

# Immune Imbalance in the Pathogenesis of Diarrhea-Predominant Irritable Bowel Syndrome: Mechanisms and Research Progress on Traditional Chinese Medicine Intervention (Postprint)

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## Abstract

Diarrhea-predominant irritable bowel syndrome (IBS-D) is a clinically common functional gastrointestinal disorder in which immune imbalance plays a crucial pathogenic role. Related immune cells include innate and adaptive immune cells, while immune cytokines encompass immunoglobulins, interferons, interleukins, tumor necrosis factors, among others. Chinese medicine monomers/compounds or formulas can regulate immunity through multi-pathway, multi-target mechanisms for IBS-D treatment. This article systematically reviews relevant literature on Chinese medicine monomers/compounds and formulas targeting immune therapy for IBS-D, examining the relationships between immune imbalance and intestinal infection, microbial dysbiosis, brain-gut axis disorder, and endocrine disorder. It simultaneously corroborates the connection between the “spleen as the defender” concept and immunity, aiming to provide insights for treating IBS-D and related diseases through immune-mediated pathways in traditional Chinese medicine.

## Full Text

### Preamble

**Review and Monograph: Mechanisms of Immune Imbalance in the Pathogenesis of Diarrhea-Predominant Irritable Bowel Syndrome and Research Progress on Traditional Chinese Medicine Intervention**

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**Abstract** Diarrhea-predominant irritable bowel syndrome (IBS-D) is a common functional gastrointestinal disorder in clinical practice, in which immune imbalance plays a crucial pathogenic role. Relevant immune cells include innate immune cells and adaptive immune cells, while immune cytokines encompass immunoglobulins, interferons, interleukins, tumor necrosis factors, and others. Traditional Chinese medicine (TCM) monomers/compounds or formulas can regulate immune function and treat IBS-D through multiple pathways and targets. This article systematically reviews and organizes relevant literature on targeted immunotherapy for IBS-D using TCM monomers/compounds and formulas, examining the relationships between immune imbalance and intestinal infection, dysbiosis, brain-gut axis disorders, and endocrine disturbances. Simultaneously, it validates the correspondence between the TCM concept of “spleen as the guard” and immune function, aiming to provide insights for TCM treatment of IBS-D and related diseases through immune-mediated pathways.

**[Keywords]** Diarrhea-predominant irritable bowel syndrome; Immune imbalance; Chinese medicine formulas; Traditional Chinese medicine monomers/compounds; Research progress

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## Introduction

Irritable bowel syndrome (IBS) is a functional disorder characterized by abdominal pain, bloating, or discomfort associated with defecation or accompanied

by changes in bowel habits<sup>1</sup>. Among its subtypes, diarrhea-predominant IBS (IBS-D) is the most common. The persistently high incidence of IBS-D severely impacts patients' quality of life and work productivity, consumes substantial clinical resources, and has become one of the major difficult diseases in the collaborative Chinese and Western medicine clinical project in China. While factors such as brain-gut axis disorders, immune imbalance, visceral hypersensitivity, intestinal infection and dysbiosis, abnormal intestinal motility and permeability, and genetics are all closely related to its pathogenesis<sup>2</sup>, immune imbalance plays a dominant role<sup>3-4</sup>. Research has confirmed that TCM monomers/compounds or formulas can regulate immunity and treat IBS-D through multiple pathways, targets, and mechanisms. This article systematically reviews relevant domestic and international research literature from recent years and provides a summary, analysis, and outlook based on the "spleen as the guard" theory, aiming to offer new evidence and ideas for the integrated treatment of IBS-D with Chinese and Western medicine.

## 1 Immune Imbalance Mechanisms in IBS-D Pathogenesis

### 1.1 Innate Immune Cells

**1.1.1 Macrophages (M)** Macrophages play a crucial role in maintaining intestinal immune homeostasis<sup>5</sup>. They can be polarized into M1 and M2 phenotypes, both of which are closely associated with inflammatory responses. In infected tissues, lipopolysaccharide (LPS) and interferon- $\gamma$  (IFN- $\gamma$ ) induce macrophage polarization toward the M1 phenotype, releasing interleukin (IL)-6, IL-12, and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) to promote intestinal inflammation. In contrast, anti-inflammatory factors such as IL-4 and IL-10 induce M2 polarization, which participates in anti-inflammatory responses and repairs damaged tissue<sup>6-7</sup>. When LPS stimulates peripheral blood CD14<sup>+</sup> monocytes from IBS-D patients, the transformation of monocytes into macrophages can be observed, indicating that IBS is in a state of immune activation<sup>8</sup>.

