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EDITED BY
Shuai Wang,
Guangzhou University of Chinese Medicine, China

REVIEWED BY
Cong Zhang,
The First People's Hospital of Foshan, China
Stanislava Stanojevic,
Institute of Virology, Serbia

*CORRESPONDENCE
Chaomin Wan
wcm0220@126.com
Zhiling Wang
wang_zhi_ling@126.com

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Toll-like receptor 4 plays a vital role in irritable bowel syndrome: a scoping review

Xuemeng Wan[1,2,3], Liyuan Wang[1,2], Zhiling Wang[1,2,3]* and Chaomin Wan[1,2,3]*

[1]Department of Pediatrics, West China Second University Hospital of Sichuan University, Chengdu, China, [2]Key Laboratory of Birth Defects and Related Diseases of Women and Children, Ministry of Education, Chengdu, China, [3]National Health Commission Key Laboratory of Chronobiology, Sichuan University, Chengdu, China

Background: Irritable bowel syndrome (IBS) is a common gastrointestinal disease. Recently, an increasing number of studies have shown that Toll-like receptor 4 (TLR4), widely distributed on the surface of a variety of epithelial cells (ECs) and immune sentinel cells in the gut, plays a vital role in developing IBS.

Objectives: We sought to synthesize the existing literature on TLR4 in IBS and inform further study.

Methods: We conducted a systematic search of the PubMed, Embase (Ovid), Scopus, Web of Science, MEDLINE, and Cochrane Library databases on June 8, 2024, and screened relevant literature. Critical information was extracted, including clinical significance, relevant molecular mechanisms, and therapeutic approaches targeting TLR4 and its pathways.

Results: Clinical data showed that aberrant TLR4 expression is associated with clinical manifestations such as pain and diarrhea in IBS. Aberrant expression of TLR4 is involved in pathological processes such as intestinal inflammation, barrier damage, visceral sensitization, and dysbiosis, which may be related to TLR4, NF-κB, pro-inflammatory effects, and CRF. Several studies have shown that many promising therapeutic options (i.e., acupuncture, herbs, probiotics, hormones, etc.) have been able to improve intestinal inflammation, visceral sensitization, intestinal barrier function, intestinal flora, defecation abnormalities, and depression by inhibiting TLR4 expression and related pathways.

Conclusion: TLR4 plays a crucial role in the development of IBS. Many promising therapeutic approaches alleviate IBS through TLR4 and its pathways. Strategies for targeting TLR4 in the future may provide new ideas for treating IBS.

KEYWORDS

irritable bowel syndrome, toll-like receptor 4, inflammation, visceral hypersensitivity, treatment

1 Introduction

Irritable bowel syndrome (IBS), a functional gastrointestinal disease, is characterized by recurring abdominal pain and alterations in stool frequency or shape (1). Using Bristol stool grade, IBS patients are classified based on their abnormal defecation patterns into diarrhea-predominant IBS (IBS-D), constipation-predominant IBS (IBS-C), mixed IBS (IBS-M) and unclassified IBS (2). Globally, the incidence of IBS varies, approximately 10.1% (9.8%-10.5%) using the Rome III criteria and 3.8% (3.6%-4.0%) using the Rome IV criteria (3). Although IBS is not associated with increased mortality (4), it significantly impacts health-related quality of life, social functioning and psychosocial factors (5–8). Moreover, it imposes a substantial social and economic burden (1, 9, 10). Most costs incurred by IBS patients are due to productivity loss, and direct healthcare expenses are driven by IBS-related comorbidities (11). However, its underlying pathophysiological mechanisms are not fully understood, possibly involving factors like gut-brain axis dysfunction, stress, visceral hypersensitivity (VHS), altered gut motility, barrier function destruction, gut microbiome disorders, intestinal inflammation, immune activation, genetic factors, etc. (1). And as a consequence, no medical therapy is proven to alter the natural history of IBS, usually focus on alleviating symptoms. Therefore, new targets for IBS prevention and treatment have become important. In recent years, increasing numbers of studies have demonstrated that the Toll-like receptor (TLR) 4 plays a pivotal role in developing IBS (12, 13).

TLRs, members of the transmembrane pattern recognition receptor family, play an essential role in innate immune responses and bridge innate and acquired immunity. TLRs are involved in mucosal immune response, barrier function, cell adhesion, cell proliferation and migration, protection from pathogens, repair of epithelial cell injury, etc. (14). Thus, dysregulation of the TLRs signaling pathway contributes to the development and progression of various diseases such as autoimmune diseases, cancer, infections and chronic inflammation (15). Almost all TLRs are expressed in the small intestine and colon intestinal epithelial cells (ECs) (16).

TLR4 is one of the earliest transmembrane pattern recognition receptor family members to be studied. TLR4 is expressed in humans and mice's colon and ileum crypts (17). TLR4 signaling is essential for maintaining intestinal homeostasis, and its hyperactivation is a crucial driver of many disease states affecting gastrointestinal function (18). Early life stress affects susceptibility to IBS by modulating TLR4 (19). One study found that TLR4 mRNA expression was associated with the intensity of abdominal pain in IBS-D patients (20). TLR4 can promote the up-regulation of pro-inflammatory cytokines by activating the transcription factors nuclear factor-kappa B (NF-κB) via the adaptor protein myeloid differentiation primary response gene 88 (MyD88), ultimately affecting the intestinal barrier, VSH (13, 21). In addition, increased intestinal permeability in IBS patients promotes the activation of TLR-dependent immune responses.

There are no relevant review articles on the role of TLR4 in the pathological mechanisms of IBS. We have collected the current essential studies on the expression of TLR4 in IBS, the mechanism of action, and the drugs targeting TLR4 for the treatment of IBS through a scoping review, which may be instructive for a complete understanding of the biological function of TLR4 in the development of IBS.

Abbreviations: IBS, Irritable bowel syndrome; IBS-D, Diarrhea-predominant IBS; IBS-C, Constipation-predominant IBS; IBS-M, Mixed IBS; VHS, Visceral hypersensitivity; TLR, Toll-like receptor; ECs, Epithelial cells; NF-κB, Nuclear factor-kappa B; MyD88, Myeloid differentiation primary response gene 88; HC, Health control; IL, Interleukin; XIST, X-inactive specific transcript; EA, Electroacupuncture; PB, Postbiotic; NLRP3, NLR family pyrin domain-containing protein 3; PI-IBS D, Post infectious IBS-D; CRD, Colorectal distension; CI, Colonic irritation; NMS, Neonatal maternal separation; WAS, Water avoidance stress; OT, Oxytocin; GSE, Grape seed extract; KD, Ketogenic Diet; LC-DG, Lactobacillus casei DG; SHE, Serpylli herba extract; MT, Melatonin; Sb, Saccharomyces boulardii; LE, Lens culinaris Medik extract; LT, Lactibiane Tolerance; MCs, Mast cells; CKF, Chang-Kang-Fang; BHS, Berberis heteropoda Schrenk roots; XYS, Xiaoyaosan; SNP, Single-nucleotide polymorphism.

