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MINI-REVIEW

Gut microbiota: a new avenue to reveal pathological mechanisms of constipation

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Abstract

Constipation is very pervasive all over the world. It is a common multifactorial gastrointestinal disease, and its etiology and pathomechanism are not completely clear. Now, increasing evidence shows that intestinal flora is closely related to constipation. Intestinal flora is the largest microbiota in the human body and has powerful metabolic functions. Intestinal flora can produce a variety of metabolites, such as bile acids, short-chain fatty acids, tryptophan metabolites, and methane, which have important effects on intestinal motility and secretion. The host can also monitor the intestinal flora and regulate gut dysbacteriosis in constipation. To explore the relationship between intestinal flora and host, the combination of multiomics technology has become the powerful and effective method. Furthermore, the homeostasis restoration of intestinal flora also provides a new strategy for the treatment of constipation. This review aims to explore the interaction between intestinal flora and host in constipation, which contributes to disclose the pathogenesis of constipation and the development of novel drugs for the treatment of constipation from the perspective of intestinal flora.

Key points

- *This review highlights the regulation of gut microbiota on the intestinal motility and secretion of host.*
- *The current review gives an insight into the role of the host on the recognition and regulation of intestinal ecology under constipation.*
- *The article also introduces some novel methods of current gut microbiota research and gut microbiota-based constipation therapies.*

Keywords Constipation · Gut microbiota · Metabolites · Gut immunity

Introduction

Constipation is common worldwide and a frequent clinical symptom. According to statistics, the global incidence of constipation ranges from 2 to 35% (Andromanakos et al. 2006; Mugie et al. 2011). Constipation can be induced by many factors, including lifestyle, diet, psychosocial factors, colonic propulsive or rectal emptying disorders, and the use of some drugs (such as anticholinergics, opioids, antihistamines, antipsychotics) (Vriesman et al. 2020). According to the duration of constipation, it is divided into acute constipation (usually lasting no more than a week) and chronic constipation (usually lasting more than 4 weeks or 3 months) (Camilleri et al. 2017). The main symptoms of constipation are dry and hard feces, difficult defecation and reduced defecation times. Currently, intestinal dysfunction is considered to be the main mechanism of the pathogenesis of constipation, including intestinal fluid transport, intestinal motility, mucus secretion and intestinal nerve conduction disorder (Zhao et al. 2021). However, a large number of recent studies showed that gut microbiota imbalance was also an important factor in the occurrence and development of constipation.

Recently, with the growing interest in the gut microbiome, our understanding of gut health and disease has been greatly advanced. The gut microbiota has been implicated in various diseases, such as type 2 diabetes, ulcerative colitis,

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and chronic kidney disease (Zhang et al. 2021a, b; Xiao et al. 2020; Zou et al. 2020). There are numerous bacteria living in the gut whose number is 10 times that of our human cells, and they even control 100 times more genetic information than ours (Fujimura et al. 2010). They play an important role on assisting host metabolism, maintaining the function of intestinal barrier and promoting the development of immune system (Obata and Pachnis 2016; Schoeler and Caesar 2019). Many studies found that the intestinal flora was disordered in constipation patients and animal models (Fan et al. 2022; Liang et al. 2019). It was shown that the beneficial bacteria were reduced and the potential pathogenic bacteria were increased in the gut of constipation (Huang et al. 2022; Mancabelli et al. 2017; Zhu et al. 2014). These phenomena suggest that the intestinal flora is involved in the occurrence and development of constipation. Although the intestinal flora imbalance is prevalent in constipation, the exact mechanism of how dysbiosis is involved in constipation needs to be fully explored.

This review focuses on clarifying the potential mechanisms by which the gut microbiota is involved in the occurrence and progress of constipation and introduces the research progress and technical replacement. Furthermore, the regulation of host on the disordered intestinal flora in the state of constipation is also introduced. Currently, the modulation of intestinal flora has become a clinical therapy for improving constipation. However, the mechanism of intestinal flora in the process of constipation is not completely clear. We hope that this review can give readers a new understanding on the relationship between intestinal flora and constipation and provide novel ideas for the research and clinical treatment of constipation.

Constipation and imbalance of intestinal flora

Increasing evidence suggests that the shift of intestinal flora is closely associated with constipation (Table 1). In the past, with culture-based methods, researchers found that constipation was usually accompanied by the disorder of gut microbiota. Zoppi et al. firstly indicated the imbalance of intestinal flora in constipation by using the culture-based microbiological method (Zoppi et al. 1998). Subsequently, Khalif et al. also used this method to conduct a similar study on adult patients. Results showed that the abundance of *Bifidobacterium* and *Lactobacillus* in patients with constipation was lower than that in the control groups, while the levels of *Enterobacteriaceae* (such as *Escherichia coli*), *Staphylococcus aureus*, and fungi increased (Khalif et al. 2005). However, this conventional detection technique underestimates about half of the colonic microbes, which limits our further understanding of gut microbiota in constipation (Quigley 2011). There are several reasons: many species are not cultured, strictly anaerobic bacteria die in aerobic conditions, and in vitro culture changes the original structure of the microflora. But, the culture-based microbiological approach made us initially understand the relationship between constipation and gut microbes.

