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REVIEW

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Novel approaches in IBD therapy: targeting the gut microbiota-bile acid axis

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ABSTRACT

Inflammatory bowel disease (IBD) is a chronic and recurrent condition affecting the gastrointestinal tract. Disturbed gut microbiota and abnormal bile acid (BA) metabolism are notable in IBD, suggesting a bidirectional relationship. Specifically, the diversity of the gut microbiota influences BA composition, whereas altered BA profiles can disrupt the microbiota. IBD patients often exhibit increased primary bile acid and reduced secondary bile acid concentrations due to a diminished bacteria population essential for BA metabolism. This imbalance activates BA receptors, undermining intestinal integrity and immune function. Consequently, targeting the microbiota-BA axis may rectify these disturbances, offering symptomatic relief in IBD. Here, the interplay between gut microbiota and bile acids (BAs) is reviewed, with a particular focus on the role of gut microbiota in mediating bile acid biotransformation, and contributions of the gut microbiota-BA axis to IBD pathology to unveil potential novel therapeutic avenues for IBD.

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1. Introduction

The human gut microbiome is essential for the digestion and breakdown of complex compounds, such as dietary fibers, and the catabolism of peptides and proteins, serving as a defensive barrier against pathogens and playing a pivotal role in immune regulation.[1] The interaction between gut microbiota and their hosts yield metabolites, such as short-chain fatty acids (SCFAs), bile acids (BAs), lipopolysaccharides (LPS), and branched-chain amino acids, which are crucial for the maintenance of intestinal integrity, peripheral tissue function, and modulation of metabolism.[2] BAs are complex enterohepatic-derived hormones with profound effects on systemic metabolism; however, their roles are not yet fully understood. Studies in humans and mice have demonstrated that BAs are involved in regulating intestinal inflammation, tumorigenesis, and immune function.[3] The dynamic interplay between gut microbiota, their BA metabolites, and the host critically affects our metabolic phenotype, immune function, and the onset of diseases such as cancer, inflammatory bowel disease (IBD), and metabolic diseases, including diabetes, nonalcoholic fatty liver disease (NAFLD), and obesity.[4–6]

IBD is a chronic, relapsing disorder of the gastrointestinal tract, encompassing conditions such as Crohn's disease (CD) and ulcerative colitis (UC). Factors influencing IBD include compromised intestinal mucosal barrier function, dysregulation of the intestinal microecology, genetics, and the response of the host immune system. However, the precise causes and mechanisms underlying IBD remain elusive.[7] Research has consistently underscored the pivotal role of gut microbiota in IBD, leading to the application of various microbial therapies such as fecal microbiota transplantation (FMT), probiotics, and engineered bacteria. However, despite their clinical use, the mechanisms by which these microbial therapies exert their effects remain insufficiently understood.[8] Recent advancements in histological techniques have provided new insights into the relationship between gut microbiota and BAs, leading to the emergence of the gut microbiota-bile acid (BA) axis as a novel target for IBD treatment.[9] This review aims to delineate interactions between gut microbiota and BAs, elucidate the mechanisms by

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which gut microbiota influence BA metabolism, and explore the changes in gut microbiota and BAs observed in IBD patients. Additionally, it provides an overview of emerging IBD therapies based on the gut microbiota-BA axis, offering new insights for clinical prevention, treatment, and diagnosis of IBD.

2. Gut microbiota-BA axis

The diverse microorganisms in the human gut facilitate the biotransformation of BAs, affecting both the composition and size of the BA pool. Conversely, changes in BAs can also alter the composition and abundance of the gut microbiota.

2.1. Gut microbiota mediates BA metabolism

Bile acid synthesis is classified into classical and alternative pathways. In humans, the classical pathway predominates, accounting for over 75% of BA synthesis. Primary bile acids (PBAs), such as cholic acid (CA) and chenodeoxycholic acid (CDCA), are produced from cholesterol with the aid of microsomal cytochrome P450 (CYPs) enzymes, including cholesterol 7α-hydroxylase (CYP7A1) and mitochondrial sterol 27-hydroxylase (CYP27A1).[10] Bile acid-CoA synthase (BACS) then converts CA and CDCA into their CoA derivatives, which are subsequently conjugated with glycine or taurine to form glycine-conjugated (predominant in humans) or taurine-conjugated (predominant in mice) BAs through formation of amide bonds catalyzed by bile acid-CoA-amino acid N-acyltransferase (BAAT).[10] The conjugated BAs are secreted from the liver into the bile ducts by the bile salt export pump (BSEP), traverse the duct walls into the gallbladder, where they are concentrated, and finally enter the duodenum.[11] In the ileum, 95% of BAs bind to plasma proteins for hepatic recirculation, while the remaining 5% are excreted in the feces as part of the enterohepatic cycle that repeats 4 to 12 times daily in humans Figure 1.[12,13] Gut microbiota modifies the BA pool composition through several enzymatic actions, including bile salt hydrolase (BSH)-mediated deconjugation to produce free BAs in the liver, *bai* gene-mediated 7α/7β-dehydroxylation to produce lithocholic acid (LCA) and deoxycholic acid (DCA) in the colon, and HSDHs-mediated oxidation and epimerization to produce isocholic acids (β-hydroxy) as well as hydroxylation at C6, such as α-muricholic acid (3α, 6β, 7α-trihydroxy-5β-cholan-24-oic acid, αMCA) and β-muricholic acid (3α, 6β, 7β-trihydroxy-5β-cholan-24-oic acid, βMCA).[14] Muricholic acids (MCAs) primarily found in mice and also detected in infant urine and feces, are among these secondary bile acids (SBAs).[14,15] Furthermore, BAs possess a cyclopentanophenanthrene steroid nucleus, with hydrophilic α-hydroxyl groups above the nucleus plane, and a hydrophobic surface below, contributing to their amphiphilic nature[16] and surface tension reduction between the oil and water phases.[16] Most of the BAs produced by the liver are taurine or glycine conjugates, which not only ensure solubility by lowering the pKa,[17] but also render them impermeable to cell membranes, reducing their critical micellar concentration. These properties of BAs are critical for solubilization of dietary lipids and lipid-soluble vitamins,[17] with the enterohepatic cycle described above enabling efficient use of BAs to maintain homeostasis.

2.2. BAs reshape the gut microbiota composition

BAs serve as potent antimicrobial agents that are essential to the innate immunity of the intestine.[18,19] Their bactericidal action is based on damaging the cell membrane, which subsequently results in leakage of intracellular contents.[20] At pH7, unconjugated BAs, such as CDCA and DCA, remain less dissociated, facilitating their passage across hydrophobic cell membranes, unlike their conjugated counterparts, such as glycocholic acid (GCA) and taurocholic acid (TCA).[20] As a result, unconjugated BAs demonstrate a more potent antibacterial effect on *Staphylococcus aureus*. [20] Dobson et al. isolated six cholic acid derivatives with significant antimicrobial activity from *Bacillus amyloliquefaciens* UWI-W23 cultures.[21] Furthermore, LCA and its derivatives have been found to exert antibacterial effects on *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, and *Pseudomonas aeruginosa*. [22] Another BA, ursodeoxycholic acid (UDCA) has also been shown to possess antibacterial and anti-inflammatory

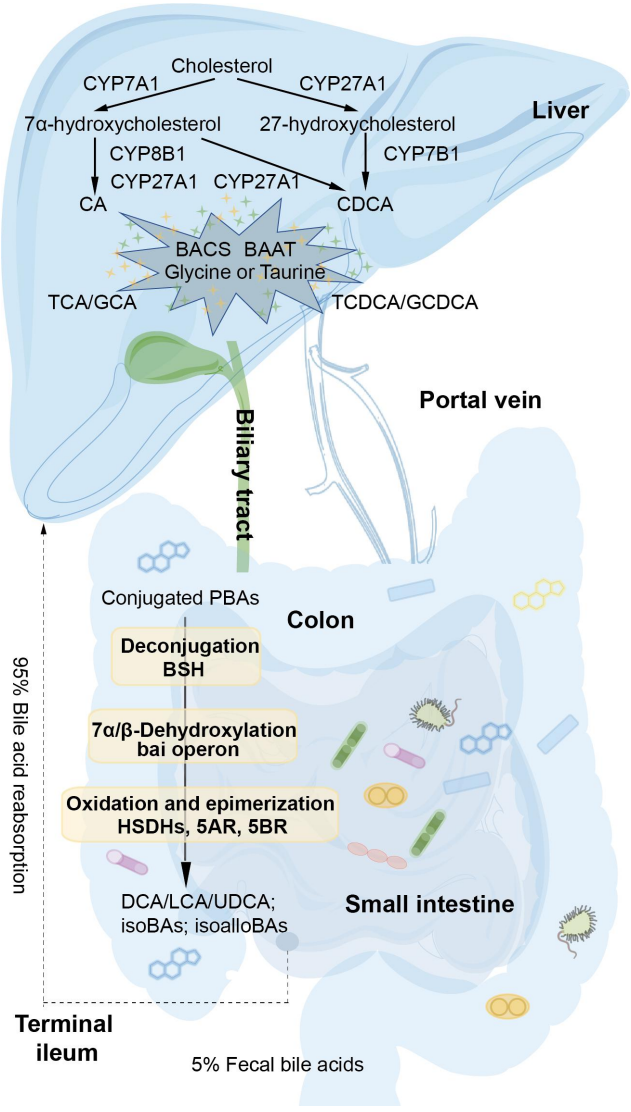

Figure 1. Gut microbiota regulates BA metabolism in the hepatic-intestinal circulation. BAs are synthesized from cholesterol catalyzed by CYPs in two ways in the liver, then conjugated with glycine or taurine catalyzed by BACS and BAAT. Conjugated PBAs then undergo a series of reactions including deconjugation, 7α/7β-dehydroxylation, oxidation and epimerization in the colon. Approximately 95% of the BAs reaching the terminal ileum are reabsorbed and thus recycled by the liver.

properties in *Helicobacter pylori*-induced gastritis.[23] Therefore, BAs and their derivatives offer potential for the development of novel antibiotics against major bacterial pathogens. Additionally, BAs may indirectly modulate the composition of the intestinal microbiota via the farnesoid X receptor (FXR), Takeda G-protein receptor 5 (TGR5), pregnane X receptor (PXR), and vitamin D receptor (VDR). For instance, the FXR agonist obeticholic acid (OCA) has been found to inhibit endogenous BA synthesis and increase the abundance of gram-positive strains, such as *Streptococcus thermophilus*, *Lactobacillus paracasei*, *Bifidobacterium shortum*, and *Lactococcus lactis*. [24] FXR activation has also been observed to induce changes in the composition of the small intestinal microbiota in response to alterations in endogenous BA concentrations.[24] A recent study revealed that the gut microbiota of centenarians (average age, 107 years old; $n = 160$) was enriched in *Bacteroides* and *Alistipes*, while the abundance of *Streptococcus* was reduced compare with older (85–89 years old; $n = 112$) and young (21–55 years old; $n = 47$).[25] Centenarians exhibited a unique bile acid profile, in particular, had significantly higher levels of isoLCA, 3-oxoLCA, alloLCA, 3-oxoalloLCA and isoalloLCA. Among them, just 2.0 μM isoalloLCA was able to potently inhibit *C. difficile* 630 production, and the researchers also tested isoalloLCA against other strains. When isoalloLCA was administered to a culture from feces obtained from young healthy volunteers, the abundance of gram-positive bacteria species (e.g., *Faecalibacterium, Bifidobacterium, Streptococcus*) was significantly reduced, while that of gram-negative species (e.g., *Bacteroides, Alistipes*) was increased, consistent with the enrichment of *Bacteroides* and *Alistipes* and depletion of *Streptococcus* species seen in the microbiomes of centenarians. This suggests that isoalloLCA has a direct influence on the composition of the gut microbiota, and may help protect the intestine from adverse effects of pathogens.[26] In summary, the metabolism of specific BAs plays a crucial role in maintaining intestinal homeostasis, and BA supplementation may affect the composition of the intestinal microbiota.[25]

3. Mechanisms of bile acid biotransformation by gut microbiota

The gut microbiota metabolizes BAs entering the colon through various processes, including deconjugation, 7α/β-dehydroxylation, oxidation/epimerization, esterification, desulfation, and reconjugation, all of which are mediated by enzymatic catalysis.

