedside. Nutrients. 2021;13(9).

30. De Boever P, Wouters R, Verschaeve L, Berckmans P, Schoeters G, Verstraete W. Protective effect of the bile salt hydrolase-active Lactobacillus reuteri against bile salt cytotoxicity. Appl Microbiol Biotechnol. 2000;53(6):709-14.

31. Begley M, Gahan CG, Hill C. The interaction between bacteria and bile. FEMS Microbiol Rev. 2005;29(4):625-51.

32. Staley C, Weingarden AR, Khoruts A, Sadowsky MJ. Interaction of gut microbiota with bile acid metabolism and its influence on disease states. Appl Microbiol Biotechnol. 2017;101(1):47-64.

33. Zhan K, Zheng H, Li J, Wu H, Qin S, Luo L, et al. Gut Microbiota-Bile Acid Crosstalk in Diarrhea-Irritable Bowel Syndrome. Biomed Res Int. 2020;2020:3828249.

34. D'Aldebert E, Biyeyeme Bi Mve MJ, Mergey M, Wendum D, Firrincieli D, Coilly A, et al. Bile salts control the antimicrobial peptide cathelicidin through nuclear receptors in the human biliary epithelium. Gastroenterology. 2009;136(4):1435-43.

35. Inagaki T, Moschetta A, Lee YK, Peng L, Zhao G, Downes M, et al. Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. Proc Natl Acad Sci U S A. 2006;103(10):3920-5.

36. Van den Bossche L, Borsboom D, Devriese S, Van Welden S, Holvoet T, Devisscher L, et al. Tauroursodeoxycholic acid protects bile acid homeostasis under inflammatory conditions and dampens Crohn's disease-like ileitis. Lab Invest. 2017;97(5):519-29.

37. Ward JBJ, Lajczak NK, Kelly OB, O'Dwyer AM, Giddam AK, Ni Gabhann J, et al. Ursodeoxycholic acid and lithocholic acid exert anti-inflammatory actions in the colon. Am J Physiol Gastrointest Liver Physiol. 2017;312(6):G550-G8.

38. Jia W, Xie G, Jia W. Bile acid–microbiota crosstalk in gastrointestinal inflammation and carcinogenesis. Nature Reviews Gastroenterology & Hepatology. 2017;15(2):111-28.

39. Hylemon PB, Zhou H, Pandak WM, Ren S, Gil G, Dent P. Bile acids as regulatory molecules. J Lipid Res. 2009;50(8):1509-20.

40. Fiorucci S, Biagioli M, Zampella A, Distrutti E. Bile Acids Activated Receptors Regulate Innate Immunity. Front Immunol. 2018;9:1853.

41. Biagioli M, Marchiano S, Carino A, Di Giorgio C, Santucci L, Distrutti E, et al. Bile Acids Activated Receptors in Inflammatory Bowel Disease. Cells. 2021;10(6).

42. Thaiss CA, Zmora N, Levy M, Elinav E. The microbiome and innate immunity. Nature. 2016;535(7610):65-74.

43. Maruyama T, Miyamoto Y, Nakamura T, Tamai Y, Okada H, Sugiyama E, et al. Identification of membrane-type receptor for bile acids (M-BAR). Biochem Biophys Res Commun. 2002;298(5):714-9.

44. Vavassori P, Mencarelli A, Renga B, Distrutti E, Fiorucci S. The bile acid receptor FXR is a modulator of intestinal innate immunity. J Immunol. 2009;183(10):6251-61.

45. Mencarelli A, Renga B, Migliorati M, Cipriani S, Distrutti E, Santucci L, et al. The bile acid sensor farnesoid X receptor is a modulator of liver immunity in a rodent model of acute hepatitis. J Immunol. 2009;183(10):6657-66.

46. Fiorucci S, Distrutti E, Carino A, Zampella A, Biagioli M. Bile acids and their receptors in metabolic disorders. Prog Lipid Res. 2021;82:101094.

47. Pols TW, Noriega LG, Nomura M, Auwerx J, Schoonjans K. The bile acid membrane receptor TGR5 as an emerging target in metabolism and inflammation. J Hepatol. 2011;54(6):1263-72.

48. Kang JH, Kim M, Yim M. FXR/TGR5 mediates inflammasome activation and host resistance to bacterial infection. Biochem Biophys Rep. 2021;27:101051.