With the maturity of sequencing technology, gut microbiome sequencing technology enables us to have deeper understanding of gut microbiota and it has been widely used to reveal the effect of gut microbiota on constipation. Zhu et al. used 16S rRNA gene pyrosequencing to show that the abundance of phylum *Bacteroidetes* in children with functional constipation (FC) was significantly reduced, while the level of several species in the phylum *Firmicutes* was markedly increased (Zhu et al. 2014). Subsequently, Kim et al. also reported that the species of *Bifidobacteria* and *Bacteroides* were notably decreased in the feces of adult patients with FC by real-time quantitative polymerase chain reaction (Kim et al. 2015). Conversely, Tian et al. found that *Bifidobacterium* was more abundance in slow transit constipation patients than in healthy controls (Tian et al. 2021). Mancabelli et al. employed 16S rRNA sequencing and whole genome sequencing to detect the intestinal microbial composition of FC patients (Mancabelli et al. 2017). Their data showed that short-chain fatty acids-producing bacteria (such as *Bacteroides*, *Roseburia*, and *Coprococcus 3*) were significantly reduced in the gut of FC patients, and the genes associated with hydrogen production, methanogenesis, and glycerol degradation had high abundance. In addition to fecal samples, some studies also examined mucosal microflora with mucosal biopsy samples. Durbán et al. found that the levels of *Bacteroides* and *Enterobacteriaceae* were elevated in the intestinal mucosa of constipated patients by 16S rRNA metagenomic analysis (V1-V2) (Durbán et al. 2012). Using this technique, Parthasarathy et al. reported that *Bacteroides* were remarkably enriched in the intestinal mucosa of constipated patients (Parthasarathy et al. 2016). Compared with the ancient culture methods, intestinal microbial sequencing can more comprehensively and accurately reflect the changes of the original structure and function of gut microbes.

Collectively, gut microbiota in constipated patients is mainly characterized by the relative reduction of beneficial bacteria (such as *Lactobacillus* and *Bifidobacterium*), the relative increase of potential pathogens, and the decrease of species richness (Ohkusa et al. 2019). However, as mentioned above, there are opposite situation that constipation patients have more beneficial bacteria in their guts than healthy controls. For example, Du et al. observed that beneficial bacteria (such as *Bifidobacterium* and *Lactobacillales*) were the predominant bacteria in the gut of Parkinson's patients with constipation (Du et al. 2022). Differences in the type of constipation may be the main reason for this situation. The environment is crucial to the shaping of the microbiome, and the

Table 1 The changes of intestinal bacteria in constipation

References	Microbiota analysis method	Biological samples	Study population	Outcomes
Zoppi et al. 1998	Microbial culture	Feces	28 children with FC (age 7.9–9.8), 14 healthy controls (age 7.9–9.8)	Constipation patients: *Bifidobacteria* ↑ *Clostridia* ↑
Khalif et al. 2005	Microbial culture	Feces	57 FC patients (age 17–70), 25 healthy controls (age ND)	Constipation patients: *Bifidobacterium* ↓ *Lactobacillus* ↓ *Clostridium* ↓ *Bacteroides* ↓ *Enterobacteriaceae* (*E. coli*) ↑ *Staphylococcus aureus* ↑ Fungi ↑
Durbán et al. 2012	16S rRNA gene sequencing (V1–V2)	Intestinal mucosa	3 IBS-C patients and 9 healthy control, age ND	Constipation patients: *Bacteroidetes* ↑ *Enterobacteriaceae* ↑
Zhu et al. 2014	16S rRNA pyrosequencing	Feces	8 children with FC (age 10.3–13.3), 14 healthy controls (age 12.5–13.9)	Constipation patients: *Blautia* ↑ *Coprococcus* ↑ *Ruminococcus* ↑ *Anaerotruncus* ↑ *Clostridium* ↑ *Prevotella* ↓
Kim et al. 2015	qRT-PCR	Feces	30 FC patients (age 30–40), 30 healthy control (age 29–35)	Constipation patients: *Bifidobacterium* ↓ *Bacteroides* ↓
Parthasarathy et al. 2016	16S rRNA gene sequencing (V3–V5)	Feces and intestinal mucosa	13 FC, 6 IBS-C and 6 mixed IBS patients (age 33–63), 25 healthy controls (age 29–49)	Constipation patients: *Bacteroidetes* ↑
Mancabelli et al. 2017	16S rRNA and shotgun metagenomics	Feces	68 FC patients (age 24–64), 79 health controls (age 15–63)	Constipation patients: *Bacteroides* ↓ *Roseburia* ↓ *Coprococcus* ↓ *Faecalibacterium* ↑

FC functional constipation, *IBS-C* irritable bowel syndrome with constipation, *ND* no description, ↑ increased abundance of bacteria, vs healthy control, ↓ decreased abundance of bacteria, vs healthy control

intestinal environment is also different for different types of constipation, resulting that the intestinal flora may be different (Coyte and Rakoff-Nahoum 2019). In addition, the relationship between gut microbiota and constipation may be bidirectional. Prolonged colonic transit during constipation may promote the expansion and colonization of slow-growing species, leading to profound changes in the structure and function of the whole microbiome. On the other hand, external factors (such as diet, drugs, and exercise) can cause the disorder of the intestinal flora and its metabolism, which contributes to intestinal dysfunction and promote the development of constipation.