**1.1.2 Mast Cells (MC)** Stimuli from the intestinal luminal microenvironment and central stress can promote mast cell activation and degranulation<sup>9</sup>, releasing various tryptase-like enzymes, histamine, 5-hydroxytryptamine (5-HT), and other inflammatory mediators and cytokines that contribute to IBS-D<sup>10</sup>. Modern research has confirmed that mast cells can induce IBS-D by increasing intestinal mucosal barrier permeability, causing mucosal immune imbalance, triggering visceral hypersensitivity, disrupting gastrointestinal motility, and activating local and central stress pathways<sup>9</sup>. LPS and trypsin induce mucosal mast cells to release prostaglandin E2 (PGE2) and cyclooxygenase-2 (COX-2), which subsequently downregulate the 5-HT transporter (SERT) and upregulate 5-HT, thereby triggering diarrhea, abdominal pain, and other symptoms<sup>11-12</sup>.

**1.1.3 Dendritic Cells (DC)** Dendritic cells are unique antigen-presenting cells that bridge innate and adaptive immune responses. Immature DCs primar-

ily reside in peripheral tissues, where they induce immune tolerance to maintain homeostasis under normal conditions. Under pathological conditions, they differentiate into mature DCs that induce differentiation of T helper cell 1 (Th1), Th2, Th17, follicular helper T cell (Tfh), and regulatory T cell (Treg) subsets, generating cellular immunity<sup>13</sup>. In IBS rats, the number of mesenteric lymph node DCs (MLN DCs) increases significantly and can stimulate CD4<sup>+</sup> T cells to promote IL-4 secretion and induce mast cell degranulation, leading to visceral hypersensitivity<sup>14</sup>.

## 1.2 Adaptive Immune Cells

**1.2.1 T Lymphocytes** T lymphocytes originate from bone marrow stem cells and mature in the thymus, maintaining immune homeostasis through cellular immunity. In IBS, abnormal immune activation of CD4<sup>+</sup> T cells occurs in peripheral blood. In post-infectious IBS (PI-IBS) mice, the expression of CD4<sup>+</sup> T cells, adenosine 2A receptor (A2AR) secretion, and Th17 polarization are promoted in the spleen. A2AR antagonists can block these processes and improve intestinal inflammation, exerting therapeutic effects<sup>15</sup>.

**1.2.2 Plasma Cells (PC)** Plasma cells, also known as effector B cells, participate in humoral immunity by releasing immunoglobulins. Moderate to significant infiltration of plasma cells, T lymphocytes, mast cells, and macrophages can be observed in the epithelial layer and lamina propria of the colonic mucosa in IBS-D patients<sup>16</sup>. The activation degree of plasma cells and IgG concentration in jejunal tissue are higher than in healthy controls, while the glycocalyx thickness is reduced<sup>17</sup>, providing experimental evidence for the involvement of humoral immune imbalance and barrier dysfunction in IBS-D pathogenesis.

## 2 Immune Imbalance and Related Factors

### 2.1 Immune Imbalance and Intestinal Infection

Numerous studies have found that low-grade mucosal inflammation caused by intestinal infection is a potential pathogenic factor for IBS-D. Statistics show that the risk of developing IBS increases significantly within one year after infectious enteritis, being 4.2 times higher than in non-infected individuals<sup>18</sup>. Intestinal infection can significantly increase the expression of Toll-like receptor 2 (TLR2), TLR4, and nucleotide-binding oligomerization domain 2 (NOD2) in intestinal epithelial cells<sup>19</sup>. Pattern recognition receptors (PRRs) composed of TLRs and NOD-like receptors (NLRs) recognize pathogen-associated molecular patterns, activating innate and adaptive immunity, promoting the production of cytokines such as IL, TNF, and IFN, and facilitating the maturation of antigen-presenting cells<sup>20</sup>, thereby maintaining intestinal homeostasis. Animal experiments have demonstrated that in PI-IBS mice, the expression of secretory immunoglobulin (sIgA) and pro-inflammatory factors IFN- $\gamma$  and IL-17 in serum is significantly elevated, while the expression of the anti-inflammatory factor IL-10 is significantly