49. Guo C, Chen WD, Wang YD. TGR5, Not Only a Metabolic Regulator. Front Physiol. 2016;7:646.

50. Perino A, Demagny H, Velazquez-Villegas L, Schoonjans K. Molecular Physiology of Bile Acid Signaling in Health, Disease, and Aging. Physiol Rev. 2021;101(2):683-731.

51. Kawamata Y, Fujii R, Hosoya M, Harada M, Yoshida H, Miwa M, et al. A G protein-coupled receptor responsive to bile acids. J Biol Chem. 2003;278(11):9435-40.

52. Wang YD, Chen WD, Yu D, Forman BM, Huang W. The G-protein-coupled bile acid receptor, Gpbar1 (TGR5), negatively regulates hepatic inflammatory response through antagonizing nuclear factor kappa light-chain enhancer of activated B cells (NF-kappaB) in mice. Hepatology. 2011;54(4):1421-32.

53. Keitel V, Gorg B, Bidmon HJ, Zemtsova I, Spomer L, Zilles K, et al. The bile acid receptor TGR5 (Gpbar-1) acts as a neurosteroid receptor in brain. Glia. 2010;58(15):1794-805.

54. Prenzel N, Zwick E, Daub H, Leserer M, Abraham R, Wallasch C, et al. EGF receptor transactivation by G-protein-coupled receptors requires metalloproteinase cleavage of proHB-EGF. Nature. 1999;402(6764):884-8.

55. Pelengaris S, Khan M, Evan G. c-MYC: more than just a matter of life and death. Nat Rev Cancer. 2002;2(10):764-76.

56. Calmus Y, Poupon R. Shaping macrophages function and innate immunity by bile acids: mechanisms and implication in cholestatic liver diseases. Clin Res Hepatol Gastroenterol. 2014;38(5):550-6.

57. Yoneno K, Hisamatsu T, Shimamura K, Kamada N, Ichikawa R, Kitazume MT, et al. TGR5 signalling inhibits the production of pro-inflammatory cytokines by in vitro differentiated inflammatory and intestinal macrophages in Crohn's disease. Immunology. 2013;139(1):19-29.

58. Haselow K, Bode JG, Wammers M, Ehlting C, Keitel V, Kleinebrecht L, et al. Bile acids PKA-dependently induce a switch of the IL-10/IL-12 ratio and reduce proinflammatory capability of human macrophages. J Leukoc Biol. 2013;94(6):1253-64.

59. Chanda D, Park JH, Choi HS. Molecular basis of endocrine regulation by orphan nuclear receptor Small Heterodimer Partner. Endocr J. 2008;55(2):253-68.

60. Biagioli M, Carino A, Fiorucci C, Marchiano S, Di Giorgio C, Roselli R, et al. GPBAR1 Functions as Gatekeeper for Liver NKT Cells and provides Counterregulatory Signals in Mouse Models of Immune-Mediated Hepatitis. Cell Mol Gastroenterol Hepatol. 2019;8(3):447-73.

61. Shin DJ, Wang L. Bile Acid-Activated Receptors: A Review on FXR and Other Nuclear Receptors. Handb Exp Pharmacol. 2019;256:51-72.

62. Yang Z, Koehler AN, Wang L. A Novel Small Molecule Activator of Nuclear Receptor SHP Inhibits HCC Cell Migration via Suppressing Ccl2. Mol Cancer Ther. 2016;15(10):2294-301.

63. Islam KB, Fukiya S, Hagio M, Fujii N, Ishizuka S, Ooka T, et al. Bile acid is a host factor that regulates the composition of the cecal microbiota in rats. Gastroenterology. 2011;141(5):1773-81.

64. Massafra V, Ijssennagger N, Plantinga M, Milona A, Ramos Pittol JM, Boes M, et al. Splenic dendritic cell involvement in FXR-mediated amelioration of DSS colitis. Biochim Biophys Acta. 2016;1862(2):166-73.

65. Ma C, Han M, Heinrich B, Fu Q, Zhang Q, Sandhu M, et al. Gut microbiome-mediated bile acid metabolism regulates liver cancer via NKT cells. Science. 2018;360(6391).

66. Willart MA, van Nimwegen M, Grefhorst A, Hammad H, Moons L, Hoogsteden HC, et al. Ursodeoxycholic acid suppresses eosinophilic airway inflammation by inhibiting the function of dendritic cells through

the nuclear farnesoid X receptor. Allergy. 2012;67(12):1501-10.