2 Method

2.1 Information sources and search strategies

Six common life-science databases (PubMed, EMBASE, Scopus, Web of Science, MEDLINE, and Cochrane Library) were applied to identify those studies that met the review criteria. In the PubMed database, we searched the eligible studies by using the following keywords: (((((((Toll-Like Receptor 4 [MeSH Terms]) OR (TLR4 [Title/Abstract])) OR (TLR-4 [Title/Abstract])) OR (Toll Like Receptor 4 [Title/Abstract])) OR (Toll-4 Receptor [Title/Abstract])) OR (Toll 4 Receptor [Title/Abstract])) OR (TLR4 Receptor[Title/Abstract])) OR (Receptor, TLR4[Title/Abstract]) AND ((((((irritable bowel syndrome [MeSH Terms])) OR (Irritable Bowel Syndromes [Title/Abstract])) OR (Syndrome, Irritable Bowel [Title/Abstract])) OR (Syndromes, Irritable Bowel [Title/Abstract])) OR (Colon, Irritable [Title/Abstract])) OR (Irritable Colon [Title/Abstract]). Reference lists of relevant publications were searched to identify more relevant studies. There were no restrictions on the language and date of publication. The literature was last searched on 7 June 2023.

2.2 Inclusion criteria

The study reports the expression of the TLR4 gene in irritable bowel syndrome compared to healthy controls, the pathogenesis involved in TLR4 in IBS, and therapeutic approaches targeting TLR4 for the treatment of IBS are included in the study. Study subjects included humans, rat rats, mouse mice, and cell cells. There are no language or publication status restrictions. Meetings and lack of full-text literature were excluded.

2.3 Literature selection

Study selection occurred in three stages: First, duplicate publications were immediately eliminated. Second, two

researchers independently reviewed titles and abstracts of the literature, and literature that did not meet the inclusion or exclusion criteria was discarded. Articles whose abstracts needed to provide more information to determine whether they were excluded were included directly in the full-text review stage. Third, full-text reviews were conducted independently by two researchers. Disagreements throughout the process were resolved by discussion or input from a third reviewer if required.

2.4 Data extraction

Two researchers worked together independently to extract data from articles that fit the topic of this study and then exchanged them for validation. A third researcher resolved the differences. Variables included clinical sample size, diagnostic criteria, study object, disease modelling method, the status of TLR4, effect on IBS, associated genes or pathways, and the main findings of the independent study.

3 Result

Six database searches identified 800 citations. After removing duplicates, 513 unique citations were screened for titles and abstracts. Of these citations, 110 met the criteria for full-text review. We excluded 70 studies because they did not have relevant data, could not be found in full text, contained insufficient information to assess the relationship between IBS and TLR4, or were conference literature. Finally, 40 studies were included. This whole process is outlined in Figure 1.

Based on the 40 included studies, we summarized the mechanisms and pathways of TLR4 involvement in IBS (Figure 2).

A total of 13 clinical research articles describing the expression of TLR4 in patients with IBS were identified in the literature search; 11 (11/13, 84.62%) articles showed that the expression of TLR4 was up-regulated in IBS compared with health control (HC), and only 2 (2/13, 15.38%) of them suggested that no change in TLR4 was found in IBS (Table 1). In addition to changes in TLR4 expression, the expression of TLR2, TLR5, TLR9, and inflammatory factors (interleukin (IL)-1α, IL-1β, IL-6 and IL-8) were up-regulated and anti-inflammatory factor (IL-10) was down-regulated (Table 1).

Ten studies reported the molecular mechanisms underlying the role of TLR4 in IBS (Table 2). The development of IBS disease was found to involve the TLR4/MyD88/NF-κB signaling pathway primarily. Activation of TLR4 and its signaling pathway can contribute to IBS development by promoting inflammation and mediating visceral sensitization and stool abnormalities (Table 2). MicroRNA was involved in IBS; research indicated that miR-16 inhibited the TLR4/NF-κB/X-inactive specific transcript (XIST) axis to relieve IBS-D (13).

We searched 18 studies that provided the data of specific treatments-targeted TLR4 for IBS (Table 3). Treatments included herbal medicine, moxibustion, electroacupuncture (EA), probiotics and its postbiotic (PB) element, diet, hormones, etc. (Table 3).

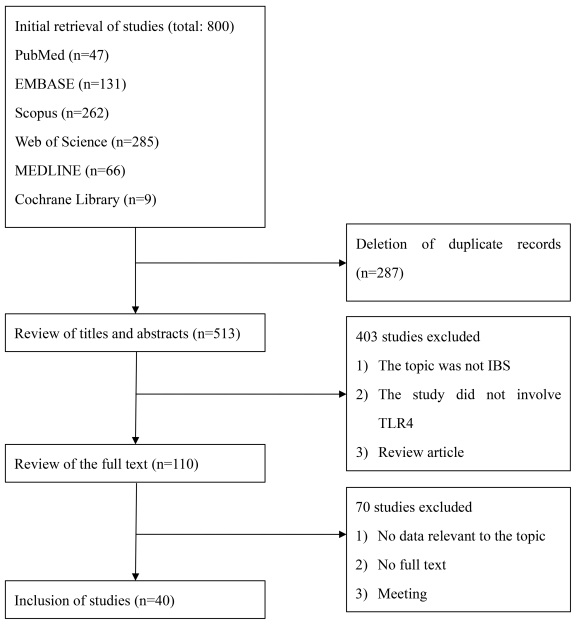

FIGURE 1
Study selection.

These treatments decreased inflammation, attenuated VHS and depressive-like behavior, and improved abnormal defecation and intestinal flora through TLR4/MyD88/NF-κB signaling pathway and TLR4/NF-κB/NLR family pyrin domain-containing protein 3 (NLRP3) pathway (Table 3).

4 Discussion

By summarizing the literature on the progress of TLR4 in IBS disease research, it is clear that TLR4 plays a vital role in developing IBS. TLR4 is involved in the pathogenesis of IBS, including low-grade inflammation, increased visceral sensitivities, intestinal barrier damage, intestinal flora dysbiosis, defecation abnormalities, etc.

TLR4 expression was upregulated in IBS patients (20, 22–28, 30–32). However, the results of TLR4 expression in different subtypes were inconsistent. Belmonte et al. reported significant differences by subtype with a 2-fold increase in IBS-M, more significant than in IBS-D (22). While Shukla et al. found the most significant increase in IBS-D (30). Five IBS-D-only publications, including one PI-IBS D, showed upregulation of TLR4 (20, 24, 26, 27, 32). Only one article on IBS-D patients suggests no change in TLR4 (29).