3.1. Deconjugation

BSH plays a crucial role in BA-mediated signaling pathways, which regulate lipid uptake, glucose metabolism, and energy homeostasis.[27] BSH catalyzes the cleavage of the amide bond in BAs, resulting in release of free BAs such as CA and CDCA, along with glycine or taurine Figure 2(a). Glycine and taurine residues may serve as nutrients for gut microbiota.[33] *Lactobacillus johnsonii (L. johnsonii)* PF01 harbors three BSH isoforms, whereas *Lactobacillus salivarius* LMG14476 contains two, named BSH1 and BSH2.[34] The BSH isoforms in *L. johnsonii* PF01 demonstrate a higher deconjugation rate for glycine-conjugated bile salts than for taurine-conjugated forms.[34] Song et al. identified BSH activity in 591 bacterial strains across 117 genera within the human gut, marking a first in this research area. The highest BSH-t3 enzyme activity was detected in *Lactobacillus*. [35] Crystal structures of BSH from various species have been resolved, including *Bacteroides thetaiotaomicron* (6UFY, blue),[28] *Ligilactobacillus salivarius* (5HKE, yellow),[29] *Bifidobacterium longum* (2HF0, green),[30] *Clostridium perfringens* (2BJF, violet),[31] and *Enterococcus faecalis* (4WL3, pink) Figure 2(b), Table 1.[32] The three-dimensional structures of proteins are crucial for their catalytic functions. BSHs can exist in monomeric, dimeric, and tetrameric forms. Structural analysis reveals that BSH proteins share a highly conserved αββα motif and two sandwiched antiparallel β-sheets, which conform to the structural characteristics of the N-terminal nucleophile (Ntn)-hydrolase family.[35] Rossocha et al. pinpointed critical residues in catalysis using *C. perfringens* BSH (2BJF) as a model Figure 2(c).[31] The active site of BSHs consists of conserved and functionally important residues, including Cys2, Arg18, Met20, Asn82, Asn175, and Arg228, which interact with reaction products like taurine and deoxycholate. Figure 2(d) shows hydrogen bonds that are formed between Cys2, Arg18 of BSH and deoxycholate. Phe61 and Ile137 flank ring A and Met20, Ala68, and Phe26 are adjacent to the isovaleric acid side. Moreover, hydrophobic interactions are observed between Ile133 and ring B, and Leu142 and ring D. Interestingly, taurine is observed in a "reversed" orientation with its sulfur group pointing toward Cys2 and leaving the active site with its amino group ahead. Taurine forms only a single hydrogen bond to a protein side chain.[31] The catalytic mechanism of BSH of the Ntn-hydrolase superfamily features an autocatalytic cleavage that reveals a nucleophilic residue at the N-terminus Figure 2(e).[33] This cleavage results in the stabilization of the tetrahedral intermediate by oxygen anion vacancies, with Arg18 potentially enhancing the nucleophilic capability of the Cys2-SH group to participate in autocatalytic processing.[33]

3.2. 7α/7β-dehydroxylation

The C-7 dehydroxylation of PBAs (CA, CDCA) to SBAs (DCA, LCA) is performed via a multistep biochemical pathway. This pathway involves the BA-inducible (*bai*) operon, encoding proteins predominantly from *Clostridium*,[43,44,73] and *Eubacterium*. [45] Despite its relatively low abundance in the human intestine, the bai operon of *Faecalicatena contorta* S122 is actively transcribed under host colonization conditions, playing a pivotal role in regulation of the 7α dehydroxylation activity.[74] The *Bai* gene has also been identified in *Eggerthella lenta*, yet its role in this bacterium awaits functional characterization.[75] Studies have demonstrated that *E. lenta* does not produce DCA or LCA.[76] These SBAs are implicated in health conditions such as obesity, gallstones, liver, and colon carcinogenesis,[77] highlighting the significance of the 7α-dehydrogenation pathway as a critical bacterial BA biotransformation process in the human gut.

The *bai* operon, encompassing baiB/CD/E/A2/F/G/H/I, is integral to the 7α-dehydroxylation metabolic pathway. Funabashi et al. isolated and characterized the enzymes involved in this pathway, achieving an *in vitro* reconstitution.[73] The cloning of six enzymes (*bai*B/CD/A2/E/F/H) from the *bai* operon of *C. sporogenes* ATCC 15,579 enabled a complete eight-step conversion of CA to DCA (Figure 3a).[73] This conversion commences with the uptake of CA by H^+-dependent active transporter (baiG), followed by thioesterification to CoA via a CoA ligase (baiB).[83,84] The resulting Cholyl-CoA is then oxidized by HSDH (baiA2) to form 3-oxocholyl-CoA.[85,86] This intermediate is further oxidized to form 3-oxo-4,5-dehydrocholyl-CoA by

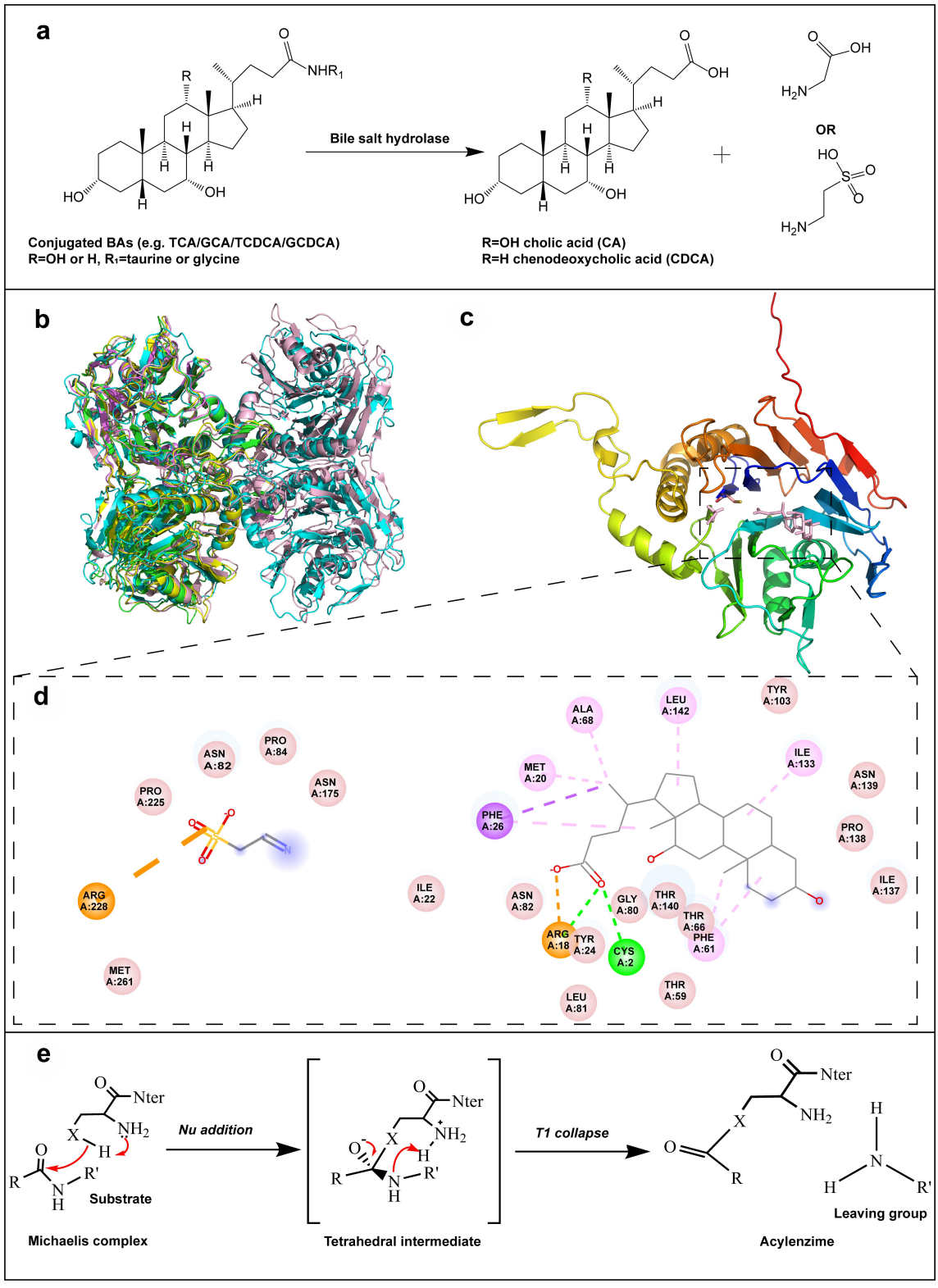

Figure 2. The deconjugation reaction and the structural features of BSH. (a) Hydrolysis of conjugated BAs by BSH to unconjugated BAs and glycine or taurine. (b) Structural homology between subunits of BSHs from *Bacteroides thetaiotaomicron* (PDB ID: 6UFY, blue),[28] *Ligilactobacillus salivarius* (PDB ID: 5HKE, yellow),[29] *Bifidobacterium longum* (PDB ID: 2HF0, green),[30] *Clostridium perfringens* (PDB ID: 2BJF, violet),[31] and *Enterococcus faecalis* (PDB ID: 4WL3, pink).[32] (c) Overall structural features of BSH, taurine and deoxycholate complex from *C. perfringens* (PDB ID: 2BJF). The reaction products taurine and deoxycholate are shown in pink. (d) The interactions between BSH and the substrate taurine, deoxycholate, mapped by Discovery Studio Visualizer software. (e) The catalytic mechanism of BSH.

Table 1. Summary of BA biotransformation by microorganisms.

Microorganisms	Enzyme/Gene	Crystallized proteins	BA transformation	Ref.
Clostridium, *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, *Listeria*, *Brucella*, *Bacteroides*, *Stenotrophomonas*	BSH	5HKE, 2HF0, 6UFY, 2BJF, 4WL3	TCA/GCA→CA, TCDCA/GCDCA→CDCA, TDCA/GDCA→DCA, TUDCA/GUDCA→UDCA, Tα/β/ωMCA→α/β/ωMCA (murine)	36–42
Clostridium clusters XIVa, *Clostridium clusters* IV, *Clostridium clusters* XI, *Clostridium scindens*, *Clostridium hylemonae*, *Clostridium hiranonis*, *Clostridium leptum*, *Eubacterium*,	7α-dehydroxylase (*bai* genes)	4L8O, 4LEH, 4L8P, 4N3V (baiE, 7α dehydratase)	CA→DCA, CDCA→LCA, MCA→MDCA (murine)	43–46
Eggerthella lenta, *Ruminococcus gnavus*, *Clostridium perfringens*	3α-HSDH	1FK8, 1FJH, 1XJB, 2DKN, 1RAL, 1IHI, 2HDJ, 4IS3, 4IS2	DCA→3-oxoDCA	32,47–53
Eggerthella lenta, *Ruminococcus gnavus*, *Peptostreptococcus*	3β-HSDH	/	3-oxoDCA→isoDCA	54
Eggerthella lenta, *Clostridium scindens*, *Escherichia coli*	7α-HSDH	1AHH, 1AHI, 3GAF, 1FMC, 5EPO, 7ENY	CDCA→7-oxoLCA	55–57
Ruminococcus gnavus	7β-HSDH	5G9T, 5FYD	7-oxoLCA→UDCA	58,59
Clostridium scindens, *Clostridium hylemonae*, *Clostridium hiranonis*, *Clostridium leptum*, *Eggerthella lenta*	12α-HSDH	/	DCA→12-oxoLCA	60
Clostridium paraputrificum	12β-HSDH	/	12-oxoLCA→epiDCA	61
Clostridium scindens ATCC 35,704, *Firmicutes*, *Bacteroidetes*, *Proteobacteria bacterium*, *Odoribacter*, *Parabacteroides*, *Alistipes*	5AR	7C83, 7BW1	3-oxoalloDCA→alloDCA, 3-oxoalloLCA→alloLCA	26,62,63
Homo sapiens	5BR	3DOP, 3G1R, 3CMF, 3COT, 3CAS, 3CAQ, 3BUV, 3BV7, 3BUR, 3CAV, 3UZY, 3UZW, 3UZX, 3UZZ	CA→alloDCA	64–67
Bacteroides, *Eubacterium*, *Lactobacillus*	Undefined	/	Unesterified BAs→Fatty acid ethyl esterified BAs	68
Clostridium, *Peptococcus*, *Fusobacterium*, *Pseudomonas*	3β-sulfate sulfohydrolyase	/	3β-hydroxy-5-cholenoic acid 3-sulfate→3α-hydroxy-5-cholenoic acid	69
Enterocloster (formerly Clostridium) bolteae, *Actinobacteria*, *Firmicutes*, *Bacteroidetes*, *Bifidobacteriaceae*, *Lachnospiraceae*, *Bacteroidaceae*	Undefined	/	Unconjugated BAs→conjugated BAs (conjugate with glutamate, glutamine, aspartate, asparagine, methionine, histidine, lysine, serine, tryptophan, valine, alanine, arginine, phenylalanine, leucine/isoleucine, or tyrosine)	70–72