Microbial signals in intestinal motility and secretion

The normal motility and secretion of the intestine can ensure the normal operation of the intestinal contents, and at the same time, it can maintain the homeostasis of the intestinal environment and provide a suitable habitat for the intestinal flora. In the past, it was believed that the motility and secretory functions of the gut were regulated by the host. For example, the loss of enteric nerve subsets and interstitial cells of Cajal, malfunction of smooth

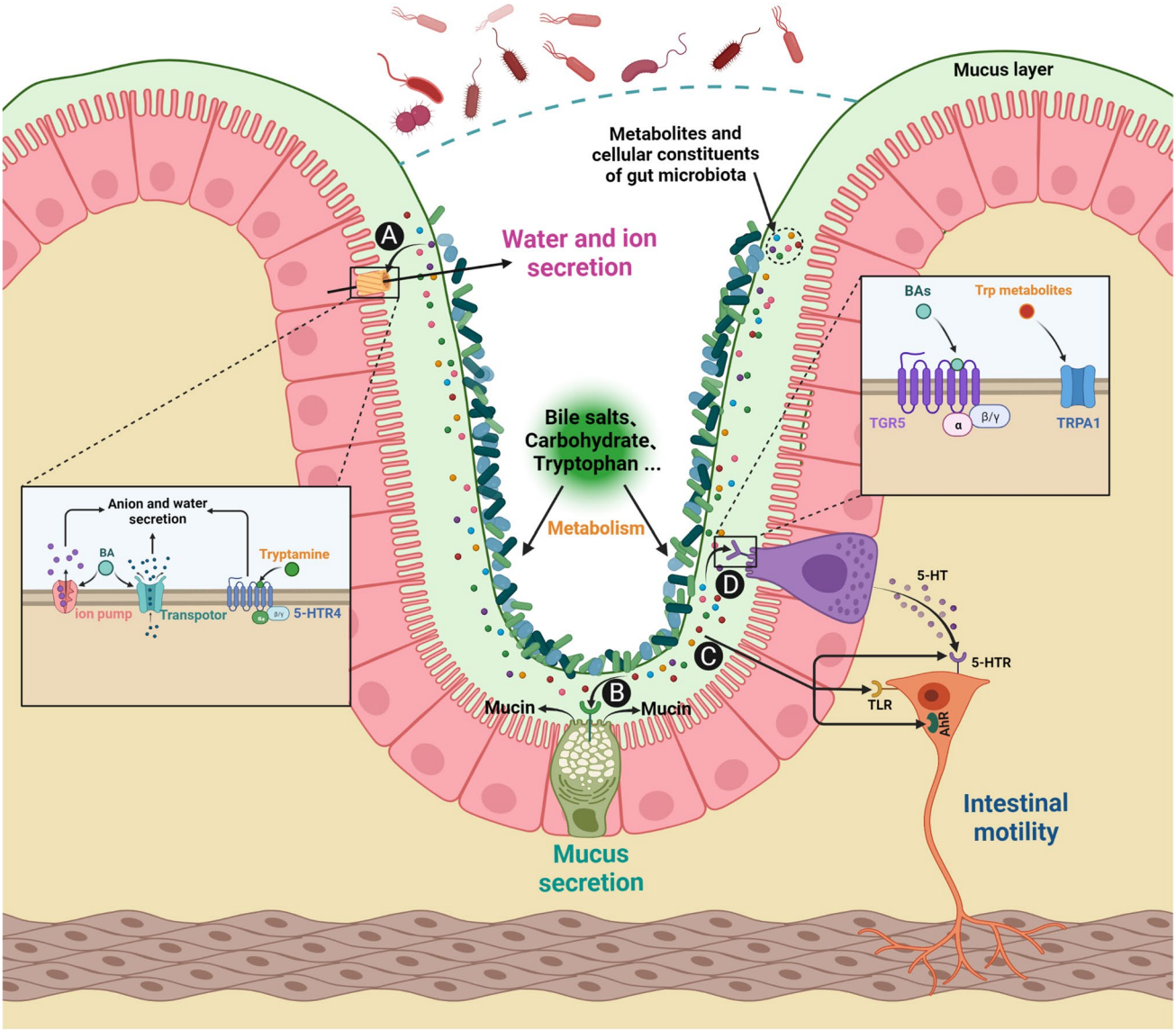

Fig. 1 Regulation of gut microbiota on host intestinal motility and secretion-related functions. **A** Stimulated by bacterial metabolites, intestinal epithelial cells can secrete water and ions into the intestinal cavity through ion pump, transporter, and exchanger. **B** Bacterial metabolites can induce goblet cells to secrete mucin and form a natural physical barrier in the intestine. In addition, intestinal microflora metabolites and cellular constituents can directly stimulate intestinal neurons (**C**) or regulate intestinal movement through intestinal endocrine cells (**D**). 5-HT, 5-hydroxytryptamine; 5-HTR, 5-HT receptor; AhR, aryl hydrocarbon receptor; BA, bile acid; TGR5, Takeda G protein-coupled receptor 5; TLR, Toll-like receptor; TRPA1, transient receptor potential ankyrin subtype 1 protein

muscle, and changes in the immune cells were regarded as the basis of internal motility obstacle (Mazzone and Farrugia 2007). However, a recent series of studies have shown that gut microbial signals can also influence gut motility and secretion (Bhattarai et al. 2020; Fukui et al. 2018; Vicentini et al. 2021). The effect of gut microbiota on gut motility and secretory function is shown in Fig. 1.

Motility

Abnormal intestinal motility is an important factor in the occurrence of constipation. Previous studies showed that the intestinal nervous system could strictly control the movement of the intestine. There are abundant receptors in the intestinal cells, which can sense physical and chemical stimuli and transmit excitation to intestinal smooth muscle through enteric nerves (Joshi et al. 2021; Steensels and Depoortere 2018). It has been found that gut microbiota-derived metabolites such as bile acids, short-chain fatty acids, and tryptophan metabolites, are also able to modulate gut motility by stimulating gut chemoreceptors (Dalziel et al. 2021).