In two clinical studies of IBS that showed no significant change in TLR4 (29, 33), the disease was diagnosed using the Rome III diagnostic criteria. One specimen was peripheral blood serum, and the other study took mucosal tissues from multiple sites in the gut. Overall, there were no significant differences from the other studies in terms of IBS diagnostic criteria, IBS subtypes, sample types, or anatomic locations. Analyze why the results of these two studies are inconsistent with those of other studies. Yoshimoto et al.'s study had the presence of taking anti-flatulent and antidepressant drugs,

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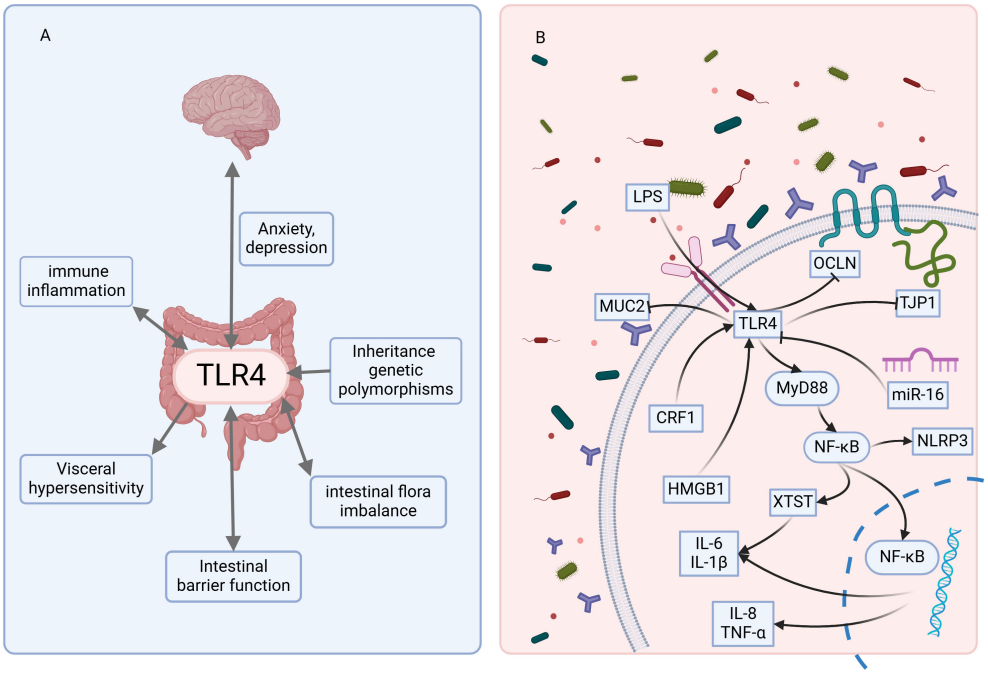

FIGURE 2
The mechanisms and pathways of TLR4 involvement in IBS. **(A)** indicates that TLR4 is involved in multiple pathogenic mechanisms of IBS. **(B)** indicates that TLR4 is involved in the molecular pathways involved in developing IBS. LPS, lipopolysaccharide; MUC2, Mucin 2; TJP1, Tight junction protein 1; OCLN, Occludin; HMGB1, High mobility group box 1; CRF1, Corticotropin-releasing factor receptor subtype 1. The figure was created using BioRender mapping software (https://BioRender.com).

TABLE 1 Studies reported the clinical findings the clinical significance of TLR4 in IBS.

Study	Publication year	Clinical sample size	Diagnostic criteria	Status of TLR4	Associated genes or pathways	Main findings
Belmonte et al. (22)	2012	48 IBS patients (37 F, mean age 49 ± 14 years) and 31 HCs (17 F, mean age 57 ± 14 years)	Rome III criteria	Activated	Increased TLR2, IL-8, and IL-1β, impaired PPARγ	Increased expression of TLR4 and TLR2 in the descending colon was observed in IBS-M patients alone, neither IBS-D nor IBS-C, resulting in enhanced expression of mucosal proinflammatory cytokines and impaired PPARγ expression.
Brint et al. (23)	2011	26 IBS patients (all F, mean age 37.9 years) and 19 HCs (all F)	Rome II criteria	Activated	Increased TLR5	The expression of TLR4 and TLR5 in IBS patient's colon mucosal showed up-regulation.
Compare et al. (24)	2017	10 post-infectious IBS-D (PI-IBS D) patients (4 F, mean age 52 years), 10 HCs (5 F, mean age 48 years)	Rome III criteria	Activated	Increased IL-1α, IL-6, and IL-8, decreased IL-10	TLR-4 protein expression was significantly higher in PI-IBS D patients than HC in both ileal and colonic mucosa.
Dlugosz et al. (25)	2017	22 IBS patients (17 F, median age, 39 [18-66] years), 14 HCs (12 F, median age, 42 [22-61] years)	Rome II criteria	Activated	Increased TLR5 and TLR9	The higher mRNA expression of TLR4, TLR5, and TLR9 in jejunum mucosa suggests the involvement of bacteria or dysregulation of the immune response to commensal flora in small bowel mucosa in IBS patients.
Guo et al. (26)	2016	20 IBS-D patients (13 F, mean age 42.9 ± 12.5 years), 20 HCs (15 F, mean age 41.1 ± 11.5 years)	Rome III criteria	Activated	Increased TLR2	Increased expression of TLR4 and TLR2 in the IBS-D's descending colon mucosal may be related to Bacteroidetes and Clostridium.

(Continued)

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TABLE 1 Continued

Study	Publication year	Clinical sample size	Diagnostic criteria	Status of TLR4	Associated genes or pathways	Main findings
Jalanka et al. (27)	2021	43 IBS-D patients (26 F, mean age 40.85 [19-69] years) and 21 HCs (16 F, mean age 42.8 [20-75] years)	Rome III criteria	Activated	Increased Myd88	Elevated TLR4 in IBS-D sigmoid colon tissue significantly correlated with MYD88.
Koçak et al. (28)	2016	51 IBS patients (43 F, mean age 38.90 ± 10.42 years) and 15 HCs (11 F, mean age 37.90 ± 10.47 years)	Rome III criteria	Activated	Increased TLR2, NO	The colonic tissue levels of TLR4 in the IBS-D and IBS-C subgroups were significantly higher, and IBS had immune dysregulation and oxidative stress.
Linsalata et al. (29)	2018	39 IBS-D patients (33 F, mean age 39.7 ± 7.2 years) and 20 HCs (13 F, mean age 40.05 ± 12.2 years)	Rome III criteria	unchanged	Increased IL-6 and IL-8, LPS not changed	There was no significant difference in the concentrations of LPS and TLR-4 in the plasma of IBS-D patients compared to the control group.
Lobo et al. (20)	2017	25 IBS-D patients (17 F, mean age 37.4 ± 2.1 years) and 16 HCs (7 F, mean age 32.1 ± 2.3 years)	Rome II criteria	Activated	TLR2 has no significant difference	TLR4 is upregulated in the jejunal mucosa of IBS-D patients, and its expression is correlated with the intensity of abdominal pain in these patients.
Shukla et al. (30)	2018	47 IBS patients (8 F, median age,34 [19-68] years) and 25 HCs (3 F, median age, 40 [20-68] years)	Rome III criteria	Activated	Increased CXCL-11 and CXCR-3, TLR2 not changed	TLR4 was upregulated in IBS patients, particularly in patients with IBS-D, and there was a positive correlation between TLR4 mRNA levels and weekly stool frequency in IBS patients.
Jizhong et al. (31)	2016	102 IBS patients (39 F) and 102 HCs (45 F), age-matched	Rome III criteria	Activated	Increased TLR2, CRF1, CRF2, IL-6, decreased IL-10	TLR4 and TLR2 were upregulated in peripheral blood samples of IBS patients, especially in IBS patients with depression.
Wang et al. (32)	2008	30 IBS-D patients (16 F, mean age 47 ± 14 years) and 12 HCs (7 F, mean age 4l ± 4 years)	Rome III criteria	Activated	Increased NF-κB	TLR4/NF-κ B signaling pathway was activated in the intestinal mucosa of IBS-D patients.
Yoshimoto et al. (33)	2022	31 IBS patients (17 F, mean age 47.0 ± 18.2 years) and 31 HCs (18 F, mean age 58.0 ± 12.0 years)	Rome III criteria	unchanged	Increased CXCL-11 in the duodenum, TLR5, CXCR-3, and IL-6 not changed	There was no significant difference in the expression levels of TLR4 in the duodenum, ileum, cecum, and rectum.