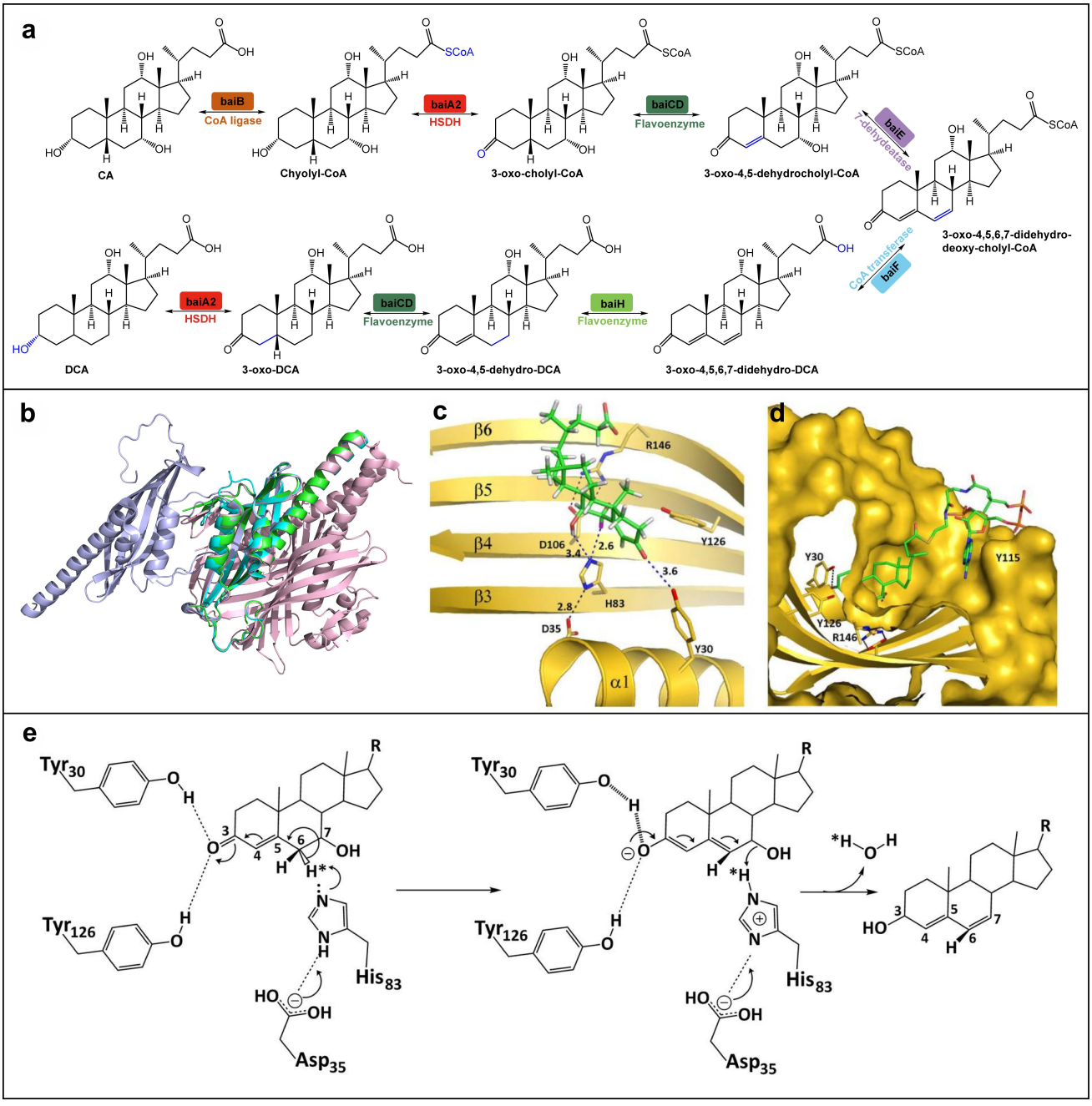

Figure 3. The dehydroxylation pathway for CA and the structural feature of 7α dehydratases. (a) Pathway of intermediate steps in 7α-dehydroxylation of CA by enzymes coded by *bai* operon genes. (b) The structure homology between BA 7α-dehydratases from *Clostridium scindens* (PDB ID: 4LEH, light pink),[78] *Clostridium hylemonae* (PDB ID: 4L8O, green),[79] *Clostridium hiranonis* (PDB: 4N3V, violet),[80] and *Peptacetobacter hiranonis* (formerly *Clostridium hiranonis*, PDB ID: 4L8P, blue).[81] (c) Predicted binding mode of 3-oxo-$^{\Delta}$4-chenodeoxylcholyl-CoA with BA 7α-dehydratases. Blue dashed lines predicted interactions of His83 and Tyr30 with substrate 3-oxo-$^{\Delta}$4-chenodeoxylcholyl-CoA.[82] Adapted with permission from ref. 55, copyright 2016, John Wiley and Sons. (d) Predicted stacking interaction between 3-oxo-$^{\Delta}$4-chenodeoxycholyl CoA and Tyr115. Carbon atoms of protein residues and product molecules are colored gold and green, respectively. H, O, N, P, and S atoms are colored in gray, red, blue, orange, and olive, respectively.[82] Adapted with permission from ref. 55, copyright 2016, John Wiley and Sons. (E) Catalytic mechanism of BA 7α-dehydratases.[82] Adapted with permission from ref. 55, copyright 2016, John Wiley and Sons.

a flavoenzyme (baiCD).[87] 7α-dehydrogenase (baiE) catalyzes the critical 7α-dehydroxylation step, resulting in the formation of 3-oxo-4,5–6,7-didehydro-deoxy-cholyl-CoA.[84] CoA is then subsequently removed by CoA transferase (baiF) to form the intermediate, and further reduced by flavoenzyme (baiH, baiCD) and HSDH (baiA2) to produce DCA.[85,87–89] BaiI appears to be non-essential in

this 7α-dehydroxylation pathway.[73] The same pathway is used for the 7α-dehydroxylation of CDCA.

The crystal structures of the enzymes encoded by the baiA2/baiB/baiE genes have been resolved.[85] BA 7α-dehydrogenase (baiE) is the rate-limiting enzyme in this pathway. Crystal structures of BaiE from *Clostridium scinden* (PDB ID: 4LEH),[78] *Clostridium hylemonae* (PDB ID: 4L8O),[79] *Clostridium hiranonis* (PDB ID: 4N3V),[80] and *Peptacetobacter hiranonis* (formerly *Clostridium hiranonis*, PDB ID: 4L8P)[81] exhibit a common twisted (α+ β barrel) fold, characteristic of the nuclear transport factor 2 (NTF2) family Figure 3(b), Table 1. Particularly, BaiE structure from *Clostridium scindens* (PDB ID: 4LEH) demonstrates trimer formations with a twisted barrel fold Figure 3(b).

Docking of the substrate, 3-oxo-Δ^4-chenodeoxycholate, into BaiE's binding pocket highlighted an active center composed of three conserved residues: Tyr30 (α1), Asp35 (α1), and His83 (β3). The side chains of Asp35 and His83 are within proximity of each other at a distance of 2.8 Å, whereas no interaction between Tyr30 and Asp35 or His83 was observed. Asp106 and Arg146 are essential for substrate binding and turnover. A unique ring arrangement formed by the residues above the active site is observed in all apo structures. The conformational flexibility of this ring may be critical for substrate binding Figure 3(c). Similarly, the interaction between the adenine group of the CoA moiety of 3-oxo-Δ4-chenodeoxycholyl CoA and baiE was also postulated, where CoA adopts a twisted conformation due to a π–π stacking interaction with Tyr115, allowing pantetheine and AMP moieties of CoA to be exposed to the solvent without any direct interaction with baiE Figure 3(d). An analysis of the catalytic mechanism revealed that Tyr30 acts as a general acid (assisted by Tyr126) to facilitate the delocalization of the π-electrons across atoms C3, C4, C5, and C6 Figure 3(e). The terminal hydroxyl group of Tyr30 could potentially protonate the oxyanion generated on the C3-oxo group to support negative charge stabilization. This leads to an electron shift that destabilizes the bond between C6 and 6αH. His83 is then strategically positioned to remove the 6αH atom, and protonate the departing C7α-hydroxy group, while Asp35 modulates the pKa of the His83-imidazole ring, aiding the deprotonation and subsequent re-protonation processes that conclude with water molecule release.[82]

3.3. Oxidation and epimerization

Gut microbiota-mediated oxidation and epimerization occur through the action of HSDHs, which catalyze the reversible oxidation and reduction of the 3α-, 7α-, or 12α-hydroxyl groups of BAs.[90,91] Microorganisms such as *Clostridium scindens*,[60] *Eubacterium*,[86] *Clostridium hiranonis*,[60] *Eggerthella lenta*,[76] *Escherichia coli*,[55] and *Bacteroides fragilis* [92] are capable of oxidizing and reducing BAs as they possess at least one HSDH. For instance, the NAD(P)H-dependent 3α-, 3β-, and 12α-HSDHs encoding gene clusters are found in *Eggerthella* sp. CAG:29815. A 12α-HSDH encoding gene is found in *Eggerthella* sp. CAG:298,[76] *Clostridium scindens, Clostridium hylemonae*, and *Peptacetobacter hiranonis*. [60] Epimerization leads to the production of more hydrophilic and less toxic isobile acids (isoBAs), which enhance bacterial resistance in the competitive and hostile intestinal environment.[93] CDCA, a hydrophobic BA, is converted to its more hydrophilic and less toxic 7β-isomer UDCA by gut microbiota.[93,94] It is worth noting that isoBAs also appear to regulate gut microbiota composition and host metabolism. *E. lenta* and *Ruminococcus gnavus* contribute to this regulatory action by producing 3-oxoDCA and isodeoxycholic acid (isoDCA), respectively, each through a specific 3α-hydroxysteroid dehydrogenase (3α-HSDH) and 3β-hydroxysteroid dehydrogenase (3β-HSDH) enzyme. IsoDCA, for instance, promotes the growth of *Mycobacterium spp*. [76]

Recent studies revealed the involvement of two types of bacterial steroid dehydrogenases, namely 5α-reductase (3-oxo-5α-steroid 4-dehydrogenase, 5AR) and 5β-reductase (3-oxo-5β-steroid 4-dehydrogenase, 5BR), in the production of homologous (5α and 5β) BAs.[62] The levels of isolithocholic acid (isoLCA), 3-oxolithocholic acid (3-oxoLCA), allolithocholic acid (alloLCA), 3-oxoallolithocholic acid (3-oxoalloLCA), and isoalloLCA were also found to be significantly higher in the centenarian group (average age, 107 years old) due to the unique gut microbiota in centenarians.[25] Analysis of centenarians' gut