Bile acids (BAs) are an important regulator of intestinal function, which are produced by the host and metabolized by

the intestinal flora. About 5% of bile salts in bile entered the colon, where they were uncoupled or metabolized into secondary bile acids by gut flora (Appleby and Walters 2014; Wahlström et al. 2016). BAs could activate G-protein coupled BA receptor of enterochromaffin cells and endogenous primary afferent neurons to stimulate the release of serotonin 5-hydroxytryptamine (5-HT) and calcitonin gene-related peptide, resulting in intestinal peristaltic reflex (Begley et al. 2006; Bunnett 2014). Several studies showed that the inhibitor of the ileal BA transporter (Elobixibat) could increase the levels of colonic BAs and improve the colonic transport (Chedid et al. 2018; Nakajima et al. 2018, 2022). Microbial transplantation study in mice revealed that the altered microflora could influence the gastrointestinal transport by affecting the uncoupling of bile salts (Dey et al. 2015; Li et al. 2021a, b). Of note, the effect of bile acids on intestinal motility requires the participation of intestinal flora, but its mechanism remains to be clarified.

Tryptamine metabolized from tryptophan by *Clostridium sporogenes* and *Ruminococcus gnavus* could reduce the colonic transport time through 5-HT receptor 4 (5-HTR4) in colonic epithelial cells (Bhattarai et al. 2018; Williams et al. 2014). Additionally, tryptamine also was a ligand for aryl hydrocarbon receptor (AhR) in intestinal cells (Vyhlídalová et al. 2020). Interestingly, Obata and his colleagues recently concluded that intestinal flora could induce the expression of AhR in intestinal neurons and make them respond to AhR ligands derived from microflora, which was beneficial to their excitation and intestinal peristalsis (Obata et al. 2020). Indeed, compared with germ-free mice, mice colonized by *Ruminococcus gnavus* showed the induction of several genes involved in tryptophan metabolism (Hoffmann et al. 2016). *Ruminococcus gnavus* was found in about 90% of adults and infants, and their ability to produce tryptamines could affect human health (Qin et al. 2010; Sagheddu et al. 2016). Ye et al. explored a new mystery that the transient receptor latent ankyrin A1 (TRPA1) could sense microbial tryptophan metabolites (indole, indoleacetic acid) to stimulate enteroendocrine cells to secrete 5-HT, and then transmit bacterial signals to the gut and vagus nerve (Ye et al. 2021). Thus, the perturbation of tryptophan metabolism, which were closely associated with the composition, diversity, and metabolism of gut microbiota, might affect intestinal transit time (Roager et al. 2016; Vandeputte et al. 2016).

Short-chain fatty acids (SCFAs) produced by intestinal bacteria from carbohydrate fermentation were the energy source of intestinal tissue and the regulator of colon motility (Vonk and Reckman 2017), among them, acetic acid, propionic acid, and butyric acid are the most common short-chain fatty acids in the human body (Li et al. 2021a, b). The stimulating effect of SCFAs on intestinal motility is hormone-dependent (Martin-Gallausiaux et al. 2021). SCFAs could regulate some gastrointestinal hormones, such as glucagon like peptide-1 (GLP-1) and peptide YY (PYY), which could regulate gastrointestinal motility, were released in response to SCFAs (Gribble and Reimann 2019). Recently, a study showed that depletion of gut microbiota led to a decrease in enteric neurons and induced intestinal hypomotility, whereas the supplementation with SCFAs could promote the recovery of enteric neurons (Vicentini et al. 2021). Butyrate was found to restore gut motility in germ-free mice in the presence of 5-HT (Vincent et al. 2018). Furthermore, propionic acid (enteral) could slow colonic movement by CO-activating free fatty acid receptors 2 and 3 (FFA2 and FFA3) through PYY and enteric nervous system pathways, respectively (Tough et al. 2018). *Lactobacillus* and *Bifidobacterium* in the intestine were proved to produce γ-aminobutyric acid (GABA), which was a derivative of SCFAs (Cui et al. 2020). GABA receptors were also expressed in intestinal neurons and could modulate the gastrointestinal motility, which was reviewed by Auteri et al. (Auteri et al. 2015). These evidences suggest that gastrointestinal hormones and enteric nerves are the pathways of SCFAs regulating intestinal motility.

The gas yielded by intestinal flora also had an important impact on intestinal motility. It was believed that methanogens could slow down intestinal movement and cause constipation (Sahakian et al. 2010). Clinical evidence showed that a significant increase in the abundance of methanogens was observed in constipation patients (Ojetti et al. 2017; Ghoshal et al. 2018). Additionally, in patients with irritable bowel syndrome dominated by constipation, antibiotic treatment could reduce methanogenic bacteria in the intestinal microflora, which led to the improvement of pathological symptoms (Low et al. 2010). *Methanobrevibacter smithii* is the most common methanogenic bacterium in human intestine (Dridi et al. 2009). A clinical study indicated that *Methanobrevibacter smithii* was overgrown in the intestines of patients with constipation and accompanied by elevated levels of methane (Takakura et al. 2022). Furthermore, symbiotic microorganisms could produce NO with nitrate or nitrite in intestinal lumen as substrate (Koch et al. 2017). NO was proved to be an inhibitory neurotransmitter, which was essential for the relaxation of gastrointestinal smooth muscle and intestinal motility (Groneberg et al. 2016).