67. Gadaleta RM, Oldenburg B, Willemsen EC, Spit M, Murzilli S, Salvatore L, et al. Activation of bile salt nuclear receptor FXR is repressed by pro-inflammatory cytokines activating NF-kappaB signaling in the intestine. Biochim Biophys Acta. 2011;1812(8):851-8.

68. Guo H, Callaway JB, Ting JP. Inflammasomes: mechanism of action, role in disease, and therapeutics. Nature medicine. 2015;21(7):677-87.

69. Guo C, Xie S, Chi Z, Zhang J, Liu Y, Zhang L, et al. Bile Acids Control Inflammation and Metabolic Disorder through Inhibition of NLRP3 Inflammasome. Immunity. 2016;45(4):802-16.

70. Yang CS, Kim JJ, Kim TS, Lee PY, Kim SY, Lee HM, et al. Small heterodimer partner interacts with NLRP3 and negatively regulates activation of the NLRP3 inflammasome. Nat Commun. 2015;6:6115.

71. Hao H, Cao L, Jiang C, Che Y, Zhang S, Takahashi S, et al. Farnesoid X Receptor Regulation of the NLRP3 Inflammasome Underlies Cholestasis-Associated Sepsis. Cell Metab. 2017;25(4):856-67 e5.

72. Jetten A, Ueda E. Retinoid-related orphan receptors (RORs): roles in cell survival, differentiation and disease. Cell death and differentiation. 2002;9(11):1167-71.

73. Jetten AM. Retinoid-related orphan receptors (RORs): critical roles in development, immunity, circadian rhythm, and cellular metabolism. Nuclear receptor signaling. 2009;7(1):nrs. 07003.

74. Hang S, Paik D, Yao L, Kim E, Trinath J, Lu J, et al. Bile acid metabolites control TH17 and Treg cell differentiation. Nature. 2019;576(7785):143-8.

75. Ma S, Patel SA, Abe Y, Chen N, Patel PR, Cho BS, et al. RORγt phosphorylation protects against T cell-mediated inflammation. Cell reports. 2022;38(11):110520.

76. Cherrier M, Sawa S, Eberl G. Notch, Id2, and RORγt sequentially orchestrate the fetal development of lymphoid tissue inducer cells. Journal of Experimental Medicine. 2012;209(4):729-40.

77. Cook DN, Kang HS, Jetten AM. Retinoic Acid-Related Orphan Receptors (RORs): Regulatory Functions in Immunity, Development, Circadian Rhythm, and Metabolism. Nucl Receptor Res. 2015;2.

78. Montaldo E, Juelke K, Romagnani C. Group 3 innate lymphoid cells (ILC3s): Origin, differentiation, and plasticity in humans and mice. Eur J Immunol. 2015;45(8):2171-82.

79. Lochner M, Ohnmacht C, Presley L, Bruhns P, Si-Tahar M, Sawa S, et al. Microbiota-induced tertiary lymphoid tissues aggravate inflammatory disease in the absence of RORgamma t and LTi cells. J Exp Med. 2011;208(1):125-34.

80. Tuong ZK, Lau P, Du X, Condon ND, Goode JM, Oh TG, et al. RORα and 25-hydroxycholesterol crosstalk regulates lipid droplet homeostasis in macrophages. PLoS One. 2016;11(1):e0147179.

81. Willson TM, Kliewer SA. PXR, CAR and drug metabolism. Nature reviews Drug discovery. 2002;1(4):259-66.

82. Pavek P. Pregnane X receptor (PXR)-mediated gene repression and cross-talk of PXR with other nuclear receptors via coactivator interactions. Frontiers in pharmacology. 2016;7:456.

83. Kast HR, Goodwin B, Tarr PT, Jones SA, Anisfeld AM, Stoltz CM, et al. Regulation of multidrug resistance-associated protein 2 (ABCC2) by the nuclear receptors pregnane X receptor, farnesoid X-activated receptor, and constitutive androstane receptor. Journal of Biological Chemistry. 2002;277(4):2908-15.

84. Gadaleta RM, Van Erpecum KJ, Oldenburg B, Willemsen EC, Renooij W, Murzilli S, et al. Farnesoid X receptor activation inhibits inflammation and preserves the intestinal barrier in inflammatory bowel disease. Gut. 2011;60(4):463-72.

85. Vavassori P, Mencarelli A, Renga B, Distrutti E, Fiorucci S. The bile acid receptor FXR is a modulator of intestinal innate immunity. The Journal of Immunology. 2009;183(10):6251-61.