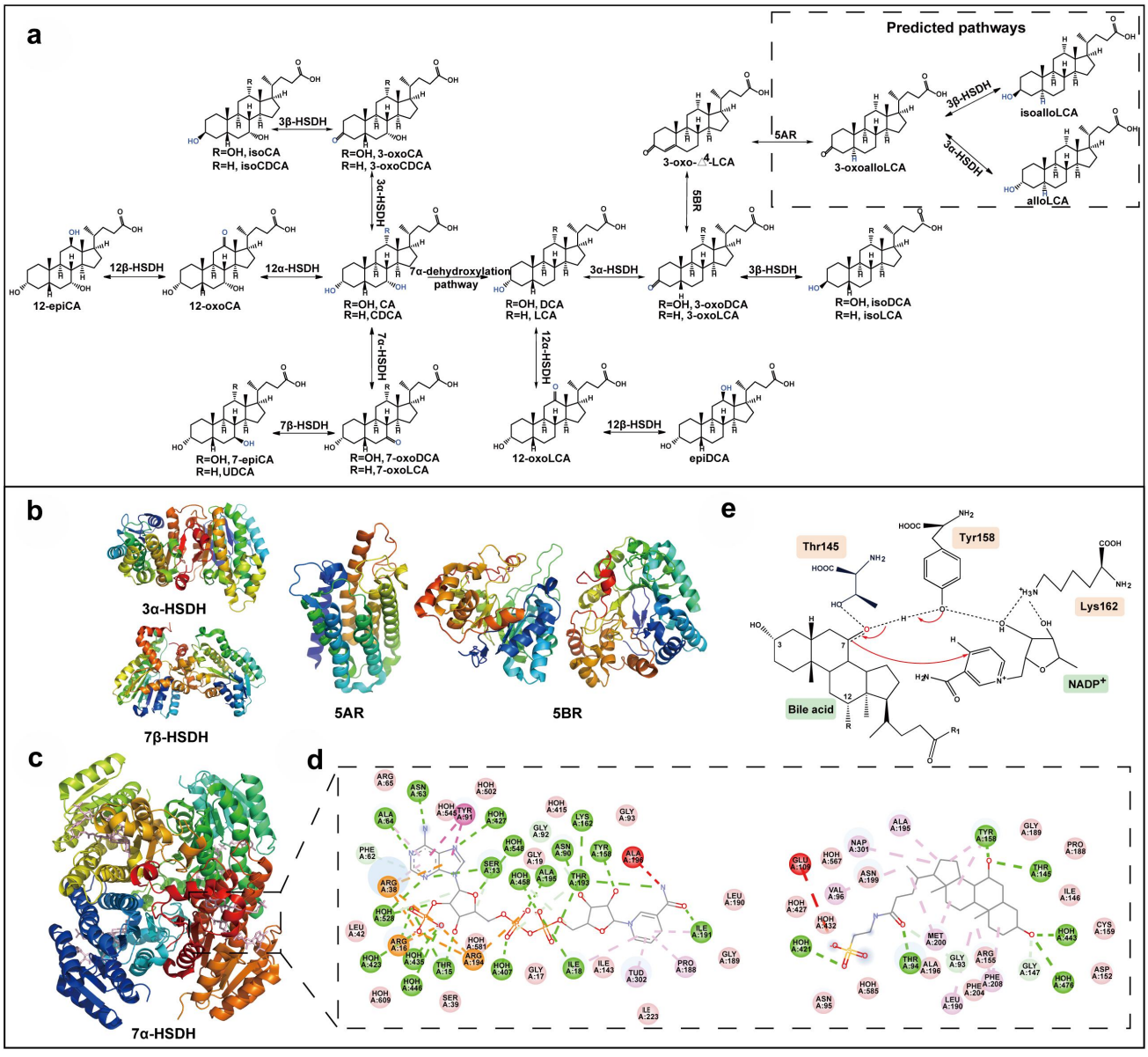

Figure 4. The oxidation and epimerization pathways of CA and CDCA and the structure features of related enzymes. (a) The pathways of CA and CDCA oxidation and epimerization. (b) Three-dimensional structures of different oxidoreductases: 3α-HSDH (PDB ID: 1FJH), 7β-HSDH (PDB ID: 5GT9), 5AR (PDB ID: 7C83), 5BR (PDB ID: 3CAQ). (c) Overall structural features of 7α-HSDH, TCDCA, and $NADP^+$ complex from *Clostridium absonum* (PDB ID: 5EPO). TCDCA and $NADP^+$ are shown in pink. (d) The interactions between *CA* 7α-HSDH and TCDCA, $NADP^+$, mapped by Discovery Studio Visualizer software. (e) The catalytic mechanism of HSDHs.

microbiota revealed the presence of many strains with strong 3α-HSDH, 3β-HSDH, 5AR, and/or 5BR activity.[25] It is plausible that 3-oxoalloLCA is generated from 3-oxo-Δ4-LCA through a 5AR homolog, and the conversion of 3-oxoalloLCA to alloLCA or isoalloLCA involves 3α-HSDH or 3β-HSDH, respectively, which is consistent with the previously-described conversion of 3-oxoDCA to DCA or isoDCA Figure 4(a).[26,95,96] The crystal structures of several enzymes involved in oxidation and epimerization have been resolved, including 3α-HSDH, 7α-hydroxysteroid dehydrogenase (7α-HSDH), 7β-hydroxysteroid dehydrogenase (7β-HSDH), 5AR, and 5BR Table 1. Figure 4(b) shows that both 5AR (PDB ID: 7C83) and 5BR (PDB ID: CAQ)[64] possess alpha-helices and loops, forming a barrel structure, while 5BR wrapping β-sheets in the middle. 3α-HSDH (PDB ID: 1FJH), 7α-HSDH (PDB ID: 5EPO), and 7β-HSDH (PDB ID: 5GT9)[58] share a common structural fold. Hence, human steroid 5BR (AKR1D1) could be converted into 3β-HSDH by a single point mutation, Glu120His (PDB ID:

3UZW).[54] The wild-type AKR1D1 specifically recognizes D4–3-ketosteroids, whereas the mutant Glu120His does not have 5β-reductase activity, but shows 3β-HSDH activity instead.[54]

We have previously identified multiple 3α-HSDHs, 7α-HSDHs, and 7β-HSDHs from the black bear gut microbial macrogenome,[97] and resolved the crystal structure of 7α-HSDH from *Clostridium absonum* (*CA* 7α, PDB ID: 5EPO).[56] *CA* 7α structure (5EPO) was chosen as the basis to investigate the catalytic mechanism of HSDHs Figure 4(c). *CA* 7α is composed of four subunits, each containing seven parallel β-strands and three α-helices on each side. The central β-sheet constitutes the active center. The interactions of *CA* 7α-HSDH with the substrate and the coenzyme NADP(H) are depicted in Figure 4(d). The SDR superfamily features a conserved active center (Asn-Ser(Thr)-Tyr-Lys) and coenzyme binding sites (Thr-Gly -X^3-Gly-X-Gly), which are primarily involved in the hydrogen bonding between the enzyme and the substrate. The orientation of NADP(H) is determined by residues including Ser13, Thr15, Arg16, Ile18, Arg38, Asn63, Asn90, Tyr158, Lys162, Ile191, Thr193, Arg194, and Ala195. Thr15, Arg16, Arg38, and Arg194 create a positively charged environment through complex hydrogen bonding interactions with the phosphate group.[98] The orientation of the substrate TCDCA in the substrate-binding site Figure 4(d) is determined by residues including Gly93, Thr94, Thr145, Gly147, Tyr158, Asn199, Phe204, and Phe208. The catalytic mechanism of HSDHs was analyzed as well Figure 4(e). The catalytic triad (Thr145-Tyr158-Lys162) was identified based on the crystal structure of *CA* 7α. Here, Thr145 was found to stabilize substrates, intermediates, and products by forming hydrogen bonds, whereas Tyr158 acts as a catalytic acid/base, and Lys162 serves as part of the proton relay and interacts with the nicotinamide cofactor.[56]

3.4. Esterification, desulfation, and reconjugation

Bacteria can also esterify BAs with alcohols, SCFAs, and long-chain fatty acids. Bacterial species from genera *Bacteroides, Eubacterium, Lactobacillus, Citrobacter*, and *Peptostreptococcus* harbor esterases that convert BAs into their C-24 ethyl esters with ethanol addition.[68,99] Methyl esters of DCA are formed by human fecal isolates; however, the mechanism is unknown. It has been hypothesized that the reaction is dependent on C1 transfer (methyl or methoxyl group) to the C24 carboxyl group rather than on methanol as a substrate.[68] Bacteria can generate BA fatty acid esters, in which C16 and C18 long-chain fatty acids, as well as SCFAs (acetate), are lined to C3 of isoDCA and isoLCA (FA-conjugated isoBAs, FA-isoBAs).[100] Esterification is a detoxification strategy to precipitate BA esters, thereby reducing the concentration of hydrophobic SBAs and toxic fatty acids and alcohols in fecal water.[101] Despite progress in liquid chromatography-mass spectrometry approaches to BA profiling, BA esters are rarely detected in fecal samples.[100] Esters of BAs account for 10% to 30% of the total BA content in human feces.[102] Unfortunately, little is known about the role of gut bacteria in BA esterification. The contribution of FA-isoBAs to intestinal physiology and their role in pathophysiologic conditions such as IBD is currently under investigated.

Sulfation increases the solubility, reduces intestinal absorption, and promotes excretion of BAs in feces and urine. BA-sulfates are also less toxic compared to their unsulfated counterparts. Therefore, sulfation is an important detoxification pathway of BAs.[103] BA sulfatase activity has been identified in *Clostridium, Peptococcus, Fusobacterium*, and *Pseudomonas* species.[69] Sulfatase activity requires a 3α/3β-sulfate group and a free C24 or C26 carboxyl group. Only the BA sulfatase from *Comamonas testosterone* has been obtained in purified form.[69]

In the presence of gut microbiota, BAs combine with amino acids to form bile acid-amino acid conjugates. The enzymes responsible for binding amino acids are currently unknown, although previous studies implied the involvement of microbial BSHs in glycine binding. *Actinobacteria, Firmicutes*, and *Bacteroidetes* species were shown to have glycine reconjugation and BSH activities, with the conjugation activities being the highest among all microbial species.[60] Recently, Guzior et al. also

found that BSH had acyltransferase activity (bile salt hydrolase/transferase, BSH/T). *Clostridium perfringens* BSH/T rapidly performed acyl transfer when provided with various amino acids and taurocholate, glycocholate or cholate, with an optimum at pH 5.3. These microbially conjugated bile acids (MCBAs) had antimicrobial properties due to have greater hydrophobicity.[104] These MCBAs can activate PXR receptor and the aryl hydrocarbon receptor.[105] The intrinsic acyltransferase activity of BSH/T greatly diversifies BA chemistry. The physiological effects of amino acid-conjugated BAs, other than those conjugated to glycine or taurine, are not yet fully understood. However, recent studies have indicated that CA conjugated to phenylalanine, leucine, and tyrosine may act as FXR agonists.[70] These conjugated BAs are also detected in IBD patients.[70] Lucas et al. also found that CDCA, DCA or CA may be conjugated with one or more glutamate, glutamine, aspartate, asparagine, methionine, histidine, lysine, serine, tryptophan, valine, alanine, arginine, phenylalanine, leucine/isoleucine, and tyrosine.[71] However, it remains unclear whether BAs conjugated to amino acids enhance intestinal absorption and enterohepatic circulation.

4. Microbiota-derived BAs in IBD and gut immunity

IBD can be influenced by various factors, including genetics, environmental influences, gut microbiota dysbiosis, immune system abnormalities, diet, and smoking. The association between gut microbiota and the development of IBD has long been recognized. Patients with IBD exhibit dysbiosis in their gut microbiota, which triggers an exaggerated immune response toward pro-inflammatory microbes, leading to chronic inflammation and tissue damage in the intestine. This dysbiosis compromises the integrity of the intestinal mucosal barrier, resulting in a reduction in mucus layer thickness and increased intestinal permeability. As a result, microbial components translocate into the intestinal tissue.[7] However, the exact role of BAs in IBD pathogenesis remains unknown.[97] Here, we summarize the changes in BA and gut microbiota composition in IBD patients as reported in recent experimental studies, along with the effects of major BA receptors on immune responses in the gut Figure 5.

4.1. Alteration of gut microbiota and microbiota-derived BAs in IBD

The diversity of beneficial gut microbiota in IBD patients is decreased, whereas that of potentially pathogenic bacteria is increased. The level of PBAs of UC patients increased, which is positively correlated with potentially-pathogenic bacteria, including *Proteobacteria*, *Escherichia coli*, and *Actinobacteria*. [106] These pathogenic bacteria cause intestinal infections and inflammation, affecting enterohepatic recycling reabsorption and BA receptor homeostasis.[107] Bile acid malabsorption (BAM), a common symptom in patients with IBD, occurs due to decreased activity of the apical sodium-dependent bile acid transporter (ASBT) in the ileum, which subsequently leads to a decrease in intestinal absorption and an increase excretion of BAs in the feces Figure 5(a). ASBT is down-regulated in active CD, active UC, and CD in remission. The ileum is often the main site of ASBT expression. Therefore, the degree of BAM may be more serious for CD with ileal resection.[108] Moreover, IBD patients who undergo ileal resection have elevated levels of PBAs, along with a decrease in microbial diversity.[109] FXR also regulates BA production via feedback inhibition of CYP7A1 by the FXR/FGF19/hepatic fibroblast growth factor receptor 4 signaling pathway.[110] Thus, BAM induced by ASBT dysfunction in IBD leads to a decrease in the BA pool, a decrease in FXR inhibition, and a subsequent increase in PBA production in intestinal epithelial cells.