Toll-like receptors (TLRs), a kind of pattern recognition receptors, which can recognize intestinal bacteria to initiate intracellular signaling affecting the gastrointestinal motility. For instance, TLR2 and TLR4 were located on the membrane of intestinal nerve cells, muscle and glial cells to regulate movement. TLR4 could recognize bacterial lipopolysaccharide (LPS), while TLR2 could recognize lipopeptides and peptidoglycans (Akira et al. 2006). In addition, bacterial microbubbles could stimulate TLR2 (Al-Nedawi et al. 2015). Reduced fecal production and longer defecation time were found in mice with TLR4 deficiency or impaired

LPS response, which confirmed that bacterial components could affect the intestinal motility (Anitha et al. 2012; Caputi et al. 2017). Recently, Yarandi et al. indicated that intestinal bacteria maintained the gastrointestinal motility via TLR2-induced intestinal neurogenesis in mice (Yarandi et al. 2020). Their results illustrated that the number of colonic myenteric neurons were markedly increased after the administration of TLR2 agonists. Emerging evidence suggested that resident intestinal flora was essential for regulating intestinal neurons and intestinal endocrine cell populations, as well as neurogenic colonic activity. The gut resident microbe *Bacteroides thetaiotaomicron* could normalize the changes in the expression of nitric oxide synthase and choline acetyltransferase in the myenteric plexus of germ-free mice (Aktar et al. 2020).

In conclusion, intestinal microbial signals can transmit information to the host to affect the gastrointestinal motility. These signals may be microbial metabolites or cellular constituents. Therefore, the microbial signal disorder caused by the imbalance of intestinal flora in the process of constipation is an important factor for the slowdown of the gastrointestinal transport. Although the dynamic balance of intestinal microflora is important for the healthy colonic transport, there is no clearly defined microflora for the optimal colonic transport. So, it is necessary to further explore the microflora under the optimal colonic transport.

Secretion

Intestinal secretion includes water, ions, and mucin, which play an important role in the smooth operation of feces in the intestinal lumen. Gut flora are also able to influence intestinal secretion through their metabolites. BAs not only affect intestinal motility and also stimulate the secretion in the colon (Keely et al. 2022). It was reported that BAs could promote chloride secretion and inhibit sodium absorption in colonic epithelial cells by regulating colonic ion pump, exchanger, and transporter to induce lumen fluid accumulation (Keely and Walters 2016). Brianna et al. found that tryptamine caused the change of short-circuit current by using Ussing chamber with the proximal mouse colon, which confirmed that tryptamine could affect the ion secretion of colonic epithelial cells (Williams et al. 2014). Additionally, tryptamine was found to act on 5-HTR4 to increase cyclic adenosine monophosphate (cAMP) to stimulate colonic anion and fluid secretion (Bhattarai et al. 2018). Several studies showed that intestinal hormone secretion was also affected by intestinal flora. Zhuang et al. reported that SCFAs produced by intestinal flora could stimulate the secretion of intestinal peptide YY in constipated rats (Zhuang et al. 2019). Wichmann et al. found that germ-free mice did not produce SCFA, resulting in a significant increase in the level of plasma glucagon like peptide-1 (GLP-1), which could inhibit intestinal peristalsis (Wichmann et al. 2013). Through fecal microbiota transplantation (FMT) in patients with irritable bowel syndrome (IBS), it was found that FMT changed the density of enteroendocrine cells in intestine in patients with IBS (Mazzawi et al. 2021). Enteroendocrine cells, as the largest hormone secreting population in the gut, expressed a diverse array of G protein coupled receptors as well as Toll- like receptors (Yu et al. 2020). Therefore, enteroendocrine cells might be the direct or indirect target of the gut microbiota influencing gut hormone secretion.

Mucin in the gut is mainly secreted by intestinal goblet cells, which is the main component of intestinal mucus layer. The intestinal mucus layer can provide the habitat for intestinal flora and lubricate the contents. A large number of studies verified that intestinal symbiotic bacteria played an important role in promoting intestinal mucus secretion (Hayes et al. 2018; Sicard et al. 2017; Bergstrom et al. 2020). For example, compared with normal mice, the number of intestinal goblet cells in sterile mice were reduced and the maturation of mucus system was slow (Johansson et al. 2015). Intestinal meprin β enzymes could cleave mucin to release mucus, but the cleavage process required the induction of intestinal flora (Schütte et al. 2014). In the intestine, some commensal bacteria such as *Bacteroides thetaiotaomicron* and *Faecalibacterium prausnitzii* could increase the differentiation of goblet cell and the expression of mucus related genes (Wrzosek et al. 2013). In fact, the outer mucous layer of the colon was inhabited by symbiotic bacteria that could degrade mucin to obtain energy, such as *Bacteroides acidifaciens* (in mice), *Bacteroides fragilis*, *Bifidobacteriaceae*, *Lactobacillus* spp., and *Akkermansia muciniphila* (in mice and humans) (Donaldson et al. 2016). The degradation of glycoprotein of outer mucous layer by symbiotic bacteria can promote the natural replacement of intestinal mucus. Although these phenomena show that intestinal flora can affect the secretion of intestinal mucus, a large number of studies are still needed to clarify its mechanism.