The fields in parentheses in this column for the study object refer to gender and age. The F represents females. PPARγ, Peroxisome proliferator-activated receptor-γ; NO, Nitric oxide; CXCL-11, C-X-C motif chemokine ligand 11; CXCR-3, C-X-C motif chemokine receptor 3.

which may have influenced the results. Disease heterogeneity is a primary reason for inconsistent results, such as concomitant symptom involvement, with one study showing that TLR4 expression was higher in IBS patients with concomitant depression (31). In addition, the sample sizes in these pieces of literature are small, which can lead to excessive random errors in the results. Future large-scale studies are needed to investigate the expression pattern of TLR4 in IBS, whether there are differences in expression between subtypes and the effect of concomitant symptoms such as anxiety and depression on TLR4 expression in IBS. This will help us to understand the disease, the relationship between the different subtypes, and the management of the disease. Other factors that may have influenced the results were genetic, environmental, etc.

4.1 TLR4 and intestinal immune and inflammatory activation

Low-grade mucosal inflammation and immune dysfunction are some of the main pathogenic mechanisms of IBS. Several clinical studies have found elevated levels of pro-inflammatory cytokines (such as IL-1α, IL-1β, IL-6, IL-8, IL-17, TNF-α, CXCL-11 and CXCR-3)and reduced levels of the anti-inflammatory cytokine IL-10 in patients with IBS (22, 24, 29–31, 59–62). There was a correlation between inflammatory factors and IBS symptoms and quality of life (60). Meanwhile, mRNA levels of TLR-4 in IBS patients were positively correlated with the inflammatory factor IL-6 (30).Activation of TLR4 induces the expression of IL-1, IL-6 and IL-8 (63). NF-κB, which TLR4 can activate, is a central mediator in

TABLE 2 Studies reported the molecular mechanisms underlying TLR4-mediated IBS.

Study	Study object	Disease modeling method	Status of TLR4	Effect on IBS	Associated genes or pathways	Main findings
Bao et al. (34)	Wistar rats (F)	Acute and chronic stress (3 weeks)	Activated	Promoted inflammation	Activated the expression of MyD88, NF-κB, IL-1β, IL-6	IBS-D may increase inflammatory factors through activating the TLR4/MyD88/NF-κB signaling pathway, resulting in disease progression.
Chen et al. (35)	SD rats (M and F)	Neonatal colorectal distension (CRD)	Activated	Promoted inflammation, mediated VHS	TLR4/MyD88/NF-κB pathway, IL-1β and TNF-α	Neonatal colonic irritation (CI) stimulated the production of IL-1β and TNF-α through the TLR4/MyD88/NF-κB signaling pathway in the spinal cord, contributing to the VHS response induced by neonatal CI in rats.
He et al. (36)	Wistar rats (F)	Acute and chronic stresses (4 weeks)	Activated	Promoted inflammation	Upregulated NF-κB (p65), MyD88, IL-8, TNF-α, downregulated IL-10	TLR4/NF-κB signal pathway played an essential role in the modulation of inflammatory responses in IBS-D.
Rodríguez-Palma et al. (37)	Swiss webster mice (M and F)	Chronic restraint stress	Activated	Promoted VHS, anxiety-like behaviors	HMGB1	Highlight the significant role of the HMGB1/TLR4 pathway in chronic stress-induced VHS, anxiety-like behaviors.
Wu et al. (38)	SD rats (M)	Neonatal rats underwent CRD	Activated	Promoted VHS	NF-κB, Pellino-1	The downregulation of Pellino-1 in the fastigial nucleus acted through the TLR4/NF-κB pathway to protect against chronic VSH in a CRD rat model.
*Xi et al. (13)	NCM460 cell/NIH mice (both genders equally divided)	LPS 2ug/ml/acetic acid instillation	Activated	Inhibited enterocyte viability, promoted apoptosis and inflammation, decreased tight junction integrity/shortened defecation intervals, raised water content in stool, promoted VHS	miR-16, XIST, IL-1β, and IL-6	*In vitro* and *in vivo* experiments have validated that miR-16 inhibited the TLR4/NF-κB/XIST axis to relieve IBS-D.
Xu et al. (39)	SD rats	Neonatal maternal separation (NMS) and Water avoidance stress (WAS)	Activated	Promoted VHS	OT receptor, TLR4/MyD88/NF-κB, TNF-α and IL-1β	Exogenous OT attenuated stress-induced VHS and Enteric glial cell activation, mediated by TLR4/MyD88/NF-κB signaling.
Yuan et al. (40)	SD rats (M)	10-d-old pups acetic acid instillation	Activated	Promoted VHS	NF-κB, CBS	Activation of TLR4 by neonatal CI upregulated CBS expression, which is mediated by the NF-κB signaling pathway, thus contributing to VHS.
Zhang et al. (41)	SD rats (M)	Neonatal CRD	Activated	Promoted VHS	MyD88/NF-κB signaling, TNF-α and IL-1β	Activation of CRF neurons and microglia in the hypothalamic paraventricular nucleus participates in the pathogenesis of VHS through TLR4/MyD88/NF-κB signaling.
Tang et al. (12)	C57BL/10JNju (Tlr4) and C57BL10/ScNJNju (Tlr4) mice	NMS	Activated	Promoted VHS	MyD88, IL-1β and TNF-α, CRF, CRF1	NMS-induced VHS is associated with increased TLR4 signaling in the PVN.

The fields in parentheses in this column for the study object refer to gender. F represents females, and M represents males. * The literature includes both cell and animal experiments, separated by a slash, with the results of the cell experiments before the slash and the results of the animal experiments after the slash. OT, Oxytocin; CBS, Cystathionine beta synthetase; CRF, Corticotropin-releasing factor; PVN, Paraventricular nucleus.