Additionally, the gut microbiota in IBD patients show impaired deconjugation and biotransformation, leading to higher levels of conjugated BAs, lower levels of SBAs, and higher levels of PBAs.[111–113] These effects have been attributed to a decrease in the abundance of BSH in the gut microbiome.[112] Meanwhile, IBD patients exhibited decreased levels of SBAs, such as DCA, LCA, and taurochenodeoxycholic acid (TLCA), indicating a depletion of SBA-producing bacteria like *Firmicutes*, *Clostridium* IV and XIVa, *Butyricicoccus*, *Clostridium*, *Faecalibacterium*, *Ruminococcaceae*, and *Roseburia*,[114–116] which are

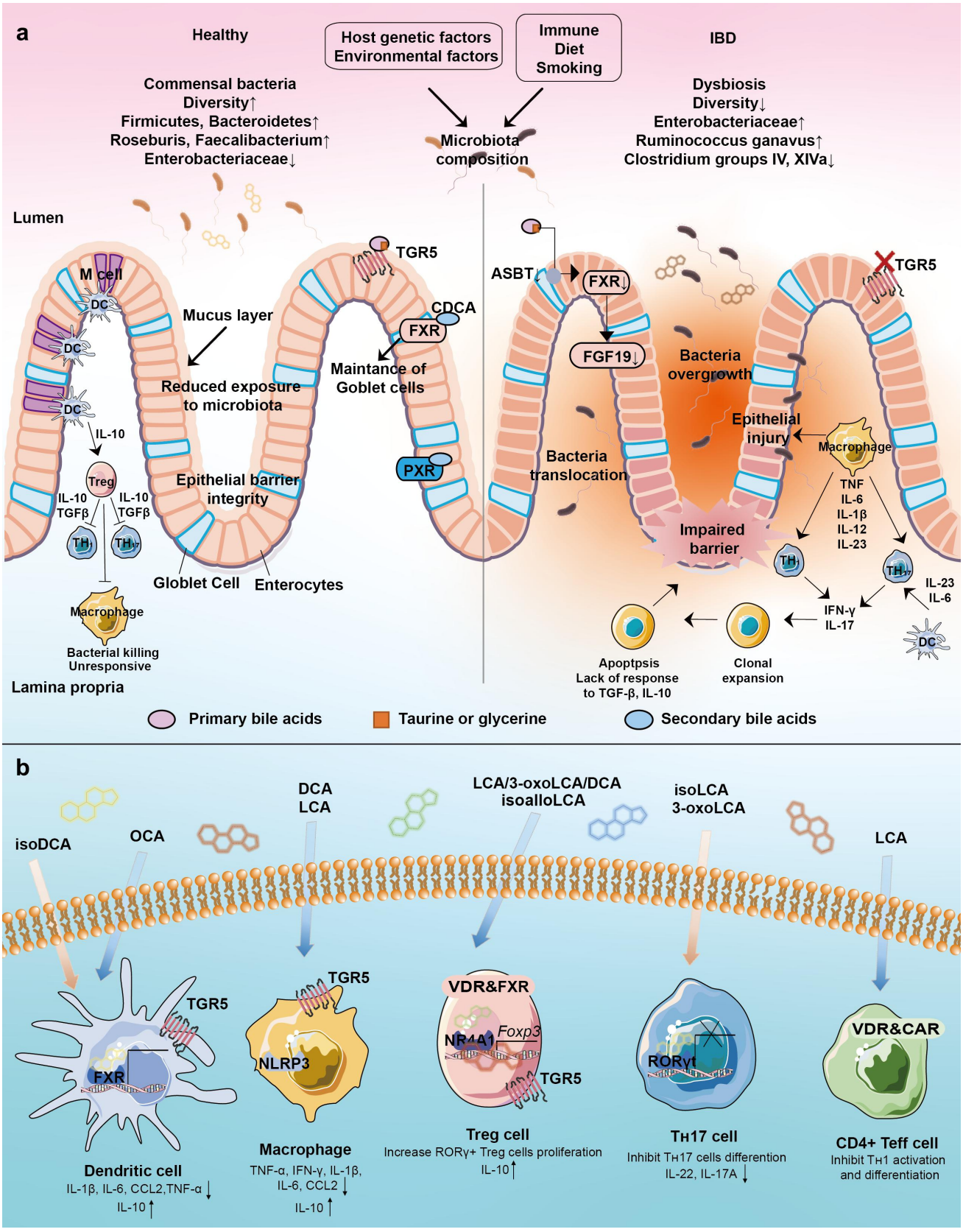

Figure 5. Interaction of gut microbiota, microbial bile acids, and host immunity. (a) Interactions between the gut microbiota and the host immune response in healthy and IBD states. Gut microbes play an important role in the maintenance of intestinal mucosal immune homeostasis, including the maintenance of an intact intestinal mucosal barrier and a modest immune response to antigens. Disturbances in the abundance and diversity of the gut microbiota can lead to impaired functioning of the host's intestinal immune system, which can lead to IBD. (b) Bile acids can activate these receptors including LCA, 3-oxoLCA, isoLCA, isoalloLCA, DCA, isoDCA,

Table 2. Gut microbes and BAs changes in IBD.

Model/Population (n)	Methods	Changes in gut microbiota	Changes of BAs	Ref.
CD (12), UC (30), HCs (29)	qPCR, HPLC	*Clostridium leptum, Faecalibacterium prausntizii* ↓ ; *Escherichia coli* ↑	Fecal conjugated and 3-OH-sulfated BAs ↑ ; SBAs ↓	[113]
IBD (15), HCs (15)	16S rDNA, UHPLC/Q-TOF-MS	*Bacteroidetes, Firmicutes* ↓ ; *Proteobacteria, Actinobacteria, Escherichia coli, Klebsiella pneumoniae* ↑	PBAs ↑	[106]
UC (32), HCs (23)	16S rRNA, Targeted metabolomics	*Firmicutes, Clostridium* IV, *Butyricicoccus, Clostridium* XIVa, *Faecalibacterium, Roseburia* ↓ ; *Enterococcus, Klebsiella, Streptococcus, Lactobacillus* ↑	TCA, CA, TCDCA, and GCDCA ↑ ; LCA, DCA, GDCA, GLCA, and TLCA ↓	[116]
CD (43), HCs (18)	16S rRNA, HPLC	*Eubacterium rectale, Bifidobacterium longum, Ruminococcus bromii* ↑ ; *Escherichia coli* ↓	PBAs ↑	[121]
CD (14), UC (12), HCs (26)	Terminal restriction fragment length polymorphism analysis, HPLC-MS/MS	*Clostridium cluster* XIVa ↓	DCA/(DCA+CA) ↓	[122]
CD (29), HCs (20)	16S rRNA, UPLC-MS	*Bifidobacteria, Clostridium* (clusters IV and XI) ↓	Unconjugated BAs ↓	[123]
UC (17), FAP (7)	Metagenomic sequencing, metabolomic analysis	Phylum *Firmicutes Ruminococcaceae* ↓	CDCA ↑ ; LCA, DCA ↓	[115]
IBD (8), HCs (8)	16S rRNA, UPLC	*Faecalibacterium Sutterella* ↑ ; *Akkermansia, Streptococcus* ↓	TBA, CA/TBA ↑ ; DCA/TBA, CDCA/TBA, LCA/CA, DCA/CA ↓	[124]
CD (67), UC (38), HCs (27)	16S rRNA, LC-MS	*Faecalibacterium prausnitzii, Roseburia hominis* ↓ ; *Clostridium hathewayi, Clostridium bolteae, Ruminococcus gnavus* ↑	PBAs and its glycine and taurine conjugates, glycochenodeoxycholate ↑ ; LCA, DCA ↓	[114]
CD (68), UC (53), HCs (34)	Shotgun metagenomic sequencing, LC-MS	*Bifidobacterium breve, Clostridium symbiosum* (UC) ↑ ; *Ruminococcus gnavus, Escherichia coli, Clostridium clostridioforme* (CD) ↑ ; *Roseburia hominis, Dorea formicigenerans, Ruminococcus obeum* (IBD) ↓	CA, TCA, GCA, CDCA, TCDCA, GCDCA ↑ ; LCA, DCA ↓	[117]
IBD (15), PSC-IBD (15)	16S rRNA, LC-MS	*Ruminococcus, Fusobacterium* ↑ ; *Dorea, Veillonella, Lachnospira, Blautia, Roseburia* ↓	TBA ↑	[125]
CD (109), UC (106), HCs (51)	16S rRNA, LC-MS	*Ruminococcus gnavus, Lachnoclostridium, Flavonifractor* (CD) ↑ ; *Lachnospira* (IBD with colorectal cancer) ↓ ; *Escherichia-Shigella* (UC with neoplasia) ↑ ; *Agathobacter* (UC with neoplasia) ↓	No differentially detected BAs (IBD vs IBD with cancer, IBD vs IBD with neoplasia)	[126]
Non-operated CD (84), CD with ileocolectomy (52), UC (30)	16S rRNA, LC-MS	*Faecalibacterium prausnitzii*, O-*acetylhomoserine aminocarboxypropyltransferase* [MetY] (CD with ileocolectomy) ↓	PBAs (CD with ileocolectomy) ↑	[109]

known to possess BSH and HSDHs enzymes responsible for the deconjugation and biotransformation of BAs.[114,117,118] Furthermore, the enzymatic activity of 7α-dehydroxylation (BSH) is inhibited at a pH below 6.5. The stool pH in IBD is acidic (pH 5.2), which therefore inhibits the catalytic action of the enzyme, and thereby prevents the conversion of PBAs to SBAs.[119] Connors et al. also found that PBAs were associated with decrease in the activities of enzymes catalyzing BA 7α-dehydroxylation.[120] The levels of LCA, DCA, and gene expression required for the conversion of PBAs to SBAs. Moreover, *Ruminococcaceae* abundance was reduced in UC compared to familial adenomatous polyposis (FAP) Table 2.[115]

Changes in ratios of certain BAs may also serve as markers of changes in the gut microbiota. For instance, the ratio DCA/(DCA+CA), which indicates 7α-dehydroxylation, is positively correlated with the presence of *Clostridium subcluster* XIVa.[122] Serum DCA/(DCA+CA) levels are significantly lower in individuals in the remission state of CD and the remission and exacerbation states of UC compared to healthy controls (HCs).[122] Furthermore, other correlations between microbiota and BAs were also observed in primary

UDCA, and OCA. IsoDCA inhibits FXR activity in DCs, resulting in the expansion of peripheral T_{reg} cells, while OCA activates FXR to decrease the secretion of TNF-α, IL-1β, IL-6, CCL2 and to increase the level of IL-10. DCA and LCA activate TGR5 on macrophage to block NLRP3 inflammatory vesicle activity, thereby suppressing intestinal inflammation. The activation of TGR5 reduces the level of TNF-α, IFN-γ, IL-1β, IL-6, and CCL2. IsoalloLCA binds to the nuclear hormone receptor NR4A1 at the Foxp3 locus and increases T_{reg} cell differentiation. The combination of LCA/3-oxoLCA increased the RORγt T_{reg} cell population and proliferation via VDR. 3-oxoLCA blocks T_H17 differentiation through RORγt and reduces IL-17A and IL-22 significantly. Besides, 3-oxoLCA and isoalloLCA regulate T_H17 and T_{reg} cell homeostasis. LCA also inhibits T_H1 activation and differentiation via VDR.

sclerosing cholangitis-IBD (PSC-IBD), where 12% of the operational taxonomic units strongly correlated with fecal BAs. In particular, significant shifts were observed within the *Firmicutes* (73%) and *Bacteroidetes* phyla (17%) at the operational taxonomic unit level.[125] Overall, these findings highlight the alterations in gut microbiota and its derived BAs in IBD, leading to potential implications for disease pathogenesis. Further research is need to fully understand these complex interaction.

4.2. Gut microbiota-derived BAs regulate intestinal inflammation and immunity

BA receptors are also involved in the regulation of BA-mediated intestinal inflammation.[127,128] Immune cells on the intestinal mucosal surface are responsible for the rapid detection and elimination of pathogenic microorganisms, while maintaining immune homeostasis.[129] Dysfunction of intestinal immune response can lead to chronic intestinal inflammation, which is a characteristic of IBD.