Regulation of dysregulated intestinal flora in patients with constipation

The microenvironment of the intestinal lumen is created by intestinal flora and intestinal cells, so the intestine also affects the microflora. Increasing evidence showed that intestinal innate immunity could shape the microbiota (Kurilshikov et al. 2017). A recent study indicated that there was an inflammatory response and flora imbalance in constipated mice, and the transplantation of the dysfunctional flora into the intestines of healthy mice could also induce the intestinal inflammation (Lin et al. 2021). Previously, colonic histopathological studies proved that there was the microscopic inflammation in the colonic tissue of patients with constipated irritable bowel syndrome, which

was mainly characterized by the increase of mast cells, intraepithelial lymphocytes, and $CD3^+$ T cells in the colonic mucosa (Barbara et al. 2004; Chadwick et al. 2002). Intestinal cells could express pattern recognition receptors (PRRs), which were used to sense microbial associated molecular patterns (MAMPs), and then promote immune response to resist pathogens (Potrykus et al. 2021). PRRs are divided into five families: Toll-like receptors (TLRs), C-type lectin-like receptors (CLRs), nucleotide binding oligomerization domain (NOD)-like receptors (NLRs), retinoic acid-induced gene-I (RIG-I)-like receptors (RLR), and recently identified absent in melanoma (AIM)-like receptors (ALR) (Agier et al. 2018; Kurilshikov et al. 2017). After PRRs sense microbial antigens, they could regulate the function of other intestinal cells against pathogens through activating intestinal immunity, and restore the intestinal ecological balance (Bonder et al. 2016; Hoving et al. 2014; Xiao et al. 2019). Therefore, in constipation, the host may activate the immune response through these PRRs to restore the balance of intestinal microbiota.

In the colon, the mucus layer, an important physical barrier, is further divided into two layers: an internal firm layer for isolating microorganisms and an external loose layer that provides the habitat for microorganisms (Zhao and Maynard 2022; Li et al. 2015; Lin et al. 2021). Intestinal mucus is mainly composed of mucin. A report suggested that the deletion of mucin 2 (MUC2) gene in mice could alter the intestinal microbiota (Wu et al. 2018). The levels of *Desulfovibrio*, *Escherichia*, *Akkermansia*, *Turicibacter*, *Erysipelotrichaceae*, and *Ruminococcaceae* increased and contents of *Lactobacilli* and *Lachnospiraceae* decreased in MUC2 deficient mice. Mucin were often modified by complex polysaccharides, and some bacterial species used glycosidases to degrade mucin to obtain polysaccharides as a carbon source (Crouch et al. 2020). It was found that O-glycans on MUC2 from the proximal colon regulate the structure and function of the microflora (Bergstrom et al. 2020). When constipation occurs, dry and hard feces stay in the colon for a long time, which is easy to take away the external loose mucus layer, creating the opportunity for bacteria to invade the internal mucus layer and cause inflammation. Excitingly, it was reported that the mediators produced by the immune system could drive the proliferation of goblet cells and increase mucus secretion (Khan and Collins 2004; Oeser et al. 2015). The increase of mucus secretion of goblet cells could help to wash away the bacteria in the inner mucus layer (Birchenough et al. 2016). However, the replenishment rate of mucus is slower than the consumption rate and the constipation cannot be relieved, which is regarded as the host-autonomous regulation of dysbiosis in constipation.

On the other hand, there are a lot of antibacterial substances (such as immunoglobulin A (IgA) and antimicrobial peptides) in the mucus layer inside the intestine, which play an important role on maintaining the balance of intestinal flora. IgA produced by intestinal lamina propria plasma cells can maintain the intestinal homeostasis through binding and interacting with mucin and intestinal bacteria in the outer mucus layer (Rogier et al. 2014). IgA not only help the host to remove pathogens but also anchor symbiotic bacteria in mucus. It has proved that symbiotic *Bacteroides fragilis* can specifically recognize IgA to promote mucosal colonization (Donaldson et al. 2018). Inatomi et al. found that the concentration of IgA was notably increased in the feces of constipated rats after the treatment with probiotics (Inatomi and Honma 2021). IgA was proved to enhance the adhesion of *Escherichia coli*, *Bifidobacterium lactis*, and *Lactobacillus rhamnosus* to epithelial cells, revealing that microbes could also benefit from IgA to establish mucosal microbial communities (Bollinger et al. 2003; Mathias et al. 2010). Furthermore, antimicrobial peptides produced by Paneth cells also play an important role on improving the intestinal dysbiosis (Suchodolski and Jergens 2016). It was found that intestinal symbiotic bacteria had stronger antimicrobial peptide resistance compare with pathogens, which was also the key for antimicrobial peptides to maintain the homeostasis of host intestinal flora (Cullen et al. 2015). Antimicrobial peptides and IgA in mucus layer are very important to maintain the balance of intestinal microflora, and their expression is regulated by intestinal immune system. Therefore, in the process of constipation, low-grade intestinal inflammation is a means for the host to perceive and maintain intestinal eubiosis (Fig. 2).