86. Mendler A, Pierzchalski A, Bauer M, Röder S, Sattler A, Standl M, et al. MAIT cell activation in adolescents is impacted by bile acid concentrations and body weight. Clinical & Experimental Immunology. 2020;200(2):199-213.

87. Hu Y-B, Liu X-Y, Zhan W. Farnesoid X receptor agonist INT-767 attenuates liver steatosis and inflammation in rat model of nonalcoholic steatohepatitis. Drug design, development and therapy. 2018;12:2213.

88. McMahan RH, Wang XX, Cheng LL, Krisko T, Smith M, El Kasmi K, et al. Bile acid receptor activation modulates hepatic monocyte activity and improves nonalcoholic fatty liver disease. Journal of Biological Chemistry. 2013;288(17):11761-70.

89. Hoekstra M, van der Sluis RJ, Li Z, Oosterveer MH, Groen AK, Van Berkel TJ. FXR agonist GW4064 increases plasma glucocorticoid levels in C57BL/6 mice. Molecular and cellular endocrinology. 2012;362(1-2):69-75.

90. Yao J, Zhou C-S, Ma X, Fu B-Q, Tao L-S, Chen M, et al. FXR agonist GW4064 alleviates endotoxin-induced hepatic inflammation by repressing macrophage activation. World Journal of Gastroenterology: WJG. 2014;20(39):14430.

91. Ma Y, Huang Y, Yan L, Gao M, Liu D. Synthetic FXR agonist GW4064 prevents diet-induced hepatic steatosis and insulin resistance. Pharmaceutical research. 2013;30(5):1447-57.

92. Jin D, Lu T, Ni M, Wang H, Zhang J, Zhong C, et al. Farnesoid X Receptor Activation Protects Liver From Ischemia/Reperfusion Injury by Up-Regulating Small Heterodimer Partner in Kupffer Cells. Hepatology communications. 2020;4(4):540-54.

93. Ho PP, Steinman L. Obeticholic acid, a synthetic bile acid agonist of the farnesoid X receptor, attenuates experimental autoimmune encephalomyelitis. Proceedings of the National Academy of Sciences. 2016;113(6):1600-5.

94. Rao J, Yang C, Yang S, Lu H, Hu Y, Lu L, et al. Deficiency of TGR5 exacerbates immune-mediated cholestatic hepatic injury by stabilizing the β-catenin destruction complex. International immunology. 2020;32(5):321-34.

95. Xiong Q, Huang H, Wang N, Chen R, Chen N, Han H, et al. Metabolite-sensing G protein coupled receptor TGR5 protects host from viral infection through amplifying type I interferon responses. Frontiers in immunology. 2018;9:2289.

96. Perino A, Pols TWH, Nomura M, Stein S, Pellicciari R, Schoonjans K. TGR5 reduces macrophage migration through mTOR-induced C/EBPβ differential translation. The Journal of clinical investigation. 2014;124(12):5424-36.

97. Li B, Yang N, Li C, Li C, Gao K, Xie X, et al. INT-777, a bile acid receptor agonist, extenuates pancreatic acinar cells necrosis in a mouse model of acute pancreatitis. Biochemical and biophysical research communications. 2018;503(1):38-44.

98. Huang R, Gao Y, Chen J, Duan Q, He P, Zhang J, et al. TGR5 Agonist INT-777 Alleviates Inflammatory Neurodegeneration in Parkinson's Disease Mouse Model by Modulating Mitochondrial Dynamics in Microglia. Neuroscience. 2022;490:100-19.

99. Hu X, Yan J, Huang L, Araujo C, Peng J, Gao L, et al. INT-777 attenuates NLRP3-ASC inflammasome-mediated neuroinflammation via TGR5/cAMP/PKA signaling pathway after subarachnoid hemorrhage in rats. Brain, behavior, and immunity. 2021;91:587-600.

100. Liang H, Matei N, McBride DW, Xu Y, Zhou Z, Tang J, et al. TGR5 activation attenuates neuroinflammation via Pellino3 inhibition of caspase-8/NLRP3 after middle cerebral artery occlusion in rats.

Journal of neuroinflammation. 2021;18(1):1-13.

101. Mencarelli A, Renga B, Palladino G, Ricci P, Distrutti E, Barbanti M, et al. Inhibition of NF-κB by a PXR-dependent pathway mediates counter-regulatory activities of rifaximin on innate immunity in intestinal epithelial cells. European journal of pharmacology. 2011;668(1-2):317-24.