Healthy human intestinal epithelial cells exhibit high expression of FXR, whereas reduced intestinal FXR signaling is seen in IBD. Impaired FXR signaling can be detrimental in IBD Figure 5(a).[130–132] Immune disturbances such as elevated macrophage infiltration were observed in $FXR^{-/-}$ mice.[133] Specifically, macrophages isolated from (trinitrobenzene sulfonic acid) TNBS-treated $FXR^{-/-}$mice were found to release more inflammatory cytokines than wild-type (WT) mice.[133] FXR exerts anti-inflammatory effects through various mechanisms, such as in the ileum, where high concentrations of conjugated PBAs have direct antimicrobial effects and mediate the antimicrobial program of FXR to prevent bacterial overgrowth.[134] In contrast, immune cells in the colon regulate immune homeostasis during microbial colonization. Gut microbiota-derived SBA isoDCA inhibits FXR activity in DCs, thus weakening the immunostimulatory abilities of DCs.[95] This anti-inflammatory phenotype acquired by DCs allows peripheral T_{reg} cells to differentiate, and help suppress the immune response during bacterial colonization.[95,135] BAs and their analogues can be used to activate FXR to treat IBD. The FXR agonist INT-747 (OCA) significantly decreases TNF-α secretion in activated human peripheral blood mononuclear cells, CD14 monocytes, DCs, and mononuclear cells in lamina propria and decrease severity of colitis in DSS-induced and TNBS- induced colitis model.[132] OCA also reduces the expression of proinflammatory cytokines (e.g., IL-1β, IL-6) and chemokines (e.g., CCL2) by activating FXR.[132] OCA-activated FXR was also shown to exert anti-inflammatory effects, including increased plasma IL-10 level and inhibition of the decrease in splenic DCs and the increase in T_{reg} cells associated with dextran sulfate sodium salt (DSS)-induced colitis Figure 5(b).[136] Phenylalanocholic acid, tyrosocholic acid, and leucocholic acid have been identified as novel FXR agonists enriched in the dysbiotic state associated with CD patients.[70] These molecules have been found to promote the expression of mouse intestinal FXR target genes *Fgf15* and *Shp*. Nonetheless, further studies are required to determine whether they induce intestinal inflammation and to elucidate FXR-related mechanisms of action.[70] Moreover, it is also crucial to carefully consider the FXR-regulating activities of human- and rodent-specific BAs, as they significantly differ from each other. This requires that attention be paid to the circulating BA pool in both experimental animal models and human patients, and how these parameters change in response to diet, inflammation, and dysbiosis is understood. Such detailed consideration is essential to fully comprehend the BA-dependent FXR functions in mucosal immune regulation.

GPBAR1 (TGR5) is a G protein-coupled receptor highly expressed in monocytes/macrophages.[137] TGR5 regulates the M1/M2 phenotype of intestinal macrophages, and inhibits the expression of inflammatory genes (TNF-α, IFN-γ, IL-1β, IL-6, and CCL2).[138] Activation of TGR5 in intestinal stem cells (ISCs) triggers the SRC/YAP signaling pathway, leading to ISC renewal and proliferation.[139] In macrophages, TGR5 activation suppresses intestinal inflammation by blocking NLRP3 inflammatory vesicle phosphorylation and ubiquitination.[140,141] DCA/LCA supplementation can reduce intestinal inflammation in DSS murine colitis model by activating TGR5.[115] Supplementation with DCA/LCA may also promote ISC stemness, inhibit NLRP3 inflammatory vesicle activity, and reduce the development of

colitis by activating TGR5. In addition, PBAs are converted to SBAs (LCA, DCA) through a series of gut microbiota-mediated reactions. LCA and DCA undergo further transformations into isoLCA, alloLCA, 3-oxoLCA, 3-oxoalloLCA, isoalloLCA, isoDCA, and 3-oxoDCA. These metabolites modulate T_H17 and T_{reg} cell differentiation, and affect intestinal inflammation.[142] IsoalloLCA enhances T_{reg} cell differentiation by activating TGR5 via binding to the nuclear hormone receptor NR4A1 at the Foxp3 locus, whereas 3-oxoLCA blocks T_H17 differentiation through inhibiting retinol-associated orphan receptor-γt (RORγt) while expression levels of IL-17A, IL-22 are decreased significantly Figure 5(b).[142] The dysregulation of T_H17/T_{reg} homeostasis in IBD patients is also modulated by 3-oxoLCA and isoalloLCA, suggesting that BA metabolites may directly regulate host immunity by modulating T_H17 and T_{reg} cell homeostasis.[143] These findings indicate that the gut microbiota-BA axis can impact the onset and progression of inflammation by altering the composition of the intestinal BA pool.

RORγt is a nuclear receptor that plays a role in various inflammatory and autoimmune diseases.[144] RORγt T_{reg} cells constitute a unique population of T_{reg} cells that contribute to the response to commensal microbes within the colon.[135] Song et al. discovered that microbial BAs, including the combination of LCA/3-oxoLCA, specifically increased the population of RORγt T_{reg} cells.[135] The proliferative effect of RORγt T_{reg} cells depends on the VDR activation state.[135] VDR activation leads to the formation of a heterodimer with the retinoid X receptor (RXR) which acts as a transcription factor. PXR acts synergistically with CAR to control BA clearance and bilirubin detoxification. LCA and its metabolites 3-oxo-LCA, glycol-LCA, and 6-oxo-LCA activate VDR.[145] Reduced VDR expression is commonly observed in mucosal biopsies of IBD patients.[146] VDR deficiency in the intestine exacerbates LCA-induced hepatotoxicity, yet intestinal transgenic expression of CYP3A4 protects mice deficient in VDR in the intestine from LCA-induced hepatotoxicity.[147] VDR is also expressed in immune cells. LCA inhibits T_H1 activation and differentiation via VDR by reducing ERK1/2 phosphorylation in primary human and mouse CD4 T_H1 cells Figure 5(b).[148] In a TNBS-induced colitis model, mice lacking VDR in the colonic epithelium exhibited increased severity of colitis, T_H1 and T_H17 responses, apoptosis of epithelial cells, and impaired intestinal permeability.[149] Thus, while the anti-inflammatory effect of VDR signaling has been demonstrated clearly, the direct effect of BAs on VDR in the context of inflammation has not been shown as clearly, prompting future investigations in this regard.

PXR is expressed across the intestine and in macrophages. PXR is activated by LCA and 3-oxoLCA, resulting in a reduction of intestinal inflammation.[150] PXR expression level (gene *NR1H4*) is an indicator of intracellular BA concentration in the liver and intestine. PXR plays a key role in regulating enterohepatic circulation. Inhibition of PXR leads to decreased expression of transforming growth factor (TGF)-β and IL-10, and increased expression of TNF-α and IL-8.[151] PXR agonists induce anti-inflammatory effects in DSS-induced colitis mice by inhibiting NF-κB and reducing cytokine and chemokine expression.[152] In a mouse model of necrotizing small intestinal colitis, administration of low-dose LCA was found to reduce intestinal IL-6 expression in a PXR-dependent manner.[150,152] Deficiency in both PXR and CAR was found to increase the relative abundance of *Lactobacillus*, which possesses BSH activity, and corresponding decreased taurine-conjugated PBAs in feces, which may lead to a higher internal burden of taurine and unconjugated BAs.[153] Taken together, these findings suggest that dysbiosis of gut microbiota and alterations in BAs may be involved in regulating inflammatory responses through BA receptors. Conversely, BA receptors can also affect the abundance of gut microbiota. Nonetheless, further investigation is needed to understand details of mechanisms of action and quantitative relationships between gut microbiota, microbiota-derived BAs, and BA receptors.

5. IBD treatments targeting the gut microbiota-BA axis

Current medical treatments for IBD typically involve the use of 5-aminosalicylates or sulfasalazine, antibiotics, corticosteroids, immunomodulators, and

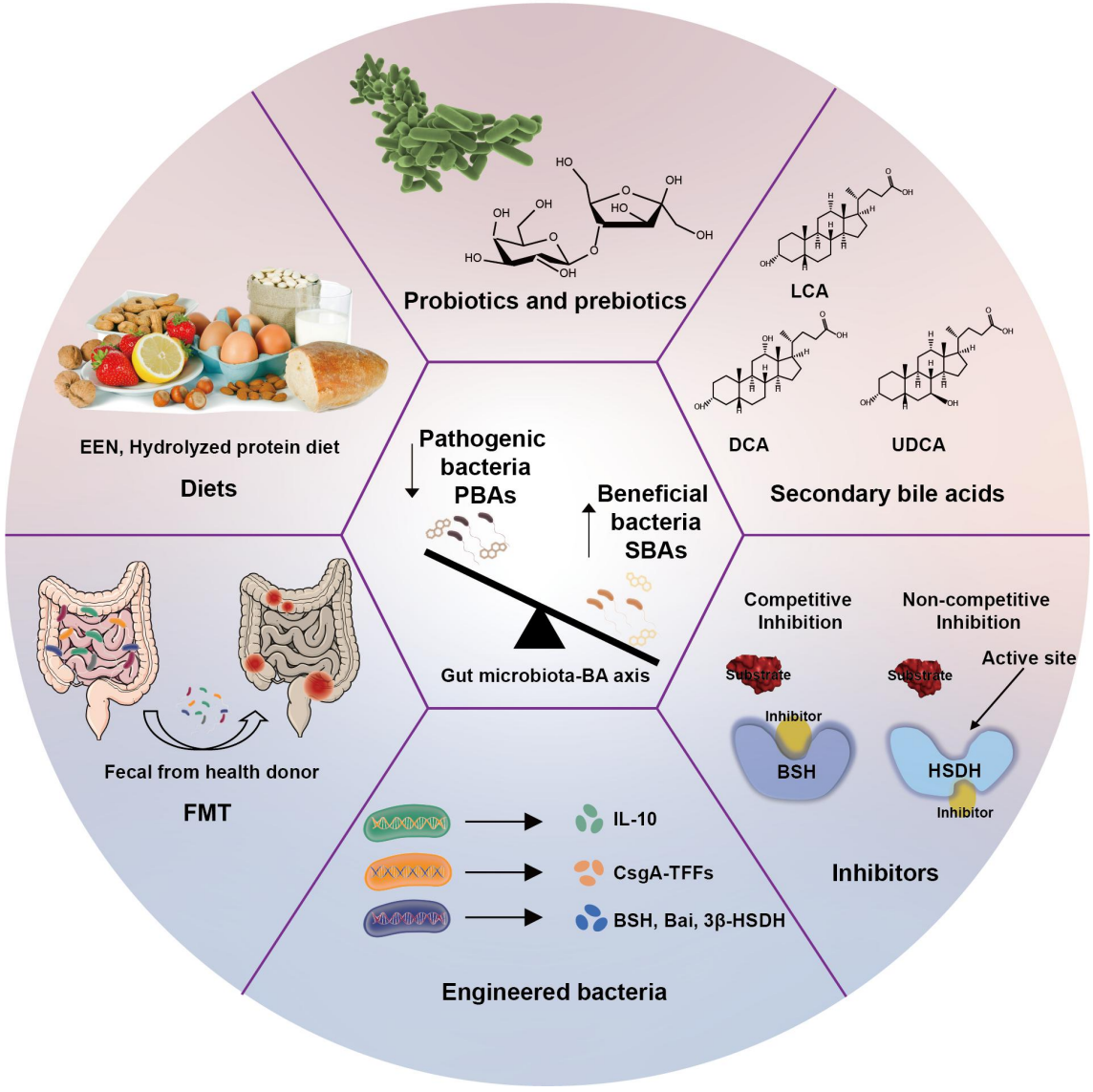

Figure 6. The emerging treatment of IBD targeting the gut microbiota-BA axis.

biologics therapies.[154,155] Considering the insights from this review on the effect of the gut microbiota-BA axis on IBD, modulating this axis may represent a promising new treatment strategy Figure 6.