Constipation therapy and research strategy based on intestinal flora

In recent years, some new constipation therapies, such as prebiotics, synbiotics, inhibitors of ileal bile acid transporters, antibiotic treatment for patients with methanogenesis, and 5-HT4 receptor agonists, have been clinically available (Choi and Chang 2015; Ford et al. 2014; Prichard and Bharucha 2018; Triantafyllou et al. 2014). Notably, microbial related agents could not only improve pathological symptoms of constipation but also regulate the host immune system (Yeşilyurt et al. 2021). For example, probiotics, especially *Lactobacillus* and *Bifidobacterium*, could stimulate immune cells (such as Th1, Th2, Th17, T regulatory cells, and B cells) and increase the production of SIgA and antimicrobial substances, which resisted pathogens and toxins in the intestine and maintained the integrity of the intestinal barrier (Dargahi et al. 2019; Shi et al. 2017). Nevertheless, studies indicated that probiotics might be a potential risk for the treatment of constipation. Especially in elderly constipated patients with impaired intestinal mucosal barrier or immunosuppressive state, probiotic therapy carried

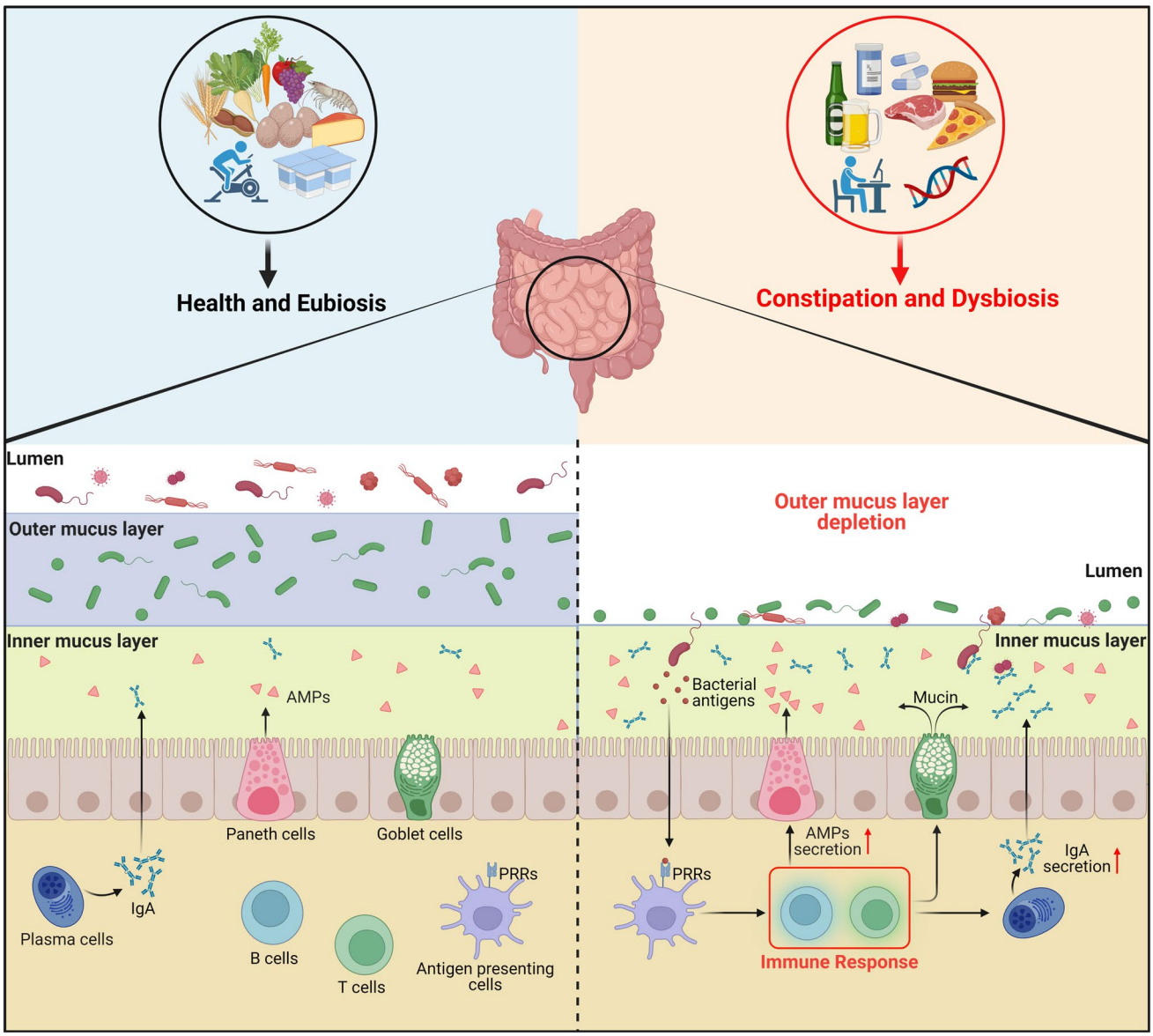

Fig. 2 Host supervision and regulation of gut microbiota in constipation. Constipation is caused by many factors, such as diet, drugs, lack of exercise, and genetic factors. Constipation is accompanied by the imbalance of intestinal flora and the consumption of intestinal mucus. The mucus layer in the intestine is divided into outer loose layer and inner solid layer. The outer loose layer provides the habitat for symbiotic bacteria. Constipation leads to the depletion of outer loose layer in the intestine, which can cause the run off of symbiotic bacteria and the invasion of pathogens and symbiotic bacteria into inner solid layer. The inner solid layer contains a large number of antibacterial substances (such as IgA and antibacterial peptides), and the entry of bacteria and their antigens into this layer will induce the immune response. The intestinal tract can recognize the pathogens and symbionts invading the internal solid layer through pattern recognition receptors to trigger the immune response, so as to regulate the synthesis of mucin, IgA, and antimicrobial peptides to resist invasion and regulate the imbalance of intestinal flora in the state of constipation