102. Shah YM, Ma X, Morimura K, Kim I, Gonzalez FJ. Pregnane X receptor activation ameliorates DSS-induced inflammatory bowel disease via inhibition of NF-κB target gene expression. American Journal of Physiology-Gastrointestinal and Liver Physiology. 2007;292(4):G1114-G22.

103. Dubrac S, Elentner A, Ebner S, Horejs-Hoeck J, Schmuth M. Modulation of T lymphocyte function by the pregnane X receptor. The Journal of Immunology. 2010;184(6):2949-57.

104. Lu D, Lan B, Din Z, Chen H, Chen G. A vitamin D receptor agonist converts CD4+ T cells to Foxp3+ regulatory T cells in patients with ulcerative colitis. Oncotarget. 2017;8(32):53552.

105. Ma Y, Khalifa B, Yee YK, Lu J, Memezawa A, Savkur RS, et al. Identification and characterization of noncalcemic, tissue-selective, nonsecosteroidal vitamin D receptor modulators. The Journal of clinical investigation. 2006;116(4):892-904.

106. Boyer JL. Nuclear receptor ligands: rational and effective therapy for chronic cholestatic liver disease? Gastroenterology. 2005;129(2):735-40.

107. Nevens F, Andreone P, Mazzella G, Strasser SI, Bowlus C, Invernizzi P, et al. A Placebo-Controlled Trial of Obeticholic Acid in Primary Biliary Cholangitis. N Engl J Med. 2016;375(7):631-43.

108. De Magalhaes Filho CD, Downes M, Evans R. Bile Acid Analog Intercepts Liver Fibrosis. Cell. 2016;166(4):789.

109. Hohenester S, Wenniger LM, Paulusma CC, van Vliet SJ, Jefferson DM, Elferink RP, et al. A biliary HCO3- umbrella constitutes a protective mechanism against bile acid-induced injury in human cholangiocytes. Hepatology. 2012;55(1):173-83.

110. Nicaise C, Prozzi D, Viaene E, Moreno C, Gustot T, Quertinmont E, et al. Control of acute, chronic, and constitutive hyperammonemia by wild-type and genetically engineered Lactobacillus plantarum in rodents. Hepatology. 2008;48(4):1184-92.

111. Jones ML, Chen H, Ouyang W, Metz T, Prakash S. Microencapsulated Genetically Engineered Lactobacillus plantarum 80 (pCBH1) for Bile Acid Deconjugation and Its Implication in Lowering Cholesterol. J Biomed Biotechnol. 2004;2004(1):61-9.

112. Li F, Jiang C, Krausz KW, Li Y, Albert I, Hao H, et al. Microbiome remodelling leads to inhibition of intestinal farnesoid X receptor signalling and decreased obesity. Nat Commun. 2013;4:2384.

113. Cummings JH, Pomare EW, Branch WJ, Naylor CP, Macfarlane GT. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. Gut. 1987;28(10):1221-7.

114. Chen X, Su W, Wan T, Yu J, Zhu W, Tang F, et al. Sodium butyrate regulates Th17/Treg cell balance to ameliorate uveitis via the Nrf2/HO-1 pathway. Biochem Pharmacol. 2017;142:111-9.

115. Consolandi C, Turroni S, Emmi G, Severgnini M, Fiori J, Peano C, et al. Behcet's syndrome patients exhibit specific microbiome signature. Autoimmun Rev. 2015;14(4):269-76.

Figure 1. BAs Synthesis. Primary BAs in hepatocytes are blue and secondary BAs in the intestines are orange. It represents sites of hydroxylation on the steroid nucleus of the most common bile acid species. Microbiota modification by enzymes or reaction steps is illustrated by (Asterisks). G, glycine-conjugated species; T, taurine-conjugated species.

Figure 2. Bile Acids Activated Receptors Regulate Innate Immunity. (Left panel) BAs are absorbed by the enterocytes through the apical transporter ASBT. After transporting through portal blood, BAs can be

transported back to the liver. Primary BAs are converted into secondary BAs by the gut microbiome through modifications such as dehydroxylation, and oxidation. (Right panel) Gut microbial mediated secondary BA regulate DCs to attenuate inflammation through TGR5. DCs induce TH1 and TH17 cells by producing pro-inflammatory cytokines such as IL1b and IL6. TGR5, a bile acid-activated receptor, is also highly expressed in NKTs and monocytes/macrophages cells.