5.1. Diets

The importance of diet in improving health is widely recognized.[156] Diets such as the 'Western' diet, which is high in fat and protein but low in fruits and vegetables, were found to be associated with IBD development.[157] A diet may trigger intestinal inflammation by altering the composition of gut microbiota and BAs, activating immune cells, and increasing intestinal permeability in IBD patients.[158] To this end, dietary therapy has shown promise in improving the health condition of IBD patients. For example, enteral nutrition (EEN) is a first-line treatment for CD in children.[159,160] EEN therapy is based on direct anti-inflammatory effects, which improve the composition of gut microbiota, reduce barrier permeability, and modulate the immune system.[161,162] Previous studies have also demonstrated that EEN has a significant and lasting impact on the composition of major intestinal bacterial groups.[163] Moreover, EEN has been found to partially restore the BA composition in patients, particularly by increasing levels of hydrophobic BA (LCA), which activates TGR5 receptors and reduces inflammation.[121] In mice

with inflammation, supplementation with fucose was found to increase *Lactobacillus* abundance and a decrease in *T*-β-MCA levels in the ileum. *Lactobacillus* exhibited the highest enzyme activity for the deconjugation of BAs. *T*-β-MCA acts as an FXR antagonist, and its reduction leads to the reactivation of the FXR-FGF15-CYP7A1 pathway, thereby reducing the BA pool and restoring the dysregulated ecology associated with colitis.[164]

Dietary fibers are essential for preserving microbial diversity, and the gut microbiota plays a pivotal role in modulating host physiology by metabolizing these fibers.[165,166] This interaction can significantly influence BA metabolism as well. For instance, soluble dietary fibers such as oligofructose can promote the conversion of PBAs to SBAs by gut microbiota.[166] Additionally, dietary resistant starch supplementation can increase gut luminal DCA abundance in mice.[167] These findings imply that dietary fibers could potentially be utilized to regulate gut microbiota and bile acid metabolism. Despite these insights, the impacts of fibers on IBD still present certain ambiguities. While epidemiological studies have indicated a link between a fiber-rich diet and a reduced incidence of IBD, numerous confounding factors complicate this relationship.[168] Individuals with IBD often exhibit intolerance to fermentable fiber-rich foods, with certain dietary items acting as triggers for gut symptoms.[169] For instance, fermentable carbohydrates such as fructans have been shown to worsen functional gastrointestinal symptoms in IBD patients.[170] Parallel complexity can be found in animal studies. For instance, feeding mice a low-fiber diet rather than a naturally fiber-rich diet predisposes mice to severe DSS-induced colitis, which is further exacerbated by the addition of highly soluble fiber inulin to the low-fiber diet. In contrast, the addition of the moderately soluble fiber psyllium to this diet provided robust protection in DSS-induced colitis and a model of T-cell transfer colitis, and psyllium fiber against colitis via activation of FXR.[171,172] Thus, the effect of fiber may be related to fiber and environment. Moreover, the influence of fiber appears to be intricately tied to an individual's microbiota composition. Specifically, Armstrong et al. found that dysregulated inulin fermentation can trigger a pro-inflammatory response through activation of the NLRP3 and TLR2 pathways in patients with IBD who lack the activity of specific fermenting microorganisms.[173] Bonazzi et al. also found that individualized microbiota determines the effect of dietary fiber on colitis sensitivity.[174] Therefore, individuals with IBD possessing a microbiota resistant to fiber may not necessarily need to eliminate all forms of soluble fiber. Conversely, those with a microbiota sensitive to fiber should meticulously assess their fiber consumption and sources as integral components of disease management. Hence, a comprehensive comprehension of how soluble fiber interacts with a specific gut microbiota is imperative for the development of individualized interventions based on fiber and microbiota, for patients with IBD as well as for healthy individuals.

Sophora alopecuroides L. is a traditional medicine known for its anti-inflammatory properties. Following treatment with *Sophora alopecuroides* L., the abundance of *Firmicutes* species was found to increase, whereas that of *Bacteroidetes* species decreased. Additionally, levels of αMCA, βMCA, ωMCA, and CA were found to increase significantly.[175] The presence of *Bacteroidetes, Proteobacteria*, and *Deferribacteres* was found to be positively correlated with αMCA, βMCA, ωMCA, and CA, whereas those of *Actinobacteria*, *Tenericutes*, *Firmicutes*, and *TM7* were negatively correlated.[175] Furthermore, the content of DCA in the intestine of DSS-induced mice was significantly reduced by the presence of *Sophora alopecuroides* L., indicating its potential to alleviate UC by reducing the cytotoxicity of DCA.[175] Another study found that dihydromyricetin (DHM), a nutraceutical supplement, was found to lead to an enrichment of beneficial genera such as *Lactobacillus* and *Akkermansia*, and increased levels of unconjugated BAs (CDCA and LCA), thereby activating FXR and TGR5 and maintaining intestinal integrity.[176] This suggests that DHM treatment in IBD targets the gut microbiota-BAs-FXR/TGR5 signaling pathway.[176] Herba Origani can also promote BA absorption and regulate BA metabolism in DSS-induced UC mice to maintain intestinal mucosal immune homeostasis. It can also enhance the abundance of the *Bacteroidota* community to restore the gut microbiota dysbiosis.[177] In summary, dietary therapy improves the disease symptoms by reducing pathogenic bacteria and

increasing the abundance of beneficial commensal bacteria. These bacteria also play a role in modulating BA composition through enzymatic catalytic activity. While dietary therapy is a relatively safe option, changing long-term lifestyle habits may be challenging.[178] Moreover, achieving effectiveness of specific diets in different populations also requires further investigation through randomized controllable trials.[179]

5.2. Probiotics and prebiotics

Probiotics such as *Bifidobacterium*, *Lactobacillus* genera, *Lactococcus* spp., *E. coli* Nissle 1917, and *Streptococcus thermophilus* are living microorganisms that are consumed as food supplements to improve gut microbiota and enhance intestinal barrier function.[180,181] Probiotics have been approved for alleviating intestinal disorders in IBD.[97] For instance, a combination supplement of *Bifidobacterium bifidum* WBIN03 and *Lactobacillus plantarum* ZDY2013 has been shown to enrich unidentified *Firmicutes* and decrease the abundance of *Bacteroidetes*, while regulating oxidative stress and inflammatory mediators to alleviate UC.[182] Similarly, *Bifidobacterium longum* (*B. longum*) CECT 7894 has been found to increase the relative abundances of beneficial bacteria such as *Bifidobacterium*, *Blautia*, *Butyricicoccus*, *Clostridium*, *Coprococcus*, *Gemmiger*, and *Parabacteroides*, while reducing the relative abundances of harmful bacteria like *Enterococcus* and *Pseudomonas*. [183] Furthermore, *B. longum* CECT 7894 has been shown to increase the abundance of SBAs such as α-MCA, β-MCA, LCA, CDCA, UDCA, HCA, isoLCA, and isoalloLCA, which were found to be positively associated with the increased abundance of BSH and 7α-dehydroxylases.[183] *B. longum* CECT 7894 was also found to improve the efficacy of infliximab (IFX) in DSS-induced colitis by modulating gut microbiota composition and BA metabolism.[183] Another study demonstrated that *Lactobacillus casei* strain Shirota (LcS) could inhibit the clinical manifestation of DSS-induced mice by increasing the relative abundance of beneficial bacterial species and reducing the relative abundance of pathobionts.[184] Additionally, the levels of taurine-conjugated PBAs (TCA, TCDCA) and taurine-conjugated SBAs (TDCA, TUDCA) were found to increase, while those of α-MCA and β-MCA decreased. These changes in the BA profile further suppressed the expression of IFN-γ and nitric oxide (NO), and increased the expression of IL-10 in colonic tissue.[184] *E. coli* Nissle 1917 was also shown to have the same efficacy and safety profile as the gold standard mesalazine in patients with UC.[185] Moreover, a mixture of multiple strains tends to be more effective than a single strain. For instance, Tursi et al. studied the effects of supplementation with VSL#3 in patients affected by relapsing UC who were already under treatment with 5-ASA and/or immunosuppressants at stable dose. Compare with placebo group, VSL#3 was found to improve rectal bleeding and decreased ulcerative colitis disease activity index (UCDAI) scores by 50% in UC.[186] VSL#3 was also shown to be efficient and safe in children with UC.[187] In contrast to traditional single probiotics or simple combinations, members of a designed bacterial consortium (GUT-103 and GUT-108) work synergistically with each other to restore microbial composition and function in IBD patients, providing DCA and LCA, SCFAs, indoles, and antimicrobial agents that are missing in IBD patients.[188] 7-α-dehydratase (7-α-DH) or 7α-HSDH activity are induced in GUT-103 and GUT-108 strains. GUT-103, which is composed of 17 strains, synergistically provides protective and sustained engraftment in the IBD inflammatory environment, preventing and treating chronic immune-mediated colitis. The therapeutic application of GUT-108 reduces the levels of colitogenic *Enterobacteriaceae* and increases beneficial resident *Clostridium* (Clusters IV and XIVa) species, especially *Lachnospiraceae* including *Dorea* species and *Lachnoclostridium* species that are not GUT-108 constituents.[188] In addition, GUT-108 strains provide additional redundancy for the synthesis of the protective LCA and DCA.[188]

Prebiotics are typically carbohydrates with various molecular structures, including polyphenols and polyunsaturated fatty acids.[189] These substances may enhance the composition and quantity of beneficial species in the gut microbiota, such as *Bifidobacteria* and *Lactobacilli*. These beneficial species utilize the nutrients from prebiotics to support their growth and proliferation.[190] In overweight dogs, inulin-type prebiotics have been found to increase the abundance of *Firmicutes* and decrease the relative abundance of *Proteobacteria*. In addition, the concentration of

fecal total BAs was observed to increase in dogs fed with prebiotics.[191] In clinical remission, fermentation of Xylo-Oligosaccharide (XOS) was found to promote the growth of beneficial bacteria such as *Roseburia, Bifidobacterium*, and *Lactobacillus*, and thereby alleviate dysbiosis in the feces of UC patients.[192] Similarly, stachyose was found to increase the abundance of beneficial microbiota species (*Akkermansia* and *Lactobacillus*) and bacterial diversity, thereby mitigating acute colitis in mice.[193] Further investigation has demonstrated that prebiotics change the metabolites of gut microbiota to treat UC. High-dose fructans have been shown to increase colonic butyrate production, significantly reduce colitis, and lead to an increased abundance of *Bifidobacteriaceae* and *Lachnospiraceae*. However, these shifts were not correlated with improved disease scores.[194]

These findings highlight the potential of probiotics and prebiotics in targeting the gut microbiota-BA axis for IBD treatment. Nevertheless, it is still challenging to arrive at definitive conclusions regarding the effectiveness of probiotic and prebiotic products due to the high heterogeneity and risk of bias in clinical studies. Large-scale and well-designed trials are necessary before probiotics and prebiotics can be adopted as definitive treatment strategies for IBD. Moreover, the selection of probiotic and prebiotic supplements should be carefully considered in individualized medicine, considering not only the disease itself but also the specific BA metabolic profile of each patient.

5.3. Fecal microbiota transplantation

FMT from a healthy donor to a patient is a therapeutic approach to restore the composition of the gut microbiota.[8] Previous studies have shown that FMT resolves 80–90% of antibiotic-resistant *Clostridium difficile* infections (CDI).[195,196] After FMT treatment of CDI, patients were found to show increased levels of SBAs, and decreased levels of PBAs, which is essential for the success of FMT treatment since PBAs (e.g., TCA) can cause the spore formation of *C.difficile*. [197] FMT also yielded positive results for IBD treatment, especially in UC patients. A clinical trial (ClinicalTrials.gov Number: NCT01545908) demonstrated nine patients with active UC without infectious diarrhea, who received fecal transplants from a single donor, and showed remission of inflammation.[198] FMT induces remission in UC by increasing microbial diversity. Patients in remission after FMT showed enrichment of *Eubacterium hallii*, *Roseburia inulivorans*, and SBAs, while patients without remission showed enrichment of *Fusobacterium gonidiaformans, Sutterella wadsworthensis*, and *Escherichia* species (ClinicalTrials.gov, Number: NCT01896635).[199] FMT with high-dose and multi-donor was also found to be more effective in active UC.[199] FMT is typically delivered via colonoscopic infusion and enemas, but orally administered FMT has recently been assessed as effective in UC as well.[200] Taken together, FMT offers potential as a therapy to achieve remission in IBD. However, FMT transplants may also fail. A recent randomized controlled clinical trial in adults with colonic or ileo-colonic CD (ClinicalTrials.gov Number: NCT02097797) showed that the enrichment of *Gammaproteo* bacteria *(Proteobacteria phylum*) such as *Klebsiella*, *Actinobacillus*, and *Haemophilus* may affect the success of donor microbiota colonization.[201] Other factors still require further consideration, including the mode of delivery, aerobic versus anaerobic stool preparation, number of donors, and the need for single or repeated FMT. To this end, larger trials are necessary to determine the feasibility of FMT as an option for IBD treatment.