reduced, suggesting that infection can affect the intestinal immune microenvironment and lead to persistent immune activation<sup>21–22</sup>.

## 2.2 Immune Imbalance and Dysbiosis

In IBS-D patients, the abundance of Dorea and Ruminococcus in the intestine increases, promoting the development of enterochromaffin cells (EC) and upregulating 5-HT expression<sup>23</sup>. 5-HT has been confirmed to have a clear immunosuppressive effect on lymphocytes at higher concentrations<sup>24</sup>. Upregulating short-chain fatty acids (SCFAs), a gut microbiota derivative, can effectively improve the Treg/Th17 imbalance, inhibit pro-inflammatory factor secretion, and alleviate colonic inflammation<sup>25</sup>. *Clostridium butyricum* can reduce the number of DCs on the intestinal mucosa of IBS-D mice, downregulate the secretion of pro-inflammatory factors IL-1 $\beta$  and IL-6, and alleviate 2,4,6-trinitrobenzene sulfonic acid (2,4,6-TNBS)-induced low-grade intestinal inflammation in IBS-D<sup>26</sup>.

## 2.3 Immune Imbalance and Brain-Gut Axis Disorders

The brain-gut axis plays a critical role in maintaining immune homeostasis. Brain-gut peptides act as messengers between the central nervous system, enteric nervous system, and immune system. During intestinal inflammation, hormones such as 5-HT, vasoactive intestinal peptide (VIP), substance P (SP), neuropeptide Y (NPY), and calcitonin gene-related peptide (CGRP) are released to stimulate immune cells and maintain intestinal immune homeostasis<sup>27</sup>. Disrupting the liver-brain-gut neural arc can reduce peripheral Th expression, leading to intestinal immune imbalance and inflammation susceptibility<sup>28</sup>. Immunofluorescence has revealed that in IBS-D patients, the immunoreactivity of temperature-sensitive channel TRPM8 on colonic mucosa colocalizes with CD11c<sup>+</sup> cells<sup>29</sup>. Stimulating TRPM8 can upregulate CGRP expression and downregulate the expression of pro-inflammatory cytokines IL-6 and TNF- $\alpha$  in CD11c<sup>+</sup> cells<sup>30</sup>, thereby inhibiting inflammatory responses.

## 2.4 Immune Imbalance and Endocrine Disorders

The endocrine pathway involved in IBS-D is the hypothalamic-pituitary-adrenal (HPA) axis. Corticotropin-releasing factor (CRF) affects the HPA axis, promoting the expression of IL-6 and TNF- $\alpha$  and increasing mast cells, lymphocytes, and monocytes, leading to comorbid depression and IBS<sup>31</sup>. Stress stimulation can promote the secretion of endogenous glucocorticoids and catecholamines under neuroendocrine regulation, decrease IgA expression, and induce intestinal inflammation, immune imbalance, and barrier damage<sup>32</sup>.

### 3 Chinese Medicine Monomers/Compounds and Immune Regulation

#### 3.1 Chinese Medicine Monomers/Compounds and T Lymphocyte Subsets

High-dose berberine can effectively downregulate Th17 cells and pro-inflammatory factors IL-6, IL-17, IL-23, IL-1 $\beta$ , and TNF- $\alpha$  expression in the spleen of IBS-D model rats, while upregulating Tregs cells, serum sIgA, and anti-inflammatory factor IL-10 expression, thereby improving intestinal inflammation and enhancing immunity<sup>33-34</sup>. Oral administration of total glucosides of paeony can inhibit IL-2 secretion and increase IL-5 secretion in serum and colonic mucosa of IBS-D rats induced by “immune induction plus psychological stress,” correcting the Th1/Th2 imbalance in intestinal mucosa and restoring immune balance<sup>35</sup>.