Table 1 The Classification and Conversions of Common BA Species.

Table 2 Summary of BARs expression and Mode of Action in Immunity are shown in Table.2, as well as the expression and role of G-protein bile acid receptor 1 (GPBAR1), Farnesoid-X-receptor (FXR), and RORγt in immune cells. GPBAR1 and FXR express on dendritic cells and macrophages. And RORγt is expressed by ILCs and T helper cells. In macrophages, triggering of these receptors by BAs activate a polarization toward the anti-inflammatory M2 phenotype with an increase of IL-10 and mediates anti-inflammatory functions. BAs act on the DCs and downregulate TNF-α and IL-12 and their maturation and differentiation. Studies have also shown that oxo-bile acid derivatives, particularly 3-oxo-LCA, can bind RORγt by acting as an inverse agonist and reduce ILC and IL-17 in T helper cells by inhibiting polarisation to ILC3 and Th17 isoforms.

Table 3 Summary of clinical projects on bile acids for the treatment of diseases.

Class	**Metabolic Conversions**	**Bile Acids**	
Primary bile acids	From cholesterol by hepatic classical (neutral) or alternative (acidic) pathways involving >17 enzymes	Humans	CA, CDCA
		Mice	CA, CDCA, UDCA, αMCA, βMCA
Secondary bile acids	From primary bile acids, through gut microbial 7-dehydroxylation	Mice	DCA, LCA, MDCA
	From primary or secondary bile acids, through gut microbial 7α/β-epimerization	Humans	UDCA
		Mice	ωMCA
	through gut microbial 3α/β-epimerization through gut microbial 5β/α-epimerization through gut microbial oxidation		iso-bile acids allo-bile acids oxo- (keto-) bile acids

Cells	**Receptors**	**Modes**	**Immune Responses**	
Macrophages	GPBAR1, FXR	Effects of BAs binding on receptors (agonism)	↓IFN gamma, IL6, IL8, IL12, TNF alpha, NLRP3/ caspase 1/ IL1b ↑ IL10	↑ Polarization to M2 phenotype
Dendritic cells	GPBAR1, FXR	Effect of BA biding on receptor (agonism)	↓IL6, IL12, TNF alpha, IL1b	↓ Maturation and differentiation
ILCs	RORγt	Effect of oxo BAs binding on receptor (inverse agonism)	↓ILC3 polarization	↓Il17 production
T Helpers cells	RORγt	Effects of Ox BA binding on RORγ t (inverse agonism)	↓ Th17 differentiation ↓ ROR γt	↑ Treg differentiation ↑ Foxp3

Reference	Intervention	Detailed Dosage	Clinical Phase	Treatment Diseases
(115)	Taursodeoxycholic Acid	250 mg four capsules by mouth, twice daily for 16 weeks.	Phase 1&2	Multiple Sclerosis
	Placebo oral capsule	Four capsules by mouth, twice daily for 16 weeks.		
(116)	OCA Tablets and UDCA	OCA 5 mg once daily in combination with UDCA for 24 weeks and then titrating up to 10 mg based on tolerability and response for remainder of double-blind period.	Phase 3	Primary Biliary Cirrhosis (PBC)
	Placebo and UDCA	Placebo once daily in combination with UDCA for 48 weeks.		
(117)	OCA	Starting dose of 10 or 50 mg administered orally once daily, followed by dose titration planned from 10 mg to 25 mg to 50 mg once daily, which could be modified for safety and tolerability issues or to achieve adequate therapeutic response.	Phase 2	
	Placebo	Matching placebo tablets were administered orally once daily.		
(118)	OCA and UDCA	OCA 10 mg, 25 mg, 50 mg once daily in combination with UDCA for 12 weeks.	Phase 2	
	Placebo	Placebo once daily in combination with UDCA for 12 weeks.		
(119)	OCA	Day -14 to Day 0 subjects will stop their usual diarrhoeal medication. Day 1 to Day 15 Obeticholic acid 25mg tablet will be administered to subjects once daily in the morning. Day 16 to day 28 normal diarrhoeal medication may be re-commenced.	Phase 2	Bile Acid Diarrhoea
(120)	OCA	10 mg OCA study medication will be administered orally, once daily, approximately 30 minutes prior to breakfast for 6 weeks.	Phase 2	Moderately Severe Alcoholic Hepatitis
	Placebo	1 tablet of placebo, taken orally daily with water, approximately 30 minutes prior to breakfast for 6 weeks.