5.4. Secondary bile acids

SBAs such as UDCA, DCA, and LCA may offer alternative treatments for IBD.[202,203] Oral administration of UDCA, TUDCA, or GUDCA has been shown to reduce intestinal ecological disorders, and increase the ratio of *Firmicutes/Bacteroidetes* in the intestine of DSS-induced mice.[204] UDCA therapy can also increase the abundance of BSH-rich bacteria, such as *Akkermansia muciniphila*, and prevent the loss of *Clostridium* cluster XIVa, bacterial species known to be particularly decreased in IBD patients.[204] Moreover, UDCA-mediated alleviation of DSS-induced colitis was dependent on the microbiota. UDCA facilitated colonization of *Akkermansia*, which was associated with the enhancement of the mucus layer upon UDCA treatment and activation of FXR receptor

in macrophages.[205] These results indicate that UDCA could be a promising treatment option for reducing dysbiosis and ameliorating inflammation in human IBD. The interaction between gut microbiota and fecal BAs is also a risk factor in colorectal cancer neoplasia. In male patients undergoing UDCA treatment, an overrepresentation of *Faecalibacterium prausnitzii* and an underrepresentation of *Ruminococcus gnavus* were more prominent in male patients without adenoma recurrence compared to those with recurrence. However, this relationship was not observed in female patients.[206] Additionally, UDCA and LCA are protective against DSS-induced increases in epithelial permeability and colonic inflammation. Specifically, UDCA administration was found to increase colonic LCA levels, while LCA administration did not alter UDCA levels.[203] Bossche et al. showed that TUDCA attenuated ileitis by alleviating the downregulation of nuclear receptors and BA transporters in $TNF^{\Delta ARE/WT}$ mice.[207] Different doses of UDCA may have different effects on patients. For example, a high-dose UDCA (50 mg/kg/day) was found to ameliorate experimental colonic inflammation, while lower doses (10, 25 mg/kg/day) showed no significant effect.[208] However, long-term use of high-dose UDCA (28–30 mg/kg/day) was also found to increase the risk of colorectal cancer in patients with UC and primary sclerosing cholangitis.[209] Therefore, maintaining the dynamic balance of BAs is crucial for intestinal homeostasis.

An important limitation of oral BAs is their rapid absorption into the hepatic-intestinal circulation of the small intestine and delivery to the liver, resulting in low concentrations within the colon. Therefore, alternative delivery methods need to be explored to achieve therapeutic concentrations in the colon. Recently, rectal administration of DCA and LCA was shown to reduce inflammation in three models of colitis in mice.[115] LCA and DCA suppress *in vitro* proinflammatory cytokine production from human peripheral blood-derived macrophages, which are key mediators of intestinal inflammation in IBD, through activation of the TGR5 receptor. In conclusion, SBAs are promising candidates for IBD treatment. However, BAs may also affect the structure of the intestinal microbial community, which plays a significant role in the development of inflammatory disorders. Therefore, the effect of BA therapy on the fecal microbiota during colitis should also be taken into account for the development of a therapy strategy.

5.5. Inhibitors

The utilization of SBA biotransformation-associated microbial elements such as BSH, HSDH, and *bai* genes, as targets for the gut-microbiota BA axis, has not been fully explored. Metagenomic analysis of the human microbiome revealed that the abundance and activity of gut BSHs are indeed correlated with IBD.[210] Parasar et al. developed chemo proteomic tools to study BSH activity in gut microbiota, and identified altered BSH activities in a DSS-induced mouse model, leading to changes in BA metabolism, and subsequently impacting host metabolism and immunity.[211] Similar to biologics that demonstrated significant improvements in inducing and maintaining remission in IBD, BSH inhibitors have also been discovered. Smith et al. discovered BSH inhibitors such as riboflavin, phenethyl caffeate, tetracycline antibiotics, and roxarsone from 2,240 biologically active and structurally diverse compounds using a high-throughput screening system.[212] Subsequently, Adhikari et al. identified a covalent pan-inhibitor compound 7 from a rational design candidate library of BSHs, leading to a decrease in the level of conjugated BAs.[28] Recently, Adhikari developed a highly potent gut-restricted inhibitor (AAA-10) with low off-target effects and durable efficacy *in vivo*. After 5 days of oral administration of AAA-10, the abundance of DCA and LCA in the gastrointestinal tract of wild-type mice was found to decrease, suggesting that AAA-10 is highly potent in inhibiting the activity of BSH, and modulating the composition of the BA pool *in vivo*.[213] Taxifolin can inhibit neurosteroid synthesis by inhibiting steroid biosynthesis steroid 5α-reductase 1 (SRD5A1) and 3α-hydroxysteroid dehydrogenase (3α-HSD, AKR1C9).[214] Similarly, a competitive inhibitor of 3β-hydroxysteroid dehydrogenase (3β-HSD) may terminate pregnancy by inhibiting the synthesis of progesterone (P4).[215] To the best of our knowledge, no inhibitor of HSDH for IBD treatment has been discovered to date. Our

knowledge regarding the role of the *bai* gene is also limited. Funabashi et al. constructed a 7α-dehydroxylation pathway including *bai* operon in *C. sporogenes*, and realized a complete eight-step conversion of CA to DCA *in vitro*. [73] SPF mice colonized with the baiH mutant S122 exhibit reduced intestinal inflammation compared to those colonized with the WT *Faecalicatena contorta* S122, indicating the potential of baiH as a therapeutic drug target for colitis.[74] These studies provide novel insights into manipulating the gut microbiota through targeting *bai* genes. However, no inhibitors of the *bai* operon have been discovered to date. In section 3, we have provided an extensive discussion of the structural features and catalytic mechanisms of the enzymes involved in the BA metabolic pathway. Structure-function studies of enzymes are vital to evaluate the molecular basis of the interaction between these enzymes and their inhibitors, in addition to providing a theoretical basis for structure-based inhibitor design. While these inhibitors are not yet ready for clinical application as new drug candidates, they can still be utilized as chemical probes to gain a better understanding of the effects of gut microbiota and BAs on host metabolism.

5.6. Engineered bacteria

Orally engineered bacteria can survive in the complex environment of the gastrointestinal tract and express desired target molecules. Engineered bacteria may then target the intestinal mucosa, synthesize and deliver therapeutic molecules on the surface of the intestinal mucosa, and thereby enhance the effectiveness of treatment compared to classical systemic therapies.[216] By using synthetic biology techniques such as the CRISPR-Cas system, researchers have developed recombinant bacterial strains that produce active therapeutic agents, such as IL-10 at mucosal sites to inhibit inflammation[217] and secrete neutralizing anti-TNF-α nanobodies to block the proinflammatory effects of TNF-α.[218] Most therapeutic approaches for IBD are aimed at modulating intestinal inflammation; however, restoring intestinal barrier function and promoting mucosal healing, which has not been studied extensively as a therapeutic strategy, may also be possible. To this end, *Lactobacillus lactis* was engineered to produce trefoil factor (TFF), then secreted TFF was absorbed in the colon to promote intestinal barrier function and epithelial repairing.[218]

Recently, *E. coli* Nissle 1917 was engineered to secrete curli-fused TFFs (CsgA-TFFs) both *in vitro* and *in vivo*. This nanofiber-composed matrix was then shown to promote mucosal healing and immunomodulation to prevent DSS-induced colitis in mice.[219] Thus, engineered bacteria may be a viable approach, although the treatments are based on animal models. The intestinal ecological dysregulation in IBD leads to reduced levels of microbial BA biotransformation and SBAs while inducing higher levels of conjugated PBAs. *In vivo*, BAs were found to be modified by the gut microbiota through a mixture of deconjugation, 7α/β-dehydroxylation, and oxidation/epimerization. In this process, the first step involves the catalysis of conjugated BAs to release free BAs by BSH. A deficiency of BSH-active bacteria plays a role in intestinal inflammation and impairs BA metabolism. Therefore, BSH-active bacteria in probiotic preparations are being actively investigated for IBD treatment.[220] The second step involves the conversion of PBAs (CA, CDCA) into SBAs (DCA, LCA) through a multistep biochemical pathway carried out by bacteria containing *bai* genes. DCA and LCA are strong TGR5 agonists with anti-inflammatory properties. Therefore, stimulating the microbial 7α-dehydroxylation (7α-DH) pathway may also promote the production of DCA and LCA, and thereby dampen intestinal inflammation.[115] Engineered 7α-DH overexpressing strains and minimal gut microbiota with 7α-DH activity are potential strategies in this regard.[221,222] Recently, Funabashi et al. constructed a 7α-dehydroxylation pathway in *C. sporogenes* based on the characteristics of the enzyme encoded by the *bai* operon and realized a complete eight-step conversion of CA to DCA *in vitro*. [73] This engineered *C. sporogenes* can be used to produce DCA and thereby regulate intestinal immunity. In addition, SPF mice colonized with the baiH mutant S122 exhibit reduced intestinal inflammation compared to those colonized with WT *Faecalicatena contorta* S122.[74] The third step involves the oxidation and epimerization of BAs via HSDHs. An engineered *Bacillus mimicus* strain with 3β-HSDH was developed to produce isoDCA, which promotes the production of colonic $RORgt^+$ T_{reg} cells in a cns1-

dependent manner. In DCs, isoDCA interacts with FXR to enhance T_{reg} induction. Recently, *Ruminococcus gnavus* with 5BR and 3β-HSDH enzyme activity was shown to epimerize DCA to 3-oxoDCA, and subsequently to 3-isoDC, which are less toxic.[75] Besides, microbiome-mediated BA metabolism can be disrupted by antibiotic-induced dysbiosis, leading to an overaccumulation of taurocholate in the colon.[223] Taurocholate can induce the germination of *Clostridioides difficile* endospores, thereby causing *C.difficile* infection (CDI).[224] As a result, *E. coli Nissle* 1917 containing recombinant bile salt hydrolase (Cbh) of *Clostridium perfringens* (EcN-Cbh) have been engineered to inhibit CDI by deconjugating taurocholate to cholate and reducing the abundance of *C.difficile* in the murine model. Additionally, EcN-Cbh can also elevate the levels of other bile salts including chenodeoxycholate, lithocholate, and deoxycholate. Therefore, EcN-Cbh can be utilized to regulate BA pool and gut microbiota, presenting promising implications for the use of such engineered microbes to treat IBD.[225] These studies suggest that targeting the gut microbiota-BA axis can alleviate intestinal inflammation, reduce BA toxicity, modulate immunity, and thus provide a treatment mechanism for IBD. Engineering bacteria to treat IBD based on the mechanism of gut microbiota catalyzing BA metabolism may also be a feasible mechanism. However, the manipulation of microbial elements associated with SBA production, including BSH, *bai*, and HSDH genes, through targeting the gut microbiota-BA axis, has not been extensively studied.[210] The treatment based on BAs also needs to be validated in humans through high-quality, randomized controlled trials before it can be implemented in clinical practice. Currently, phase II clinical trials are underway to verify whether UDCA reduces inflammation and improves the quality of life in UC pouch patients (ClinicalTrials.gov, Number: NCT03724175).[115]

6. Conclusion and perspective

Numerous studies to date have investigated the relationship between gut microbiota, microbiota-derived BAs, and IBD. While many reviews have focused on the role of BAs and their receptors in driving immune and microbial changes within the intestines, the interplay between alterations in abundance and function of gut microbiota, BA concentrations, recently discovered metabolic pathways *in vivo*, the diverse biological roles of BAs, and the specific mechanisms of enzymatic conversion in metabolic pathways need to be elucidated. In this review, we discussed the role of gut microbiota in the metabolism of BAs, with a particular emphasis on providing insights into the structure and catalytic mechanism of key enzymes found in the gut microbiota. Advancements in synthetic biology and understanding of the related catalytic mechanisms allow for the design of engineered bacteria. These bacteria, characterized by high activity and robust thermostability, can be tailored for various pathological and physiological contexts. However, manipulation of SBA transformation-related microbial elements, including BSH and *bai* genes, remains an underexplored avenue in the gut-microbiota BA axis. We have also discussed changes in the gut microbiota and BAs in IBD, and interactions between the gut microbiota and its metabolized BAs through different BA receptors and immune cells. Thus, targeting the gut microbiota-BA axis holds significant potential for IBD treatment. To this end, further research is necessary to deepen our understanding of the underlying mechanisms and validate their efficacy in clinical practice.

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