#### 3.2 Chinese Medicine Monomers/Compounds and Immunoglobulins

Patchouli oil can effectively upregulate serum sIgA, IL-4, and IL-10, as well as colonic tissue tight junction proteins (ZO-1) and occludin expression in PI-IBS rats induced by “acetic acid combined with phosphate buffer enema,” increasing intestinal mucosal barrier integrity and thereby enhancing the intestinal immune barrier<sup>36</sup>. Yupingfeng polysaccharide can effectively restore intestinal mucosal immune function in cyclophosphamide-induced mice, primarily by promoting activation of intestinal epithelial cells and submucosal Peyer’s patches, upregulating IL-2, TGF- $\beta$ 1, IL-6, and sIgA expression, regulating the Th1/Th2 drift state, and repairing the intestinal immune barrier<sup>{37}</sup>.

#### 3.3 Chinese Medicine Monomers/Compounds and Mast Cells

*Saposhnikovia* water extract can regulate the 5-HT signaling pathway by upregulating 5-HT3 receptor (5-HT3R) and tryptophan hydroxylase 1 (TPH1) mRNA and protein expression while downregulating 5-HT4R mRNA and protein expression in colonic tissue of IBS-D rats induced by “maternal separation combined with restraint stress,” thereby reducing the number and positive area of mucosal mast cells and improving mast cell function. The experimental results can be validated using the 5-HT3 receptor antagonist ondansetron<sup>38</sup>. Different compatibility ratios of Wu Ji Wan extracts (alcohol extract of Coptis, alcohol extract of Evodia, and water extract of Paeonia) can reduce mast cell density in colonic tissue of IBS rat pups induced by “percutaneous transluminal coronary angioplasty (PTCA) balloon stimulation.” Among them, a ratio of Coptis:Evodia:Paeonia of 12:2:3 can effectively reduce serum 5-HT, plasma SP and SS, and colonic SS-positive expression<sup>39</sup>. Costunolide can alleviate intestinal dysfunction and depressive behavior in chronic stress-induced IBS mice, with the underlying mechanism potentially related to inhibiting colonic mast cell activation, tight junction protein (Claudin2) expression, and 5-HT metabolism while upregulating occludin expression<sup>40</sup>.

### 3.4 Chinese Medicine Monomers/Compounds and Macrophages

Kurarinone can alleviate visceral hypersensitivity and maintain intestinal barrier function in TNBS-induced IBS-D mice, presumably by mediating the aryl hydrocarbon receptor (AhR) to inhibit macrophage activation and myeloperoxidase (MPO), IL-6, TNF- $\alpha$ , and IL-1 $\beta$  expression while upregulating anti-inflammatory cytokine IL-10 expression<sup>41</sup>.

In summary, Chinese medicine monomers/compounds can target different immune mediators to treat IBS-D by reducing intestinal inflammation, repairing intestinal mucosal barrier, decreasing visceral hypersensitivity, and regulating immune disorders. Details are summarized in Table 1 .