TABLE 3 Studies reported the treatments targeted by TLR4 in IBS.

Study	Study object	Disease modeling method	Intervention or treatment	Status of TLR4	Effect on IBS	Associated genes or pathways	Main findings
Arie et al. (42)	SD rats (M)	WAS	Grape seed extract (GSE)	Inhibited	Decreased VHS	NA	GSE ameliorated repeated WAS-induced visceral allodynia and colonic hyperpermeability via inhibition of TLR4.
Chimienti et al. (43)	Newborn Wistar rats	NMS	Ketogenic Diet (KD)	Inhibited	Inhibited inflammation	COX-2, SOD1, SOD2, Beclin-1 and LC3 II	Feeding animals with KD elicited compensatory mechanisms that reduced inflammation and oxidative stress, restored mitochondrial function, and restored baseline autophagy.
Chu et al. (44)	SD rats (M)	Chronic restraint combined with gavage of Senna-leaf solution	Moxibustion at "Tianshu" (ST25) and "Shangjuxu" (ST37)	Inhibited	Improved diarrhea symptoms and VHS, alleviated inflammation	TLR4/MyD88/NF-κB signaling pathway	Moxibustion may alleviate IBS-D symptoms by inhibiting the TLR4/MyD88/NF-κB signaling pathway and reducing the expression of inflammatory factors.
Compare et al. (24)	10 PI-IBS D patients (4 F, mean age 52 years),10 HC (5 F, mean age 48 years)	Ex-vivo organ culture model	Lactobacillus casei DG (LC-DG) and its PB	Inhibited	Attenuated mucosal inflammatory response	IL-1α, IL-6, IL-8, IL-10	LC-DG and its PB attenuated the inflammatory mucosal response in an ex-vivo organ culture model of PI-IBS D.
Ding et al. (45)	SD rats (M)	Acetic acid enema plus restraint stress	Wumei Pill	Inhibited	Improved abnormal bowel movements and VHS	TLR9, NF-κB	Wumei Pill had a significant therapeutic effect on IBS-D by restraining the transmission of the TLR4/9-NF-kB signaling pathway.
Elbadawi, M et al. (46)	Mouse intestinal organoid	Exposure to CRF	STW 5-II	Inhibited	Reduced inflammation	TLR4/MyD88/NF-κB	STW 5-II may alleviate inflammation through inhibited TLR4/MyD88/NF-κB pathway.
Huang et al. (47)	rats	NMS combined with *Campylobacter jejuni* (ATCC81-176) treatment	QingHuaZhiXie prescription	Inhibited	Reduced diarrhea index and intestinal hypersensitivity	TLR4/MyD88/NF-κB pathway	QingHuaZhiXie prescription reversed the imbalance of cytokines by acting on the TLR4/MyD88/NF-κB pathway, contributing to the release of IBS-D symptoms.
Ruiz-Malagón et al. (48)	SD rats (M)	Deoxycholic acid enema	Serpylli herba extract (SHE)	Inhibited	Improved VHS and immune status	NA	SHE may reduce IBS mucosal inflammation by inhibiting TLR4.
Lin et al. (49)	ICR mice (M)	Restraint stress (10h)	Melatonin (MT)	Inhibited	Mitigated colonic microbiota dysbiosis and intestinal inflammation	NF-κB	MT may mitigate "restraint stress"-induced colonic microbiota dysbiosis and intestinal inflammation by inhibiting the activation of the TLR4/NF-κB pathway.
Liu et al. (50)	Wistar rats (M)	Repeat WAS	Saccharomyces boulardii (Sb)	Inhibited	Improved colonic hypermotility and decreased inflammation	Inhibited IL-6, IL-1β, and IFN-γ, increased IL-10	Sb improved repeated WAS-induced colonic hypermotility and decreased TLR4 expression in the colon.
Maqoud et al. (51)	Caco-2 cells	LPS	Lens culinaris Medik extract (LE)	Inhibited	Reduced inflammation	Inhibited NF-κB	LE suppressed TLR4 and NF-κB and inhibited inflammation.
Mylene et al. (52)	C57/Bl6 mice (M)	WAS	Probiotic combination Lactibiane Tolerance (LT)	Inhibited	Prevented epithelial barrier impairment	NA	LT prevention of WAS-induced epithelial barrier integrity loss could be linked to this treatment's ability to decrease WAS-induced TLR4 up-regulation in the colonic mucosa.

(Continued)

TABLE 3 Continued

Study	Study object	Disease modeling method	Intervention or treatment	Status of TLR4	Effect on IBS	Associated genes or pathways	Main findings
Xia et al. (53)	SD rats (M)	WAS	Apigenin	Inhibited	Mitigated VHS and colonic hypermotility	TLR4/MyD88/ NF-κB pathway	Apigenin inhibited Mast cells (MCs) activation through the TLR4/Myd88/NF-κB pathway, which improved visceral sensitivity.
Zhang et al. (54)	SD rats	Restraining stress and acetic acid enema	Sancao Lichang Decoction	Inhibited	Improved abnormal defecation, inhibited the inflammatory response, and improved intestinal mucosal barrier function	TLR4/MyD88/ NF-κB	Sancao Lichang decoction may reduce IBS-D by inhibiting the TLR4/MyD88/NF-κB pathway.
Zhang et al. (55)	SD rats (M)	NMS in combination with restraint stress and gavage of senna decoction	Chang-Kang-Fang (CKF)	Inhibited	Attenuated VHS, reduced the number of fecal pellets, improved inflammatory response and colonic barrier function	TLR4/NF-κB/ NLRP3 pathway	CKF might mitigate the symptoms of IBS-D rats by inhibiting the TLR4/NF-κB/NLRP3 pathway.
Zhu et al. (56)	Wistar rats (both genders at a ratio of 1:1)	Gavage of senna decoction plus restraint stress	Berberis heteropoda Schrenk roots (BHS)	Inhibited	Reduced VHS, improved abnormal defecation and intestinal flora	NF-κB, TLR2	BHS downregulated the mRNA expression of TLR2, TLR4, and NF-κB in brain and colon tissues, improving IBS-D.
Zhu et al. (57)	SD rats (M)	Chronic restraint stress	Xiaoyaosan (XYS)	Inhibited	Improved depressive-like behavior and weight loss	TLR4, MyD88, NF-κB, NLRP3	XYS can improve depressive-like behavior in rats by suppressing the activation of the TLR4/NLRP3 inflammasome signaling pathway.
Yang et al. (58)	SD rats (newborn M)	Acetic acid instillation	EA	Inhibited	Decreased VSH	IL-8, IL-1β	The potential mechanism underlying the reduction in visceral hypersensitivity through the stimulation of Zusanli using EA involves suppressing TLR4 expression in the MCs of colonic tissues.