risks such as microbial translocation, opportunistic pathogen infection, D-lactic acidosis, and loss of bioactivity of antimicrobial or antifungal agents (Camilleri 2019; Rao et al. 2018; Suez et al. 2019). Fortunately, evidence indicated that the use of inactivated probiotics or probiotic metabolites could eliminate the risk of probiotic treatment of constipation (De Marco et al. 2018; Żółkiewicz et al. 2020). Because they are not living microorganisms, bacterial translocation and detrimental metabolic activities do not occur (Sotoudegan et al. 2019; Rossi et al. 2022). Additionally, based on the effect of gut microbiota on gut function, fecal microbiota transplantation (FMT) therapy for constipation has also received widespread attention (Liu et al. 2021). Researchers have carried out some clinical trials on FMT for the treatment of constipation, which obtained certain clinical effects (Johnsen et al. 2018; Zhang et al. 2021a, b; Kuai et al. 2021). However, the microbial species of FMT are very complex, including bacteria, fungi, viruses, and other components, and it is difficult

to determine its risk source, which hinders its clinical application (Blaser 2019). Therefore, a large number of studies are still needed to prove the safety of FMT to promote the promotion of FMT in the clinical treatment of constipation.

In Southeast Asia, plant laxatives or herbs including traditional Chinese medicines (TCMs) are usually used to relieve constipation. Anthraquinones (such as senna, aloe, rhubarb, frangula, and cascara) are the most commonly used plant laxatives, which can improve constipation mainly by stimulating fluid secretion and intestinal motility, especially for the short-term treatment of tension constipation, acute constipation, and before lower gastrointestinal endoscopy (Cirillo and Capasso 2015; Wang and Yin 2015). Interestingly, TCM formulas are proved to treat diseases (including constipation) through restoring the normal composition and function of gut microflora in clinical or animal studies (Tong et al. 2018; Xu et al. 2015). Zengye decoction (ZYD) composed of Sophorae Flavescentis Radix, Ophiopogonis Radix, and Rehmanniae Radix Praeparata notably decreased the abundance of harmful microbes (such as *Desulfovibrio*, *Prevotella*, and *Ruminococcus*), whereas it markedly increased the abundance of *Oxalobacter*, *Clostridium*, and *Roseburia* in aged rats with constipation (Liu et al. 2019). Additionally, the latest findings in our lab illustrated that rhubarb could markedly improve various pathological symptoms in constipated rats by restoring the homeostasis of intestinal flora and ameliorating the disorder of its metabolism (Yang et al. 2022). However, TCM formulas usually exerted their effects by multitargets and multipathways, while their mechanisms related to gut microflora have not been adequately investigated.

During the past two decades, with the implementation of the human genome project and the development of a new generation of sequencing technology, researches on intestinal microorganisms have received increasing attention and achieved fruitful results. In fact, these microbial detection technologies tend to reflect differences in composition and cannot obtain accurate results of the functional changes of gut microflora. However, the combination of multiomics and alternative medical technology complement the study of intestinal microflora. As an example, intestinal microbiota sequencing could identify new functional genes, microbial pathways, and dysfunctions of intestinal microbiome and determine the interaction and coevolution between microflora and host (Wang and Yin 2015). However, due to different primers and GC contents, each gene may not be amplified with the same efficiency during the PCR reaction, resulting in sequencing bias (Wensel et al. 2022). Furthermore, taxonomy accuracy relies on the integrity of the reference database, and the quality and quantity of databases determine the accuracy and resolution of taxonomy classifications (Jo et al. 2016). Metabolomics provided important help for us to understand the metabolic differences of intestinal flora in the disease state (including constipation), identify new metabolic markers, and uncover the functional changes of intestinal flora (Bauermeister et al. 2022). Furthermore, environmental transcriptomics and proteomics also provided an important supplement from the gene and protein levels for the study of intestinal microbial function (Bashiardes et al. 2016; Verberkmoes et al. 2009). Simultaneously, the newly developed intestinal chip and microfluidic technology enable us to have a clearer understanding of the interaction between intestinal flora and host, because these in vitro techniques can help us eliminate some interference factors and can more easily achieve variable control (Puschhof et al. 2021). Therefore, we can explore the effect of gut microflora on composition by regulating intestinal motility and secretion function based on integrated technologies and approaches, so as to clarify the intestinal flora related pathogenesis of constipation and develop new therapeutic drugs.

Conclusion

In the process of constipation, the imbalance of intestinal flora has been adequately confirmed. The intestinal flora is in the closed environment of the host intestine and is affected by the external environment and the host. In terms of external factors, constipation inducements such as diet, drugs, living habits, and social pressure will trigger the disorder of intestinal flora, while the damage of intestinal mucus caused by constipation and the disorder of intestinal hormones can also destroy the habitat and environment of intestinal symbiotic bacteria. In addition, intestinal flora can affect intestinal motility and secretion. There are abundant chemoreceptors in the intestine, which can sense the metabolites and cellular components from intestinal bacteria. In the state of constipation, the disorder of intestinal flora and its metabolism can result in the abnormality of gut motility and secretion. At the same time, the host has a regulatory role on the intestinal flora by some pattern recognition receptors. When the flora is disordered, the host activates the intestinal immune system and mucus to resist the pathogen and assist the colonization of probiotics. Therefore, the study of constipation based on intestinal flora can give us a comprehensive understanding of the pathogenesis of constipation and contribute to the development of new therapies. However, due to the structural complexity and functional diversity of intestinal flora, it is an appropriate strategy to uncover the relationship between constipation and intestinal flora through multiomics approaches.

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Declarations

Ethical approval This article does not contain any studies with animals performed by any of the authors.

Conflict of interest The authors declare no competing interests.

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