## 4 Chinese Medicine Formulas and Immune Regulation

### 4.1 Chinese Medicine Formulas and T Lymphocyte Subsets

Wang et al.<sup>42</sup> applied Jiawei Chaishao Liujun Granules (Bupleurum 10 g, Codonopsis 15 g, Paeonia, Poria, and stir-fried Atractylodes 15 g each, Citrus peel 6 g, Pinellia 10 g, Licorice 6 g, with modifications according to syndrome) to treat IBS-D patients with liver depression and spleen deficiency for 4 weeks, compared with a Western medicine group (Bifico capsules, twice daily, 4 capsules each time). The results showed that the TCM group effectively reduced Traditional Chinese Medicine syndrome scores, visual analog scores, and stool form scores, increased the proportion of CD3<sup>+</sup> and CD4<sup>+</sup> in peripheral blood, and decreased the CD8<sup>+</sup> proportion, thereby improving immune disorders. Su et al.<sup>43</sup> used Wenshen Jianpi Formula (Nutmeg 15 g, Psoralea 30 g, Schisandra 9 g, Evodia 9 g, Codonopsis 30 g, Atractylodes 15 g, Curcuma 18 g, Ginger 2 g, Jujube 2 g) to treat IBS-D rats induced by “acetic acid enema + senna leaf gavage + tail clamping,” compared with the Sishen Pill group. The study confirmed that this formula could reduce CD45<sup>+</sup>, regulate visceral hypersensitivity and immune imbalance, with effects significantly superior to the control group. Tan et al.<sup>44</sup> administered Fuzi Lizhong Decoction (light-processed aconite slice 6 g, dried ginger 3 g, Codonopsis 10 g, Atractylodes 10 g, roasted licorice 3 g) via gavage to IBS-D rat models replicated by “restraint stress combined with senna leaf gavage.” The results showed significantly reduced expression of colonic mucosal NALP-3 inflammasome, CD4<sup>+</sup> cells, and mast cell numbers, confirming that Fuzi Lizhong Decoction could alleviate intestinal inflammation and regulate immunity by modulating colonic mucosal mast cell and T lymphocyte functions. Ma et al.<sup>45</sup> showed that Wumei Pill combined with acupoint application (Wumei Pill: Wumei and Codonopsis 15 g each, Phellodendron, dried ginger, light-processed aconite slice, cinnamon twig, and Angelica 10 g each, Sichuan pepper and Coptis 5 g each, Asarum 3 g, with modifications; herbal paste formula: white mustard seed, Evodia, cinnamon, Magnolia bark, and Bupleurum in equal parts, ground into fine powder, mixed with mature vinegar, applied to Shenque (CV8) and Zhongwan (CV12) acupoints, once daily for 6 hours each time) achieved a cure rate of 32.5% for IBS-D with

cold-heat complex syndrome, significantly higher than the pinaverium bromide group (12.5%). The main mechanism involves downregulating IFN- $\gamma$  and IL-2 while upregulating IL-4 and IL-10, shifting the Th1/Th2 balance rightward and restoring intestinal immune balance. Zhao et al.<sup>46</sup> found that Tongxie Anchang Formula (stir-fried *Atractylodes* 15 g, blast-fried ginger 9 g, *Paeonia* 12 g, *Wumei* 9 g, Citrus peel 6 g, Cicada slough 6 g, *Coptis* 6 g) could improve stool characteristics, water content, and abdominal withdrawal reflex in IBS-D mice with liver depression and spleen deficiency by reducing Th17 polarization and increasing Treg polarization in mesenteric lymph nodes, thereby decreasing the Th17/Treg ratio, with medium and high-dose groups showing more significant efficacy.

#### 4.2 Chinese Medicine Formulas and Immunoglobulins

Wu et al.<sup>47</sup> applied Fuzi Lizhong Decoction (*Codonopsis* 10 g, *Atractylodes* 10 g, dried ginger 3 g, light-processed aconite slice 6 g, roasted licorice 3 g) to treat IBS-D rats replicated by “restraint stress combined with senna leaf purgation.” The results showed increased serum CD4<sup>+</sup> and CD4<sup>+</sup>/CD8<sup>+</sup> ratio and decreased IgA and IgG concentrations, confirming that Fuzi Lizhong Decoction regulates systemic immunity through both humoral and cellular immunity.

#### 4.3 Chinese Medicine Formulas and Mast Cells

Si Ni San (*Bupleurum*, *Paeonia*, *Aurantium* fruit, Licorice, dosage not specified) can improve intestinal dysfunction in chronic stress-induced IBS-D rats, with the main mechanism closely related to inhibiting the generation of enterochromaffin cells and mast cells in the ileum<sup>48</sup>. Wei et al.<sup>49</sup> used Tongxie Yao Fang Granules (medicinal ingredients: *Saposhnikovia* 31.25 g, stir-fried Citrus peel 46.875 g, *Paeonia* 62.5 g, stir-fried *Atractylodes* 93.75 g) to treat IBS-D rats with liver depression and spleen deficiency induced by “tail clamping combined with senna leaf gavage.” The results showed significantly reduced expression of corticotropin-releasing hormone receptor 1 (CRH-R1) and mast cell numbers in intestinal mucosa, confirming that Tongxie Yao Fang can regulate intestinal immunity and visceral hypersensitivity by inhibiting CRH-R1 expression and affecting the CRH pathway.