The fields in parentheses in this column for the study object refer to gender and age. F represents females, and M represents males. COX-2, cyclooxygenase; SOD, superoxide dismutase; NA, Not available.

the induction of pro-inflammatory genes and plays a role in both innate and adaptive immune cells (64). Three studies showed that inflammatory factor production may be caused by the TLR4/MyD88/NF-κB pathway in an animal model of IBS-D, which promotes the development of IBS-D (34–36). Inflammatory factor (IL-6, IL-1β) expression was found to be attenuated by inhibition of the TLR4/NF-κB/XIST pathway in LPS induced damage to human normal colonic ECs (13). Furthermore, Belmonte et al. found that the imbalance between elevated levels of TLR4 and the impaired expression of PPARγ, a potential inhibitor of colonic inflammation, suggests an altered response to luminal bacteria leading to colonic inflammation (22).

Then how does TLR4 specifically participate in IBS disease progression through immune cells? In the intestine, TLR4 is expressed on antigen-presenting cells (e.g., macrophages and dendritic cells) and lymphocytes (65). In normal physiology, immune cell expression of TLR4 is required for B cell recruitment, dendritic cell maturation, and triggering of T cell responses to invading pathogens (18). A Mendelian randomization study shows a significant genetic correlation between immune cell phenotype and IBS (66). In IBS, alterations in lymphocyte populations, including B and T lymphocyte counts and activation levels, are associated with increased colonic MCs in IBS patients (67). Colonic mast cells are more numerous in IBS, and their activation degranulation can modulate visceral sensitivity and epithelial barrier function by releasing neuroactive mediators (53, 55, 68, 69). In clinical trials, mast cell stabilizers or histamine 1 receptor antagonists improved IBS symptoms and quality of life (20, 70). In an experiment of IBS supernatant-induced degranulation of BMMCs (bone marrow-derived MCs (BMMCs)), it was found that TLR4 activation led to degranulation and histamine production and that a TLR4 inhibitor (TAK-242) attenuated degranulation of BMMCs (71). In animal experiments, the potential mechanism of visceral hypersensitivity has also been found to involve the expression of TLR4 in MCs of colonic tissues (58). It indicates that TLR4 may influence intestinal function and visceral sensitization responses by regulating mast cell degranulation. However, no evidence of TLR4 regulation of other immune cells in IBS was found. Most of the studies were unclear in

their observation of cell specificity and failed to provide a distinction between epithelial cells and immune cells expressing TLR4, thus preventing in-depth analysis of the specific mechanism (18).

4.2 TLR4 and intestinal barrier function

Increased intestinal permeability in patients with IBS ranges from 2% - 62% (72). The increased intestinal permeability in IBS is associated with abdominal pain and visceral sensitivity and exposes neurological and immune components to luminal microbes (73, 74). In IBS patients, TLR4 is strongly associated with barrier function-related genes, including protease-activated receptor 2, OCLN, and TJP1, suggesting potential functional relationships (75). Xi et al. found that overexpression of TLR4 led to down-regulation of TJP1 and OCLN, whereas inhibition of TLR4 expression led to up-regulation of TJP1 and OCLN in the IBS-D cell model (13). Singh P. et al. found that a high-FODMAP diet leads to colonic barrier loss and mast cell activation and that TLR4 receptors on MCs are critical for this high-FODMAP mediated loss of the colonic barrier (76). *In vivo* and *in vitro* experiments have revealed that activation of TLR4 increases intestinal permeability by down-regulating phosphorylated OCLN expression in the intestinal epithelial barrier, increasing myosin light chain kinase protein expression and kinase activity (77, 78). MUC2, a glycoprotein, forms the mucus layer of the intestinal barrier (79). *In vitro* experiment, silencing of TLR4 in the TLR4-expressing rat intestinal epithelioid cell line 6 induced MUC2 production, whereas overexpression of TLR4 in human Caco-2 cells, which generally do not express TLR4, resulted in the loss of their normal MUC2-producing phenotype (80). In the same *in vivo* experiments, increased permeability of the gut in villin-TLR4 mice (increased TLR4 signaling), about the significantly lower expression of epithelial cell-cell adhesion genes in colonic ECs, including junctional adhesion molecule A and cadherin-1, and a decreased depression of TJP1 although not significantly (81). In conclusion, aberrant expression of TLR4 is closely related to intestinal barrier function and is involved in barrier damage in IBS.

4.3 TLR4 and intestinal flora

In a healthy state, the gut microbiota interacts closely with intestinal epithelial cells and the immune system to regulate inflammation and maintain the development of intestinal barrier and immune system (82, 83). Intestinal dysbiosis, an imbalance in the intestinal microbiota due to various internal and external factors, is an important causative factor in IBS (72, 84). Several Meta-analyses of altered intestinal flora in patients with IBS have shown the presence of intestinal dysbiosis in patients with IBS, mainly characterized by lower levels of *lactobacilli* and *bifidobacteria* compared to healthy controls (85, 86). Flora dysbiosis may cause loss of intestinal integrity and increased intestinal permeability, which can lead to penetration of the epithelial barrier by bacterial products and metabolites, thereby triggering an inflammatory response (87). Increased intestinal permeability may also increase bacterial dissemination (81). In addition, intestinal dysbiosis affects intestinal motility, increases VHS, and regulates the gut-brain axis (88, 89). TLRs recognize specific microbial components of commensal and pathogenic bacteria and play a role in immune tolerance to commensal bacteria and defense against pathogens (90). Altered microbiota profiles may affect TLR expression and immune activation in IBS (30). Guo et al. found that the diversity of intestinal mucosal colonizing flora and the two dominant bacterial genera (*Mycobacterium avium* and *Clostridium* spp.) were significantly reduced in IBS-D patients compared with that of healthy people, while the mucosal immune-related receptors TLR2 and TLR4 were significantly over-expressed, and there was a correlation between the reduction of these two genera and the high expression of TLR (26). Similarly, another study found that TLR4 expression in IBS-D was negatively associated with the microbial relative abundance of the *Lactobacillus* and *Escherichia/Shigella* genera, whereas it was positively associated with the relative abundance of the genera *Megasphera* and *Sutterella* and the class *Betaproteobacteria* (27). Both studies suggest a correlation between TLR4 and gut flora. Bacterial invasion can activate pro-inflammatory responses through TLR4-induced TIRAP/MyD88 and TRAM/TRIF signaling cascades (91). Thus, intestinal dysbiosis may induce immune disorders by activating the natural and acquired immune systems by activating intestinal mucosal TLR4 proteins, triggering inflammation and ultimately leading to IBS-D (26). Targeting TLR4 may benefit by restoring epithelial function and changing the microbiota (81). Future studies are needed to explore the mechanisms of gut flora dysbiosis and the role of the TLR4 pathway in IBS disease.