In summary, Chinese medicine formulas can effectively treat IBS-D by regulating the dynamic balance of T lymphocyte subsets, reducing immunoglobulin levels, and decreasing mast cell numbers, thereby modulating immunity, suppressing inflammation, and reducing visceral hypersensitivity. Details are summarized in Table 2 .

### 5 Summary and Outlook

The main symptoms of IBS-D, such as diarrhea and abdominal pain, can be classified under the categories of “diarrhea” and “abdominal pain” in Traditional Chinese Medicine. The disease location is in the intestines, and the primary

affected organ is the spleen, with the pathogenesis being spleen deficiency and dampness excess<sup>1, 50</sup>. The therapeutic principles of TCM for IBS-D consistently involve “harmonizing the spleen” through methods such as strengthening the spleen, tonifying the spleen, activating the spleen, and awakening the spleen. The *Huangdi Neijing · Lingshu* states: “Among the five viscera and six bowels, the heart is the master...the spleen is the guard, and the kidney is the exterior.” The spleen is the source of qi and blood generation. When food enters the stomach, it undergoes digestion by the stomach and transformation by the spleen to produce essential substances. The clear and refined components transform into nutritive qi, while the vigorous and slippery components transform into defensive qi. When the spleen and stomach function robustly, qi and blood are generated abundantly, ensuring defensive qi is plentiful, circulates and distributes throughout the body, warms the body, regulates the interstitial spaces, and expels pathogenic factors, thereby protecting the body and resisting external evils. This concept aligns perfectly with immune system function in modern medicine. The “spleen” in TCM encompasses not only its anatomical location and physiological functions but also includes the spleen, pancreas, gastrointestinal tract, and the digestive, immune, endocrine, and hematological systems centered around it. The concept of “spleen as the guard” highly corresponds to body immunity. Through systematic review of the pathogenic mechanisms of immune imbalance in IBS-D and the immunomodulatory effects of Chinese medicine, the correspondence between Chinese and Western medical theories is once again confirmed. Chinese medicine can exert therapeutic effects by intervening in different immune mediators, enhancing the “spleen as the guard” function, restoring immune homeostasis, reducing inflammatory responses, decreasing visceral hypersensitivity, slowing intestinal motility, and repairing the mucosal barrier.

This article has organized the important mechanisms of immune imbalance in IBS-D pathogenesis related to intestinal infection, dysbiosis, brain-gut axis disorders, and endocrine disturbances, as well as the targets of Chinese medicine immunotherapy for this disease, summarizing them based on the “spleen as the guard” theory. TCM has made meaningful progress in treating IBS-D; however, formulas are not simple additive combinations of drug components but rather organic compositions. On this basis, modern techniques such as metabolomics, liquid chromatography, and mass spectrometry can be employed to further screen the components and efficacy of Chinese medicines for IBS-D treatment, identifying optimal component combinations and dosage ratios.

Nevertheless, research on TCM treatment of IBS-D through immune regulation still has certain limitations. First, when evaluating different syndrome-type IBS-D animal models, there are no specific, quantitative, or recognized indicators and assessment standards; models are evaluated solely based on modeling methods and external animal manifestations, lacking accuracy. Second, mechanistic exploration of Chinese medicine intervention in immune therapy for IBS-D based on the “spleen as the guard” theory remains limited in scope and superficial in depth, with insufficient literature. Although gene modification, histone

modification, methylation, microRNA (miRNA), long non-coding RNA, and autophagy are all involved in IBS-D pathogenesis to varying degrees, related TCM intervention studies are still scarce. Meanwhile, this points the direction for further clinical exploration of the correlation between the TCM spleen and immunology and genetics, expanding the clinical application of classic formulas, clarifying precise dosage modifications and compatibility, studying specific drug effects and mechanisms, and laying the foundation for the inheritance, innovation, and modernization of Chinese medicine.

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