4.4 TLR4 and visceral hypersensitivity

VHS, which refers to internal organs like the gastrointestinal tract exhibiting amplified perception of pain in response to stimuli, is a frequent complaint among individuals with IBS. The inflammatory consequences of TLR activation on glial cells (mainly microglia and astrocytes), sensory neurons, and other cell types affect injury perception processing and lead to pain (92). TLR4 expression in colonic tissues is associated with VHS reactions (58). Correlation analysis shows that TLR4 mRNA expression correlates with the intensity of abdominal pain in IBS-D patients (20). LPS Activation of TLR4 increases the production of pro-inflammatory cytokines, which activate visceral sensory neurons to induce visceral hypersensitivity (93). The results of eight studies have shown that VHS in IBS may be mediated through the TLR4, TLR4/NF-κB, TLR4/MyD88/NF-κB, TLR4/NF-κB/CBS, HMGB1/TLR4 pathways (12, 13, 35, 37–41). Genetically altered (i.e., TLR4 knockout and point-mutant) mice and rats with down-regulated TLR4 expression exhibit analgesia and low expression levels of cytokines, such as TNF-α and IL-1β (94). Tang et al. found that MS was associated with increased VHS, microglial TLR4, and inflammatory factors IL-1β and TNF-α expression in Tlr4 +/+ mice; however, MS did not alter VHS, IL-1β and TNF-α expression in Tlr4 -/- mice (12). Increased IL-1β and TNF-α proteins released

by microglia via the TLR4/MyD88/NF-κB signaling pathway induced neonatal stress-induced VHS and pain (35). Administration of OT peripherally reduced VHS or visceral pain in human samples and animal models (39). OT pretreatment inhibited these increases as TLR4 signaling elicited a cellular response that released the downstream effectors MyD88, NF-κB, IL-1β, and TNF-α, thereby inducing VHS (39). TLR4 signaling and the pro-inflammatory cytokines TNF-α and IL-1β may be involved in neuroglial interactions in the pathogenesis of VHS reactions (41). TLR4 deficiency reduced visceral pain and prevented the development of chronic psychosocial stress-induced VHS. Administration of TLR4 antagonists, such as TAK-242 and CLI-095, counteracted chronic stress, neonatal colonic inflammation, and neonatal CRD-induced VHS (38, 40, 95). Furthermore, rats with a FODMAP diet resulted in impaired gut barrier function and increased sensitivity to colorectal distension, its LPS or fecal supernatants induced VHS, and this was blocked by small interfering RNA inhibition of TLR4 mRNA, suggesting that TLR4 activation by fecal LPS could mediate VHS (96). In conclusion, the generation of VHS is closely related to TLR4, NF-κB, and pro-inflammatory effects, which may be an essential way to improve abdominal pain in IBS patients.

4.5 TLR4 and defecation abnormalities

Abnormal defecation is one of the main symptoms of IBS, including changes in fecal water content and bowel motility. TLRs, especially TLR2 and TLR4, significantly affect post-infection and lipopolysaccharide-mediated regulation of gastrointestinal motility (97). The muscle contractility induced by acetylcholine was significantly lower in TLR2 (-/-) and TLR4 (-/-) concerning WT mice (98). Gastrointestinal motility was significantly delayed in mice that do not express TLR4 or Myd88 compared to wild-type mice (99). These studies suggest that TLR4 is involved in intestinal motility. In patients with IBS, a positive correlation was found between mRNA levels of TLR-4 and weekly stool frequency in IBS patients (30). High expression of TLR4 in the IBS model results in shorter bowel intervals, higher fecal water content, and greater urgency (13, 27).

4.6 TLR4 and psychosocial

IBS affects psychosocial factors, including general and gut-related anxiety, depression, and somatization (8, 100). Some of these affections are bidirectional, and psychosocial factors can aggravate IBS symptoms and the disease progression, and vice versa (101, 102). Up to the minute, two Mendelian randomization studies revealed a bidirectional causal relationship between IBS and cerebral cortex structures, confirming the two-way communication along the brain-gut axis (103, 104). Moreover, there are longitudinal interactions from childhood into adulthood. A Swedish prospective longitudinal birth-cohort study found that health-related quality of life deterioration and psychological distress of adolescence are associated with new cases of adult IBS, and undergoing an abdominal pain–related adolescent gut-brain interaction disorder is associated with new-onset adult psychological distress (105). Growing evidence suggests that TLRs are associated with the pathophysiology of major depressive disorder, among which multiple linear regression analysis revealed that TLR4 was an independent risk factor relating to the severity of major depression (106, 107). TLRs (including TLR4) are upregulated in major depressive disorder patients, while antidepressant treatment downregulates TLRs expression, suggesting that TLRs are critical mediators for antidepressant therapy (108). Clinical studies have found that patients with IBS and depression have higher levels of TLR4 expression and IL-6, accompanied by a decrease in IL-10, which indicates that TLR participates in the inflammation reaction in IBS and depression (31). Erick J et al. found that chronic restraint stress induces anxiety-like behaviors, while blockade of the HMGB1/TLR4 pathway reverses chronic restraint stress-induced anxiety-like behaviors (37). Some drugs can treat depression through TLR4 and its signaling pathway. XYS can improve depressive-like behavior in rats by suppressing the activation of the TLR4/NLRP3 inflammasome signaling pathway (57). In the LPS-induced depression model, the use of raspberry ketone supplementation can alleviate depressive behavior through mitigated gut inflammation by inhibiting the TLR-4/NF-κB pathway (109).

4.7 Corticotropin-releasing factor signaling systems and IBS

The development and worsening of symptoms in irritable bowel syndrome is known to be closely related to stress, which induces visceral hypersensitivity and altered colonic motility and plays a vital role in the pathophysiology of disease development (110, 111). CRF, expressed in the brain and colon, is a significant mediator of the brain-gut axis stress response and mediates stress-induced enhancement of colonic motility and VHS, suggesting that CRF is a critical component of IBS (112). CRF receptor subtype 1 (CRF1), CRF2, and TLR4 were found to be upregulated in peripheral blood samples of IBS patients, especially in patients with concomitant depression (31). Persistent activation of the CRF1 system at central or peripheral sites may be one of the underlying causes of diarrhea and abdominal pain symptoms in IBS (112). Colonic TLR4 expression is downregulated in CRF-deficient mice and is more susceptible to colitis (113). One study found that CRF induces VHS and colonic hyperpermeability via TLR4 and cytokine systems, and these changes are dependent on CRF1 (114). They also found that LPS-induced VHS is mediated through CRF, TLR4, and pro-inflammatory factor pathways (115). Moreover, LPS increases colonic CRF expression at the gene and protein level (116) and activates peripheral CRF receptors (115). The CRF-TLR4-inflammatory cytokine system also affects the gut microbiota. Evidence suggests that activation of the CRF-TLR4-inflammatory cytokine system is followed by impairment of the intestinal barrier, which may alter the microbiota (93). In conclusion, CRF and TLR4-pro-inflammatory cytokine signaling generates a vicious cycle of mutual activation, leading to intestinal barrier damage and

ecological dysregulation, affecting intestinal motility, and inducing a visceral hypersensitivity response that leads to IBS symptoms.

4.8 MiRNAs involved in TLR4 regulation in IBS

MiRNAs regulate the pathophysiological mechanisms of IBS, and searching for relevant miRNA biomarkers as diagnostic and therapeutic candidates for IBS is a hot topic (117, 118). Only one publication reported that miR-16 in IBS regulates defecation intervals, stool water content, and VHS by targeting the TLR4/NF-κB/XIST pathway (13). It has been reported that miR-16 targets and inhibits TLR4 in the LPS-induced inflammatory pathway, and this alteration can be reversed by the lncRNA SNHG16 (119). In addition, miR-16 can down-regulate the expression of NF-κB, NLRP3, and other inflammatory factors by targeting TLR4, thereby attenuating inflammation in the LPS-induced acute lung injury model (120). Given that most miRNAs have many-to-many relationships with target genes, more studies are needed to clarify the molecular mechanisms of miRNAs in IBS on the TLR4 pathway.

4.9 TLR4 Single-nucleotide polymorphism and IBS

Genetics contributes to the development of IBS disease. As early as 2001, it was proposed that identical twins have a significantly higher concordance of IBS than dizygotic twins (121). SNPs are the most common type of sequence variation in genomes. It has been shown that TLR9 rs5743836 (A/g) gene polymorphism may be associated with IBS-D phenotype (122). However, this review did not retrieve complete text reports on TLR4 gene polymorphism in IBS. A study of SNPs in the TLR4 gene in inflammatory bowel disease shows that TLR4 D299G polymorphism is significantly associated with inflammatory bowel disease in North Indian populations and regulates the transcription of inflammatory cytokines during ulcerative colitis, leading to abnormal immune responses (123). The results suggest that SNPs in TLR4 are associated with immune inflammation, which is the primary pathogenesis of IBS. Its role in IBS needs to be clarified, and future studies can look for its role in IBS regarding TLR4 gene polymorphisms.

4.10 TLR4's feasibility as a potential therapeutic target

We included 18 studies in this review, which reported specific treatments for IBS by targeting TLR4. All these studies found that inhibition of TLR4 was one of the essential mechanisms underlying the improved IBS exerted by specific treatments. Multiple studies have found that chronic psychosocial stress or early-life stress impacts susceptibility to IBS by modulating TLR4, etc. (19, 37, 124, 125). KD might be beneficial in psychiatric disorders like stress, anxiety, depression, mood disorders, etc., given its ability to remodel the gut microbiota and antioxidant and anti-inflammatory effects, consequently impacting the brain-gut axis (126, 127). Chimienti et al. found that feeding animals with KD can reduce inflammation and oxidative stress, restore mitochondrial function and baseline autophagy, and thus reduce the harmful effects of stress in an animal model of IBS (43). In addition to diet therapy, EA and moxibustion therapy also have significant therapeutic effects on IBS (128, 129). These therapies may be practical to decrease patients' pain, and fewer side effects will be sought.

Regarding the mechanism, the study showed that moxibustion improved diarrhea symptoms and VHS and alleviated inflammation of IBS-D through inhibited TLR4/MyD88/NF-κB signaling pathway (44). At the same time, EA-reduced visceral sensitivity of IBS may be involved in the suppression of TLR4 expression in the MCs of colonic tissues, which inhibits mast cell activation in colonic tissues and reduces the levels of inflammatory factors in serum that participate in the process of VHS (58). Multiple systematic reviews and meta-analyses have shown that herbal medicine effectively relieves IBS symptoms (130–132). TLR4 was the most critical type of TLRs regulated by phytochemicals (133). Herbal compound prescriptions, such as Wumei Pill, STW 5-II, QingHuaZhiXie Prescription, Sancao Lichang Decoction, CKF, Xiaoyaosan, improvement of inflammation, defecation abnormalities, VHS and depressive behavior in IBS, via suppressed TLR4, TLR4/MyD88/NF-κB or TLR4/NF-κB/NLRP3 signal pathway (45–47, 54, 55, 57). Flavonoids, mostly found as natural pigments in fruits, vegetables, and seeds of edible plants, have significant therapeutic activities, such as anti-inflammatory and antioxidant effects, and enhance intestinal barrier function (133–135). GSE, LE, and Apigenin, natural flavonoids, could diminish inflammation, maintain tight junction integrity, and improve visceral sensitization and colonic hypermobility with IBS by inhibiting TLR4 and TLR4/MyD88/NF-κB pathway (42, 51, 53). Other herbs that act on TLR4, such as SHE and BHS, may also improve IBS symptoms (48, 56). Increasingly, evidence has shown that gut microbiota dysbiosis plays a vital role in IBS pathogenesis (86, 136). Probiotic use may offer particular utility in managing IBS through its metabolic activity, immunomodulatory, and cross-feeding effects (137). LC-DG and its PB, Sb, and probiotic combination LT could decrease TLR4, attenuate inflammation, improve colonic hypermotility, and prevent epithelial barrier impairment of IBS (24, 50, 52). In addition to probiotics, MT is also closely linked to the gut microbiota, mitigated colonic microbiota dysbiosis, and intestinal inflammation in IBS animal models by inhibiting the activation of the TLR4/NF-κB pathway (49). Overall, the 18 studies suggest that multiple therapies targeting TLR4 can reduce IBS-related symptoms. TLR4 is viable as a potential therapeutic target.

4.11 The relationship between TLR4 and IBS

TLR4 may play a vital role in the pathogenesis of IBS, and its abnormal activation may lead to dysregulation of the intestinal inflammatory response, which in turn affects the intestinal function, leading to hypersensitivity of the intestinal nervous system and aggravation of abdominal pain and discomfort. The structure of the

intestinal flora of patients with BS may change, and the overgrowth of certain bacteria may lead to the activation of TLR4, which in turn may further affect the balance of the intestinal flora, forming a vicious circle and exacerbating the symptoms of IBS. Polymorphisms in the TLR4 gene may be associated with the risk of developing IBS. In addition, environmental factors such as diet and stress may also indirectly affect the development and symptoms of IBS by influencing the expression and function of TLR4. From the current evidence, changes in TLR4 and the development of IBS may be causative, and more clinical and basic studies are needed to elucidate the exact causal relationship between TLR4 and IBS.

5 Conclusion

TLR4 is significantly up-regulated in IBS, correlating with clinical manifestations, and is accompanied by up-regulation of pro-inflammatory factors and down-regulation of anti-inflammatory factors. Pathogenesis involved in IBS, such as intestinal barrier damage, intestinal dysbiosis, abnormal intestinal peristalsis, increased visceral sensitization, and anxiety behaviors, are related to the interactions among TLR4, the NF-κB pathway, the pro-inflammatory effects, and the CRFs. Various therapies such as herbs, acupuncture, probiotics, and their associated PB can treat IBS by targeting TLR4 and its pathway. In conclusion, TLR4 may be a promising target for treating IBS, and more clinical studies will be needed to evaluate therapeutic approaches targeting this pathway.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author/s.

Author contributions

XW: Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft. LW: Supervision, Writing – review & editing. ZW: Data curation, Formal analysis, Methodology, Writing – original draft. CW: Conceptualization, Supervision, Writing – review & editing, Writing – original draft, Investigation.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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